MAPAN-JOURNAL ,
Publisher: METROLOGY SOC INDIA , NPL PREMISES,
Address: DR K S KRISHNAN MARG, NEW DELHI, INDIA,
ISSN / eISSN:0970-3950 / 0974-9853
Volume 25-Issue 1-(2025)
https://doi-001.org/1025/17624120463991

OPEN ACCESS

PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITIES OF METHANOLIC AND AQUEOUS EXTRACTS OF *Rosmarinus Officinalis* L.

Naima Layachi [1, 2*]

[1]Laboratory of Organic Chemistry and Interdisciplinary, Department of Biology, Faculty of Natural and life Sciences, Mohamed Cherif Messaadia University, Souk-Ahras 41000, Algeria

Email: layachinaima@yahoo.fr

Received : 10/04/2025 ; Accepted : 25/09/2025

Abstract

This research aimed to assess the phytochemical composition, and antioxidant potential of methanolic and aqueous extracts of Rosemary (*Rosmarinus officinalis* L.). Qualitative phytochemical screening of the extracts revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids and steroids. Quantitative determination of total phenolic contents (TPC) and total flavonoid contents (TFC) were conducted using Folin Ciocalteu method and aluminum chloride method. The methanolic extracts exhibited the highest TPC and TFC, with TPC valuing from 30.57±1.41 to 78.45 ± 2.6 mg GAE/g of dry extract for polyphenols, and TFC valued from 16.22±0.27 to 23.62±1.27 mg RE/g dry extract for flavonoids. Antioxidant activity was evaluated using DPPH• (2,2-diphenyl-1-picrylhydrazyl),and ABTS•+ (2, 2'azino bis- [3-ethylbenzthiazoline-6-sulfonic acid]) radical scavenging assays assay. The results highlight the methanolic extract exhibited superior antioxidant activity in both assays, where the IC50 values ranging from 64.6 ± 0.23 to 126.7 ± 0.43 µg/ml by DPPH method and 88.5 ± 0.31µg/ml to 93.4 ± 0.65µg/ml by ABTS$^{\bullet+}$ assay method. These findings highlight the potent antioxidant capacity of Rosmarinus officinalis, corroborating its traditional therapeutic applications and underscoring its potential for use in pharmaceutical and nutraceutical formulations.

Keyword: *Rosmarinus officinalis*, Total phenolics ,Total flavonoids, Antioxydant activity

Introduction

In the recent decades, multiple synthetic antioxidant compounds have demonstrated toxic and carcinogenic effects. As a result, naturally antioxidants has garnered such a considerable research attention, that they are considered safer alternatives with significant roles in regulating oxidative stress and preventing chronic diseases such as cancer, cardiovascular, and neurodegenerative disorders (Karobari et al., 2022). Medicinal Plants contain various kinds of phytochemicals which are potential sources of natural antioxidants such as phenolic acids, flavonoids, tannins and phenolic diterpenes (Dawidowicz, Wianowska, & Baraniak, 2006). These compounds are believed to have the capacity to regenerate endogenous atocopherol within phospholipid bilayer of lipoprotein particles which restores it to its active antioxidant form (Rice-Evans, Miller, & Paganga, 1996).

Rosemary, scientifically known as Rosmarinus officinalis L, is among the plants exhibiting high antioxidant potentials (Al-Sereiti et al., 1999; Zhang et al., 2012; Vallverdú-Queralt, 2014). It is an evergreen sclerophyll species highly adapted to the limitations of the Mediterranean climate (Rahbardar & Hosseinzadeh,2024). Rosemary species are excellent sources of iron, calcium, glutathione, enzymes, phenolic compounds, vitamins B-6 and E and C (Chetouani et al., 2019). Extracts derived from rosemary are extensively used in culinary practices, food preservation, cosmetics, and phytotherapy as a result of their antimicrobial and anti-inflammatory properties, in addition to their antioxidative potential . (Senanayake, 2018; Anselmi et al.;,2004 ; Erkan et al.,2008). Given the criticality of the numerous natural agents play through their antioxidant properties in preventing oxidative-induced diseases, the evaluation of these compounds seems rational and of fundamentally significant. The present study aims to identify phytochemicals groups present in the methanolic and aqueous extracts of rosemary (*Rosmarinus officinalis* L.) leaves , and evaluate the antioxidant activity ,as well as their total phenolic and flavonoid contents.

2. Materials and Methods

2.1. Plant material and extraction

The leaves rosemary were obtained from souk-ahras herbal medicinal market. The dried plant material was ground to pure powder, which macerated in distilled water and methanol 90% (50 g of plant material per 250 ml of solvent) at room temperature for 48 h, with the process repeated to the point of exhaustion (of the material). subsequently, the crude extracts were filtered, evaporated and dried under reduced pressure to yield both the aqueous and methanol extract of Rosemary.

2.2. Qualitative phytochemical analysis:

A Qualitative phytochemical analysis was conducted to determine the presence of some active compounds such as phenols, flavonoids, tannins, saponins, alkaloids and steroids in the extracts.

Standard procedures described by methods (Harborne, 1984.; Harborne ,1980; Shihata., 1951; Stahl,1969; Al-Abid, 1985).

2.3. Phytochemical content determination

2.3.1. Determination of total phenolic compounds (TPC)

Total phenolic content (TPC) was assessed using the Folin–Ciocalteu colorimetric method, referring the procedure described by Singleton and Rossi (1965). 500 µL of each extract was mixed with 2500µL of Folin–Ciocalteu reagent (diluted 1:9 with distilled water), and 200 µL of 7% sodium carbonate (Na_2CO_3). The result (mixture) was incubated in the dark at room temperature for 15 minutes. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid was used as the standard, and results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract).

2.3.2. Determination of Total flavonoids Content

Total flavonoid content (TFC) was determined using the colorimetric method assay described by Koolen et al. (2013). A volume of 500 µL of each extract was mixed with 1.5 mL of distilled water and 150 µL each of 5% sodium nitrate ($NaNO_2$) and 10% $AlCl_3$ and 500 µL of 1 M sodium hydroxide (NaOH). solution. After 5 min, 150 µL of 10% aluminum chloride ($AlCl_3$) was added. After 6 min of incubation, at room temperature, 500 µL of 1 M sodium hydroxide (NaOH) were added. The mixture was incubated at room temperature for 15 minutes and measured the absorbance at 510 nm using a UV-visible spectrophotometer. Quercetin was used as standard and the results were expressed as milligram Quercetin Equivalents per Gram of Dry Matter (mg QE/ g extract).

2.4. Antioxidant capacity

2.4.1. DPPH Free radical scavenging activity

DPPH radical scavenging activity was evaluated according to the method of Meriga et al.,(2012). A volume of 4 mL of DPPH solution in methanol (0.1 mM) was mixed with 1 mL of varying concentrations (of extract solutions). The mixture was vigorously shaken and incubated at room temperature for 30 minutes. Absorbance was measured at 517 nm. The percentage of inhibition was calculated, and IC_{50} values (concentration required to inhibit 50% of DPPH radicals) were derived from the dose-response curves.

2.4.2. ABTS radical scavenging assay

ABTS Radical Scavenging activity was assessed following the method of Re et al.(1999) with some modifications. A solution of ABTS radicals was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulfate and the mixture were allowed to stand in dark at room temperature for 24 h

before use. The solution was diluted with methanol to achieve an absorbance of 0.70 ± 0.02. Subsequently, 50 µL of each sample was combined with 1000 µL of ABTS solution and incubated at room temperature for 30 min, the absorbance of reaction mixture was measured at 734 nm and the percentage inhibition was calculated.

2.5. Statistical analysis

Data were represented as mean ± standard error of mean (SEM) of three replicates. Statistical analysis were performed descriptively, and IC_{50} values were calculated using non-linear regression models in GraphPad Prism 5.0. A significance level of $p < 0.05$ was considered as significant.

Results and discussion

Extraction yields

The extraction yield revealed that the *Rosmarinus Officinalis* Methanolic extraction process achieved a yield of 29.94 % whereas the *Rosmarinus Officinalis* aqueous exhibited a lower yield of 16.7% (v/w). The result of this study is in agreement with the extraction yields of some medicinal plant (Sultana et al.,2009) reported in previous studies. These findings are not surprising, given that most of the active components in plant are saturated organic molecules which are non-polar. Thus, methanol being a less polar solvent than water could be more effective in extracting higher yield of non-polar biological compounds from plant material.

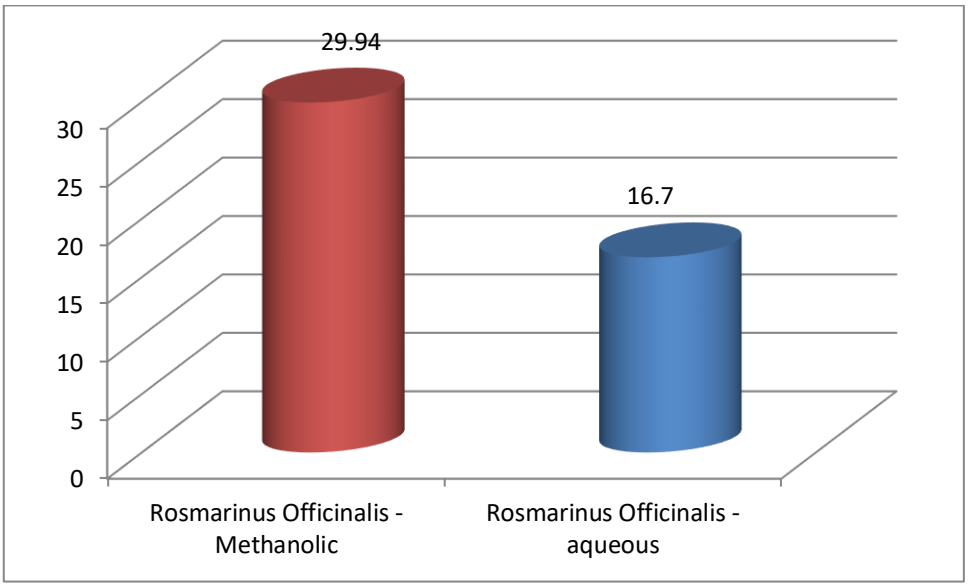

Figure 1. Yields, of both aqueous and methanolic extracts of rosemary

Phytochemical Analysis

The qualitative phytochemical analysis of aqueous and methanolic extracts showed that the rosemary plant was positive for a plethora of secondary metabolites such as phenols, flavonoids, tannins,

saponins, alkaloids, and Steroids (Table 1). These findings are consistent with many studies (Al-Samarrai et al.,2017; Salih and Hamed,2022).

Table 1: Qualitative analysis of extracts of rosemary leaves

Secondary metabolite	Aqueous extract	methanolic extract
Phenols	Positive	Positive
Flavonoids	Positive	Positive
Tannins	Positive	Positive
Saponins	Positive	Positive
Alkaloids	Positive	Positive
Steroids	Positive	Positive

Total phenolic and flavonoid contents of both aqueous and methanolic extracts of rosemary

The phenolic compounds are considered the major contributors to the antioxidant activity of plants (Pereira et al. 2009). This activity was believed to be a result of phenolic compounds redox properties, which allowed them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and have also metal chelating properties.

Total phenolic content values of the tested extracts were shown in figure 02. As is indicated in the table above, the highest TPC was found in methanolic extract as 78.45±2.6 mg Gallic acid equivalent per gram of dry extract, followed by aqueous extract as 30.57±1.41 mg Gallic acid equivalent per gram of dry extract. Similarly, the highest total flavonoid content was also obtained from methanolic extract as 23.62±1.27 mg Quercetin equivalent per gram of dry extract, while aqueous extract was found as 16.22±0.27 mg Quercetin equivalent per gram of dry extract (Figure.03). In general, methanolic extracts gave the best results for quantitative assays. Efficiency of extraction is affected by the solvent used. This latter strongly affects the amounts of total phenolic, and total flavonoids. In addition, the used of hydroalcoholic solvent provides a much higher reproducibility (Safafar et al., 2015). Because of its polarity and its low vapor pressure, the methanol can lead to the maximum of secondary metabolites. This result is in agreement with that of Quy Diem et al. (2014).

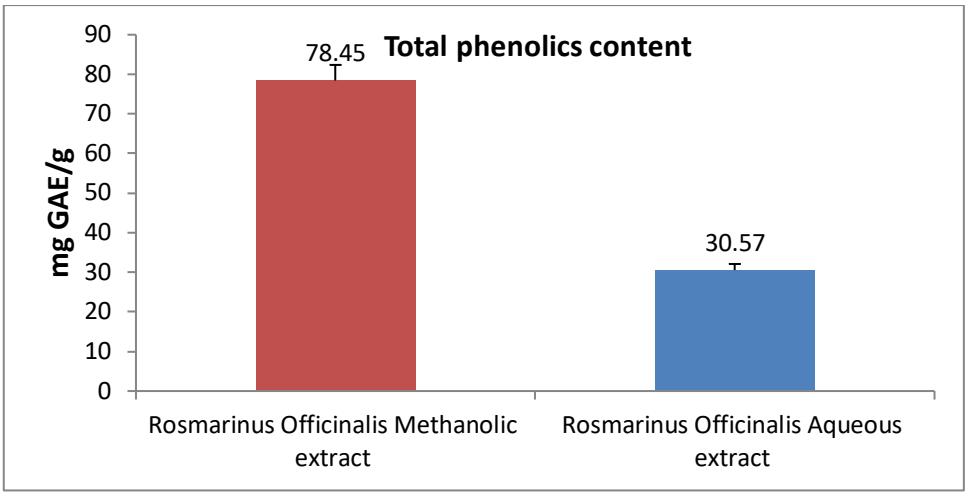

Figure 02. Total phenolic content in aqueous and methanol extracts of rosemary

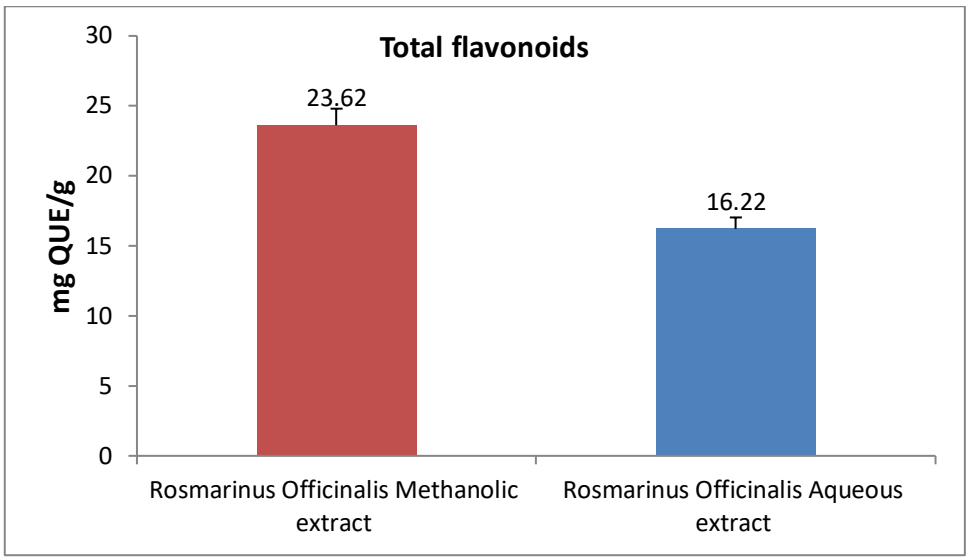

Figure 03. Total flavonoid content in aqueous and methanol extracts of rosemary

Antioxidant capacity

The antioxidant capacity of the methanolic and aqueous extracts of officinalis was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS).

DPPH is a stable nitrogen-centered free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH scavenging activity is a widely used model to evaluate antioxidant activity of plants, and this property is largely attributed to their phenolic contents (Mao,2006). As shown in table1, the methanolic extract exhibited a stronger scavenging activity compared to the aqueous extract. The IC_{50} value for the alcoholic extract was 64.6 µg\ml, whereas the aqueous extract showed a value of 126.7µg\ml. The higher DPPH scavenging activity of methanolic extract may be attributed to its high phenolic content because they are effective hydrogen

donors capable of neutralizing the DPPH. In contrast, the xeaker activity observed in the aqueous extract is likely due to a lower efficiency of water in extracting certain hydrophobic or less polar phenolics critical for DPPH activity.

In contrast, the ABTS assay results revealed that aqueous extract surpassed methanolic one in scavenging the ATBS+ radical cation. The ABTS assay measures antioxidant efficacy against both hydrophilic and lipophilic radical, making it more sensitive to water soluble antioxidants like tannins or glycosides, which may be more abundant or extractable in the aqueous phase (Al Jaafreh,2024). In this study, aqueous extract reached ABTS value of approximately 93.4 µg\ml, indicating robust activity despite generally lower total phenolics compared to methanolic extract. This complementarity between DPPH and ABTS assays highlights the diverse antioxidant capacity, underpinning rosemary's potential as a natural source of bioactive compounds for pharmaceutical and functional food applications.

Table 2. Antioxidant activity of both aqueous and methanol extracts of rosemary by DPPH, and ABTS test.

Extracts	**DPPH** (µg\ml)	**ABTS** (µg\ml)
Rosmarinus Officinalis Methanolic	64.6± 0.23	88.5± 0.31
Rosmarinus Officinalis Aqueous	126.7±0.43	93.4± 0.65

Conclusions

In conclusion, this study demonstrated *Rosmarinus Officinalis* Methanolic extract of the leaves had a high content of total polyphenols, and flavonoids, which also had an antioxidant effect. This suggests that the *Rosmarinus Officinalis* Methanolic extract plays a protective role from oxidative stress. These results support the beneficial utilization of this plant as a natural antioxidant in food and folk medicine.

References

- Ahmad M. Al Jaafreh. (2024). Evaluation of Antioxidant Activities of Rosemary (*Rosmarinus officinalis* L.) Essential Oil and Different Types of Solvent Extractions . *Biomed. & Pharmacol. J,* Vol. 17(1), 323-339.
- Al-Abid MR. (1985). "ZurZusammemsetrung der Absuchlu B memtramein phoenix dactilyfrawuzburg", Univ. Wurzburg F. R. of Germany, 1985.
- Al-Samarrai, Rafaa Razouk Hamid, Al-Samarrai, Othman Rashid, Ibrahim, Ali Sarhan.(2017) . A comparative study of the types of flavonoids identified using HPLC technology in raw

rosemary leaves and the flavonoids isolated from it. Journal of Educational and Scientific Studies - Chemistry. pp. 166-178.

- Al-Sereiti, M.R., Abu-Amer, K.M., Sen, P.(1999). Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian J. Exp. Biol. 9, 37, 124-130.
- Anselmi, C., Centini, M., Granata, P.,Sega, A., Buonocore, A., Bernini, A., Facino, R.M. **(**2004).Antioxidant activity of ferulic acid and alkyl esters in a heterophasic system: A mechanistic insight. Journal of Agricultural and Food Chemistry, *52*, 6425–6432.
- Chetouani M, Mzabri I, Aamar A, Boukroute A, Kouddane N, Berrichi A .(2019). Morphological-physiological and biochemical responses of Rosemary (*Rosmarinus officinalis*) to salt stress, Materials Today: Proceedings 13 752-761.
- Dawidowicz, A. L., Wianowska, D., & Baraniak, B. (2006). The antioxidant properties of alcoholic extracts from Sambucus nigra L. (antioxidant properties of extracts). Lebensmittel-Wissenschaft und Technologic, 39, 308–315.
- Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM. (2009). Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas y Aceites. 60(4):405-12.
- Erkan, N., Ayranci, G., Ayranci, E.(2008). Antioxidant activities of rosemary extract, blackseed essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chemistry, *110*, 76–82.
- Harborne JB.(1973).“Phytochemical Methods”, Science paperbacks, 1980, Chapman Hall, London, 1973.
- Harborne JB.(1984). “Phytochemical Methods. A guide to modern technique of plant analysis”, Chapman Hall, London.
- Karobari, MI., Adil, AH., Assiry, AA. (2022). Herbal medications in Endodontic and its application-a review of literature, Materials, 15(9), 3111.
- Koolen H.H.F. , da Silva F.M.A. , Gozzo F.C. , de Souza A.Q.L. , de Souza A.D.L. (2013). Antioxidant, antimicrobial activities and characterization of phenolic compounds from buriti (*Mauritia flexuosa* L. f.) by UPLC–SI-MS/MS. Food Res Int, 51 , pp. 467-473
- Mao, L.C., Pan, X., Que, F., Fang, X.H., (2006). Antioxidant properties of water and ethanol extracts from hot air-dried and freeze-dried daylily flowers. Eur. Food Res. Technol. 222, 236–241.
- Meriga, B.; Mopuri, R.; MuraliKrishma, T. (2012): Insecticidal antimicrobial and antioxidant activities of bulb extracts of Allium sativum. Asian Pacific Journal of Tropical Medicine .5: 391-395.

- Pereira RP, Fachinetto R, de Souza PA, et al. (2009). Antioxidant efects of diferent extracts from Melissa ofcinalis, Matricaria recutita, and Cymbopogon citratus. Neurochem Res. 34(5):973–83.

- Quy Diem D, Artik EA, Phuong LT-N, Lien HH, Felycia ES, SI, Yi-Hsu J (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic*. Journal of food and drug analysis 22: 296-302
- Rahbardar, M.G.; Hosseinzadeh, H.(2024). Toxicity and safety of rosemary (Rosmarinus officinalis): A comprehensive review. Naunyn-Schm Arch. Pharm. 398, 9–23.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C.(1999) Antioxidant activity applying an improved ABTS radical cation decoloration assay. Free Radic Biol Med.26:1231–1237.
- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine, 20(7), 933–956.
- Safafar H, van Wagenen J, Møller P, Jacobsen C (2015). Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. Marine drugs 13:7339–7356
- Salih HT, Hamed AH.(2022). Extraction and determination of tannic acid in rosemary, anise, and cinnamon by reversal phase RP-HPLC. Eurasian Chem. Commun.4:94-102.
- Senanayake S. N. (2018). Rosemary extract as a natural source of bioactive compounds. Journal of Food Bioactives, **2**, 51-57 .
- Shihata IM. (1951).“A Pharmacological study of *Anagallis arvensis*”, MSc. D. Vet. Thesis, Cairo University, p: 43.
- Singleton, V.L.; Rossi, J.A. (1965).Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Jour-nal of Enology and Viticulture 16(3):144-158.
- Stahl E. (1969).“Thin-Layer-Chromatography, a Laboratory Hand book”, 2nd. Translated by Ashworth, M. R. F. Spring. Verag, Berlin, Heidelberg, New York.
- Sultana, B, Anwar F, Ashraf M.(2009). Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts. Molecules ;14:2167e80.
- Vallverdú-Queralt, A., Regueiro, J., Martínez-Huélamo, M., Rinaldi Alvarenga, J.F., Leal, L.N., & Lamuela-Raventos, R.M.(2014). A comprehensive study on the phenolic profile of widely used culinary herbs and spices: Rosemary, thyme, oregano, cinnamon, cumin and bay. Food Chem., 154, 299-307.

- Zhang, Y., Smuts, J.P., Dodbiba, E., Rangarajan, R., Lang, J.C., Armstrong, D.W.(2012). Degradation study of carnosic acid, carnosol, rosmarinic acid, and rosemary extract (*Rosmarinus officinalis* L.) assessed using HPLC. J. Agric. Food Chem. 60, 9305-9314.