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Phytochemical Analysis and Antimicrobial Activity of *Rosmarinus officinalis* L. Growing in Saudi Arabia: Experimental and Computational Approaches

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Abstract: *Rosmarinus officinalis* L. is widely distributed in Saudi Arabia. However, only a few studies have been reported regarding this species. In this study, we investigated the phytochemical analysis of *R. officinalis* essential oil using GC-MS analysis in order to identify its major components; the in vitro antimicrobial activity of the essential oil was also evaluated using disc diffusion assay against gram-positive (*S. aureus*) and gram-negative bacteria (*E. coli*), the antimicrobial activity was also assessed with molecular docking against several microbial proteins; TyRS, DNA gyrase and DHFR. The GC-MS analysis has indicated the presence of 18 constituents, representing 99.93 % of the total oil content. The major compounds detected were Bornyl acetate (26.59%), Eucalyptol (17.38%), Camphor (10.42%), Borneol (9.78%), Beta-Caryophyllene (7.80%) and α-pinene (3.85%). The antimicrobial study showed that *R. officinalis* has strong antimicrobial activity against *S. aureus* with *an* inhibition zone of 30 mm and *E. coli* with an inhibition zone of 25 mm. The affinities of molecular docking (TyrRS: between −4.8 and −4.9 Kcal/mol against −8.2 Kcal/mol obtained with Clorobiocin; DNA gyrase: between −4.5 and −4.9 Kcal/mol against −9.1 Kcal/mol obtained with Clorobiocin). However, strong affinities were obtained with the molecules when tested against DHFR (DHFR: between −5.8 and −6.0 Kcal/mol against −6.3 Kcal/mol obtained with SCHEMBL2181345). As a consequence, the pharmaceutical industry may use the essential oils from this plant to develop cutting-edge synthetic drugs to treat this illness infection.

Keywords: *Rosmarinus officinalis*; essential oils; antimicrobial; gas chromatography

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1. Introduction

Nowadays, medicinal and aromatic plants are widely recognized for their therapeutic properties. Various preparations were utilized throughout time, ranging from cold aqueous alcoholic to hot decoction and hydro-distillated essential oils (EOs). Essential oils are concentrated and potent plant extracts that have been utilized in various fields since the dawn of civilization, including agriculture, medicine, and cosmetics. Because of their intense taste, they are employed in culinary preparation [1]. The Lamiaceae plant family is renowned for having an abundance of essential oils. It belongs to the flowering plant family that is sometimes referred to as the mint, sage, or deadnettle family. Numerous members of this family of herbs, including marjoram, mint, oregano, lavender, and many more, are used regularly [2]. Rosmarinus, a genus (Rosemary), is a genus of three endemic to the Mediterranean area, wild-growing fragrant shrubs (*Rosmarinus eryocalix*, *officinalis*, and *tomentosus*) [3]. *R. officinalis* is the most popular species of this genera, and it is widely utilized for both culinary and therapeutic applications all over the world [4,5]. *R. officinalis* is often used as an analgesic, anti-inflammatory, anti-rheumatic, antispasmodic in renal colic and dysmenorrhoea, carminative, and choleretic in conventional as well as complementary alternative medicine [6]. There is evidence that rosemary includes many bioactive compounds with a range of pharmacological effects, most notably antioxidative [7], anti-inflammatory [8], antidiabetic [9], and antibacterial [10] effects. Moreover, multiple in vivo [11,12] and in vitro [13–15] investigations have shown that rosemary extracts have promising anticarcinogenic properties. Additionally, the plant is effective in treating bacterial infections, particularly those of the stomach. *H. pylori* is a common and deadly bacteria that may cause a stomach ulcer, but rosemary has been demonstrated to help limit its growth. Additionally, rosemary has been linked to the prevention of *Staphylococcal* infection, which kills thousands each year [16]. Between 1.0 percent and 2.5 percent of essential oil may be found in rosemary leaves (EO). In most situations, the rosemary's essential oil is light yellow to pale, with a highly unique and reviving scent [17]. Rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid, and carnosol are only a few of the phytochemicals that it contains [18]. The molecular structure of *R. officinalis* essential oil may vary depending on a number of conditions, including temperature, soil, sun exposure, and extraction method. However, the most usually documented chemotypes are cineoliferum, which is predominantly comprised of 1,8-cineole, and camphoric acid, where camphor predominates [19]. We present here the results of the phytochemical analysis of *Rosmarinus officinalis* L. essential oil using GC-MS analysis to determine its major constituents. Additionally, we assessed the essential oil's in vitro antimicrobial activity using a disc diffusion assay and its molecular activity against several microbial proteins.

2. Materials and Methods

2.1. Plant Material and Preparation of the Volatile Oil

Rosmarinus officinalis L. aerial portion was taken in March from Altaif city in Saudi Arabia's southern area during the spring season (2022). The plant was harvested, dried in the shade at room temperature, and powdered. *R. officinalis* L. ground aerial component (100 g plant material in 1 L tap water) was hydrodistilated for 3 h using Clevenger-type equipment. The volatile oil produced was stored at 4 °C until tested and evaluated.

2.2. Gas Chromatographic Analysis

The GC-MS analysis was carried out using a Perkin Elmer Clarus 600 T gas chromatograph linked to a Turbomass spectrometer at the Research Center, College of Pharmacy, King Saud University, Riyadh, in accordance with the technique described by Khali et al. [20]. The *R. officinalis* essential Oil (ROEO) chemical composition was determined by comparing the mass spectra obtained with the mass spectra from Adams Library [21] and the Wiley GC/MS Library [22].

2.3. Antimicrobial Activity Determination

2.3.1. Test Microorganisms

The microbial strains used in this study were gram-positive bacteria *Staphylococcal aureus* (ATCC 25923) and the gram-negative bacteria *Escherichia coli* (ATCC 25922).

2.3.2. Disk Diffusion Assays

The disc diffusion technique was used to determine the antibacterial activity of *R. officinalis* essential oil. A total of 100 µL of suspensions containing 10^7 CFU/mL of bacteria in exponential growth phase was spread on Mueller–Hinton agar medium [23]. Filter paper disks (6 mm in diameter) were impregnated with 20 µL of each sample (2 mg/disc) which was previously dissolved in DMSO and placed on the inoculated Petri dishes. Petri dishes were then incubated for 24 h at 37 °C. Antimicrobial activity was evaluated by measuring the inhibition zone (mm) against the studied microorganisms, including disc diameter.

2.4. Molecular Docking

2.4.1. Preparation of the Ligand

The SDF format of Camphor (CID: 2537), Eucalyptol (CID: 535040), and Bornyl acetate (CID: 6448) were obtained from PubChem. The SDF file was converted to the PDBQT format using AutoDock Tools. For the final ligand preparation, Gasteiger partial charges were added, rotatable bonds were defined, and the nonpolar hydrogen atoms were merged.

2.4.2. Preparation of the Receptors

The PDB file for each receptor was retrieved from the protein data bank website. The X-ray crystal structures of the receptors were chosen for their completeness, resolution, and compliance with our research objective. Table 1 describes the identified receptors in detail.

Table 1. Description of the studied receptors.

Receptors	ID	Resolution (Å)	Class
TyRS	1jij	3.20 Å	Ligase
DNA gyrase	1KZN	2.30 Å	Isomerase
DHFR	3fyv	2.20 Å	Oxidoreductase

3. Results and Discussion

3.1. Phytochemical Analysis of *R. officinalis* L. Essential Oil

The outcome of the essential oil's chemical composition analysis of *R. officinalis* by CG-MS is shown in Table 2 and Figure 1. From this oil, 18 constituents were identified, representing 99.93 % of the total oil content. The major compounds detected were Bornyl acetate (26.59%), Eucalyptol (17.38%), Camphor (10.42%), Borneol (9.78%), Beta-Caryophyllene (7.80%), and α-pinene (3.85%). Several studies from different regions in the world revealed a wide change in the chemical constituents of rosemary essential oil.

Table 2. GC-MS analysis of *R. officinalis*.

Compound Name	Chemical Formula	Molecular Weight (g/mol)	RT (min)	% Area
Alpha-Pinene	$C_{10}H_{16}$	136.23	6.094	3.85
Camphene	$C_{10}H_{16}$	136.23	6.339	4.1
Beta-Pinene	$C_{10}H_{16}$	136.23	6.784	3.41
Eucalyptol	$C_{10}H_{18}O$	154.25	7.654	17.38
Gamma-Terpinene	$C_{10}H_{16}$	136.23	8.079	0.28
Camphor	$C_{10}H_{16}O$	152.23	9.469	10.42
Borneol	$C_{10}H_{18}O$	154.25	9.809	9.78
Terpinen-4-ol	$C_{10}H_{18}O$	154.25	9.951	1.17
Alpha-Terpineol	$C_{10}H_{18}O$	154.25	10.15	3.77
Bornyl acetate	$C_{12}H_{20}O_2$	196.29	11.511	26.6
Beta-Caryophyllene	$C_{15}H_{24}$	204.35	13.365	7.8
Alpha-Caryophyllene	$C_{15}H_{24}$	204.35	13.79	1.06
Cadina-1(10),4-diene	$C_{15}H_{24}$	204.35	14.613	0.32
Caryophyllene oxide	$C_{15}H_{24}O$	220.35	15.416	1.19
Bicyclo [4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	$C_{15}H_{24}$	204.35	16.05	0.33
Linoleic acid	$C_{18}H_{32}O_2$	280.4	20.995	0.5
Gamma-Sitosterol	$C_{29}H_{50}O$	414.7	27.33	0.89
Lupeol	$C_{30}H_{50}O$	426.7	28.559	7.18

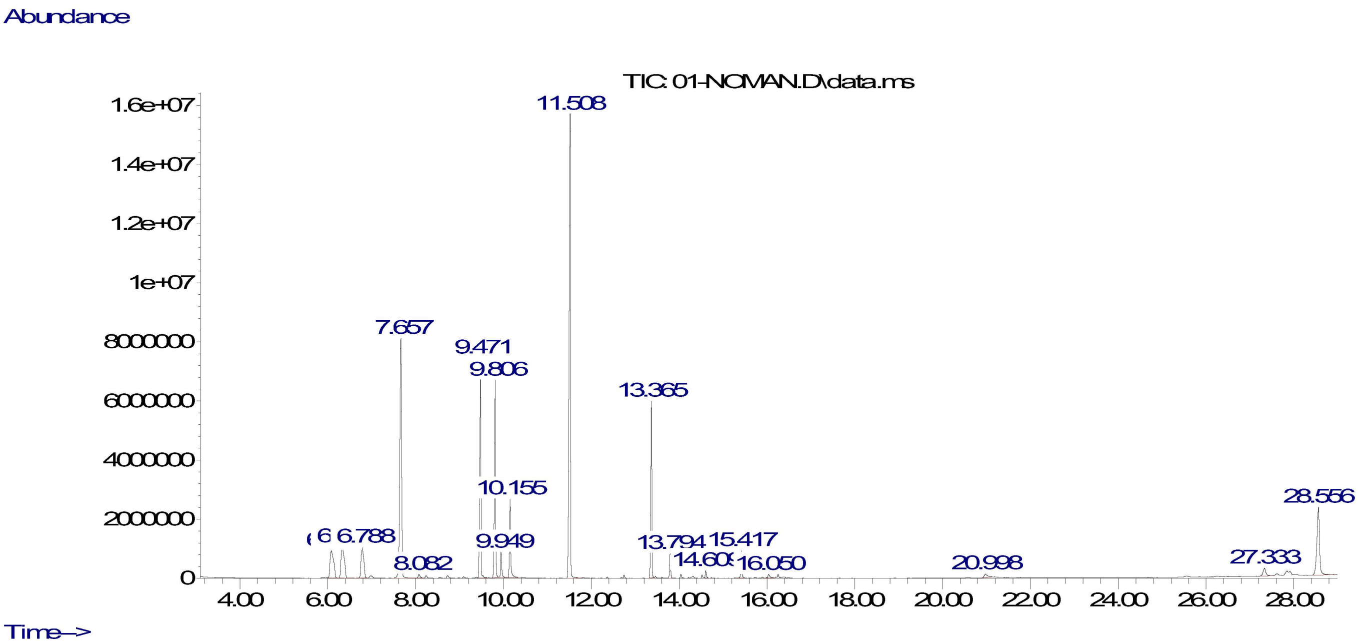

Figure 1. GC-MS chromatogram of *R. officinalis*.

Algerian rosemary essential oil, for example, has 52.4% 1,8-cineole, 12.6% camphor, 5.7% β-pinene, and 5.2% α-pinene [24]. Camphor (18.9%), verbenone (11.3%), α-pinene (9.6%), β-myrcene (8.6%), 1,8-cineole (8.0%), and β-caryophyllene (5.1%) are all found in the Brazilian rosemary leaves [25]. Concerning Turkish EOs of rosemary, as examined by Ozcan and Chalchat et al. [26], it mostly contains p-cymene (44.02%), followed by linalool (20.5%), γ-terpinene (16.62%), and thymol (1.81%). However, The oils extracted from Chinese rosemary leaves, according to GC as well as GC-MS analyses, comprise 1,8-cineole (26.54%), followed by α-pinene (20.14%), then camphor (12.88%), camphene (11.38%) as well as β-pinene (6.95%) as major components [27]. These differences in the chemical compositions of oils could be attributed to climatic effects on the plants [28]; other factors may also influence the differences in genotypic and environmental differences within species, such as sample extraction time and extraction technique used to obtain the rosemary oil or extract.

3.2. Antimicrobial Activity

The antimicrobial activity of ROEO was assessed by the presence or absence of inhibitions zones and zone diameters. Figure 2 shows the essential oil inhibition zones found using the diffusion approach on a solid medium for one Gram-positive and one Gram-negative bacteria. The diameters of the zones of inhibition for the extract were assessed after 24 h of incubation. The findings indicate that ROEO has potent antibacterial properties. The Gram-positive bacteria *S. aureus* inhibition zone (30 mm) was more sensitive than the Gram-negative *E. coli* inhibition zone (25 mm), which was likewise susceptible to these oils. Camphor has been observed to be particularly sensitive to *E. coli* bacteria. Camphor is easily absorbed via the skin and creates a cooling sensation comparable to menthol, as well as acting as a mild local anesthetic and antibacterial agent [29]. This explains the strong antimicrobial activity of *R. officinalis* against *E. coli*. Moreover, among the major compounds identified in our study, some were reported to have antimicrobial activity, including Bornyl acetate [30], α-pinene [31], camphor [32], borneol [33], and β-caryophyllene [34]. However, other additional components of the essential oil can also have antimicrobial effects [35].

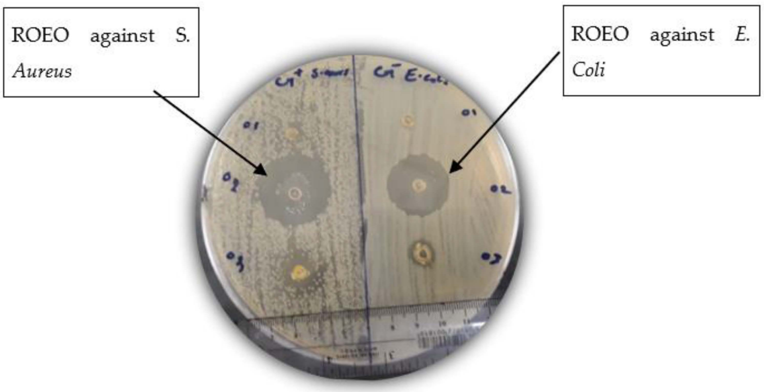

Figure 2. Inhibition zones of *R. officinalis* against S. aureus and *E. Coli*.

3.3. Molecular Docking

Table 3 displays the findings of the affinities between the chosen receptors and compounds. To compare the outcomes of the molecules with the TyrRS and DNA gyrase receptors, chlorobiocin was employed as a positive control, while SCHEMBL2181345 was utilized to test against the DHFR. From the results summarized in Table 3, poor affinities were made between the three selected molecules Camphor, Eucalyptol, and bornyl acetate when compared with those of TyrRS and DNA gyrase (TyrRS: between −4.8 and −4.9 Kcal/mol against −8.2 Kcal/mol obtained with Clorobiocin (Figure 3); DNA gyrase: between −4.5 and −4.9 Kcal/mol against −9.1 Kcal/mol obtained with Clorobiocin (Figure 4). However, strong affinities were obtained with the molecules when tested against DHFR (DHFR: between −5.8 and −6.0 Kcal/mol against −6.3 Kcal/mol obtained with SCHEMBL2181345 (Figure 5)).

Table 3. Affinity results of the selected receptors and molecules.

	Affinities (Kcal/mol)		
	TyrRS	*DNA Gyrase*	**DHFR**
Camphor	−4.8	−4.5	−6.0
Eucalyptol	−4.8	−4.6	−5.8
Bornyl acetate	−4.9	−4.9	−6.0
Clorobiocin	−8.2	−9.1	-
SCHEMBL2181345	-	-	−6.3

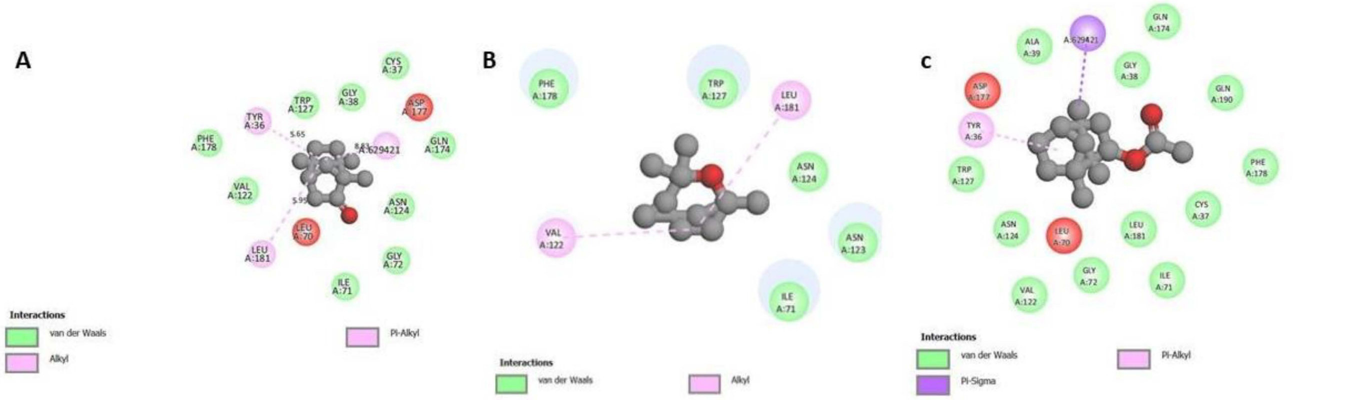

Figure 3. 2D scheme of TyrRS interaction with the tested ligands (**A**) Eucalyptol; (**B**) Camphor; (**C**) Bornyl acetate.

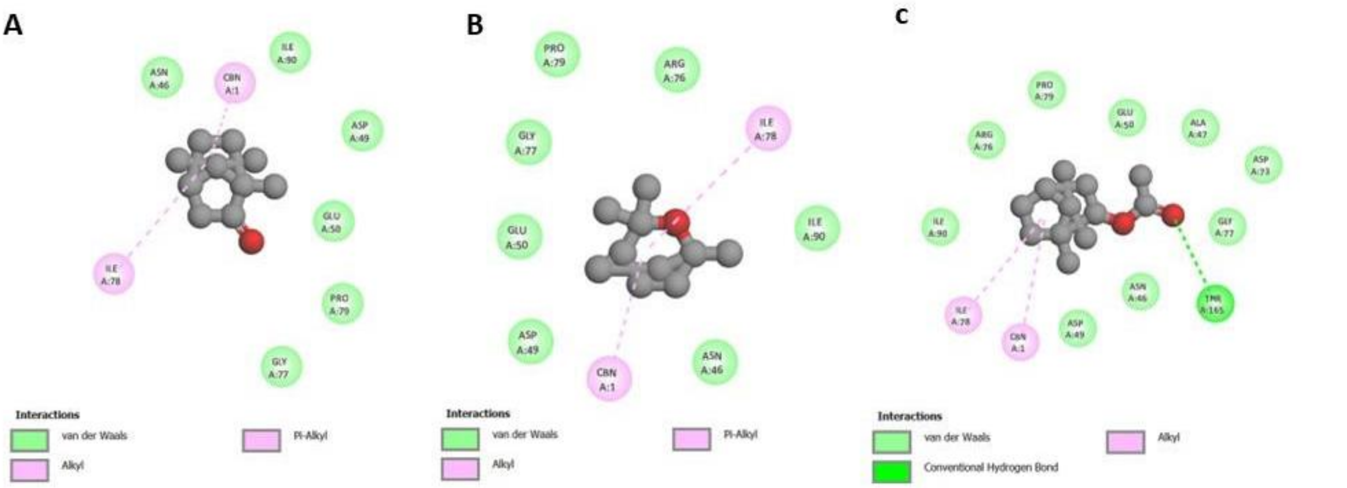

Figure 4. 2D scheme of DNA gyrase interaction with the tested ligands (**A**) Eucalyptol; (**B**) Camphor; (**C**) Bornyl acetate.

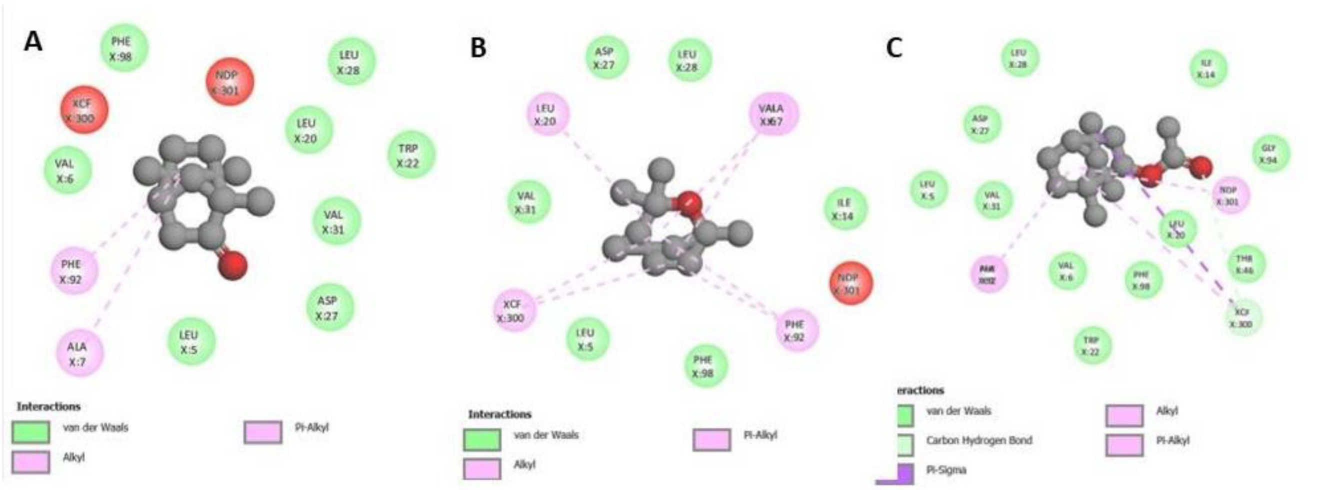

Figure 5. 2D scheme of DHFR interaction with the tested ligands (**A**) Eucalyptol; (**B**) Camphor; (**C**) Bornyl acetate.

4. Conclusions

The chemical content of *Rosmarinus officinalis* L. essential oil may vary depending on a number of conditions, including temperature, soil, solar exposure, and extraction method, *R. officinalis* growing in Saudi Arabia revealed a high concentration of phytochemical components as well as good antibacterial activity against both +ve and −ve bacteria. The molecular docking demonstrated a high affinity for microbial proteins. As a result, the essential oils of this plant may be employed in the pharmaceutical sector to create novel synthetic medications for the treatment of this illness infection.

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