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# CHARACTERIZATION OF PHYTOCHEMICALS AND PHYSICOCHEMICAL PROPERTIES OF *ROSMARINUS OFFICINALIS*

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## ABSTRACT

Herbal plants contain a diverse array of bioactive compounds with medicinal properties and have been widely used for the treatment of various health issues for decades. *Rosmarinus officinalis* is a well-known medicinal plant commonly employed to treat different health conditions and enhance memory. In present study rosemary was analyzed for its compositional, physicochemical, and phytochemical properties. Proximate composition analysis revealed the following values: moisture content (9.54±0.7%), ash content (7.37±0.3%), crude fiber content (19.38±0.77%), crude fat content (14.99±0.44%), crude protein content (5.23±0.26%), and nitrogen free content as (43.47±1.75%). Among the minerals rosemary contains Potassium (1669.71±2.09 mg/100g), Calcium (1073.14±2.93 mg/100g), Magnesium (39.98±0.49 mg/100g), Phosphorous (457.72±2.45 mg/100g), Iron (36.01±0.05 mg/100g) and Sodium (81.79±1.08 mg/100g). Furthermore, ethanolic extract exhibited highest value of total phenolic content (242.58±2.98 mg GAE/g), total flavonoid content (296.95±2.04 µg CE/g) and DPPH assay (78.04±2.33%) as compared to aqueous and methanolic extracts. These results strongly suggest the nutritional and pharmacological potential of rosemary, supporting its use in both therapeutic and nutritional formulations.

**Keywords:** *Rosmarinus officinalis*, Bioactive compounds, Antioxidants, Nutritional profile, Medicinal plant

## 1. INTRODUCTION

Rosemary, a perennial shrub belonging to the family Lamiaceae, is originally instinctive to the Mediterranean region but is now cultivated worldwide due to its numerous beneficial properties. It is extensively utilized as a food flavoring and preservative agent, and it also serves as a medicinal plant (Hamidpour *et al*., 2017). *Rosmarinus officinalis L.* is the scientific name of rosemary which is combination of two Latin words ros and marinus which means dew and sea respectively. So in Latin Rosemary means ‘dew of the sea’ (Begum *et al*., 2013). Another potential explanation for the name's origin is the combination of the Greek words "rhos" and "mirrinos," signifying shrub and aromatic, respectively, reflecting its distinctive characteristics (Borges *et al*., 2019). The species name

*"officinalis"* denotes its use as a medicinal plant. Among the ancient Greeks, it earned the names 'antos,' representing the flower for brilliance, or 'libanotis,' attributing to its fragrance (Pintore *et al*., 2002).

It is an evergreen shrub with distinctive fragrant needle-shaped dark green leaves featuring curved edges and small purple, white, blue, or pink flowers. Thriving best in light, dry, and sandy soil, it serves not only as an ornamental plant but also as a commercially grown herb. There are approximately 20 varieties of rosemary, each distinguished by differences in calyx, corolla, presence of glandular trichomes, leaf size, and inflorescence (Hanson, 2016). Rosemary *(Rosmarinus officinalis L.)* holds significance in the European market, utilized in various forms including fresh or dried, as oil, or oleoresin, owing to its well-established culinary and medicinal properties. The plant can grow up to 2 meters in height and features an extended flowering season from April to August (Macedo *et al*., 2020). Rosemary essential oil serves as a seasoning for food items such as meat, salami, and sauces. Beyond its culinary uses, it is employed as an antioxidant for food preservation, as well as an antibacterial and antifungal agent against certain spoilage organisms (Ancuţa *et al*., 2008).

In the past, dysmenorrhea and renal colic have been treated medically using rosemary. It was additionally utilized to promote hair growth and treat the symptoms of respiratory problems. These days, anxiety-related disorders are frequently treated with rosemary extracts in aromatherapy to boost attentiveness. The components of rosemary that have been researched the most include rosmarinic acid and its derivative, caffeic acid. These substances are being studied as possible treatments for inflammatory diseases, cancer, and hepatotoxicity because they may have antioxidant qualities (Ulbricht *et al*., 2010). The food sector worldwide has been using rosemary extract, one of the most widely utilized antioxidants obtained from natural sources. It is widely used across various food applications, such as meat products, snacks, roasted nuts, frying oils, and fish oils (Xie *et al*., 2017).

The efficacy of pharmacological drugs decline over time and may also cause harmful side effects on the body. Due to these reasons scientists look for herbal medicinal plants to synthesized pharmacological drugs. Investigation of plant phytochemicals is essential for this purpose and various phytochemicals underwent clinical studies to explore their efficacy (Shankar *et al*., 2024). Rosemary offers not only culinary uses but also pharmacological properties highlighting its application in medicinal field. The bioactive components of rosemary including flavonoids, rosmarinic acid and carnosic acid exhibit 90% of antioxidant potential, which plays a key role in health promotion (Rahbardar and Hosseinzadeh, 2020). Thus, the present study was planned to analyze physicochemical and phytochemical properties of rosemary.

## 2. MATERIALS AND METHODS

### 2.1 Procurement and preparation of raw material

Rosemary leaves were obtained from the botanical garden of University of Agriculture, Faisalabad. The leaves were thoroughly washed and sun-dried for one week. After drying, they were ground into fine powder using a laboratory grinder and stored in airtight jars for further analysis.

### 2.2 Proximate analysis of rosemary powder

Rosemary leaves powder was analyzed for crude protein, moisture, ash, crude fiber, crude fat, and nitrogen free extract in triplicate in accordance to the procedures of AOAC (2019).

### 2.2.1 Moisture content

The moisture content of rosemary leaves powder was measured according to method as described in AOAC (2019). Samples from powder were taken and placed in clean china dish and weight was measured. China dish containing a sample of powder was placed at 105$^{o}$C for 24 hours in hot air oven for drying. After 24 hour samples were taken out of oven and weight was measured.

$$\text{Moisture \%} = \frac{\text{Weight of powdered sample} - \text{Weight of dried sample}}{\text{Weight of powdered sample}} \times 100$$

### 2.2.2 Ash content

AOAC (2019) procedure was followed to analyze the ash content of rosemary leaves powder. 3g of rosemary powder was weighed precisely and taken in crucible. Crucible having sample was exposed to flame to make smoke-less. Then the crucible was placed in muffle furnace for 5 hours at 550°C until a white grayish residue sample was obtained. After removing the sample from the muffle furnace it was immediately cooled in desiccators and weighed. The below given formula was used to calculate ash content:

$$\text{Ash content (\%)} = \frac{(\text{Weight of crucible with ash}) - (\text{Weight of crucible})}{\text{Weight of powdered sample}} \times 100$$

### 2.2.3 Crude protein

The percentage of nitrogen content of rosemary leaf extract powder was measured according to AOAC (2019) by using Kjeldhal apparatus. 2 g of powdered sample was placed into the flask and 5g digestion mixture was also incorporated in it. Then 30 ml of 98% conc. sulfuric acid was also added in flask. The digestion was done through heating the sample and digested for about 2-3 hours till dull greenish color appeared. After the digestion same mixture was taken in 250 ml capacity flask and distilled $H_2O$ was added to make the volume complete. From diluted sample 10ml solution was taken in flask of Kjeldhal apparatus and about 10 ml NaOH was added. Steam was provided to release ammonia in gaseous form and ammonia gas was trapped in boric acid (4%) solution which contained ethylene blue and methyl red indicator. Amount of $NH_3$ trapped in boric acid titrated with sulphuric acid solutions (0.1 N) till purplish end point obtained. The formula given below was used for calculation:

$$\text{Nitrogen (\%)} = \frac{\text{Volume of 0.1 N H2SO4 used} \times 250 \times 0.0014}{\text{Weight of sample} \times 10} \times 100$$

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

### 2.2.4 Crude fat

The crude fat content was determined by succeeding the procedure described by AOAC (2019). Fat was measured by using Soxhlet apparatus. 5 g of powder was wrapped in filter paper and then sample was put in thimble. Hexane was used at condensation rate of approximately 2-3 drops per second for about minimum 16 hours. After distillation of excess hexane, the extraction flask containing residue was dried for 30 minutes at 100°C to obtain constant weight. Crude fat was determined by given below formula:

$$\text{Crude fat (\%)} = \frac{\text{Weight of extracted fat}}{\text{Weight of powdered sample}} \times 100$$

### 2.2.5 Crude fiber

The powder samples underwent fiber content analysis following the procedure outlined in AOAC (2019). Initially, 2 g of fat-free samples were placed in a 500 ml beaker and 1.25% sulfuric acid was added to the beaker. The contents were allowed to stand for a complete 30 minutes and were then subjected to filtration and acid washing. After the sample was acid-free filtered, residues were shifted to another beaker containing 1.25% NaOH and boiled again for half an hour for digestion. After complete digestion of sample contents were filtered and washing was done for 2-3 times to make sample alkali free. The sample residues were shifted in China dish for drying in oven at 100°C until constant weight was obtained. Then sample was made completely smoke free and kept in muffle furnace for approximately 4 hours at 550°C until a white grayish residue sample was obtained. The difference in both of weights was calculated by using given equation:

$$\text{Crude fiber (\%)} = \frac{\text{Weight loss on ignition}}{\text{Weight of powdered sample}} \times 100$$

### 2.2.6 Nitrogen free extract

It was assessed by the difference method: moisture content, ash content, crude protein, crude fat and crude fiber subtracted from total(hundred) as following formula

Nitrogen free extract%= 100 – (moisture% in sample +crude fiber%+crude fat%+crude protein%+ash residues %)

### 2.3 Mineral analysis

The mineral profiling of rosemary powder was carried out using the AOAC (2019) procedure. Specifically, potassium (K) and sodium (Na) levels were analyzed using a Flame photometer (Sherwood Model 410), while the measurement of iron, calcium, phosphorous, and magnesium was conducted using an atomic absorption spectrophotometer (Perkin Elmer).

### 2.4 Extract preparation

Rosemary extract was prepared using ethanol, methanol, and water, with slight modifications to the orbital shaker method described by Anjum et al. (2021). 20g of sample and 200 mL of solvents were added to flasks and they were secured with foil paper to prevent solvent evaporation. The flasks were shaken continuously at a steady 250 rpm speed in a temperature controlled orbital shaker for 8 hours. The resulting mixture was then concentrated in a rotating vacuum evaporator (Eyela OSB-2100) at 40-$^0$C after being filtered through Whatman filter paper no. 4. Then these extracts were further dried in hot air oven. The resultant extracts were stored for further analysis.

### 2.5 Phytochemical screening

#### 2.5.1 Total phenolic content (TPC)

The total phenolic content (TPC) of the extracts was determined by Folin-Ciocalteu (FC) technique as described by Naz *et al*. (2016) with minor alterations. Different concentrations of Gallic acid were used to prepare the calibration curve. Gallic acid (GA) solutions were prepared in methanol, with concentrations ranging from 0.01 to 0.10 mg/mL. These solutions were then mixed with 5 mL of Folin-Ciocalteu reagent (diluted tenfold) and 4 mL of 20% sodium carbonate. After allowing an hour for the reaction, the absorbance was measured at 765 nm. A calibration curve was established by plotting absorbance against concentration. Simultaneously, the same reagent was added to 1 mL of rosemary extract (0.001 g/mL), and after 60 minutes, the absorbance of the obtained blue color complex was measured at 765 nm. To quantify the total amount of phenolic compounds in rosemary extracts, Gallic acid (GA) was used as the standard, and final calculation was done by applying the formula provided by Sharif *et al.* (2018) to calculate Gallic acid equivalents (GAE).

#### 2.5.2 Total flavonoid content (TFC)

The procedure described by Rehman *et al*. (2013) was employed to analyze the total flavonoid content (TFC) of plant extracts. In summary, a mixture comprising 2 mL of distilled water, 0.15 mL of 5% $NaNO_2$ solution, and 0.5 mL of plant extract was prepared. The mixture underwent a six-minute incubation period. Subsequently, after introducing 0.15 mL of a 10% $AlCl_3$ solution, the mixture underwent another six-minute incubation period before the addition of a 4% NaOH solution. To achieve a final volume of 5 mL, methanol was added and thoroughly mixed with the reaction mixture. Following a 15-minute incubation, the absorbance of the resulting mixture was measured at 510 nm, as specified by Ayub *et al*. (2017). The total flavonoid contents (TFC) of the extracts were quantified by expressing them as microgram catechin equivalents per mL of the plant extract, utilizing the catechin linear regression curve.

### 2.6 Anti-oxidant assay

#### 2.6.1 Free radical scavenging ability (DPPH assay)

The anti-oxidant activity of plant extracts was evaluated through the DPPH radical scavenging assay, following the procedure outlined by Shahid *et al*. (2014). In accordance with this method, 3 mL of rosemary extract was mixed with 1 mL of a freshly prepared 0.004% DPPH solution in methanol. The mixture was then kept in darkness for thirty minutes, and the absorbance was subsequently measured at 517 nm. A reaction mixture displaying low absorbance indicates a high capacity to scavenge free radicals. The anti-oxidant activity of ascorbic acid and BHT was also determined for

reference. Additionally, a solution without plant extract was used as a control (Naseem *et al*., 2020). This process was repeated three times, and the percentage inhibition of DPPH radical samples was calculated using the following formula

DPPH Inhibition (%) = $A_0$-$A_1$/$A_0$*100

Where: $A_1$ = Absorbance of the sample $A_0$ = Absorbance of the blank

## 3. RESULTS AND DISCUSSION

### 3.1 Proximate composition

The compositional analysis has an authoritative effect for determining quality features and for usage in functional food production, as well as for understanding the essential role of raw material in research (Ganogpichayagrai and Suksaard, 2020). The mean values of the proximate analysis parameters are given in Table 1 including, the moisture content (9.54±0.7%), ash content (7.37±0.3%), crude fiber content (19.38±0.77%), crude fat content (14.99±0.44%), crude protein content (5.23±0.26%), and nitrogen free content (NFE) as (43.47±1.75%).

The results of current study for rosemary leaves powder chemical composition analysis are in harmony with earlier results of Sharma and Dhuria (2021). They determined the composition of rosemary leaf powder firstly by determining the dry matter and then analyzed other parameters of proximate nutrient analysis. The results showed dry matter as 91.5%, crude protein 5.15%, crude fiber 4.52%, total ash 7.50%, ether extract 15.20%, NFE 67.63% and acid insoluble ash 1.7%. The results are also in synchronization with the results of Attia (2018) which indicated that dry matter, ash, crude protein, ether extract, crude fiber, and nitrogen free extract were as 91.59%, 6.53%, 11.12%, 18.50%, 9.20% and 46.24%. Similarly, the outcomes are also in line with the previous findings of ELnaggar *et al*. (2016), who stated moisture content as 8.62%, crude protein content 5.08%, ether extract 16%, crude fiber content 18.94%, ash content 7.52% and nitrogen free extract 43.84%. The variations in composition may be attributed to differences in geographical location, stage of maturity, soil characteristics, and environmental conditions.

**Table 1: Proximate composition of rosemary leaves powder**

| **Parameters** | **Quantity %** |
|---|---|
| **Moisture** | 9.54±0.7 |
| **Ash** | 7.37±0.3 |
| **Crude fiber** | 19.38±0.77 |
| **Crude Fat** | 14.99±0.44 |
| **Crude protein** | 5.23±0.26 |
| **NFE** | 43.47±1.75 |

### 3.2 Mineral analysis

Mineral profiling in the current study covered the determination of Phosphorous (P), Magnesium (Mg), Potassium (K), Sodium (Na), Calcium (Ca), and Iron (Fe). Mineral composition of leaves is given in Table 2. The results indicate that rosemary contains Potassium (1669.71±2.09 mg/100g), Calcium (1073.14±2.93 mg/100g), Magnesium (39.98±0.49 mg/100g), Phosphorous (457.72±2.45 mg/100g) in high amounts in comparison with other mineral like Iron (36.01±0.05 mg/100g) and Sodium (81.79±1.08 mg/100g). The fallouts of present study are in accordance with the results of Ali *et al*. (2021) who confirmed the amounts of Sodium, Potassium, Calcium, Phosphorus, Magnesium and Iron in amounts as 92.31 ± 0.577mg/100g, 2035.51 ±3.89mg/100g, 1246.35 ± 4.49 mg/100g, 477.29 ± 5.51 mg/100g, 45.95 ± 1.05 mg/100g and 45.36 ± 1.09 mg/100g. Their results indicated that rosemary leaves contains abundant amount of Potassium and Calcium in case of macro-minerals. While in the case of micro-minerals Iron and Zinc are present in high amounts.

The fallouts are also strengthened by the earlier findings of Arslan and Ozcan (2008) who analyzed the mineral content of fresh, sun dried, oven dried, and microwave oven dried. The results of sun dried rosemary minerals like Calcium, Iron, Potassium, Magnesium, Sodium and Phosphorus are as 12343.27 ± 794.15, 534.70 ± 29.14, 19612.54 ± 1002.55, 2617.90 ± 25.08, 4377.31 ± 151.16, 2105.74

± 129.11 mg/kg. The fallouts are also in collaboration with the effort of Zeroual *et al*. (2021) who determined the macro and micro minerals present in wild and cultivated rosemary. The fallouts indicated that Calcium, Potassium, Sodium, Magnesium and Iron are as 3.005 ± 0.003, 3.024 ± 0.002, 0.150 ± 0.002, 1.920 ± 0.003 and 0.005 ± 0.001 mg/g respectively in cultivated rosemary.

**Table 2: Mineral Analysis of rosemary leaves powder (mg/100g)**

| Mineral Composition | Quantity (mg/100g) |
|---|---|
| **K** | 1669.71±2.09 |
| **Ca** | 1073.14±2.93 |
| **Mg** | 39.98±0.49 |
| **P** | 457.72±2.45 |
| **Fe** | 36.01±0.05 |
| **Na** | 81.79±1.08 |

### 3.3 Phytochemical screening

#### 3.3.1 Total Phenolic content (TPC) and Total Flavonoid content (TFC)

Mean values regarding total phenolic content of methanolic, ethanolic and aqueous extracts (196.57±1.01, 242.58±2.98 and 217.94±1.44 mg GAE/g, respectively) and total flavonoid content of methanolic, ethanolic and aqueous extracts (200.87±4.66, 296.95±2.04 and 102.83±2.02 µg CE/g respectively) of rosemary leaves are provided in Table 3. The comparison of mean values showed that they are statistically significant (Table 3). The fallouts showed that ethanolic extract contains the highest total flavonoid and phenolic content.

The results are in collaboration with the fallouts of Saini *et al*. (2020) who extracted rosemary by using 70% ethanol and they analyzed total phenolic content as 136.66±7.41 mg of Gallic acid/g and total flavonoid content as 37.13±6.04 mg rutin/g. Chan *et al*. (2012) determined the TPC of fresh rosemary as 1440 ± 94 mg GAE/100 g and TFC as 340 ± 75 mg QE/100 g. Jalloul *et al*. (2022) quantified the total phenolic and total flavonoid content of aqueous extract of rosemary and they obtained the results as 32.65±0.46 mg GAE/g and 14.56±0.78 mg CE/g, respectively. In another study conducted by Salamatullah *et al*. (2021) total phenolic and total flavonoid content of rosemary was measured after boiling for different times. They boiled rosemary for 5,10 and 15min and obtained TPC as 122.84 ± 5.79, 119.24 ± 2.47 and 140.43 ± 4.44 mg GAE/g dw and TFC as of 78.36±1.55, 86.85±2.80, and 109.73±0.33 (mg CE/g dw), respectively.

**Table 3: Comparison of mean values for TPC, TFC and DPPH**

| Extracts | TPC (mg GAE/g) | TFC (µg CE/g) | DPPH (%) |
|---|---|---|---|
| **Ethanolic** | 242.58±2.98$^{A}$ | 296.95±2.04$^{A}$ | 78.043±2.33$^{A}$ |
| **Methanolic** | 196.57±1.01$^{C}$ | 200.88±4.66$^{B}$ | 75.543±2.54$^{A}$ |
| **Aqueous** | 217.941.44$^{B}$ | 104.16±2.02 $^{C}$ | 22.1571.93$^{B}$ |

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05)

Similarly, Zeroual *et al*. (2021) extracted wild and cultivated rosemary leaves by using different solvents i.e methanol, ethanol, hexane and ethyl acetate. They obtained total phenolic content of extract of wild rosemary as 34.72 ± 1.65, 30.88 ± 1.20, 15.00 ± 1.93 and 25.13 ± 1.11 mg GAE/g respectively, while in case of cultivated rosemary's extract TPC was 33.59 ± 0.63, 26.43 ± 1.55, 12.48 ± 1.17 and 23.56 ± 3.05 mg GAE/g respectively. They obtained total flavonoid content of methanolic extract of wild rosemary as 25.02 ± 1.53, 16.55 ± 2.06, 9.35 ± 2.34 and 12.19 ± 1.24 mg QE/g respectively while in case of farmed rosemary's methanolic extract TPC was 22.96 ± 1.88, 14.77 ± 1.58, 8.01 ± 2.64 and 10.69 ± 1.58 mg QE/g respectively. Zheng *et al*. (2019) determined that methanol gives better extraction yield as compared to ethanol but the residue is toxic that's why ethanolic extracts are more suitable.

### 3.4 Free radical scavenging ability (DPPH assay)

The results regarding DPPH free radical scavenging activity (% inhibition) elucidated that peak value of DPPH Assay 78.04±2.33% was observed in ethanolic extract followed by methanolic (75.54±2.54) and aqueous extract (22.15±1.93%). Additionally, the mean values of DPPH assay concerning ethanolic and methanolic extracts were not statistically different (Table 3).

The findings regarding DPPH assay are reinforced by the outcomes of Wanga *et al*. (2018), they extracted rosemary leaves by using 80% ethanol for 15, 30, 60, 120 and 180 minutes and obtained the DPPH scavenging ability as 94.07 ± 0.05, 94.84 ± 0.17, 94.79 ± 0.20, 94.59 ± 0.09 and 94.68 ± 0.10%. While in terms of $EC_{50}$ of DPPH radical scavenging activity the result was 1.90 ± 2.51 µg/mL which indicated the strong anti-oxidant potential of rosemary ethanolic extract.

Zeroual *et al*. (2021) assessed DPPH activity of rosemary on the basis of $IC_{50.}$ The moles of phenolic compounds divided by the moles of DPPH required to reduce by 50% the absorbance of DPPH. So the lower value of $IC_{50}$ shows higher anti-oxidant activity. In this study they extracted the rosemary leaves by using methanol, ethanol, ethyl acetate and hexane and their $IC_{50}$ values are 50.02 ± 0.08, 120.17 ± 0.35, 190.03 ± 0.45 and 265.00 ± 0.20 respectively. So, the results explained that ethanol and methanol have more anti-oxidant ability as compared to other two solvents. The findings of Muzolf-Panek and Stuper-Szablewska (2021) indicated that DPPH of aqueous extract of rosemary was 12.71±0.53 µmol TE/g after the extraction period of half hour. While after the extraction period of 24 hours the DPPH was 69.28±11.40 µmol TE/g.

## CONCLUSION

A comprehensive analysis of *Rosmarinus officinalis* showed the presence of number of bioactive compounds, highlighting its potential as a powerful medicinal and nutritional tool. This herb can be effectively incorporated into the formulation of different dietary supplements, nutraceuticals, and functional foods because of its rich nutritional profile and potential to treat different health problems. These findings pave the way for future research to explore its specific health benefits and elucidate the underlying mechanisms.

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