International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 12 No. 1, 2026
https://theaspd.com/index.php

Assessment of Antioxidant and Hemolytic Activities of *Rosmarinus officinal* L. From Rural Region of Algeria: A Comparative Study of Extraction Methods

Fadila Cherifi[1*], Aicha Bouhafsoun[1], Chaima Benaouda[2] ,Fouzia Belmokhtar[2], Nouria Belhadj[2] , Abderezek Djabeur [1]

[1]Laboratory of Plant and Microbial Production and Valorization (LP2VM), Department of Biotechnology, University of Science and Technology of Oran Mohamed Boudiaf, B.P. 1505, El M'Naouar, Oran 31000, Algeria.
[2] Hemobiology and Blood Transfusion Laboratory, Oran University Hospital Center.
* Corresponding author's Email: fadila.cherifi@univ-usto.dz
Received Date: 17-04-2026 Revised Date: 14-05-2026 Accepted Date: 20-05-2026

Abstract
In the present study, the phytochemical composition, antioxidant activity and toxic effects of aqueous leaf extracts of Rosmarinus officinalis L.were evaluated. The preparation of the extracts was conducted using decoction and maceration procedures and the phytochemical composition and activities were determined using various methods. The extraction residues obtained by maceration and decoction exhibited similar total phenol content values (26.7 µg GAE/mg and 26.5 µg GAE/mg, respectively) and lowest flavonoid content (1.56 µg CE/mg and 1.54 µg CE/mg, respectively). The maceration extracts exhibited high antioxidant potential (93.02% with an IC50 of 253.30µg/mL) and high hemolytic activity (73.01% with an IC50 of 112.5µg/mL). Conversely, the decoction extract demonstrated low DPPH scavenging activity (88% with an IC50 of 209.90 µg/mL) and low hemolytic activity (57.18% with an IC50 of 367.87 µg/mL).Ours finding indicated that rosemary extracts have been demonstrated to induce significant hemolysis effects as a consequence of polyphenol pro-oxidation despite their well-documented antioxidant properties. The pro-oxidant effect perturbs membrane phospholipids and their structural precipitating witch conduces to the cellular damage. It is imperative that the present result be given due consideration when incorporating rosemary leaves into daily use by native population, in order to avert any toxic effects on human health.
Keywords: *Rosmarinus officinalis L., phytochemical composition, antioxidant activity, toxicity*

1. INTRODUCTION

In recent years, medicinal plants have been used as an important source of phytochemicals that can replace synthetic molecules in the pharmaceutical industry (Habtemariam, 2016). Many phenolic compounds, including phenolic acids, flavonoids, tannins and coumarins identified in medicinal plants, are known for their pharmacological uses. Despite these properties, uncontrolled use at high dose can cause toxicity. Despite these properties, uncontrolled use and high doses can cause toxicity,for exemple the *Teucrium polium* extracts cause hemolysis and renal failure at high doses; conversely ,their use at lower doses is generally considered safe for users (Gholami et al., 2025).In the same context, a study was conducted by Boumediou and Addoun (2017) on the use of aromatic plants in a rural community, and the results showed that 20 of these plants are toxic herbs. At the same time, they were used in 60 medicinal preparations. They noted also that 73% of consumers of these plants had no information on the toxicity of the preparations .These relevant findings prompted us to assess the toxicity of high-dose rosemary (*Rosmarinus officinalis*), especially with the frequent use of aromatic plants as a natural remedies. *Rosmarinus officinalis* L., commonly known as rosemary, is a plant that is known by people across the globe on a daily basis. It is employed in a variety of culinary preparations and as a spice, and is a constituent of most cuisines. Belonging to the family Lamiaceae, this plant is frequently used in the form of dried leaves or leaf powder in the preparation of meals. It is also consumed in the form of an infusion or decoction to treat various health problems. Numerous studies have been conducted to investigate the biological activities of rosemary (*Rosmarinus officinalis* L.)leaves, revealing their antioxidant, anticancer, anti-infectious and anti-inflammatory properties, which are linked to their high content of phenolic compounds and essential oils (Masumoto et al., 2018; Alagawany et al., 2023; Gómez-Rivera et al.,2022; Nehal et al., 2022; Saleh et al.,2022).The leaf extracts of rosemary contain phenolic substances, including coumaric acid and rosmarinic acid (Mena et al., 2016) and triterpenes as well as monoterpens and essential oil fraction (Nehal et al., 2022 ;

Saleh et al., 2022;).Upon reviewing the studies on rosemary, we found that few of them addressed its

hemolytic effects when used in high doses. To supplement this research, we have established the following hypotheses: Do rosemary extracts cause hemolysis of red blood cells in vitro? Can the antioxidant activity protect cells from hemolysis when the extracts are used in high doses? Does the extraction method affect antioxidant and hemolytic activity of extract? To answer these questions, we have focused on determination of the phytochemical composition and assessing the antioxidant and hemolytic activities of rosemary leaves, obtained using the maceration and decoction methods, and then establishing the relationship between these two biological activities.

2. MATERIALS AND METHODS

2.1. Plant material and sample preparation

Rosmarinus officinalis L. leaves were collected in February 2025 from a mature plant growing in the Ighil-Ali locality (Bejaia), which is situated in north-east Algeria (36.34°N, 4.69°E).The fresh leaves were collected from the upper and middle parts of the plant. After sampling, 200 g of fresh leaves were washed then left to drain in a ventilated oven set at 25 °C for four days. The leaves were then divided into two parts. The first part was ground into a fine powder using a RETSCH-type knife grinder, while the second part was stored for extraction using the decoction process.

2.2. Extraction by maceration

Rosemary leaf powder was used to prepare the maceration aqueous extract, following the method described by Cherifi and Kaid-Harche (2019) with some modifications.50g of the powder was dissolved in 1L of distilled water and stirred continuously for 24 hours in the dark at room temperature. After stirring, the solution was centrifuged at 3800 rpm for 20 min and the supernatant was collected and kept at 4°C.

2.3. Extraction by decoction

50 g of rosemary leaves were mixed with 1 L of distilled water. The mixture was then heated on a hot plate set at 100 °C for 15 minutes. After heating, the solution was filtered and the filtrate was stored at 4°C until use.

2.4. Preparation of test solutions

The aqueous extract obtained both by decoction and maceration was dried separately using a rotary evaporator set at 40 °C. The drying residues were weighed and used to prepare the test solutions by dissolving 0.2 mg of residue in 1 ml of distilled water. The same procedures were followed to prepare the 0.4, 0.6, 0.8 and 1 mg/ml solutions. Homogenized solutions were used in the antioxidant and hemolytic activity tests.

2.5. Phytochemical screening

Phytochemical screening was performed using simple chemical test procedures. Each test gives a color that indicates the presence or absence of phytomolecules.

Coumarin detection

The presence of coumarins was detected by the following steps: The extract was incubated in a water bath and covered with filter paper containing 1 ml of NaOH (1 N).After 20 minutes, the filter paper was observed under UV light (366 nm).The presence of coumarins was confirmed by the detection of yellow fluorescence (Bruneton,1999).

Tannin detection

Tannins were detected by mixing 5 ml of the extract solution with 1 ml of a 1% ferric chloride solution ($FeCl_3$). If the solution turned blue, gallic tannins were present; if it turned green-black, catecholic tannins were present (Karumi et al., 2004).

Saponin detection

To evaluate the presence of saponins, the foam index was estimated quantitatively by the following method: 10 mL of aqueous extracts were put in a glass tube and shaken for 5 minutes. If foam remained at the top of the tube for 10 minutes, the saponin test was considered positive (N'Guessan et al., 2009).

Alkaloid detection

The presence of alkaloids was determined using Dragendorff's reagent test. A few drops of acetic anhydride were added to the aqueous plant extracts, followed by two drops of concentrated sulphuric acid.

The appearance of a red-blue color that turned green indicated the presence of alkaloids (Mojab et al., 2003).

2.6. Total phenol content determination

The concentration of total polyphenol of solutions test was determined according to the method of Folin– Ciocalteu adapted by Li et al. (2007). Briefly, 200 µl of each solution test was added to 1000 µl of Folin-Ciocalteu (0.1M), and then (after 5minutes) 800µl of aqueous sodium carbonate Na_2CO_3 (7.5 %) was added to the mixture which was thereby incubated for 2 hours for being used for the total phenols measurement by spectrophotometer (UVIKON UV/VIS), at 765 nm. The standard curves were prepared using 20 to 200µg/mL of gallic acid solution dissolved in methanol. Total phenol values are expressed in terms of gallic acid equivalent (mg. g–1 of dry mass), a common reference compound.

2.7. Total Flavonoids content determination

The flavonoid concentrations of solutions test were measured follow Kim et al. (2003) method, 500 µl of methanol and aqueous solutions were mixed separately with 1500 µl of distilled H_2O and 150 µl of a 5% $NaNO_2$ solution. After 5 min, 150 µl of 10% $AlCl_3$ solution was added to each tube. Finally, 500 µl of 1 M NaOH were added to the mixture, the intensity of pink color was measured at 510 nm using the spectrophotometer.The flavonoid concentrations was calculated using catechin as a standard and flavonoid contents were expressed as mg catechin equivalents per g of dry extract.

2.8. In Vitro Antioxidant Activity Assessment (DPPH Assay)

The antioxidant activity of leaf extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method following the method described by Athamena et al. (2012).

In this experiment, serial dilutions were performed to obtain concentrations of 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL,0.8 mg/mL and 1mg/mL.1 ml of each extract at different concentrations was added to 1 ml of a 0.1 M DPPH solution .The mixture was well mixed and then incubated in the shade for 30 minutes. After incubation, the absorbance was measured at 517 nm and. The percentage of DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{Ac-As}{Ac} \times 100$$

- **Ac**: Absorbance of control containing only DPPH
- **As**: Absorbance of the sample containing DPPH
-

2.9. Determination of hemolytic activity

Blood samples were obtained from healthy volunteers at the University Hospital Centre of Oran (Algeria). An erythrocyte suspension was prepared according to the protocol described by Yang et al (2017), with modifications. Initially, 2 ml of blood was centrifuged in tubes at 800 rpm for 10 minutes. The supernatant was washed twice with 1 ml of PBS (pH 7.4). Each washing step involved centrifugation at 800 rpm for 10 minutes to remove the supernatant. Finally, the erythrocyte pellet was suspended in 1 mL of PBS (pH 7.4) and used immediately.

The hemolytic activity of the extracts was assessed using the method described by Meziti et al (2012). Briefly, 2,950 µL of a freshly prepared erythrocyte suspension was mixed with 50 µL of an aqueous leaf extract at different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg/mL). The mixture was then incubated for one hour in a water bath at 37 °C. A positive control was created by incubating the erythrocytes in 1% SDS solution under the same conditions (considered to be 100% hemolysis).After incubation, 1.5 ml of PBS was added to each test tube and the reaction was stopped by placing the tubes in a glass bath for 10 minutes. The samples were then centrifuged at 800 rpm for 10 minutes. The supernatant was collected and the hemolysis of the blood was determined by measuring the absorbance of the supernatant at 548 nm using a Jenway 6700 spectrophotometer, which corresponds to hemoglobin release.

A negative control tube was prepared under the same experimental conditions. This contained only 3,000 µl of erythrocyte suspension and 80 µl of PBS buffer solution. In contrast, a positive control sample corresponding to 100% hemolysis was prepared as follows: 1 ml of erythrocyte suspension was mixed with 9 µl of SDS. Finally, hemolysis percentage was calculated using the following formula:

$$\textbf{Hemolysis rate (\%)} = \frac{\text{As– A negative control}}{\text{A positive control}} \times 100$$

- **As**: Absorbance of the erythrocytes suspension with solution test.
- **A negative control**: Absorbance of erythrocytes suspension without solution test.
- **A positive control**: Absorbance of the erythrocytes suspension with SDS solution.

2.10. Statistical analysis

The results are expressed as an average of three experiments. The differences between the levels of polyphenols and flavonoids are evaluated by the one-way ANOVA test with a threshold $p < 0.05$ and $p < 0.01$.On the other hand the Pearson's correlation test was applied to analyze the correlation between antioxidant and hemolytic activities.

3. RESULTS AND DISCUSSION

3.1. Results

Phytochemical determination

Qualitative screening of phyto-compounds indicated the presence of alkaloids, tannins, and saponins in the decoction and maceration extracts of rosemary leaves. Conversely, coumarins were not detected in either extract. The results of the phytochemical investigations are summarized in Table 1.

Table 1: Results of phytochemical screening of *Rosmarinus officinalis* L. leaf extracts.

Parameters	Leaf extracts	
	Decoction	**Maceration**
Tannins	+	++
Alkaloids	+	+
coumarins	-	-
Saponins	+	+++

Note: ++ positive, + medium, - negative

Polyphenol and flavonoid determinations

The determination of polyphenol concentrations in rosemary extracts obtained through maceration and decoction methods showed a low content of polyphenols and flavonoids in both extracts. However, concentrations increased with the concentration of test solutions ranging from 0.2 to 1 mg/mL (Table 2).

Table 2: Total phenol and flavonoid con tents in aqueous extracts.

Test Solutions mg/mL	Polyphenol concentrations GAE/mg		Flavonoid Concentrations CE/mg	
	Aqueous decoction extract	Aqueous maceration extract	Aqueous decoction extract	Aqueous maceration extract
0.2	3.58 ± 0.45	3.68 ± 0.7	0.40 ± 0.67	1.41 ± 0.5
0.4	10.48 ± 0.09	10.65 ± 0.91	0.82 ± 0.5	0.83 ± 0.8
0.6	15.68 ± 0.1	16.08 ± 0.1	0.86 ± 0.5	0.88 ± 0.41
0.8	20.96 ± 0.39	22.08 ± 0.4	1.23 ± 0.1	1.24 ± 0.6
1	26.2 ± 0.1	26.7 ± 0.25	1.54 ± 0.5	1.56 ± 0.5
Significance	*Not significant*		*Not significant*	

Note: *no significant difference between the means both for polyphenols and flavonoids content*

Furthermore, the findings revealed that flavonoids constitute only a small proportion of the total polyphenols in our extracts. However, we also noticed that, for concentrations of polyphenols ranging from 26.2 to 26.7 µg, we obtained low concentrations of flavonoids ranging from 1.54 to 1.56 µg, respectively.

Antioxidant activity results

The results of the antioxidant activity evaluation indicate that the maceration extract exhibits higher activity than the decoction extract (Table 3), with respective percentages ranging from 79.06% to 93.02% at test solution concentrations of 0.2 to 1 mg/mL. Conversely, the activity of the decoction extract is lower, estimated to be between 44.1% and 88%. We also noticed that the IC_{50} values were 253.35µg/mL

and 209.09µg/mL for the aqueous decoction and maceration extracts respectively (Figures 1 and 2).

Table 3: Antioxidant activity of test solutions

Test Solutions	***Antioxidant percentage (%)***	
mg /mL	**Aqueous decoction extract**	**Aqueous maceration extract**
0.2	44.1	79.06
0.4	62.5	80.02
0.6	67.4	82.5
0.8	74.5	89.5
1	88	93.02
IC50	253.30 µg /mL	209.09 µg/mL

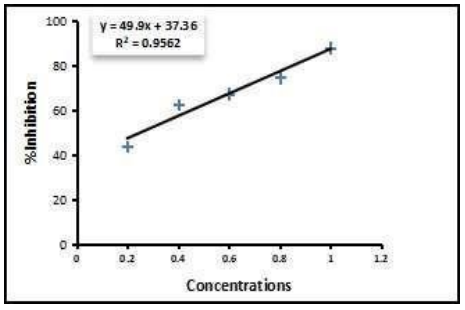

Figure 2: Calibration curve that shows the inhibition percentage of DPPH by aqueous decoction extract at different concentrations

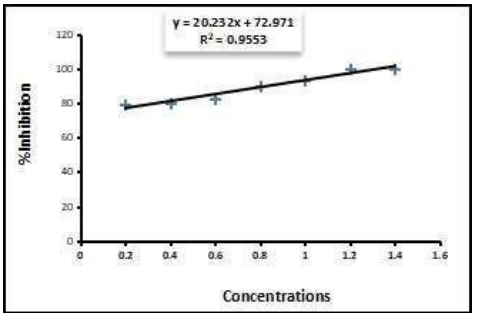

Figure 2: Calibration curve that shows the inhibition percentage of DPPH by aqueous maceration extract at different concentrations

Hemolytic activity results

As shown in Table 4, the hemolysis activity results indicated that the maceration extract produced the highest hemolysis rate compared to the decoction extract. The recorded hemolysis percentage ranged from 48.53% to 57.18% for the aqueous decoction extract and from 73.01% to 68.9% for the aqueous maceration extract. In addition, the hemolytic effect increased progressively with the increasing of the test solution concentrations, as reflected by the IC_{50} values of 367.78µg/mL and 112.5 µg/mL for of the aqueous decoction extract and the aqueous maceration extract respectively (Figures 3 and 4).

Table 4: Hemolytic activity of test solutions

Test Solutions	***Hemolysis percentage (%)***	
mg /mL	**Aqueous decoction extract**	**Aqueous maceration extract**
0.2	48,53	68,9
0.4	50,3	70,35
0.6	52,07	71,18
0.8	54,26	71,91
1	57,18	73,01
IC50	367.78 µg /mL	112.5µg/mL

IC50*: the concentration of a drug required for 50% inhibition of the hemolytic activity*

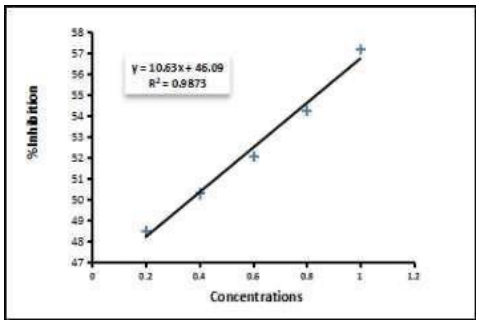

Figure 3: Calibration curve that shows the Hemolysis percentage of Aqueous decoction extract at different concentrations

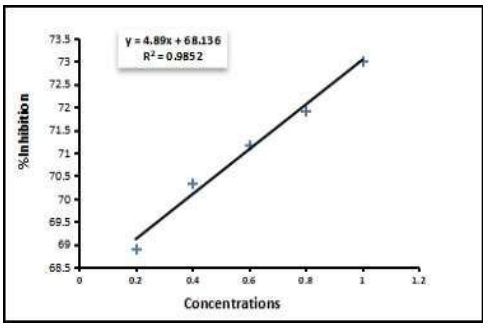

Figure 4: Calibration curve that shows the Hemolysis percentage of Aqueous maceration extract at different concentrations

The statistical study of the relationship between hemolytic and antioxidant activity showed a positive correlation between the two activities. The respective correlation coefficients were 0.94 and 0.92 for aqueous decoction and maceration extracts (Table 5).

Table 5: Correlation coefficient between hemolytic and antioxidant effects

	Aqueous decoction extract	**Aqueous maceration extract**
Correlation *Coefficient*	0,94	0,92

3.2. DISCUSSION

Much attention has been focused on the antioxidant potential of natural substances; antioxidants play an important role in combating oxidative stress, which can damage cells. This study aimed to evaluate the antioxidant activity of rosemary leaves and their ability to prevent red blood cell hemolysis *in vitro*. Our aim was also to assess the impact of extraction techniques on the chemical composition and biological activity of the obtained extracts.

The phytochemical screening tests revealed the presence of the follows secondary plant metabolites: alkaloids, tannins and saponins. Ours results are consistent with those reported in several studies, the most dominant classes of phenolic compounds in rosemary leaves are phenolic acids and flavonoids .The quantification of total phenol content show that the results were nearly similar to those reported by Santiago and Carlos (2020) estimations which indicated the presence of 22 µg/mg for phenol in their extract. However ,a contrasting results was observed by Klancnik et al.(2009),who reported 198µg/g of phenols in the aqueous extract. The differences observed in the chemical composition of rosemary extracts may be linked to several factors, such as geographical impact on the plant, drought, humidity, altitude, UV radiation intensity and soil. All these factors influence metabolism and the accumulation of secondary metabolites (Ahuja et al., 2010; Cherifi and Kaid Harche, 2019). Secondary metabolites accumulate in the wall of the plant cell to minimize water losses and protect the plant against intense UV radiation, particularly under arid conditions, this is considered as a survival strategy .In addition, the extraction solvents as well as the extraction methods can modulate the yield of active substances with regard to their solubility in different polarity and according to the conditions of the extraction methods.

The first activity explored was antioxidant activity using free radical scavenging test which it determine the reaction of *R. officinalis* aqueous extracts (various concentrations) with DPPH. Both decoction and maceration extracts exhibited significant free radical scavenging activity, with percentages ranging from 44.1% to 93.02%. Several studies was highlighting their potential as natural sources of antioxidants, rosemary leaf extracts are known for their antioxidant properties linked to the presence of two groups of compounds: a volatiles (diterpens such us as carnosic acid, carnosol and rosmanol) ,phenolic compounds (flavonoids, rutin , catechin, rosmarinic acid and some phenolic acids such as ferulic, rosmarinic, chlorogenic and caffeic acids witch play a key role in the antioxidant mechanism (Campo et al, 2000 ;Majid et al., 2022; Nehal et al,.2022 ; Rašković et al ., 2014;Gaya et al., 2013;Andrade et al., 2018; Mena et al., 2016).Chemically, phenolic acids, in particular rosmaniric acid have four hydroxyl groups linked to the phenyl ring, this structure rich in hydrogen atoms represents a capacity to bind free radicals and neutralize them thanks to their antioxidant power. (Scalbert et al.,2005).On the other hand, the antioxidant power of these molecules can be influenced by the nature of the reaction medium, their

chemical polarity and their degree of solubility, however Athamena et al.(2012) confirmed that the hydroethanolic extract of rosemary showed a low antioxidant activity (79.34%) compared to the aqueous extract.In order to better evaluate the antioxidant activity of the two extracts, we proceeded with the calculation of the IC50 which corresponds to the concentration of plant extract with a capacity. It is defined as the concentration of plant extract with 50% of activity compared to the original activity. The estimation using calibration curve equation showed IC50 values of 253.30 µg /mL and 209.09µg/mL respectively for decoction and maceration extracts. By comparing aqueous extract with the ethanol extracts tested by Manyou et al.(2021). we can deduce that water allows for better solubility of the active molecules, the IC50 value is low so the aqueous extract is considered more active. Despite, their biological activities, the rosemary extracts caused red blood cells lyse, we noticed different percentages of hemolysis, related to the type of extract and the concentration of polyphenols in the extracts. In contrast, several studies reported that there is no hemolytic effect of rosemary extracts on human erythrocytes (Tawfeeq et al., 2018).In addition Pérez-Fons et al.(2010) and Wang et al.(2018) suggested the protective effect of carnosic acid carnosol, rosmadial, genkwanin, and rosmarinic acids of rosemary on red cells this molecules have the ability to scavenge peroxyl radicals and prevent the erythrocytes membrane rupture.

The results of the statistical analysis indicate a positive correlation between hemolytic and antioxidant activities (r= 0.94 for ADE and r= 0.92 for AME), suggesting that the antioxidant power can paradoxically increase the lysis of the plasma membrane of red blood cells.

The antioxidant activity of aqueous extracts of rosemary leaves is linked to the presence of carnosic acid, carnisol, rosmadial and rosmarinic acid; these molecules interact with membrane phospholipids by altering the order, organisation and fluidity of the membrane bilayer; all these changes can affect membrane permeability and their stability (Pérez-Fons et *al* (2010). Based on this finding, we suggest that similar interactions may occur in the membranes of red blood cells upon contact with our extracts, these interactions thereby causing the rupture and release of hemoglobin and hemolysis, noting that these molecules have been identified in rosemary leaves from various locations around the world.

The toxicity of *Rosmarinus officinalis L* extracts has also been reported in a more recent study. Darra et al (2025) demonstrate in their results that aqueous extracts of rosemary leaves exhibit cytotoxic and antiproliferative activities against tumour cells; these activities are due to the presence of monoterpenes and phenolic acids capable of altering the membranes of the tested cells. Other compounds can inducing hemolysis such us saponins detected previously in ours extracts. The saponins have surfactant-like power and can cause hemolysis by rupturing the membranes of erythrocytes .In plant, the saponins accumulate in leaf mesophyll, their extraction need maceration procedures. Whereas, certain flavonoids located closely in leaf surface and more easily extracted by decoction which may explain the different level activities of decoction and maceration extracts. Additionally, the localisation of phenolic compounds must be considered during the phytotherapeutic preparation using rosemary leaves to avoid toxicity risk. The findings of the present study confirm that extraction techniques do indeed influence the biological activity of rosemary extracts; the extraction technique has a significant impact on the chemical composition of rosemary extracts as well as their biological activity.

4. CONCLUSION

The results of this study revealed the chemical richness of rosemary in plant compounds with antioxidant activity, the most important of which are flavonoids, alkaloids, tannins, and saponins in both decoction and maceration extracts. As for antioxidant potential, both extracts caused hemolysis of red blood cells. The hemolytic activity observed in our study is considered as a manifestation of this membrane toxicity of rosemary extracts, particularly it was due to the chemical properties of some phenolic acids that are able to change the structure of cellular membranes and disrupt the stability of phospholipids, causing the rupture of red blood cells and the release of haemoglobin. Additionally, the extraction methods affected the activity of the extract, as maceration was more effective for polyphenol extraction consequently the hemolytic activity was higher compared to decoction extract, which confirms and explains the change in their chemical composition. We suggest that the positive correlation between the two activities may are related to the presence of the same components specially the diterpens compounds and phenolic acids. Finally, we emphasize that although this plant is used in our daily lives, we must use it with caution and in lower concentrations to avoid its toxic effect. These results require a complementary study in order to isolate the active substances and examine their activity individually, in order to better understand their biological activity

Acknowledgements

The authors acknowledge the financial support of Algerian General Directorate of Scientific Research and technological development (DGRSDT) and the Algerian Ministry of Higher education and Scientific Research (MESRS).

REFERENCES

[1] Ahuja, I.,De Vos, R. C., Bones, A. M., and Hall, R. D,2010," Plant molecular stress responses face climate change",Trends Plant Sci.,15(12).pp.664–674. https://doi.org/10.1016/j.tplants.2010.08.002.

[2] Alagawany, M., El-Hack, M. E. A., and Farag, M. R., 2023, "The therapeutic roles of rosemary (Rosmarinus officinalis L.) and its bioactive components", Frontiers in Pharmacology, 14, 1124580. https://doi.org/10.3389/fphar.2023.1124580.

[3] Andrade, J. M., Faustino, C., Garcia,C., Ladeiras, D., Reis, C. P.,and Rijo, P ,2018, "*Rosmarinus officinalis L*. An update review of its phytochemistry and biological activity" Future Sci OA, 4(4).https://doi.org/10.4155/fsoa-2017-0124.

[4] Asha D.,Mathew L., Iyer R. S., and Bose L. V,2017."Isolation and Characterization of a Novel Saponin from Rosmarinus officinalis L. (Lamiaceae)", Int.J.Adv.Res, 5(2). https://dx.doi.org/10.21474/IJAR01/3336.

[5] Athamena S., Athamena M., Laroui S.,Louaer S.,and Segueni N.,2012,"Evaluation of the antioxydant activity of the *Rosmarinus officinalis* L.extracts",*Sci and Tech*, 36,pp.45-50. https://revue.umc.edu.dz/c/article/view/87.

[6] Bakri M.M., Alghonaim M .I, Alsalamah S.A, Yahya R.O., Ismail K. S., and Abdelghany T.M,2024,"Impact of Moist Heat on Phytochemical Constituents, Anti-*Helicobacter Pylori*, Antioxidant, Anti-Diabetic, Hemolytic and Healing Properties of Rosemary" ,Plant Extr in Vitro,15, pp.4965–4979.

[7] Boumediou A. and Addoun S.,2017, Etude ethnobotanique sur l'usage des plantes toxiques, en médecine traditionnelle, dans la ville de Tlemcen (Algérie).*University of Tlemcen Chetouane*, Algeria ,p .119.

[8] Campo, J. D., Amiot M. J., and Guyen C.N-The., 2000,"Antimicrobial effect of rosemary extracts". J. Food Protect, 63,pp.1659–1368. https://doi.org/10.4315/0362-028X-63.10.1359.

[9] Chang, S.S., Ostric-Matijasevic B., Hosieh, O.A.L.,and Huang, C.L.,1977,"Natural antioxidants from rosemary and sage",J.Food.Sci,42,pp.1102–1106. https://doi.org/10.1111/j.1365-2621.1977.tb12676.

[10] Cherifi F., and Kaid Harche M.,2019,"Evaluation of leaf extracts from Algerian *Argania spinosa.*L .Skeels: (Sapotaceae) trees: Total phenol content, protein content, and Antibacterial activities against six against clinical phytopathogenic bacteria",South.Asian. j exp bio,9 (3),pp.104-113. https://doi.org/10.38150/sajeb.9(3).p104-113.

[11] Darra R., Majdalawieh A.F., Mahasneh A., Rah B., Mawieh Hamad M.,Kanan S.,2025, Anti-proliferative effects of *Rosmarinus officinalis L.* (Rosemary) against human breast and liver carcinoma cells,Food Biosci,65, 106164.

[12] Gaya M., Repetto V., Toneatto J., Anesini C., and Piwien-Pilipuk ,2013,"Antiadipogenic effect of carnosic acid, a natural compound present in *Rosmarinus officinalis*, is exerted through the C/EBPs and PPARγ pathways at the onset of the differentiation program",Biochim and Biophysica Acta,1830(6),pp.3796- 3806. https://doi:10.1016/j.crvi.2008.07.024.

[13] Gholami, L., Ahmadi, S. M., and Pursusan, S.,2025," Acute kidney failure and hemolysis secondary to high-dose Teucrium polium (Lamiaceae):Case report",Clin Case Rep, 13(3),e70294. https://doi.org/10.1002/ccr3.70294.

[14] Habtemariam, S., 2016," The therapeutic potential of rosemary (*Rosmarinus officinalis*) diterpenes for Alzheimer's disease", Evid. Based Complement. Alternat. Med, 14. https://doi.org/10.1155/2016/2680409

[15] Karumi, E W.,2013,"Phytochemical, Anthelmintic And Antimicrobial Investigation Of Hagenia Abyssinica (bruce) Gmelin.*Doctorat thesis"*.USA. http://hdl.handle.net/11295/62684**.**

[16] Kim D.O., Chun O.K., Kim Y.J., Moon H.Y. and Lee C.Y.2003.Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agri.Food.Chem,*51*, 6509 - 6515.https://doi.org/10.1021/jf0343074.

[17] Klancnik A.,Guzej B.D.,Hadolin kolar M., Abramovic H.B.,and Smole Mozina S.,2009,"*In Vitro* Antimicrobial and Antioxidant Activity of Commercial Rosemary Extract Formulations",J.Food Protect.,72(8),pp.1744–1752.https://doi.org/10.4315/0362-028x-72.8.1744.

[18] Li HB., Cheng K W., Wong CC., Fan KW., Chen F., and Jiang Y.2007.Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry, 102: 771 -776.https://doi.org/ 10.1016/j.foodchem.2006.06.022 .

[19] Manyou Y. , Irene G. , João R., Ana I R N A B., 2021 ,"Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources",Sci Rep,11(1),pp.10041.https://doi.org/ 10.1038/s41598-021-89437-4.

[20] Masumoto N., Yuzo N., Naoki S., Kyoko S., 2018," Phytochemical profiling of rosemary extract products distributed as food additives in the Japanese market", Jpn. J. Food Chem. Safety, 25(2),pp. 105-113.

[21] Mena P., Cirlini M., Tassotti M., Herrlinger K A., Dall'Asta C., and Del Rio D. ,2016,"Phytochemical profiling of flavonoids, phenolic acids, terpenoids, and volatile fraction of a rosemary (*Rosmarinus officinalis L.*)extract ". Molecules, *21*(11), pp.1576. https://doi.org /10.3390/ molecules21111576.

[22] Meziti A., Meziti H., Boudiaf K., Mustapha B. and Bouriche H.,2012,"Polyphenolic profile and antioxidant activities of Nigella Sativa seed extracts *in vitro* and *in vivo".Inter. J. Biotech. and Bioeng,*pp.109–117.

[23] Mojab F., Kamalinejad M., Ghaderi N and Vahidipour H. R.,2003,"Phytochemical Screening of Some Species of Iranian Plants".Iran. J. Pharm. Res., pp. 77-82.

[24] Nehal I., Abbas H., El-Sayed N.S. and Gad H.A.Scientific.,2022,"*Rosmarinus officinalis* L.hexane extract: phytochemical analysis, nanoencapsulation, and in silico, *in vitro,* and *in vivo* anti-photoaging potential evaluation".Reports,(12):13102. ,https://doi.org/10.1038/s41598-022-16592-7 .

[25] N'Guessan K., Kadja B.,Zirihi G.N., Traoré D. and Aké-Assi L,2009,"Screening Phytochimic of Some Medicinal Herbs of the Ivory Coast Used in Country Krobou (Agboville, Coast-of Ivory)".Sci.Nat,1(6),pp.154-159. https://doi.org/10.4314/scinat.v6i1.48575.

[26] Pérez-Fons L.,Garzon M .T., and Micol V .,2009,".Relationship between the Antioxidant Capacity and Effect of Rosemary (*Rosmarinus officinalis* L.) Polyphenols on Membrane Phospholipid Order", J. Agric.Food Chem.,58(1). https://doi.org/10.1021/jf9026487

[27] Rašković A.,Milanović I.,Pavlović N., Cebović T., Vukmirović S., and Mikov M,2014,"Antioxidant activity of rosemary (*Rosmarinus officinalis L.*) essential oil and its hepatoprotective potential". BMC. Complement. Altern. Med.,14:225. https://doi.org/10.1186/1472-6882-14-225.

[28] Saleh A., Al Kamaly O.,Alanazi A.S., and Noman O.,2022,"Phytochemical Analysis and Antimicrobial Activity of Rosmarinus officinalis L.Growing in Saudi Arabia Experimental and Computational Approaches",Processes,10(11),422. https://doi.org/10.3390/pr10112422.

[29] Santiago A. and Carlos Alías J.,2020," Quantification of the Antioxidant Activity of PlantExtracts: Analysis of Sensitivity and Hierarchization Based on the Method Used Natividad Chaves", *Antioxidants,* 9(76). https://doi.org/10.3390/antiox9010076.

[30]Scalbert, A., Johnson I. T., and Saltmarsh M.,2005,"Polyphenols: antioxidants and beyond",Ame. J.Clin. Nutr.,81,pp.215–222.https://doi.org/10.1093/ajcn/81.1.215

[31] Singleton V. L., and Rossi J. A., 1965, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents",Am.J. Enol. Vitic., 16, pp.144-158.

[32] Tawfeeq A.A., Mahdi M.F., Abaas I.S. and Alwan A.H.,2018,"Isolation, quantification, and identification of rosmarinic acid, gas chromatography-mass spectrometry analysis of essential oil, cytotoxic effect, and antimicrobial investigation of *Rosmarinus officinalis* leaves",*Asian j.Pharm. Clin. Res.*, 11(6),pp.126-132. https://doi:10.22159/ajpcr.2018.v11i6.24134.

[33] Yang, H.L.,Korivi, M., Lin, M.K., Chang, H.C.W., Wu, C.R., Lee, M.S. and Hseu, Y.C.,2017," Antihemolytic and antioxidant properties of pearl powder against 2, 2′-azobis (2- amidinopropane) dihydrochloride-induced hemolysis and oxidative damage to erythrocyte membrane lipids and proteins", *J.Food.Drug.Anal.*,*25*(4),pp.898-907. https://doi.org/10.1016/j.jfda.2016.10.007

[34] Wang, Y., Zhang, X.,and Li, H.,2023,"Protective effects of carnosic acid against oxidative stress-induced hemolysis in human erythrocytes", *Food Chem.Toxicol., XXXX*. https://doi.org/10.1016/j.fct.2023.113790.