**Anti-lipid Peroxidation And Antioxidant Activities Of Deodorized Rosemary Leaf Extract (Rosmarinus officinalis L.)**

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### Abstract

The objectives of this study were to evaluate the antioxidant and anti-lipid peroxidation activities of rosemary residue extracts. Those were previously eliminated essential oil using two different methods, hexane extraction (HE) and steam distillation (SD). The 1-hour-steam distillation residue (1h-SD-Et ext) extracted by ethanol reached the highest antioxidant capacity (IC50 = 5.1 1.4 *µ*g/mL) in reducing free radical DPPH. Simultaneously, the malondialdehyde (MDA) produced during lipid peroxidation in peanut oil was moderate (TBARS value = 6.0 0.1 mg MDA/kg sample). The high antioxidant activity of this extract was due to the high concentration of polyphenolic compounds, containing 0.0128 0.6 (w/w dried leaves) of carnosic acid and 0.0075 0.2 (w/w dried leaves) of carnosol, which were analyzed by HPLC. In addition, the scent of the extracts from distillation residues also became pleasant to use. The results from this study indicated the prospect of replacing synthetic additives with rosemary extract, mainly when used on the base of low-polarity products, such as vegetable oils, fats, and oil-based cosmetics. It also broadens the potential of utilizing rosemary wastes from the essential oil industry into valuable products, which aims to achieve sustainable production by non-polluting and conserving natural resources.

# Introduction

The rosemary (Rosmarinus officinalis L.) of the Lamiaceae family [[1](#_bookmark15)] was recorded as the first explored in the hills along the Mediterranean [[2](#_bookmark14)], and has been grown more commonly on all continents. Rosemary is a unique spice used in the food industry as an antioxidant [[3](#_bookmark13)], and also have antibiotic effects on human cells [[4](#_bookmark12)].

The antioxidant and anti-lipid peroxidation activities of rosemary belonging to the polyphenol family have been widely published in the scientific literature through the recognition method by high-performance liquid chromatography (HPLC) [[5](#_bookmark11)]. In particular, the most characteristic is the presence of carnosic acid and carnosol [[6](#_bookmark10)], and rosmarinic acid [[7](#_bookmark9)]. Compared with synthetic antioxidants (such as propyl gallate

* PG, hydroxytoluene butylated – BHT, hydroxyanisole butylated – BHA, *α*-tocopherol – Vitamin E), those compounds naturally show a better effect in enhancing stability in oils [[8](#_bookmark8)].

Rosemary extract (E392) was evaluated in 2008 for the safety of use as a food additive by the European Food Safety Authority (EFSA) and the Council on Food Additives, Flavorings, and Food Processing Aids (AFC) [[9](#_bookmark7)]. Following this assessment, in 2016, the FAO/WHO International Expert Committee on Food Additives (JECFA) established a temporarily acceptable daily consumption (ADI) for E392 at 0 – 0.30

mg (carnosic acid and carnosol)/kg of adult body weight (BW) (Panel et al., 2018).

Despite their benefits, these food additives remain drawbacks as containing the flavor of rosemary essential oil that might make up adverse effects on the taste of foods. Consequently, scientists have recommended some methods to remove volatile compounds from rosemary leaves, constituting two main directions: the steam distillation method [[10](#_bookmark6)] and solvent extraction techniques [[11](#_bookmark5)]. Following the re- search of Raul N. C. Jr. et al. [[12](#_bookmark4)], crushed rosemary leaves were extracted by hexane under reflux for 3 hours. The yield was determined at 1.07% (mass of volatile compounds/mass of dry raw materials), which was as high as its yield of extracts by hydro-distillation, accounting for 1.43%.

This study aimed to use rosemary leaves, which were deodorized through the steam distillation process and hexane extraction, to evaluate the antioxidant property of those extracts. Total polyphenols content (TPC), IC50 (DPPH method), and the concentration of malondialdehyde produced in peanut oil (TBARS method) were used as criteria to evaluate the antioxidant and lipid peroxidation activities of the extracts. The results of this work were expected to develop on a large scale to be used as the food additive in vegetable oils, oil-based cosmetics, and pharmaceuticals.

# Materials and Methods

## Materials

Rosemary leaves were cultivated on January 2022 in Lam Dong Province, Vietnam (11.78451,108.25821). It was an ideal time for the active compounds to reach a relatively high concentration. The leaves were then dried in a greenhouse solar dryer with a polyethylene cover with ultraviolet protection. After being dried, those leaves were crushed to about 0.5 – 2 mm. The humidity of crushed materials needed to be less than 12%.

## Deodorizing process

### Steam distillation

Crushed rosemary leaves with a mass of 200 g were distilled at intervals of 60 minutes, 120 minutes, and 180 minutes through the steam distillation system. The distillation time began when the first drop of essential oil condensed into the container. After that, the essential oil was preserved for other purposes. Meanwhile, the odorized rosemary residues were dried in the air to avoid direct exposure to ultraviolet sunlight, preparing for the following extraction procedure.

### Hexane extraction

Dried rosemary was deodorized with hexane solvents by using the material/solvent ratio is 1:30 (g/mL), and the extraction is performed over 4 hours using the Soxhlet extraction system. According to the research by Ludmila et al. [[13](#_bookmark3)], hexane extraction aims to eliminate less polar and odorous compounds, such as the components of rosemary essential oil. As a result, it enables other compounds, such as polyphenols, especially carnosic acid and carnosol, to be released better at the next extraction stage. After deodorizing by hexane extraction, the hexane extracts (Hex–ext.) were collected to evaluate the antioxidant activity. Meanwhile, the residue materials were dried in the air for the following extraction procedure.

## Extraction process

Extraction is a necessary process to separate the natural compounds contained in the plant from dry raw materials. Regarding the ethanolic extracts, this research followed the optimal extraction conditions

which were investigated in previous research by Cuong et al. [[14](#_bookmark2)]. Following this, the deodorized rosemary residues were extracted with 65% ethanol solvent (v/v) with a solid-to-liquid ratio of 1:7.5 (g/mL). Then, the extract was stirred at 350 rpm under heating conditions of 60 5*o*C. After that, those extracts were evaporated in the vacuum evaporation machine. Then the concentrated ones were stored in dark glass bottles at temperatures below -18*o*C.

*±*

Besides ethanol extraction, this research also evaluated the antioxidant effect of the ethyl acetate extracts, which were conducted through the Soxhlet extraction system following the research of Yeddes et al. [[15](#_bookmark1)]. The reason why choosing ethyl acetate as the second investigating solvent for extraction was that the results from the research of L. Trojakova et al. [[13](#_bookmark3)] illustrated that the best yield of rosemary extracts was obtained with solvents of medium polarity. Meanwhile, ethyl acetate is also a medium polar solvent and has been widely used in the food industry [[16](#_bookmark0)] due to its non-toxicity and cost-efficiency. Regarding the ethanol extracts (ext) from steam distillation (SD) residue, those were named following the structure as [time distillation] – [SD-Et] [ext]. For example, the ethanol extract from 1 h – Steam distillation residue would be named as 1h-SD-Et ext. In addition, other extracts from hexane deodorization were named as [Hex] – [Et] [ext] or [Hex] – [EA] [ext] according to the use of solvent extraction was ethanol or ethyl acetate, respectively.

## Analytical methods

### Extraction yield (PY

The Extraction yield (PY) was immediately indicated after the extraction experiment by a ratio of the quantity of the concentrated extract (dry basis) to the total solid content (dry basis) in the material.

*Extraction yield* (%) =

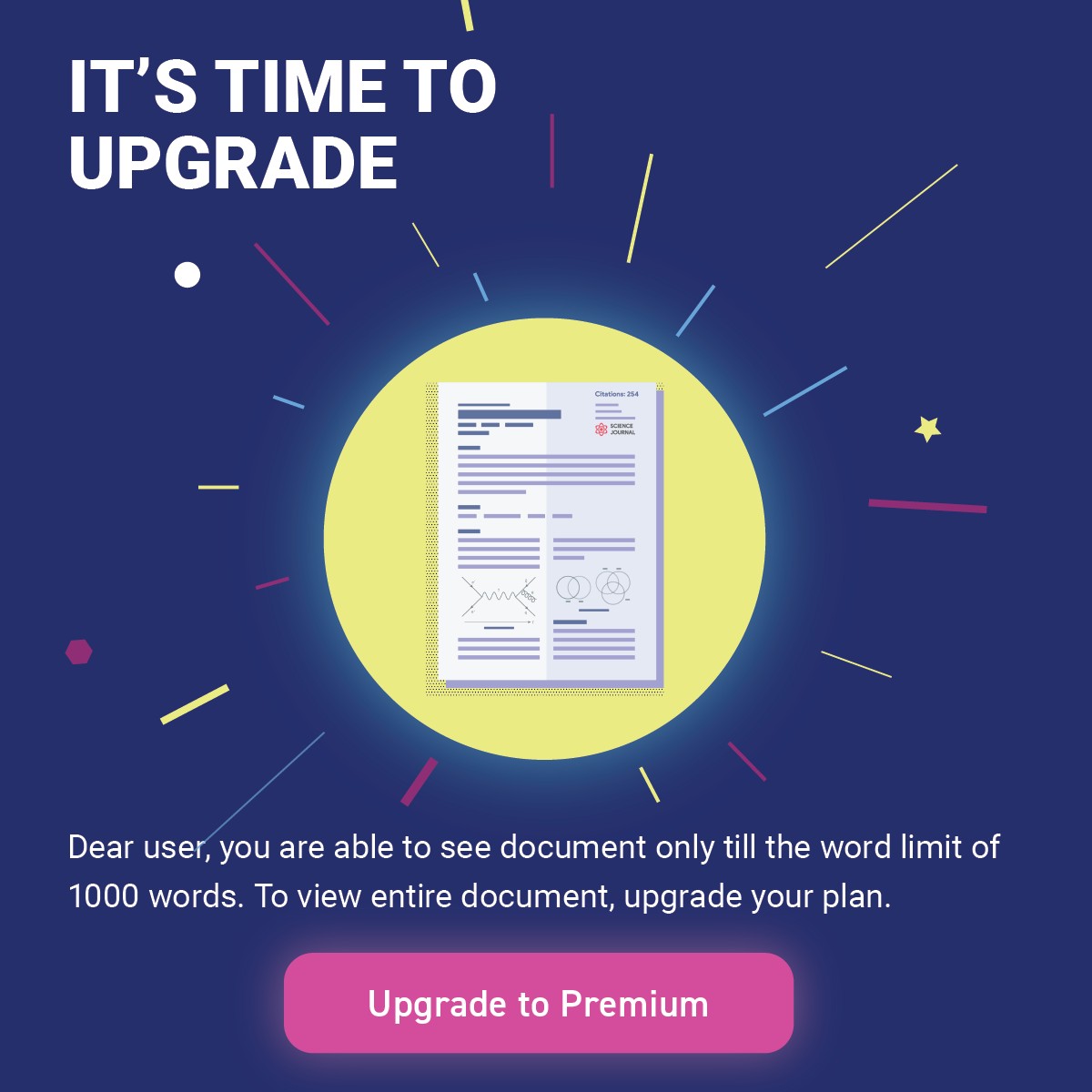
*weight of the dried concentrated extract* (*g*)

*weight of the dried material* (*g*) *×* 100

%

### Total Polyphenol

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# Acknowledgments

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# Appendix

[Table 1 about here.]

1h-SD-Et ext: The ethanol extract from 1 h - steam distillation residue

Hex-Et/EA ext: The ethanol/ethyl acetate extract from hexane deodorized residue

[Table 2 about here.] [Table 3 about here.] [Table 4 about here.]

[Figure 1 about here.]

# List of Tables

* 1. [Comparison of extraction yield of the extracts from deodorized rosemary leaves by hexane extraction and steam distillation](#_bookmark16) 7
  2. [Total polyphenols content of the extracts from deodorized rosemary leaves by hexane ex- traction and steam distillation](#_bookmark17) 8
  3. [IC50 values of DPPH free radical scavenging of the extracts from deodorized rosemary leaves by hexane extraction and steam distillation](#_bookmark18) 9
  4. [TBARS values of the extracts from deodorized rosemary leaves by hexane extraction and steam distillation](#_bookmark19) 10

Table 1: Comparison of extraction yield of the extracts from deodorized rosemary leaves by hexane extraction and steam distillation

No. Assay Extraction method Extraction yield (% dry basis)

* + 1. Ethanol extract (Et ext) – Control sample 34.0 0.1
    2. Deodorization by hexane extraction (Hex ext) 21.0 0.8
    3. Hexane – Ethanol extract (Hex-Et ext) 32.7 0.9
    4. Hexane – Ethyl acetate extract (Hex-EA ext) 18.3 0.3
    5. Deodorization by steam distillation in 1 hour (1h)

1h – SD – Ethanol extract (1h-SD-Et ext) 33.6 0.1

* + 1. Deodorization by steam distillation in 2 hours (2h)

2h – SD – Ethanol extract (2h-SD-Et ext) 35.5 0.1

* + 1. Deodorization by steam distillation in 3 hours (3h)

3h – SD – Ethanol extract (3h-SD-Et ext) 25.5 0.2

Table 2: Total polyphenols content of the extracts from deodorized rosemary leaves by hexane extraction and steam distillation

No. Assay Extraction method TPC (mg GAE/g dried extract)

1. Ethanol extract (Et ext) – Control sample 220.7 17.1
2. Deodorization by hexane extraction (Hex ext) 85.5 0.4
3. Hexane – Ethanol extract (Hex-Et ext) 200.2 7.5
4. Hexane – Ethyl acetate extract (Hex-EA ext) 121.6 5.1
5. Deodorization by steam distillation in 1 hour (1h)

1h – SD – Ethanol extract (1h-SD-Et ext) 205.8 4.3

1. Deodorization by steam distillation in 2 hours (2h)

2h – SD – Ethanol extract (2h-SD-Et ext) 184.5 4.5

1. Deodorization by steam distillation in 3 hours (3h)

3h – SD – Ethanol extract (3h-SD-Et ext) 123.4 3.4

Table 3: IC50 values of DPPH free radical scavenging of the extracts from deodorized rosemary leaves by hexane extraction and steam distillation

No. Assay Extraction method IC50 ( g/mL)

* 1. Ethanol extract (Et ext) – Control sample 5.0 0.1
  2. Deodorization by hexane extraction (Hex ext) 9.16 0.04
  3. Hexane – Ethanol extract (Hex-Et ext) 7.68 0.1
  4. Hexane – Ethyl acetate extract (Hex-EA ext) 8.02 0.5
  5. Deodorization by steam distillation in 1 hour (1h)

1h – SD – Ethanol extract (1h-SD-Et ext) 5.1 1.4

* 1. Deodorization by steam distillation in 2 hours (2h)

2h – SD – Ethanol extract (2h-SD-Et ext) 7.4 0.1

* 1. Deodorization by steam distillation in 3 hours (3h)

3h – SD – Ethanol extract (3h-SD-Et ext) 10.4 0.5

Table 4: TBARS values of the extracts from deodorized rosemary leaves by hexane extraction and steam distillation

No. Assay Extraction method TBARS (mg MDA/kg sample)

1. Butylated hydroxytoluene (BHT) 3.6 0.2
2. Deodorization by hexane extraction (Hex ext) 7.1 0.02
3. Hexane – Ethanol extract (Hex-Et ext) 5.7 0.2
4. Hexane – Ethyl acetate extract (Hex-EA ext) 7.0 0.2
5. Deodorization by steam distillation in 1 hour (1h)

1h – SD – Ethanol extract (1h-SD-Et ext) 6.0 0.1

1. Deodorization by steam distillation in 2 hours (2h)

2h – SD – Ethanol extract (2h-SD-Et ext) 7.0 0.1

1. Deodorization by steam distillation in 3 hours (3h)

3h – SD – Ethanol extract (3h-SD-Et ext) 8.0 0.2

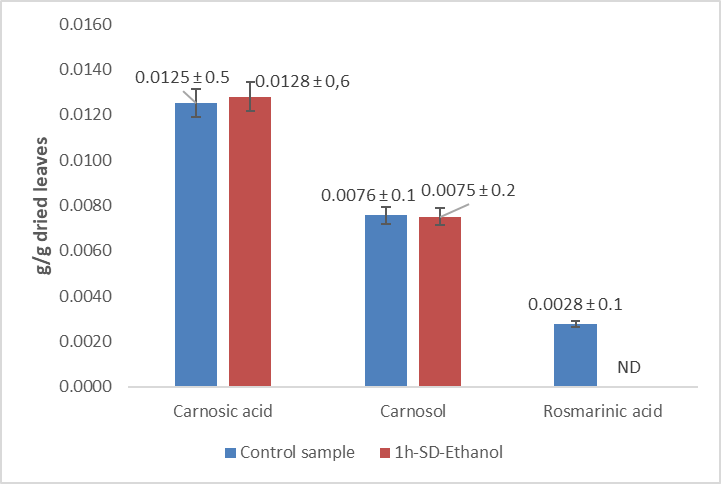


Figure 1: The percentage of polyphenol compounds in rosemary dried leaves (ND: Not detected)