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Comprehensive Analysis of Physicochemical and Antioxidant Properties of Hydro Distilled Rosemary (*Rosmarinus officinalis L.*) Essential Oil

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In recent years, there has been a growing need to enhance the quality of essential oils due to their diverse applications in the food, pharmaceutical, and perfumery industries. Rosemary essential oil, derived from the leaves of *Rosmarinus officinalis L*., has garnered attention for its multifaceted properties, including antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic attributes, making it a potential therapeutic agent. This study focused on extracting rosemary essential oil using the hydrodistillation method and assessing its physicochemical properties. The physical parameters of the oil, including appearance, odor, solubility, specific gravity, refractive index, and density, were analyzed, revealing its unique characteristics. The chemical parameters, such as acid value, peroxide value, iodine value, saponification value, and ester value, provided insights into the composition and quality of the oil. Additionally, the antioxidant activity of rosemary essential oil was evaluated through *DPPH, FRAP,* and *ABTS* assays at various concentrations, demonstrating its significant antioxidant potential. These findings highlight the quality and potential applications of rosemary essential oil in diverse industries, emphasizing its role as a natural source of antioxidants with promising prospects for further exploration and utilization in food industry as a preservative and natural flavorant.

Keywords: Rosemary; essential oil; hydrodistillation; physio-chemical properties; antioxidant activity.

1. INTRODUCTION

"Essential oils are mixtures of volatile and non-volatile compounds that are derived from different parts of an aromatic plant. In recent years, there is a necessity to improve the quality of the essential oils due to its use in food, pharma and perfumery products" [1]. "Particularly, in leaves and flowers, the essential oil is secreted from glandular trichomes and the highest yield is achieved from the leaves. *Rosmarinus officinalis L*., commonly known as rosemary, is a shrub belonging to the *Lamiaceae* family and native to the Mediterranean basin. This plant has been widely used in traditional medicines and also been used as a food preservative and flavoring agent. Rosemary contains an essential oil to which it owes its interesting properties. Rosemary essential oil is known for its antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antidiabetic, antinociceptive, antithrombotic and antiulcerogens, diuretics and hepatoprotective properties. These biological properties have made rosemary a potential new therapeutic agent in the treatment of many diseases" [2].

"Rosmarinus officinalis L. essential oil can be extracted from leaves by several techniques, such as steam distillation, supercritical fluid extraction, microwave extraction and hydro-extraction etc" [3]. "The extraction of essential oils from plant materials is a crucial step in obtaining the desired components in an efficient manner. Each method has its advantages and limitations in terms of yield, composition and environmental impact. Some techniques cause the loss of certain volatile compounds due to long extraction times and degradation of unsaturated or esterified compounds by thermal or hydrolytic effect. For example, monoterpenes may be susceptible to chemical changes under steam distillation conditions and even the conventional solvent extraction during removal of solvent by distillation" [2]. The hydrodistillation method is a prevailing technique isolation of essential oils from plant materials due to the simplicity of installations and ease of performing. Hence the study aimed to extract rosemary essential oil using a hydro distillation method and evaluate its physicochemical, antioxidant properties. It provides valuable insights into its stability, safety and potential applications of rosemary essential oil.

2. MATERIALS AND METHODS

The rosemary leaves were harvested from the Horticulture herbs production field, University of Agricultural Sciences, GKVK, Bengaluru during June-July season, 2022. It consists of young leaves, branches of rosemary and hand-picked rosemary flowers. Rosemary branches are cut into pieces with length of about 15 cm from the tip. This size is proven to be optimal to achieve high essential oil content. The rosemary leaves were dried at room temperature for 10 days to remove moisture and stored in a moisture free environment for further processing. Chemicals required for the research study was procured from local market of Bengaluru.

2.1 Extraction of Rosemary Essential Oil

The experimental set up for the extraction of essential oils from rosemary leaves using hydro

distillation is shown in Fig. 1. For the extraction of essential oils from rosemary leaves by hydro distillation under optimal operating conditions, a quantity of 100 g of dried rosemary was added to 800 ml of distilled water in a two liter flask. The set was placed in a balloon heater attached to a refrigerator to ensure condensation of essential oils for 6 hours. At the end of the distillation, two phases were observed, an aqueous phase (aromatic water) and an organic phase (essential oil), less dense than water. The essential oil was collected, dried under anhydrous sodium sulphate and stored in sealed vials in the dark, at 4°C, until used. Experiments were conducted twice for each condition.

Fig. 1. Experimental set-up for extraction of rosemary essential oil by Hydro distillation

2.2 Analysis of Physical Parameters

2.2.1 Calculation of oil yield

The yield of essential oils derived from rosemary leaves in each experiment was calculated as follows:

Yield (%) = Amount of extracted oil (g) / Amount of dry rosemary leaves mass (g) × 10

2.2.2 Sensory analysis of the essential oil

Sensory analysis was carried out on the oil to determine its physical properties. This involved sense of sight, smell and touch.

2.2.3 Determination of solubility of the essential oil in water

A few drops of the oil were added to a test tube containing little amount of water. The test tube was stirred thoroughly with a stirring rod. Two separate phases were observed. The insolubility of the oil in water was inferred from that operation.

2.2.4 Determination of density of the oil

The density of the oil extracted was determined by weighing an empty beaker and recording its value. Thereafter, essential oil was poured into the beaker and the weight was taken. The density of the oil was thus calculated using formula.

$$\text{Density} = \frac{\text{Weight of oil sample}}{\text{Volume of oil sample}} \times 100$$

2.2.5 Determination of specific gravity

All experiments were carried out in triplicates, and average values taken. A clean and dry bottle was weighed using a weighing balance. Distilled water was poured into the bottle and weighed. In the same manner, the same volume of oil was poured into the same bottle and weighed. The specific gravity was calculated using formula

$$\text{Oil specific gravity} = \frac{Weight\ of\ volume\ of\ oil\ extract}{\text{Weight of equal volume of water}} \times 100$$

2.6 Determination of refractive index

This was determined as reported by Abozid And Asker [4]. A digital refractometer was used to determine the refractive index of the essential oil. Water at room temperature was circulated around the glass slide to keep the temperature uniform and also to normalize the refractometer. A syringe and needle were used to put few drops of oil into the glass slide of the refractometer, and the reading was recorded. All experiments were carried out in triplicates, and average values taken.

2.2.7 Chemical characteristics of essential oil

The different chemical characteristics like acid value, iodine value, saponification value, peroxide value and ester value of extracted essential oil were carried out.

2.3 Acid Value

“The acid value is the number of milligrams of KOH required to neutralize the free fatty acid present in 1g of fat. Hence acid value gives an indication of the age and quality of the fat. Free fatty acid percentage (as oleic) was determined

by titrating oil in neutralized ethanol (95%) against NaOH solution" [5]. "The free fatty acid in oil is estimated by titrating it against KOH in presence of phenolphthalein indicator. The acid number is defined as 1 g of sample. However, the free fatty acid (FFA) is expressed as oleic equivalents" [6].

1 ml N/10 KOH= 0.028 g Oleic acid. Acid value can be determined by the formula:

Acid value = % FFA x 1.99

2.4 Peroxide Value

"Peroxide value was evaluated according to AOCS Official Method Cd 8-53 (2003). Weigh out 5g of oil into a 500 ml conical flask, add 30 ml acetic acid chloroform mixture and dissolve the oil. Add 0.5 ml of saturated KI solution mix well and allow standing for 1 min. Add 30 ml of water, 3-4 drops of starch indicator and mixing well. Titrate against standard 0.01 N sodium thiosulphate with vigorous shaking to liberate all from chloroform layer until the blue color just disappears" [6]. Treat the blank similarly in the absence of oil.

$$\text{Peroxide value (meq } O_2\text{/kg oil)} = \frac{(\text{Blank reading} - \text{Sample reading}) \text{x N of sodium thiosulphate}}{Weight\ of\ the\ sample} \times 100$$

2.5 Iodine Value

"The iodine value of the sample was determined by AOAC methods was used. Accurately 0.4gm of the sample was weighed into a conical flask and 20ml of carbon tetra chloride was added to dissolve the oil. Then 25ml of iodine monochloride solution in glacial acetic (Wij's solution) was added to the flask using a safety pipette in fume chamber. Stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours and 30 minutes. At the end of this period, 20ml of 10% aqueous potassium iodide and 125ml of water were added using a measuring cylinder. The content was titrated with 0.1M sodium-thiosulphate solutions until the yellow color almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking" [6]. The same procedure was used for blank test and other samples. The iodine value is given by the expression: [5].

$$\text{Iodine value} = \frac{(\text{B}-\text{S})\text{X N X } 126.9}{W} \times 100$$

Where, Iodine value = g of iodine absorbed per 100 g of sample, B = Volume of titrant (ml) for blank, S = Volume of titrant (ml) for sample, N = Normality of Na2S2O3 (mol/1000 ml), 126.9 = MW of iodine (g/mol), W = Sample mass (g).

2.6 Saponification Value

"The saponification value is the number of milligram of KOH required to neutralize the fatty acids present as a result of the complete hydrolysis of 1g fat. Accurately weighed 2 g of oil into a 250 ml of conical flask, add 25 ml of alcoholic KOH and dissolve the oil completely. Connect air condenser to the flask and boil for about 30 min on a boiling water bath. Cool to room temperature, add 2 drops of phenolphthalein indicator and mix. Titrate against standard 0.5 N HCl until the pink color disappears. Treat blank similarly in absence of oil" (A.O.A.C, 2000).

2.7 Ester Value

Ester value is the number of milligrams of potassium hydroxide (KOH) necessary to saponify esters present in 1 g of the substances. Ester value of oil was calculated by using following relation:

Ester value = Saponification value – Acid value

2.8 Antioxidant Activity

The antioxidant activity rosemary essential oil was determined by free radical scavenging effect on DPPH, FRAP and ABTS assay.

2.9 DPPH (2,2-diphenyl-1-picryl hydrazyl) Radical Scavenging Activity

Free radical scavenging ability of the essential oil was tested by DPPH radical scavenging assay as described by Sultana *et al*. (2017). The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. The test sample with different concentration viz., 0.2, 0.4, 0.6, 0.8 and 1.0 ml is mixed with the 0.1 mM of freshly prepared DPPH

solution with different concentration of methanol (3.8, 3.6, 3.4, 3.2 and 3.0 ml) respectively. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard. Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = \frac{A0 - A1}{A0} \times 100$$

Where, A0: absorbance of the control
A1: absorbance of the extractives/standard.

Then % of inhibition was plotted against concentration and from the graph IC50 was calculated. The experiment was repeated three times at each concentration.

2.10 Ferric Reducing Antioxidant Power Assay (FRAP)

"Total antioxidant activity of the extracted essential oil was determined using the ferric reducing antioxidant power analysis" as described by Chu *et al*. (2000). "Different concentration of the essential oil (0.2, 0.4, 0.6, 0.8 and 1.0 ml) was mixed with methanol from which 1 mL of the methanolic extract was taken in a test tube. It was then mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v). Followed by that the mixture was kept in boiling water bath at 50˚C for 25 minutes and 2.5 mL of trichloro acetic acid (10% w/v) was added to all the test tubes from which 2.5 ml of working sample was pipetted out and added with 2.5 mL of distilled water. At last 500µL of ferric chloride solution (0.1% w/v) was added and kept for an incubation time of 30 minutes. The observance value of the prepared solution was read at 700 nm using UV-visible spectrophotometer. Higher the absorbance, higher will be the reducing activity of the antioxidant compounds present in the essential oil" [7].

$$\text{FRAP percentage (\%) inhibition} = \frac{A0 - A1}{A0} \times 100$$

Where, A0: absorbance of the control and A1: absorbance of the extractives/standard.

2.11 ABTS (2,2′-azino-bis- 3-ethylbenzothiazoline-6-sulfonate)

The radical scavenging ability of the extracted essential oil and standards were measured using ABTS free radical (2,2'azinobis-3-ethylbenzothiazoline- 6-sulphonate) as reported by Re *et al*. [8]. Reactive ABTS solution was made by mixing 7 mM of ABTS at pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration), followed by storing it for 16 hours at room temperature in the dark. The mixture was then diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using UV visible- spectrophotometer. For each sample (0.2, 0.4, 0.6, 0.8 and 1.0 ml) the diluted methanol solution of essential oil was allowed to react with the ABTS solution (2700 µL) for 6 minutes after initial mixing, and then the absorbance was measured. As a control, ascorbic acid was employed. .

$$\text{ABTS percentage \% Inhibition} = \frac{A0 - A1}{A0} \times 100$$

Where, A0: absorbance of the control
A1: absorbance of the extractives/standard.

2.12 Statistical Analysis

The obtained data was tabulated and analysed by keeping in view of the objectives and parameters of the study. All the analyses were performed in triplicate, the data was subjected to one way and three way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) for testing the significance of variation using OP STAT software developed by O. P. Sheoran, Professor, Statistics at COBS and HCCS HAU, Hisar.

3. RESULTS AND DISCUSSION

3.1 Physical Parameters of Rosemary Essential Oil

The Table 1 presents a comprehensive overview of the physical parameters of Rosemary essential oil in comparison to a reference oil, providing valuable insights into its characteristics and potential applications.

3.2 Yield

The yield of Rosemary essential oil was found to be 4.3%, signifying a successful extraction process, although no specific yield information was available for the reference oil.

3.3 Appearance and Odor

Rosemary essential oil exhibited a unique white transparent appearance, distinct from the yellowish appearance of the reference oil. Both oils shared an aromatic odor, characteristic of essential oils and suggestive of their potential use in aromatherapy and fragrance applications.

3.4 Solubility

Both oils displayed similar solubility behavior, being immiscible in water but readily miscible in ethanol, methanol, and alcohol. This solubility profile is in line with the typical attributes of essential oils, which find utility in various industries due to their limited solubility in aqueous solutions.

3.5 Specific Gravity, Refractive Index, and Density

The specific gravity of Rosemary essential oil was marginally higher at 0.8910 g/cm³ compared to the reference oil's 0.8891 g/cm³. Similarly, the refractive index of Rosemary essential oil (1.4682) was slightly lower than that of the reference oil (1.4697). Furthermore, Rosemary essential oil exhibited a slightly lower density of 0.88 g/cm³ in contrast to the reference oil's density of 0.90 g/cm³. These subtle variations in specific gravity, refractive index, and density highlight distinctions in the physical properties of the two oils, which may be attributed to differences in their chemical compositions.

The comprehensive analysis of Rosemary essential oil's physical parameters, as presented in Table 1, underscores its unique characteristics and potential applications. These findings provide essential information for quality control, product characterization, and decision-making in diverse industries, including cosmetics, aromatherapy, and food processing. Further exploration of the chemical constituents of these oils will contribute to a deeper understanding of their distinct attributes and potential benefits in various applications.

3.6 Chemical Parameters of Rosemary Essential Oil

The Table 2 provides an in-depth analysis of the chemical parameters of rosemary essential oil in comparison to a reference oil, shedding light on the chemical composition and potential applications of the oil.

3.7 Acid Value (mg KOH/g)

Rosemary essential oil exhibited a significantly lower acid value of 1.12 compared to the reference oil's value of 3.37. A lower acid value suggests reduced levels of free fatty acids, indicating the superior quality and stability of Rosemary essential oil.

3.8 Peroxide Value

The peroxide value of Rosemary essential oil (1.24) was notably lower than that of the reference oil (5.76). This lower peroxide value indicates better resistance to oxidation and rancidity, highlighting the oil's potential for extended shelf life and use in food and cosmetic products.

3.9 Iodine Value (g/100g)

Both Rosemary essential oil (89.45) and the reference oil (94.12) displayed similar iodine values, suggesting comparable degrees of unsaturation in their fatty acid compositions.

Table 1. Physical parameters of rosemary essential oil

Parameters	Reference Oil (Commercially available oil)	Rosemary essential oil
Yield	--	3.3 %
Appearance	Yellowish	White transparent
Odour	Aromatic	Aromatic
Solubility	Immiscible in water Miscible in ethanol, methanol and alcohol	
Specific gravity (g/cm³)	0.8891 ± 0.01	0.8910 ± 0.02
Refractive index	1.4697 ± 0.01	1.4682 ± 0.03
Density (g/cm$^{3)}$	0.90 ± 0.01	0.88 ± 0.04

Table 2. Chemical parameters of rosemary essential oil

Parameters	Reference Oil	Rosemary essential oil
Acid value (mg KOH/g)	3.37 ± 0.12	1.12 ± 0.02
Peroxide value	5.76 ± 0.13	1.24 ± 0.02
Iodine value (g/100g)	94.12 ± 0.26	89.45 ± 0.26
Saponification value (mg KOH/g)	70.20 ± 0.02	96.20 ± 0.11
Ester value (mg KOH/g)	167.69 ± 0.11	66.83 ± 0.11

3.10 Saponification Value (mg KOH/g)

Rosemary essential oil demonstrated a higher saponification value of 96.20, whereas the reference oil had a lower value of 70.20. The saponification value provides insights into the average molecular weight of the fatty acids in the oil, with higher values indicating the presence of larger, potentially more complex, molecules in Rosemary essential oil.

3.11 Ester Value (mg KOH/g)

Rosemary essential oil exhibited a significantly lower ester value of 66.83 compared to the reference oil's value of 167.69. The ester value is indicative of the ester content in the oil, with lower values suggesting fewer ester compounds in Rosemary essential oil.

The comprehensive chemical analysis presented in Table 2 highlights the distinct chemical parameters of Rosemary essential oil compared to the reference oil. The oil's low acid and peroxide values, indicative of high quality and stability, make it a promising candidate for various applications, including in the food and cosmetic industries. The saponification and ester values provide further insights into the chemical composition of the oil, which can be valuable for product formulation and quality assessment. These findings contribute to a better understanding of Rosemary essential oil's chemical characteristics and its potential utilization in diverse industrial applications.

3.12 Total Antioxidant Activity of Rosemary Essential oil by DPPH Assay

Table 3 presents the total antioxidant activity of rosemary essential oil, ascorbic acid (a known antioxidant), and a reference oil, measured using the *DPPH* assay at various concentrations (in µl). The results are expressed as mean values with standard deviations (±) for each concentration. At a concentration of 200 µl, rosemary essential oil exhibited a total antioxidant activity of 48.17, while ascorbic acid had a lower activity of 47.06 and the reference oil showed the lowest activity of 28.17.

As the concentration increased to 400 µl, rosemary essential oil still maintained its relatively high antioxidant activity, with a value of 51.82. Ascorbic acid increased slightly to 48.04, while the reference oil also improved but remained lower at 31.82. At 600 µl, rosemary essential oil continued to exhibit strong antioxidant activity, with a value of 56.44. Ascorbic acid increased to 51.96, and the reference oil showed an increase as well (36.44). The trend continued at 800 µl, with rosemary essential oil demonstrating the highest antioxidant activity at 60.48, followed by ascorbic acid at 52.94 and the reference oil at 41.48. Finally, at the highest concentration of 1000 µl, rosemary essential oil maintained its superior antioxidant activity, with a value of 62.17. Ascorbic acid showed an increase to 55.88, while the reference oil remained the least effective at 48.17.

The results of the DPPH assay clearly indicate that rosemary essential oil possesses significant antioxidant activity at all concentrations tested, surpassing both ascorbic acid and the reference oil. Several key observations and discussions can be made based on these findings: concentration-dependent activity: the antioxidant activity of rosemary essential oil increased in a concentration-dependent manner. This suggests that the oil contains compounds with antioxidant properties that become more effective as their concentration rises. This concentration-dependent effect is a common characteristic of many natural antioxidants.

Table 3. Total Antioxidant activity of rosemary essential oil by DPPH assay

Concentration (µl)	Ascorbic acid	Reference Oil	Rosemary essential oil
200	47.06± 0.16	28.17±0.18	48.17±0.16
400	48.04±0.12	31.82±0.09	51.82±0.21
600	51.96±0.23	36.44±0.12	56.44±0.11
800	52.94±0.13	41.48±0.24	60.48±0.23
1000	55.88±0.21	48.17±0.19	62.17±0.22
Factors	**F value**	**SEm±**	**CD @5%**
A	*	0.233	0.671
B	*	0.261	0.750
A×B	*	0.522	1.500

Ascorbic acid, a well-known antioxidant, exhibited antioxidant activity close to or slightly lower than rosemary essential oil at most concentrations. This highlights the potency of rosemary essential oil as a natural antioxidant source, potentially comparable to or even better than the commonly used ascorbic acid. The reference oil consistently showed the lowest antioxidant activity among the three substances tested. This underscores the importance of selecting the right natural product for antioxidant applications, as not all oils or substances exhibit strong antioxidant properties.

The superior antioxidant activity of rosemary essential oil, even at lower concentrations, suggests its potential use in various applications, such as in the food industry to extend shelf life, in cosmetics to prevent oxidative damage, or in pharmaceuticals for its potential health benefits. However, further studies are needed to identify and isolate the specific compounds responsible for this activity and to assess its safety and efficacy in different contexts. The results of this study indicate that rosemary essential oil possesses strong antioxidant activity, which may have practical applications in various industries. Further research is warranted to explore the mechanisms of this antioxidant activity and to determine the specific compounds responsible for its effectiveness.

This can be explained by Teneva et al. [9], that hydrodistillation method of extraction is less likely to introduce external substances, such as solvents, which could potentially dilute the natural antioxidants present in the oils.

3.13 Total Antioxidant Activity of Rosemary Essential oil by *FRAP* Assay

Table 4 presents the total antioxidant activity of rosemary essential oil, ascorbic acid (a known antioxidant), and a reference oil, measured using the *FRAP* (Ferric Reducing Antioxidant Power) assay at various concentrations (in µl). The results are expressed as mean values with standard deviations (±) for each concentration. At a concentration of 200 µl, rosemary essential oil exhibited a total antioxidant activity of 49.19, while ascorbic acid had a slightly higher activity of 50.26±0.16, and the reference oil showed an activity of 29.18. At 400 µl, rosemary essential oil continued to display good antioxidant activity with a value of 52.02, surpassing both ascorbic acid (52.15) and the reference oil (32.12). At 600 µl, rosemary essential oil maintained its strong antioxidant activity with a value of 58.44. Ascorbic acid exhibited an activity of 53.16, and the reference oil showed 35.14 in this concentration. At 800 µl, rosemary essential oil demonstrated high antioxidant activity at 64.48, while ascorbic acid also increased to 55.34. The reference oil showed 40.38 in this concentration.

At the highest concentration of 1000 µl, rosemary essential oil still exhibited significant antioxidant activity with a value of 69.17, surpassing both ascorbic acid (66.18) and the reference oil (43.27).

The results obtained from the *FRAP* assay, which measures the ferric reducing antioxidant power of substances, provide important insights into the antioxidant activity of rosemary essential oil, ascorbic acid, and the reference oil: similar to the previous assays, the FRAP assay confirms that the antioxidant activity of rosemary essential oil increases with higher concentrations. This suggests the presence of compounds in rosemary essential oil that can effectively reduce ferric ions, indicating strong antioxidant potential. In the *FRAP* assay, ascorbic acid exhibited high antioxidant activity, especially at the highest concentration. However, rosemary essential oil consistently displayed competitive and robust antioxidant power, particularly at lower concentrations. This underscores the potential of rosemary essential oil as a natural alternative to synthetic antioxidants like ascorbic acid.

Table 4. Total antioxidant activity of rosemary essential oil by *FRAP* assay

Concentration (µl)	Ascorbic acid	Reference Oil	Rosemary essential oil
200	50.26± 0.16	29.18±0.18	49.19 ±0.16
400	52.15±0.12	32.12±0.09	52.02^{c} ±0.21
600	53.16±0.23	35.14±0.12	58.44±0.11
800	55.34±0.13	40.38±0.24	64.48±0.23
1000	66.18±0.21	43.27±0.19	69.17±0.22
Factors	**F value**	**SEm±**	**CD @5%**
A	*	0.240	0.690
B	*	0.268	0.771
A×B	*	0.537	1.542

The reference oil continued to demonstrate lower antioxidant activity compared to both rosemary essential oil and ascorbic acid. This reinforces the importance of selecting appropriate natural products or oils for their antioxidant properties. The results of the *FRAP* assay further emphasize the strong antioxidant potential of rosemary essential oil, especially at lower to moderate concentrations. This suggests its potential use in various industries where antioxidants are required to prevent oxidative damage and extend product shelf life, such as in the food, cosmetic, and pharmaceutical sectors.

The FRAP assay results support and validate the findings from previous assays, highlighting the significant antioxidant activity of rosemary essential oil. The concentration-dependent nature of this activity suggests that rosemary essential oil could serve as a valuable natural source of antioxidants, potentially offering benefits in various applications. Further research is needed to identify and characterize the specific antioxidant compounds within rosemary essential oil and to assess its suitability for practical use in different contexts.

Abdelwahab et al. [10] reported that variation in antioxidant activity can be attributed to the differences in the natural antioxidant components present in these oils. Hydrodistilled essential oils, which involve a process of extracting essential oils through water, hence exhibited the highest antioxidant activity.

3.14 Total Antioxidant Activity of Rosemary Essential oil by *ABTS* Assay

Table 5 presents the total antioxidant activity of rosemary essential oil, ascorbic acid (a known antioxidant), and a reference oil, measured using the ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay at various concentrations (in µl). The results are expressed as mean values with standard deviations (±) for each concentration. At a concentration of 200 µl, rosemary essential oil exhibited a total antioxidant activity of 53.21, while ascorbic acid had a slightly higher activity of 53.16. The reference oil showed an activity of 30.28. At 400 µl, rosemary essential oil continued to display strong antioxidant activity with a value of 57.12, surpassing both ascorbic acid (56.25) and the reference oil (33.02).

At 600 µl, rosemary essential oil maintained its robust antioxidant activity with a value of 59.42. Ascorbic acid exhibited an activity of 59.36, and the reference oil showed 35.14 in this concentration. At 800 µl, rosemary essential oil demonstrated high antioxidant activity at 68.18, while ascorbic acid also increased to 62.44. The reference oil showed 40.38in this concentration. At the highest concentration of 1000 µl, rosemary essential oil still exhibited significant antioxidant activity with a value of 70.17, surpassing both ascorbic acid (70.28) and the reference oil (43.27).

The results from the *ABTS* assay, which measures the ability of substances to scavenge ABTS radicals, provide valuable insights into the antioxidant activity of rosemary essential oil, ascorbic acid, and the reference oil. The *ABTS* assay confirms that the antioxidant activity of rosemary essential oil increases with higher concentrations, reinforcing the presence of effective antioxidant compounds within the oil. In the *ABTS* assay, ascorbic acid and rosemary essential oil showed comparable antioxidant activity at most concentrations, with rosemary essential oil even surpassing ascorbic acid at some concentrations. This highlights the potent antioxidant potential of rosemary essential oil, which could serve as a natural alternative to ascorbic acid in certain applications.

Table 5. Total antioxidant activity of rosemary essential oil by *ABTS* assay

Concentration (µl)	Ascorbic acid	Reference Oil	Rosemary essential oil
200	53.16±0.19	30.28±0.18	53.21±0.16
400	56.25±0.14	33.02±0.19	57.12±0.23
600	59.36±0.13	35.14±0.11	59.42±0.13
800	62.44±0.17	40.38±0.14	68.18±0.23
1000	70.28±0.11	43.27±0.21	70.17±0.22
Factors	**F value**	**SEm±**	**CD @5%**
A	*	0.220	0.631
B	*	0.246	0.706
A×B	*	0.491	1.412

The reference oil consistently demonstrated lower antioxidant activity compared to rosemary essential oil and ascorbic acid. This reaffirms the importance of selecting appropriate natural products or oils for their antioxidant properties. The *ABTS* assay results suggest that rosemary essential oil possesses significant antioxidant activity across a range of concentrations. This underscores its potential use in various industries where antioxidants are needed to counteract oxidative damage and extend product shelf life, such as in food, cosmetics, and pharmaceuticals. In conclusion, the *ABTS* assay results provide further evidence of the strong antioxidant potential of rosemary essential oil. Its concentration-dependent activity and comparable or superior performance to ascorbic acid in this assay suggest that it may be a valuable natural source of antioxidants for various applications. Further research is necessary to identify and characterize the specific antioxidant compounds in rosemary essential oil and to evaluate its suitability for practical use in different contexts [11-13].

4. CONCLUSION

The study successfully extracted rosemary essential oil using the hydrodistillation method and comprehensively analyzed its physicochemical properties. The oil exhibited distinctive physical characteristics, including a pleasant odor, solubility, specific gravity, refractive index, and density, which collectively contribute to its marketability and utilization. Furthermore, the chemical parameters assessed, including acid value, peroxide value, iodine value, saponification value, and ester value, provided valuable insights into the composition and quality of the rosemary essential oil. One of the standout findings of this study is the remarkable antioxidant potential of rosemary essential oil, as demonstrated through *DPPH, FRAP*, and *ABTS* assays. This suggests its capacity to effectively combat oxidative stress, a property of great interest for the food, pharmaceutical, and cosmetic industries, where natural antioxidants are in high demand. Overall, the results of the research highlight the significant promise of rosemary essential oil as a natural source of antioxidants and underscore its potential applications in various sectors. The findings contribute to the growing body of knowledge regarding the beneficial properties of rosemary essential oil, paving the way for further research and development in harnessing its potential for therapeutic and commercial purposes. As consumer interest in natural and sustainable products continues to rise, rosemary essential oil emerges as a valuable and versatile candidate for future exploration and incorporation into a wide range of products.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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