

Phytochemical Screening and in Vitro Antimicrobial and Anticancer Activities of Different Extracts of *Rosmarinus officinalis* (Rosemary): A Comparative Study

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Abstract Various medicinal plants are found in the dense forest of Albaha region, southwest of Saudi Arabia. These plant species are natively utilized for the prevention and treatment of various diseases. This study was designed to analyze the chemical composition of ethanolic, petroleum ether, chloroform, and methanolic extracts of *Rosmarinus officinalis* (rosemary) collected from Albaha region and evaluate the antimicrobial and cytotoxic activities of these extracts. Fresh aerial parts of *R. officinalis* (stem and leaves) were used for extraction. Then the crude extracts were investigated by using gas chromatography- mass spectrometry (GC-MS) technique to determine their chemical constituents. Antimicrobial assays were performed using *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria), and *Candida albicans* (fungus) to determine the antimicrobial activities. MTT assay was applied to MCF-7 (human breast cancer cell line) as well as on HCT-116 (human colon cancer cell line) to calculate the IC₅₀ of different plant extracts. The GC-MS analysis showed that only petroleum ether extract has an abundance of cyclohexane compounds including 46.5% methyl-cyclohexane. Significant antibacterial and antifungal actions against the tested strains were shown by the petroleum ether and chloroform extracts in antimicrobial assay. Antibacterial activity against *S.*

aureus (SA) and *E. coli* (EC) was exhibited by methanolic extract, whereas no effect was observed on *B. subtilis* (BS) and *C. albicans* (CA). In MTT assay, the petroleum ether extract showed the greatest cytotoxic activity against MCF-7 (3.77 µg/mL) and HCT-116 (3.09 µg/mL) cells. The extract of chloroform also displayed significant cytotoxic effect but only against MCF-7 with IC₅₀ values of 12.7 µg/mL. The present study showed that the *R. officinalis* petroleum ether extract contains significant antimicrobial and cytotoxic activities which can be accredited to the plentiful manifestation of methyl-cyclohexane, methylbenzene and other cyclohexane derivatives, and it may be used to develop new antimicrobial and anticancer drugs.

Keywords *Rosmarinus officinalis*, Antimicrobial Activity, Cytotoxicity, Medicinal Plant

1. Introduction

Due to a sudden rise in the number of contagious diseases and the development of antimicrobial resistance against current drugs, drug development studies are vital to discovering novel medicinal compounds [1]. Nowadays,

therapeutic research focuses on medicinal plants due to their perceived effectiveness, lower side effects, and lower cost compared to that of synthetic drugs [2]. Several medicines are produced from plants, for instance quinine from the cinchona tree, aspirin is secreted from a willow tree, whereas, opium poppy provides vital medicine known as morphine [3]. Medicinal plants have shown to be beneficial in the function of various systems in the human body. For example, several plant extracts have strong antioxidant, anti-inflammatory, antimicrobial, antitumor, and immunostimulatory properties [4]. Hence, the search for new and effective medicinal plants from around the world has gained importance.

The Albaha region of Saudi Arabia, located in the southwest between Makkah and Aseer, is surrounded by forests and agricultural land; therefore, it is an ideal habitat to diverse flora, including several medicinal plants [5]. *Rosmarinus officinalis* (rosemary), which belongs to the *Lamiaceae* family, is a woody medicinal plant that originated from the Mediterranean region [6]. The medicinal uses of *R. officinalis* including treatment for gastrointestinal diseases, memory enhancement, antidepressant, and as an anti-inflammatory agent have been known for centuries [7–10]. In addition, *R. officinalis* extracts show a high antioxidant activity and can be used to increase the shelf life of perishable food items [11,12].

The antimicrobial activity of *R. officinalis* extracts is also widely reported [11,13–15]. It has been found that the leaf extract of rosemary inhibits the growth of two of the main species, *Shigella sonnei* and *E. coli* [14]. The presence of different types of phenolic compounds in rosemary extracts makes it an effective antioxidant and imparts antimicrobial action against various microbes [11].

The cytotoxic and antitumor effects of rosemary extracts have been demonstrated in several studies [13,16–20]. It has been reported that rosemary has a high content of phenolic compounds which contribute to its antitumor activity [21]. Polyphenols are phytochemicals that primarily modulate cell growth and inhibit tumor development [22]. The most common polyphenols present in *R. officinalis* are phenolic acids (rosmarinic acid and caffeic acid), diosmin, and apigenin [16,23].

A literature survey indicated that no study had previously evaluated the antimicrobial and anticancer activities of *R. officinalis* growing in the Albaha region. In the present study, we aimed to identify the chemical constituents that are present in different extracts of *R. officinalis* using GC-MS analysis and evaluate the antimicrobial and anticancer activities of these extracts and compare their efficiencies.

2. Materials and Methods

2.1. Chemicals

Streptomycin, Muller-Hinton agar, penicillin, Dulbecco's modified Eagle's medium (DMEM), dimethyl sulfoxide (DMSO), glutamine, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), high-glucose medium fetal bovine serum (FBS), methanol, ethanol, petroleum ether and chloroform were purchased from Sigma chemicals Co., USA.

2.2. Plant Materials

Rosemary plants were gained from a local farmer located in Albaha, Saudi Arabia in March 2019. The botanical identification of the plant was authenticated by Dr. Haider, Department of Biology, Albaha University. The obtained specimen was deposited in the Botany Laboratory of the Biology department in Albaha University, Albaha, Saudi Arabia.

2.2.1. Extraction of Crude Extract

The fresh aerial parts of the rosemary plant (stem and leaves) were air dried at room temperature for approximately 15 days. Around 250g of the dried material was powdered using grinder machine (Pulverizer HR-30B, USA) and soaked in 500 mL ethanol with shaking for three days. Then they were filtrated using filter paper (Whatman no1). The residues were air dried and extracted into various fractions by using solvents with increasing polarity: petroleum ether, chloroform, and methanol. All filtrates were concentrated using a rotary evaporator (IKA RV-10, Germany) and air dried to dryness [24].

2.3. GC-MS Analysis

A Perkin Elmer model Clarus 600 T, together with single quadrapole mass spectrometer, was applied for GC-MS analysis [25], with certain modifications. The chromatographic column used was an Elite 5-MS column (30 m × 0.25 mm × 0.25 µm film thickness). High-purity helium was used as the gas carrier, at a flow rate of 1 mL/min. The injector temperature was 280 °C and the split ratio was 20:1. At the beginning of the procedure a 40 °C temperature was applied for 1 min, and subsequently raised to 150 °C at 10 °C min⁻¹ for 1 min, afterwards a further increase to 300 °C at 10 °C min for 1 min was applied. The injector volume for each sample extract was 1 µL. The temperature of ion source was 220 °C whereas the inlet line temperature was at 240 °C. The sample was analyzed by applying a scan range between 40 to 600 m/z at electron energy of 70 eV, and the solvent delay of 4 min. Finally, NIST 2005 (National Institute of Standard and Technology library) and Wiley 2006 library were utilized to identify unknown compounds as previously described by Mosbah *et. al.* [26].

2.4. Antimicrobial Activity Screening

Four different extracts (ethanol, petroleum ether, chloroform, and methanol) of rosemary were tested against specific strains of American type culture collection (ATCC) included gram-positive bacteria: *Staphylococcus aureus* 29213 and *Bacillus subtilis* 6633, gram-negative bacteria: *Escherichia coli* 35218, and the fungus: *Candida albicans* 76615; the strains were obtained from the Microbiology Laboratory of King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Agar diffusion technique was conducted for the fundamental screening of the antimicrobial activity [27]. Briefly, petri dishes with 90 mm diameter were filled with 25mL of Muller-Hinton agar containing 1 mL culture (1×10^6 CFU/mL) of each strain. A sterile borer was used to create wells with diameter of 4 mm in seeded agar plates. Afterwards, the wells were permeated with 50 μ L of each of the rosemary extract (10 mg/mL) while DMSO of 10% was used as a negative control. Then the plates were nurtured at 37 °C for 24h. The inhibitory effect was determined as the non-appearance of microbial growth in the field around the wells.

Independent experiments were performed against each of the tested microorganisms in triplicate. After incubation, a caliper was used to measure the broadness of the appearing growth inhibition zones. Subsequently, with the obtained measurements the average diameter was calculated and the mean values were accordingly tabulated.

2.5. Cell Lines and Culture Medium

Human breast cancer cell line (MCF-7) and colorectal cancer cell line (HCT-116) were provided by Dr. Thikryat, Pharmacology and Toxicology Laboratory, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. The cells were cultured in 75 cm² flasks of DMEM/high-glucose medium supplemented with 10% (v/v) FBS, 10,000 units/mL penicillin/streptomycin, and 1% (v/v) glutamine and kept under a temperature of 37 °C in a humidified incubator of 5% CO₂ as described by Cheung *et. al.* [20].

2.5.1. Cytotoxicity Assay

To indicate the cytotoxic activity of the rosemary extracts, the MTT colorimetric assay was observed [28]. MCF-7 and HCT-116 cells (1×10^5 cells/mL) were implanted in 96-well plates in triplicate while they were incubated whole night at 37 °C in a humidified incubator where CO₂ percentage was 5% as previously described [29] with certain modifications. Rosemary extracts (ethanol, petroleum ether, chloroform, and methanol) at seven

concentrations (10, 25, 50, 100, 250, 500, and 1000 μ g/mL) were added to the cells in triplicate and were further incubated at 37 °C in a CO₂ concentration of 5% for 72 h. The extracts were dissolved in 0.1% DMSO as a vehicle. Appropriate control wells using untreated and DMSO-treated cells were prepared at the same time. As a reference drug (positive control), Paclitaxel was used. Subsequently, the medium of each well of all plates was detached and replaced with 100 μ L of complete medium (FBS and antibiotic) with 10% of MTT (10 mg/mL). These plates were incubated at 37 °C in 5% CO₂ for 4 h. The supernatants were removed after incubation and 100 μ L of DMSO was combined to dissolve the purple formazan crystals obtained by the applicable cells. These cells were incubated for another 5 min at 37 °C in 5% CO₂. The dissolved solutions at 570 nm were measured by using the SpectraMax M3 plate reader (Molecular Devices, San Jose, CA, USA). The cell viability was calculated using the following formula:

Cell viability (%) = (A of treated cells/A of control cells) \times 100, where A = absorbance at 570 nm.

2.6. Statistical Analysis

Data is expressed as a mean of three replicates \pm standard deviation. The half-maximal inhibitory concentration (IC₅₀) was estimated using the ED50 GraphPad Prism software 5.0 (GraphPad Software, Inc., CA, USA).

3. Results

3.1. GC-MS Analysis

Tables 1–4 list the chemical compositions of the four *R. officinalis* extracts. GC-MS analysis showed that the ethanolic extract contained 3,7-dimethyl-2,6-octadien-1-ol (22.59%), bicyclo [3.1.1] hept-3-en-2-one (18.94%), 1,8-cineole (14.64%), carnosol (10.95%), and 2-(5-tert-butyl-4-hydroxy-2-methylphenyl) benzoic acid (7.39 %); the petroleum ether extract contained methyl-cyclohexane (46.51%), methyl-benzene (12.58%), berbenone (7.03%), 1,8-cineole (6.11%), and trans-geraniol (4.32%); the chloroform extract contained bicyclo [3.1.1] hept-3-en-2-one (19.84%), trans-geraniol (13.43%), endo-borneol (7.96%), 1,8-cineole (9.4%), and vitamin E (5.24%); the methanolic extract contained bicyclo [3.1.1] hept-3-en-2-one (14.82%) caryophyllene diepoxide (10.5%), 1,2,3-propanetriol (6.3%), 5-(hydroxy)-2- furancarboxaldehyde (6.27%), and borneol (5.1%) is the most abundant components with a concentration higher than 5%.

Table 1. Chemical composition of *Rosmarinus officinalis* ethanolic extract

| | Name of Compound | RT ^a | Area % | Area |
|----|---|-----------------|--------|---------|
| 1 | CIS-OCIMENE | 6.4 | 0.53 | 29825 |
| 2 | 1,8-CINEOLE | 8.01 | 14.64 | 820450 |
| 3 | ALPHA.-TERPINOLENE | 9 | 1.12 | 62921 |
| 4 | CAMPHOR | 9.82 | 0.89 | 49615 |
| 5 | 1,7 BICYCLO[2.2.1]HEPTAN-2-OL | 10.2 | 2.53 | 141634 |
| 6 | 2-BICYCLO[3.1.1]HEPTAN-3-ONE | 10.26 | 0.32 | 18157 |
| 7 | 4-TERPINEOL | 10.3 | 0.3 | 16880 |
| 8 | 3-CYCLOHEXENE-1-METHANOL | 10.52 | 1.25 | 70094 |
| 9 | 6,6-DIMETHYL-BICYCLO[3.1.1] HEPT-2-ENE-2-ETHANOL | 10.62 | 0.37 | 20545 |
| 10 | BICYCLO[3.1.1]HEPT-3-EN-2-ONE | 10.73 | 18.94 | 1061125 |
| 11 | 2-HYDROXY-2-METHYL-BUT-3-ENYL 2-METHYL-2(Z)-BUTENOATE | 10.81 | 1.16 | 65070 |
| 12 | BETA.-CITRONELLOL | 10.88 | 0.53 | 29453 |
| 13 | 3,7-DIMETHYL-2,6-OCTADIEN-1-OL | 11.23 | 22.59 | 1265704 |
| 14 | (R-1,C-4)-P-MENTH-8-EN-1-OL | 11.3 | 0.31 | 17360 |
| 15 | BORNYL ACETATE | 11.77 | 1.7 | 95437 |
| 16 | TRANS-CARYOPHYLLENE | 13.96 | 0.52 | 29405 |
| 17 | FORMIC ACID | 14.06 | 0.37 | 20907 |
| 18 | PHENYLMETHYL ESTER OF (-)-CARYOPHYLLENE OXIDE | 16.31 | 0.27 | 15353 |
| 19 | HEXADECANOIC ACID | 20.43 | 0.29 | 16240 |
| 20 | 3,7,11,15-TETRAMETHYL 2-HEXADECEN-1-OL | 21.9 | 0.48 | 26712 |
| 21 | 6(E),9(Z),13(E)-PENDECTRIENE | 22.14 | 0.53 | 29433 |
| 22 | 11,14,17-EICOSATRIENOIC ACID | 22.4 | 0.23 | 12755 |
| 23 | (5.BETA)-CHOL-7-ENE-12,24-DIOL | 23.07 | 0.3 | 17013 |
| 24 | (+)-.BETA.-COSTOL | 23.84 | 0.3 | 16788 |
| 25 | FERRUGINOL | 23.94 | 0.5 | 28110 |
| 26 | 3,8-DIHYDROXY-1,4,6-TRIMETHYL DIBENZO[B, E][1,4]DIOXEPIN-11-ONE | 24.87 | 3.2 | 179414 |
| 27 | ISOCARNOSOL | 25.11 | 0.5 | 27850 |
| 28 | CARNOSOL | 25.27 | 10.95 | 613529 |
| 29 | ISOCARNOSOL | 25.46 | 0.25 | 14249 |
| 30 | FERRUGINOL | 25.54 | 0.69 | 38464 |
| 31 | 2-(5-TERT-BUTYL-4-HYDROXY-2-METHYLPHENYL) BENZOIC ACID | 26.02 | 7.39 | 414265 |
| 32 | 2-ALLYL-1,4-DIMETHOXY-6-METHYL BENZENE | 26.11 | 2.8 | 156808 |
| 33 | CARNOSOL | 26.4 | 0.6 | 33474 |
| 34 | (-)-ALPHA-COSTOL | 27.05 | 0.99 | 55377 |
| 35 | 3,7,11-TRIDECATRIENENITRILE | 27.52 | 0.83 | 46385 |
| 36 | DOCOSANE | 28.1 | 0.59 | 32789 |
| 37 | SPINASTERONE | 28.52 | 0.25 | 13831 |

^aRetention time (as minutes).

Table 2. Chemical composition of *Rosmarinus officinalis* petroleum ether extract

| | Name of Compound | RT ^a | Area % | Area |
|----|---|-----------------|--------|----------|
| 1 | 2,2,4-TRIMETHYL-PENTANE | 3.23 | 0.26 | 67760 |
| 2 | METHYL-CYCLOHEXANE | 3.33 | 46.51 | 12081537 |
| 3 | ETHYL-CYCLOPENTANE | 3.43 | 0.78 | 202159 |
| 4 | CYCLOPENTANE | 3.51 | 0.39 | 100798 |
| 5 | 1,2,4-TRIMETHYL-TETRAHYDRO GERANIOL | 3.6 | 0.2 | 52528 |
| 6 | 2,3-DIMETHYL-HEXANE | 3.72 | 0.2 | 52658 |
| 7 | METHYL-BENZENE | 3.83 | 12.58 | 3267939 |
| 8 | 3-METHYL-HEPTANE | 3.9 | 0.51 | 132387 |
| 9 | 1,4-DIMETHYL-CYCLOHEXANE | 4.03 | 1.75 | 454445 |
| 10 | METHYL-CYCLOHEPTANE | 4.15 | 0.16 | 42265 |
| 11 | 2,4-DIMETHYL-HEPTANE | 4.27 | 1.87 | 485756 |
| 12 | 1,2-DIMETHYL-CYCLOHEXANE | 4.32 | 0.21 | 55091 |
| 13 | 1,3-DIMETHYL-CYCLOHEXANE | 4.41 | 0.11 | 29116 |
| 14 | 2,6-DIMETHYL-HEPTANE | 4.7 | 0.15 | 39763 |
| 15 | ETHYL- CYCLOHEXANE | 4.84 | 0.2 | 53235 |
| 16 | 1,2-DIMETHYL-BENZENE | 5.37 | 0.56 | 146510 |
| 17 | ALPHA.-PINENE | 6.43 | 0.28 | 71872 |
| 18 | VERBENENE | 6.76 | 0.27 | 70230 |
| 19 | 1,8-CINEOLE | 8.05 | 6.11 | 1587119 |
| 20 | LINALOOL | 9.04 | 0.59 | 153253 |
| 21 | 2,6-DIMETHYL-3,5-HEPTADIEN-2-OL | 9.43 | 0.11 | 29111 |
| 22 | (+.-)-CAMPHOR | 9.86 | 0.44 | 115010 |
| 23 | PINOCARVONE | 10.07 | 0.11 | 27581 |
| 24 | DELTA.-TERPINEOL | 10.18 | 0.14 | 35744 |
| 25 | BORNEOL | 10.24 | 1.95 | 505392 |
| 26 | BICYCLO[3.1.1]HEPTAN-3-ONE | 10.29 | 0.15 | 37908 |
| 27 | 4-METHYL-1-3-CYCLOHEXEN-1-OL | 10.34 | 0.3 | 78997 |
| 28 | 3-CYCLOHEXENE-1-METHANOL | 10.56 | 0.9 | 234079 |
| 29 | BICYCLO[3.1.1]HEPT-2-ENE-2-ETHANOL | 10.66 | 0.22 | 57693 |
| 30 | BERBENONE | 10.77 | 7.03 | 1827293 |
| 31 | 4-METHYL-3-PENTEN-2-ONE | 10.85 | 0.4 | 105119 |
| 32 | 3,7-DIMETHYL-6-OCTEN-1-OL | 10.91 | 0.12 | 30172 |
| 33 | TRANS-GERANIOL | 11.27 | 4.32 | 1123131 |
| 34 | 1-VINYL-1-(4-METHYL)PENTAN-3-ENE | 11.33 | 0.08 | 19854 |
| 35 | ACETA-Z-CRYSANTHENYL | 11.73 | 0.19 | 48091 |
| 36 | 1,7,7-TRIMETHYL-BICYCLO[2.2.1]HEPT-2-YL ESTER ACETIC ACID | 11.81 | 1.58 | 411378 |
| 37 | TRANS-FARNESOL | 11.92 | 0.43 | 112082 |
| 38 | NERYL ACETATE | 13.1 | 0.22 | 57353 |
| 39 | 1,2-DIMETHOXY-4-(2-PRBENZENE | 13.48 | 0.07 | 17646 |
| 40 | TRANS-CARYOPHYLLENE | 14 | 0.26 | 67407 |
| 41 | CARYOPHYLLENE OXIDE | 16.33 | 0.05 | 13733 |
| 42 | N-HEXADECANOIC ACID | 20.46 | 0.08 | 21366 |
| 43 | 9-OCTADECENOIC ACID | 20.88 | 0.1 | 26928 |
| 44 | 3,7,11,15-TETRAMETHYL 2-HEXADECEN-1-OL | 21.91 | 0.3 | 79085 |
| 45 | OXIRANEMETHANOL | 22.16 | 0.17 | 44454 |
| 46 | (-)-OBTUSANE | 23.6 | 0.04 | 10951 |
| 47 | (-)-CARYOPHYLLENE OXIDE | 23.84 | 0.04 | 11129 |
| 48 | FERRUGINOL | 23.95 | 0.28 | 73522 |
| 49 | VITAMIN E | 24.35 | 0.62 | 161554 |
| 50 | 1-(1,2-DICYANOETHENYL)[6](1,4)-NAPHTHALENOPHAN -1,2-CYCLOPROPANE | 24.6 | 0.87 | 226226 |
| 51 | 4,4-DIMETHYL-2-((Z)-[2-(N-P-TOLYLAMINO)-4-METHYL]PENT-1-ENYL)-2-OXAZOLINE | 24.88 | 0.39 | 101217 |
| 52 | 2-HYDROXY-1 OCTADECANOIC ACID | 25.28 | 1.19 | 310016 |
| 53 | 13-ISOPROPYLPODOCARPEN-12-OL-20 AL | 25.54 | 0.12 | 30868 |
| 54 | 2-ALLYL-1,4-DIMETHOXY-6-METHYL BENZENE | 26.11 | 0.48 | 123703 |
| 55 | TRITETRACONTANE | 26.69 | 0.4 | 104941 |
| 56 | TETRATETRACONTANE | 28.08 | 1.88 | 487837 |

^aRetention time (as minutes).

Table 3. Chemical composition of *Rosmarinus officinalis* chloroform extract

| | Name of Compound | RT ^a | Area % | Area |
|----|--|-----------------|--------|--------|
| 1 | VERBENENE | 6.76 | 0.78 | 34849 |
| 2 | METHYL(1-METHYLETHYL)-BENZENE | 7.9 | 0.67 | 29954 |
| 3 | 1,8-CINEOLE | 8.04 | 9.4 | 418656 |
| 4 | LINALOOL | 9.04 | 2.14 | 95457 |
| 5 | CHRYSANTHENONE | 9.43 | 0.34 | 15123 |
| 6 | CAMPHOR | 9.86 | 1.17 | 52075 |
| 7 | BICYCLO[2.2.1]HEPTAN-3-ONE | 10.07 | 0.34 | 15052 |
| 8 | 3-CYCLOHEXENE-1-METHANOL | 10.17 | 0.68 | 30251 |
| 9 | ENDO-BORNEOL | 10.23 | 7.96 | 354651 |
| 10 | PINOCAMPHONE | 10.29 | 0.6 | 26773 |
| 11 | 4-TERPINEOL | 10.34 | 1.24 | 55258 |
| 12 | LINALYL PROPIONATE | 10.55 | 3.25 | 144673 |
| 13 | B6,6-DIMETHYLICYCLO[3.1.1] HEPT-2-ENE-2-ETHANOL | 10.65 | 1.14 | 50601 |
| 14 | BICYCLO[3.1.1]HEPT-3-EN-2-ONE | 10.76 | 19.84 | 883454 |
| 15 | 2-HYDROXY-2-METHYL-BUT-3-ENYL | 10.84 | 1.21 | 53862 |
| 16 | 3,7-DIMETHYL-6-OCTEN-1-OL | 10.91 | 0.48 | 21453 |
| 17 | TRANS-GERANIOL | 11.26 | 13.43 | 598086 |
| 18 | (+)-TRANS-CARAN-TRANS-2-OL | 11.33 | 0.27 | 11908 |
| 19 | 1,7,7-TRIMETHYL-BICYCLO[2.2.1] HEPT-2-YL ESTER ACETIC ACID | 11.81 | 2.16 | 96168 |
| 20 | NEROLIC ACID | 12.61 | 0.81 | 36187 |
| 21 | NERYL ACETATE | 13.09 | 0.67 | 29892 |
| 22 | (-)-CARYOPHYLLENE OXIDE | 16.33 | 0.49 | 21870 |
| 23 | TETRADECANAL | 16.55 | 0.19 | 8578 |
| 24 | (+)-.BETA.-COSTOL | 17.24 | 0.37 | 16650 |
| 25 | GLOBULOL | 17.41 | 0.12 | 5507 |
| 26 | NEOPHYTADIENE | 19.65 | 0.24 | 10760 |
| 27 | XYCAINE | 19.81 | 1.16 | 51518 |
| 28 | HEXADECANOIC ACID | 20.45 | 0.73 | 32616 |
| 29 | 1-TETRADECENE | 20.87 | 0.34 | 14987 |
| 30 | 3,7,11,15-TETRAMETHYL 2-HEXADECEN-1-OL | 21.9 | 0.89 | 39725 |
| 31 | 3.BETA ERGOSTA-7,22-DIEN-3-OL | 23.1 | 0.19 | 8541 |
| 32 | TETRACOSANE | 23.37 | 3.15 | 140177 |
| 33 | 12-CHLOROMERCURIOTOTARA-8,11,13-TRIEN-13-OL | 23.95 | 0.78 | 34715 |
| 34 | 9-OCTADECENAMIDE | 24.1 | 1.18 | 52608 |
| 35 | ISOCARNOSOL | 24.27 | 0.51 | 22532 |
| 36 | VITAMIN E | 24.35 | 5.24 | 233497 |
| 37 | CARNOSOL | 25.26 | 4.13 | 184040 |
| 38 | 5.ALPHA.STIGMAST-24(28)-ENE | 26.1 | 2.72 | 120907 |
| 39 | PENTATRIACONTANE | 27.82 | 2.92 | 130105 |
| 40 | TRITETRACONTANE | 28.07 | 4.95 | 220319 |
| 41 | (-)-ALLOAROMADENDRONE | 28.51 | 0.46 | 20356 |

^aRetention time (as minutes).

Table 4. Chemical composition of *Rosmarinus officinalis* methanolic extract

| | Name of Compound | RT ^a | Area % | Area |
|----|---|-----------------|--------|--------|
| 1 | 2-FURANMETHANOL | 5.11 | 0.34 | 6066 |
| 2 | DL-GLYCERALDEHYDE DIMER | 5.73 | 3.79 | 68546 |
| 3 | 2-HYDROXY-2-CYCLOPENTEN-1-ONE | 6.22 | 0.42 | 7615 |
| 4 | 2,4-DIHYDROXY-2,5-DIMETHYL-3(2H)-FURAN-3-ONE | 7.15 | 1.02 | 18436 |
| 5 | 1-BUTOXY-2-PROPANOL ACETATE | 7.29 | 1.85 | 33377 |
| 6 | 1,8-CINEOLE | 8.04 | 2.47 | 44618 |
| 7 | HYDROXY DIMETHYL FURANONE | 8.34 | 0.61 | 11051 |
| 8 | 3-HYDROXY-2-METHYL-4H-PYRAN-4-ONE | 8.64 | 1.12 | 20207 |
| 9 | 2-ACETYL-5-METHYLFURAN | 8.86 | 0.47 | 8425 |
| 10 | 3-AMINO-2-OXAZOLIDINONE | 9.53 | 0.82 | 14881 |
| 11 | CARYOPHYLLENE DIEPOXIDE | 9.73 | 10.52 | 190193 |
| 12 | BORNEOL | 10.23 | 5.1 | 92248 |
| 13 | Z-3-HEXENYL 2-METHYLPROPANOATE | 10.38 | 0.67 | 12076 |
| 14 | 1,2-BENZENEDIOL | 10.47 | 3.62 | 65373 |
| 15 | 3-CYCLOHEXENE-1-METHANOL | 10.54 | 1.3 | 23449 |
| 16 | (-)-CARYOPHYLLENE OXIDE | 10.64 | 0.57 | 10257 |
| 17 | BICYCLO[3.1.1]HEPT-3-EN-2-ONE | 10.76 | 14.82 | 267852 |
| 18 | 5-(HYDROXY)-2-FURANCARBOXALDEHYDE | 10.88 | 6.27 | 113269 |
| 19 | 1,2,3-PROPANETRIOL | 11.13 | 6.3 | 113800 |
| 20 | 2,7-OCTADIENE-1,6-DIOL | 11.57 | 1.45 | 26172 |
| 21 | 2,3-DIHYDRO-3-HYDROXY-4H-PYRAN-4-ONE | 11.69 | 2.61 | 47161 |
| 22 | EXOBORNYL ACETATE | 11.81 | 0.96 | 17275 |
| 23 | 4-ACETOXY-3-METHOXYSTYRENE | 12.16 | 1.55 | 28005 |
| 24 | 2,6-DIMETHOXY-PHENOL | 12.66 | 0.84 | 15229 |
| 25 | 4-ETHYL-1,3-BENZENEDIOL | 13.18 | 0.73 | 13144 |
| 26 | 2-HYDROXY-6-METHYL BENZALDEHYDE | 14.1 | 10.64 | 192328 |
| 27 | (Z)-NON-2-EN-6,8-DIYNOIC ACID | 14.58 | 0.24 | 4263 |
| 28 | D-ALLOSE | 14.91 | 3.38 | 61092 |
| 29 | 4-HYDROXY-3-METHYL-BENZOIC ACID | 15.79 | 0.58 | 10482 |
| 30 | 3-DEOXY-D-MANNOIC LACTONE | 16.34 | 4.32 | 78104 |
| 31 | DECANAL | 16.59 | 1.57 | 28325 |
| 32 | GINGEROL | 16.96 | 0.55 | 9932 |
| 33 | 4-((1E)-3-HYDROXY-1-PROPENYL)-2-METHOXYPHENOL | 18.1 | 0.79 | 14315 |
| 34 | DL-3,4-DIMETHYL-3,4-HEXANEDIOL | 18.36 | 0.77 | 13843 |
| 35 | 4-HYDROXYL-PROLINE | 19.83 | 1.39 | 25078 |
| 36 | HEXADECANOIC ACID | 20.44 | 0.66 | 11964 |

^aRetention time (as minutes).

3.2. Antimicrobial Activity

The *in vitro* antibacterial and antifungal assays were performed using the ethanol, petroleum ether, chloroform, and methanol extracts obtained from *R. officinalis*. Results showed that chloroform, methanol, and petroleum ether extracts exhibited significant antimicrobial activity towards most of the selected strains (Table 5). The highest activities were observed for petroleum ether extract with

zones of inhibition of 22 mm, 14 mm, 21 mm, and 22 mm, followed by the chloroform extract with zones of inhibition of 18 mm, 12 mm, 15 mm, and 12 mm against SA, BS, EC, and CA strains, respectively. The methanol extract showed significant activity against SA with a zone of inhibition of 20 mm and a moderate activity against EC with a zone of inhibition of 7 mm. Notably, the ethanol extract did not exhibit any activity against the tested strains.

Table 5. Antibacterial and antifungal activities of *Rosmarinus officinalis* extracts

| Extracts | Zone diameter ^a (mm) | | | |
|-----------------|---------------------------------|--------------------------|-------------------------|-------------------------|
| | Gram-positive bacteria | | Gram-negative bacteria | Fungus |
| | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> | <i>Escherichia coli</i> | <i>Candida albicans</i> |
| Chloroform | 18 ± 0.05 | 12 ± 0.07 | 15 ± 0.04 | 12 ± 0.04 |
| Methanol | 20 ± 0.04 | - | 7 ± 0.12 | - |
| Ethanol | - | - | - | - |
| Petroleum ether | 22 ± 0.04 | 14 ± 0.14 | 21 ± 0.09 | 22 ± 0.05 |

3.3. Cytotoxic Activity

To discover new agents capable of hindering the propagation of human breast cancer as well as human colorectal cancer cell lines, different extracts (ethanol, petroleum ether, chloroform, and methanol) of rosemary were tested at seven concentrations against MCF-7 and HCT-116 cells. The IC₅₀ values of the extracts were determined to evaluate their effectiveness. As shown in Table 6, the IC₅₀ for MCF-7 ranged from 3.77 to 76.2 µg/mL, whereas the IC₅₀ for HCT-116 ranged from 3.09 to 324 µg/mL. Petroleum ether extracts showed the greatest cytotoxic effect against MCF-7 and HCT-116 cells with IC₅₀ values of 3.77 and 3.09 µg/mL, respectively. Chloroform as well as methanol extracts also displayed significant cytotoxic effects against MCF-7 cells with IC₅₀ values of 12.7 and 23.59 µg/mL, respectively. In contrast, ethanol extract displayed a moderate cytotoxic effect with an IC₅₀ of 76.2 µg/mL against MCF-7. The chloroform extract showed a moderate cytotoxic effect against HCT-116 cells with an IC₅₀ of 59.1 µg/mL, whereas methanol as well as ethanol extracts exhibited weak cytotoxic effects against HCT-116 cells with IC₅₀ values of 259 and 324 µg/mL, respectively.

Table 6. The IC₅₀ of *Rosmarinus officinalis* extracts against tested human cancer cell lines

| Compound | IC ₅₀ ^a (µg/mL) MCF-7 | IC ₅₀ ^a (µg/mL) HCT-116 |
|----------------------------|--|--|
| Chloroform extract | 12.7 ± 5.4 × 10 ⁻⁶ | 59.1 ± 1.8 × 10 ⁻⁶ |
| Methanol extract | 23.59 ± 0.27 × 10 ⁻⁶ | 259 ± 4.7 × 10 ⁻⁵ |
| Ethanol extract | 76.2 ± 2.2 × 10 ⁻⁵ | 324 ± 3.37 × 10 ⁻⁵ |
| Petroleum ether extract | 3.77 ± 1.3 × 10 ⁻⁶ | 3.09 ± 2.28 × 10 ⁻⁶ |
| Standard drug (paclitaxel) | 0.23 ± 2.2 × 10 ⁻⁶ | 0.32 ± 5.7 × 10 ⁻⁶ |

4. Discussion

GC-MS was performed to investigate the phytochemical composition of each rosemary extract. The petroleum ether extract contained the most constituents (56 compounds). Approximately 39 of these constituents are unique, including the bioactive compounds

methyl-cyclohexane (46.5%), methylbenzene (12.58%), and alpha-pinene (0.28%), which are not found in other *R. officinalis* extracts of this study.

The common bioactive compound that was determined in all extracts was 1,8-cineole. Terpinol and camphor were found in ethanol, petroleum ether, and chloroform extracts, excluding the methanolic extract. The (-)-caryophyllene oxide and borneol were found in methanol, petroleum ether, and chloroform extracts, but not in the ethanolic extract. Geraniol and vitamin E were found in the petroleum ether and chloroform extracts. Few differences were observed in the ingredients and combinations of the extracts in comparison to those which were mentioned before in *R. officinalis* collected from various geological areas, which may be credited to certain elements as follows: climate, time of collection, and mode of extraction [30,31].

The microorganisms tested are morphologically and physiologically different. Petroleum ether extracts exhibited a great antimicrobial activity against all tested strains, followed by chloroform extracts. The strong antimicrobial effect of petroleum ether and chloroform extracts can be attributed to the presence of the phytoconstituents geraniol and borneol that show antimicrobial action against various species [32,33]. Our findings are consistent with various studies that highlight the antibacterial and antifungal actions of extracts obtained from *Rosmarinus* species. For instance, oil extracts from *R. officinalis* have displayed how to prohibit the development of bacteria such as EC, *Listeria monocytogenes*, and SA [34]. In addition, it has been reported that *R. officinalis* oil extract inhibits the activity of CA by preventing the adhesion of this fungus via denaturation of the cellular structures and alteration of membrane permeability [35].

The cytotoxic of *R. officinalis*' extracts impacts on MCF-7 and HCT-116 cell lines were investigated by performing the MTT assay. The petroleum ether extract demonstrated the greatest cytotoxic effect against MCF-7 and HCT-116 cells with IC₅₀ values of 3.77 and 3.09 µg/mL, respectively. Chloroform and methanol extracts exhibited significant cytotoxic effects against MCF-7 cells with IC₅₀ values of 12.7 and 23.59 µg/mL, respectively, but not against HCT-116 cells. The National Cancer

Institute (USA) plant screening program states an IC_{50} of $< 20 \mu\text{g/mL}$ as a threshold for an extract to be considered as anticancer, following an incubation period of 48 and 72 h [36,37]. The findings of the current study are consistent with previous anticancer studies that report the potent cytotoxic effects of *R. officinalis* extracts on colon cancer cell lines [38]. Similarly, previous studies have demonstrated a significant cytotoxic effect of *R. officinalis* extract on breast cancer cell lines [39].

Remarkably, the petroleum ether extract showed the highest antimicrobial and anticancer activities compared to those of other *R. officinalis* extracts. This may be associated with the existence of a high number of methyl-cyclohexane, methylbenzene, and other cyclohexane derivatives. Cyclohexane and its practically alternative derivatives are vital compounds showing distinct biological actions involving antioxidant, anticancer, cytotoxic, analgesic, anti-inflammatory, and antithrombin activities [40–46].

5. Conclusions

Various cancers and infectious diseases contribute to morbidity and mortality worldwide. This situation requires the development of new cost-effective therapies that are more effective than the present therapies. In this aspect, medicinal plants are a useful source of novel therapeutic substances, and several drugs are composed of plant-derived chemicals. The current study was carried out to analyze the phytochemical contents of different extracts of *R. officinalis* obtained from the Albaha region and evaluate the antimicrobial and anticancer properties of each extract. The petroleum ether extract demonstrated significant antimicrobial and anticancer activities. This may be linked to the presence of a high amount of methyl-cyclohexane, methylbenzene, and other cyclohexane derivatives that are already in consideration to display antimicrobial and anticancer activities. Advanced research is important to evaluate which components are mainly responsible for these antimicrobial and anticancer activities and their mechanism of action.

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