Characterization of the essential oil extracted from rosemary.

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Abstract

Rosemarinus **was dried by tunnel dryer at 40°C, 50°C, 60°C and decreasing temperature, essential oil was extracted from it by petroleum oxide solvent (Soxhlet method) with 8.5% yield, then this oil was analysed in order to know its proprieties (physicochemical, radical and microbiological analysis), also it was characterised by spectroscopy using UV-visible spectrophotometer and chromatography using GC-Ms method was used identify all organic components of this oil.**

Keywords: *Rosmarinus*/Rosemary, Drying, Essential oil, Analyses, Chromatography.

Introduction

Medicinal aromatic plants are rich in bioactive molecules that protect against the development of dangerous diseases (cancer, cardiovascular diseases, inflammatory diseases, gastrointestinal diseases, etc.) [1].

Research on the antioxidant, radical-scavenging, antiviral, and antibacterial activities of plants or oils extracted from these plants is ongoing, leading to new discoveries, with applications in medicine, pharmacy, and the food industry [2].

Medicinal aromatic plants such as rosemary can be dried either traditionally or industrially at temperatures that do not compromise the quality of the dried products. A kinetic study can provide information about the reliability, speed, and moisture content of the desired plant. However, the extraction and analysis of essential oils can reveal their effects and compositions [3].

Materials and Methods

Drying of Rosmarinus

Sample preparation: The drying of rosemary required the following steps:

- The stems of the plant were collected from rosemary plants on the Itha mountain.
- They were transported to the laboratory.
- The leaves were peeled and stored in the refrigerator.
- A mass of 30 g was weighed and placed in a drying chamber.
- The instantaneous mass loss was monitored to record all kinetic parameters.

Airflow: This is a humid air blower mounted on a frame, where the temperature, speed, and humidity of the air can be controlled. It is a type of convective tunnel dryer operating on electrical energy. It includes:

- A square-shaped test duct (25 cm × 25 cm) with a convergent section at the inlet for better speed distribution and a divergent section at the outlet to avoid detachment. The duct includes a space for securing samples and inlets for thermocouples and a Pitot tube.
- A water humidifier, supplied with variable-temperature water through a heater regulated by a humidity probe, with the following characteristics: a) Installed power: 12 kW; b) Water flow rate: 1.4×10^{-4} $m^3.s^{-1}$; c) Maximum relative humidity: 80%
- A heating battery (24 kW) regulated by a temperature probe.
- An air treatment unit with a variable-speed fan, allowing average air speeds in the test duct to range from 4 to 12 $m.s^{-1}$. For lower speeds required during the drying process, an air intake system is used. Data acquisition is manual. The blower is controlled by a control cabinet that includes: A heater regulator; A humidity regulator; A ventilation speed controller; An electrovalve control for the humidifier circuit

Measured parameters include: The wet and dry air temperatures, air speed, wet and dry product masses, and the relative humidity of the drying air [4].

Drying kinetics: The drying kinetics reflects the instantaneous change in the dried mass, moisture content of the material, or the drying rate of the product. This study was carried out at different drying temperatures: 40°C, 50°C, 60°C, and decreasing from 60°C to 40°C. The change in temperature and moisture content of the material is usually balanced by a heat

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and mass transfer between the surface of the product and the drying agent [5].

The absolute moisture of rosemary is determined by weighing the product before and after drying. For this, an electronic balance is connected to the blower to instantly record the product mass, with a mass loss measurement every 10 minutes [6].

The absolute moisture is calculated using the formula:

Mh=Wet mass of the product (kg)

MS=Dry mass of the product (kg)

X=Moisture content of the product (kg water/kg MS)

The instantaneous drying rate is calculated as:

Xp=Experimental drying rate (kg/h/kg/s)

Where:

- X0X_0X0 is the initial moisture content at time t=0t=0t=0 (kg/h/kg)
- XnX_nXn is the moisture content at time t=nt=nt=n (kg/h/kg)

The drying is faster at higher temperatures than at lower temperatures, with speeds decreasing linearly after a short time to stabilize. At the critical moisture values, the drying rate becomes stable: at 40°C, the rate stabilized at 0.00158 g/(g of dry matter min), while at 60°C, it reached 0.00087 g/(g of dry matter min) after 200 minutes [7].

Results and Discussion

Extraction

Preparation and extraction technique: A Soxhlet apparatus consists of a round-bottom flask, a condenser, and an extractor. The extractor has a tube system for draining the reservoir, which varies in volume depending on the model. The system is completed with a cellulose cartridge placed in the reservoir to receive the compound to be extracted.

The dried leaves are finely ground using a porcelain laboratory mortar. 20 g of ground rosemary are introduced into the extractor's cartridge [8].

The extraction is performed using petroleum ether as a solvent (boiling point 60°C, density 0.64).

Extraction yield: The dried samples are subjected to extraction under the same conditions:

- Same mass of dried rosemary (40 g)
- Same solvent volume (150 ml) and same heating temperature (60°C)

The yield can be tested on a sample of rosemary dried at 40°C, as bibliographical research confirms that this temperature is the most severe for extraction.

Analysis of extracted rosemary oils

Peroxide index: This represents the amount of hydroperoxides present in the oil, formed during storage due to autoxidation. It is expressed in milliequivalents of active oxygen per kilogram of fat [9].

The peroxide index is determined by weighing 1g of oil, adding 12.5 ml of a mixture (5 ml chloroform and 7.5 ml acetic acid), dissolving by stirring, and adding 0.5 ml of KI. After 5 minutes, the reaction is stopped by adding 37.5 ml of water, followed by the addition of iodine indicator. The purple color indicates the presence of peroxide, and the dosage of sodium thiosulfate is carried out until the purple color disappears from both the sample and blank.

The results of physicochemical analysis are tabulated, showing that acidity increases with decreasing temperature, and the peroxide index is lower at higher temperatures (Figures 1-3).

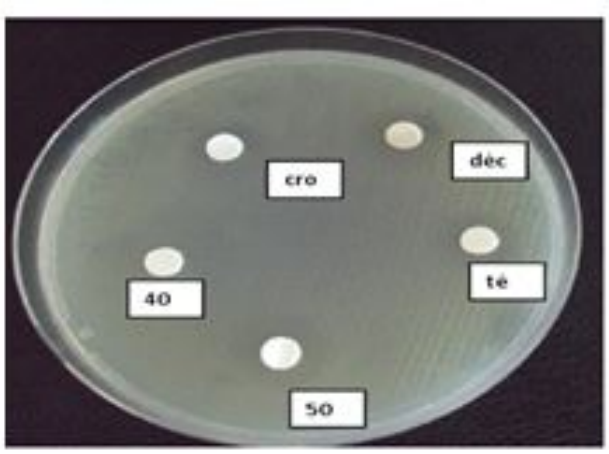


Figure 1. *Results with Escherichia coli ATCC25922.*

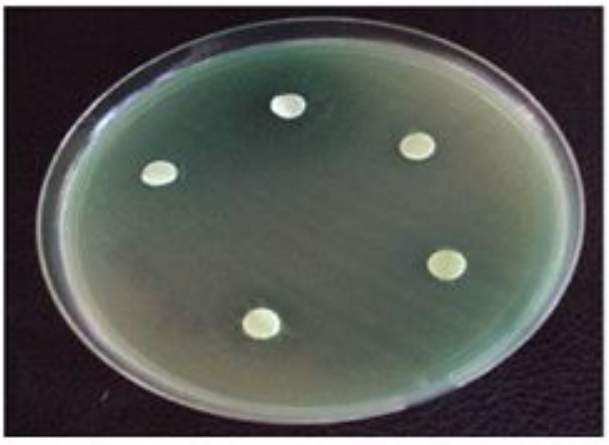


Figure 2. *Results with Pseudomonas aeruginosa ATCC 27853.*

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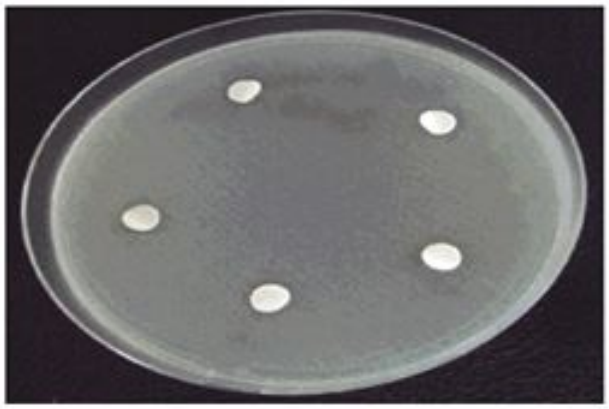


Figure 3. *Results with Salmonella enteritidis DMB 560.*

Antibacterial activity

The antibacterial activity of rosemary essential oil extract against five bacteria was demonstrated by the diffusion method on a solid medium, particularly using the disc method.

Results indicate that rosemary oils extracted at 60°C show the highest antibacterial activity against *Escherichia coli*, while *Pseudomonas aeruginosa* shows resistance to rosemary oils.

Chromatographic identification of rosemary oil components

The essential oil is analyzed by gas chromatography using an HP 6890 series apparatus with a Flame Ionization Detector (FID), an Electronic Pressure Control (EPC) injector, and a polar HP Innowax column.

The major components identified in the oils at different drying temperatures include α- and β-pinene, camphene, 1,8-cineole, camphor, β-caryophyllene, borneol, and geraniol, with variations in percentages depending on the drying conditions (Tables 1-3).

Table 1. *Results of chromatographic characterization by GC-MS.*

Identification	**T=40°C**	**T=50°C**	**T=60°C**	**Decreasing time**
Retention time	**Peak area**	**Retention time**	**Peak area**	**Retention time**
Tricyclene	4.65	13.444	4.663	5.285
α-Pinene	5.067	680.33	5.074	325.27
α-Thujene	5.252	29.244	5.264	11.71
Camphene	6.462	375.43	6.472	161.2
β-Pinene	8.288	583.84	8.294	278.75
Sabinene	9.211	7.202	9.21	0
Myrcene	12.03	7.312	12.05	4.143
α-Terpinene	12.52	88.772	12.52	44.45
Limonene	14.21	135.821	14.2	84.22
Cineole-1,8	14.64	2571.06	14.6	1261
β-Ocimene	15.9	0	15.9	0
γ-Terpinene	17.16	97.56	17.16	66.4
3-Octanone	17.95	6.493	17.95	0
p-Cymene	18.58	40.605	18.59	31.87
Terpinolene	19.29	30.816	19.29	20.19
3-Octanol	24.92	3.282	24.95	0
1-Octen-3-ol	27.57	1.542	27.57	0
Ylangene	29.25	32.411	29.25	13.27
Camphor	30.692	519.733	30.691	189.92
Linalool	31.1	0	33.123	27.099
Bornyl acetate	33.125	59.39	33.123	27.099
Terpinene-1-ol-4	33.99	171.4	33.99	131.5
β-Caryophyllene	34.62	122.46	34.63	152.86

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δ-Terpineol	36.61	0	36.61	0
α-Humulene	37.66	8.282	37.66	11.29
α-Terpinolene	38.15	30.965	38.15	22.385
Borneol	39.2	162.52	39.2	123
Verbenone	39.638	2.687	39.63	2.63
Geraniol	41.37	623.32	41.37	684.7
Caryophyllene oxide	49.42	14.262	49.42	27.39

Table 2. *Operating parameters of GC-MS.*

Component	Parameters	Value or Program
Column	Stationary phase	HP-5MS 5% phenylmethyl siloxane
Dimension	30 m × 250 mm × 0.25 mm	
Temperature control parameters	Temperature program	40°C for 10 minutes, then increase at 3°C/min to 205°C for 10 minutes
Injection parameters	Injection volume	1 ml
Injector temperature	250°C	
Injection mode "Split"	Split ratio 60:1	
Transfer line	Temperature	280°C
Mobile phase	Carrier gas	Helium (He)
Detector parameters	MS source temperature	230°C
Quadrupole temperature (Analyzer)	150°C (<200°C)	
Scan mode interval used	50.0 amu-550.0 amu	

Table 3. *Main components of the oils (%).*

Compounds	Rosemary oil dried at 40°C	Rosemary oil dried at 50°C	Rosemary oil dried at 60°C	Rosemary oil dried at decreasing temperature
α and β-pinene	10.597 and 9.094	8.771 and 7.517	10.579 and 6.651	10.913 and 8.885
α and γ-terpinene	1.383 and 1.52	1.119 and 1.791	1.223 and 0.913	1.298 and 1.588
Camphene	5.848	4.347	6.179	5.948
Cineole-1.8	40.046	34.008	43.14	36.852
Camphor	8.095	5.121	9.619	8.817
Terpin-1-en-4-ol	2.67	3.546	0	3.165
β-Caryophyllene	1.907	4.122	1.833	3.756
Borneol	2.531	3.336	2.958	2.772
Geraniol	9.709	18.462	7.243	10.072

Conclusion

In this study, rosemary was dried under four different temperature conditions and essential oils were extracted using Soxhlet apparatus. The oils exhibited significant physicochemical characteristics, antibacterial activities, and substantial antimicrobial properties, especially against *Escherichia coli*.

The chromatographic analysis revealed that the essential oils contained various molecules with important phytotherapeutic properties. These properties support the potential use of rosemary essential oils as antimicrobial agents in the food or pharmaceutical industries.

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