

Research Article

Phytochemical profiling, antimicrobial and anticancer potential of *Rosmarinus officinalis* growing in Kashmir Himalayan region

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Abstract

Currently, medicinal plants are gaining importance in pharmaceutical and scientific communities. Medicinal plants are the most abundant natural source of valuable phytochemicals, which can help treat human diseases. The present study aimed to do phytochemical profiling and assess the antimicrobial and anticancer activity of the methanolic leaf extract of *Rosmarinus officinalis*. The phytochemical profiling of *R. officinalis* leaves was done by GC-MS analysis. Twenty-six compounds were identified from the leaf extracts with great significance in pharmaceutical science for therapeutically efficient formulations to combat various diseases. The antimicrobial activity was done by the well diffusion method, while the anticancer potential against the A549 lung cancer cell line by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The highest zone of inhibition was seen against *Escherichia coli* ATCC 11229 (21 ± 0.7 mm), *Enterococcus faecalis* ATCC 29212 (19 ± 0.8 mm), *Candida albicans* ATCC 10231 (18 ± 0.6 mm) followed by *Staphylococcus aureus* ATCC 25923 (18 ± 0.8 mm) and *Pseudomonas aeruginosa* (11 ± 0.5 mm) with MIC values ranging from 128 to 256 µg/mL. *R. officinalis* demonstrated significant ($p \leq 0.05$) anticancer activity against the A549 cancer cell line with IC₅₀ values of 39.70 and 33.60 µg/mL for 24 and 48 hours, respectively. The methanolic extract of *R. officinalis* can be a potential antimicrobial and anticancer agent and a vital resource for developing new drugs.

Keywords: Antimicrobial activity, Anticancer activity, GC-MS analysis, Medicinal plants, *Rosmarinus officinalis*

INTRODUCTION

The plant kingdom is a valuable source of potential drugs, and the interest in medicinal plants has been

growing rapidly in recent years (Aruna *et al.*, 2022). Medicinal plants contain diverse phytochemicals that can potentially treat various human and animal diseases (Sofi *et al.*, 2022a). As per the WHO report, 80% of

the population living in developing countries depend upon traditionally used medicinal herbs as a source of drugs (Bodeker *et al.*, 2022). Medicinal plants have been proven to be the least expensive, successful strategy for discovering potential drugs. Researchers have recognized the potential of medicinal plants as a source of new drugs, and there has been a shift towards developing plant-derived drugs over the past few decades (Yuan *et al.*, 2016; Sofi *et al.*, 2022 (b); Harahap *et al.*, 2022). This is partly due to the limitations of conventional therapy, including the side effects of synthetic drugs and their high cost. Chemotherapeutic drugs, in particular, can cause various side effects, including nausea, vomiting, hair loss, and fatigue. These side effects can be severe and impact a patient's quality of life (Atanasov *et al.*, 2021; Greenwell and Rahman, (2015). Additionally, the high cost of some chemotherapeutic drugs can limit their availability to patients, particularly those in low-income countries (Ocran *et al.*, 2021). By developing plant-derived drugs, researchers hope to address some of these limitations. Plant-derived drugs can be less toxic than synthetic drugs, have fewer side effects, and be more affordable to produce (Newman and Cragg, (2020).

Furthermore, the emergence of drug-resistant microorganisms has become a major global health threat (Gogry *et al.*, 2022). Synthetic antibiotics are becoming less effective, and there is a need for new and innovative therapeutic strategies. Plants and their products have been used for medicinal purposes for centuries and continue to be an important source of new drugs. They contain various compounds with potential therapeutic properties, including antimicrobial, antiviral, and anticancer activities (Akhouri *et al.*, 2020; Qanash *et al.*, 2022). Research has shown that plant-derived compounds can be effective against drug-resistant microorganisms, making them a promising alternative to traditional antibiotics. Moreover, plant-based medicines have a long history of use, are generally considered safe, and have fewer side effects than synthetic drugs (Vaou *et al.*, 2021; Khameneh *et al.*, 2019)

R. officinalis is an important medicinal plant that belongs to the Lamiaceae family and genus *Rosmarinus*. It is native to the Mediterranean region and is cultivated worldwide (De Oliveira *et al.*, 2019). The herb has a long history of being used in traditional medicine as an antibacterial, a great reliever of rheumatic pain, carminative in nature, and the best in analgesic properties. Further, it is also used as an expectorant, diuretic, and antispasmodic in renal colic (Boelens (1985); Hussain (1989). The extracts and essential oils from flowers and leaves of the rosemary are also used to treat fever, wounds, dyspepsia, and rashes, including circulatory problems. The biological activities of rosemary extracts include, anticonceptive and anti-inflammatory

(González *et al.*, 2007; Juhás *et al.*, 2016), anticarcinogenic (Moore *et al.*, 2016) antioxidant and hepatoprotective (R ašković *et al.*, 2014). The Kashmir Himalayan region is known for its unique flora, and the study of *R. officinalis* from this region will help to understand the chemical composition and medicinal properties of this herb. The main aim of the present work was to investigate the chemical composition, antimicrobial and anticancer properties of *R. officinalis*.

MATERIALS AND METHODS

Chemicals

Faetal Bovine serum was purchased from Sigma-Aldrich (St. Louis, MO, USA). 0.25 % trypsin-EDTA and Antibiotic Antimycotic Solution (100×) with 10,000 units Penicillin, 10 mg streptomycin and 25 µg/mL amphotericin Mueller Hinton Agar and methanol from HiMedia Laboratories Pvt. Ltd (Mumbai). Dulbecco's Modified Eagle Medium from Gibco (USA).3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide Sisco Research Laboratories Pvt Ltd (SRL chemicals), India All consumables and cell culture wares were obtained from Tarsons, India.

Collection of plant material

Leaf samples of *R. officinalis* plant (Fig. 1) were collected from the Daksum (Altitude 2438 m, Latitude 33° 36'43"N & longitude 75°26'6"E (Fig. 2) Anantnag, Jammu and Kashmir, India. The specimen was identified at the Centre for Biodiversity and Taxonomy, University of Kashmir, Srinagar (J &K). The specimen was deposited in the Kashmir University Herbarium under voucher No. 2839 Herbarium-(KASH). The fresh leaves were shade dried for about 10 days and then powdered using an electric blender.

Preparation of plant extract

10 grams of rosemary leaf powder was soaked in 200 ml of methanol and then kept in a shaker overnight.



Fig. 1. Showing *R. officinalis* plant

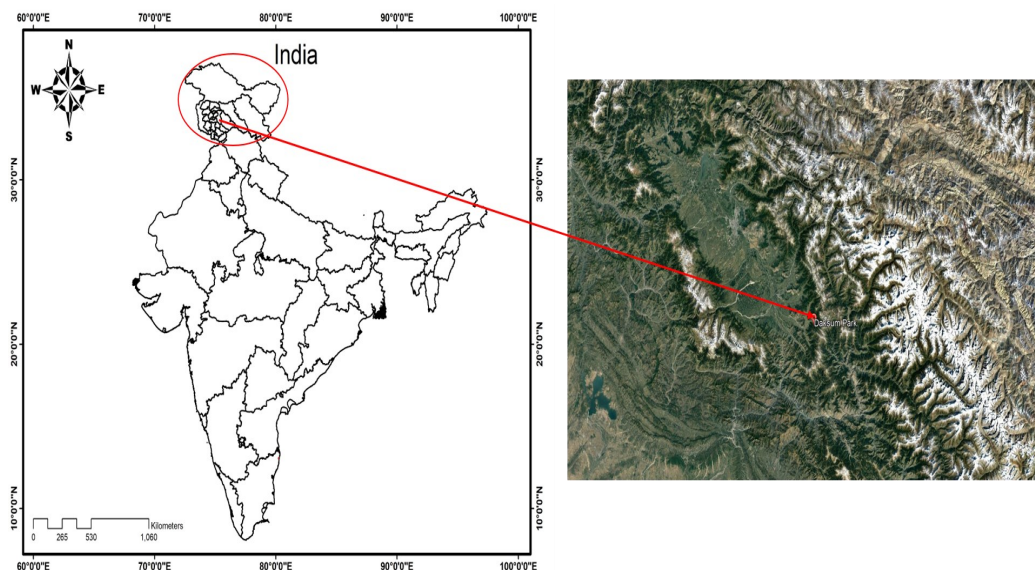


Fig. 2. Showing the site Daksum Anantnag, Kashmir

The procedure was repeated twice. Each time the extract was filtered using Whatman filter paper No.1. Finally, the extract was concentrated by a Rotary evaporator and used for further analysis.

GC-MS analysis

The phytochemical profiling was done according to (Vasantharaja *et al.*, 2019) using GC-MS Shimadzu – QP2010 Ultra analyzer. The sample was injected in a split mode into the capillary column, with helium as a carrier gas, column flow rate adjusted to 1mL/minute, and the overall run time was 50 minutes. The column oven temperature started from 80 °C initially to 200 °C at a rate of 3 °C /minute, then finally raised to 260 °C at 10 °C /minute, held for 5 minutes.

Microbial strains used for the study

The microorganisms used for this study were gram-positive bacteria such as *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 and gram-negative bacteria such as *Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 11229, and a fungus, *Candida albicans* ATCC 1023. All these microbes were procured from HiMedia, Laboratories Pvt. Ltd, Mumbai, India.

Antimicrobial assay

In this study, the antibacterial susceptibility of *R. officinalis* methanolic leaf extract was tested using well diffusion assay. First, sterile Muller Hinton Agar medium was poured into sterile Petri dishes and solidified. Then, the test microbes were swabbed onto the surface of the medium at concentrations ranging from 10^4 to 10^6 CFU/mL. To create wells on the surface of the medium, a cork borer was used. Four wells were filled with *R. officinalis* extract at concentrations of 25 μ L, 50 μ L,

75 μ L, and 100 μ L from a stock solution of 10 mg/mL. The fifth and sixth wells were filled with positive and negative controls, respectively. After incubation at 37°C for 16-18 hours, the inhibitory zones were measured. The inhibitory zones represent the area of growth inhibition around the well containing the plant extract. Standard antibiotics such as Ciprofloxacin, Fluconazole, and Ampilox were used as positive controls to compare the effectiveness of the *R. officinalis* extract. Dimethyl sulfoxide was used as a negative control.

Determination of minimum inhibitory concentration

The experiment was carried out in a 96-well plate, according to (Gabrielson *et al.*, (2002). 5 μ L of a 12-hour-old microbial culture was added to the first ten wells of the plate. The culture was likely diluted in a suitable growth medium. Serial dilutions of *R.officinals* extract were prepared, ranging from 512 to 1 μ g/mL, and added to the first ten wells of the plate. The plate was incubated at 37 °C for 16 to 18 hours. This allowed the microbes to grow and potentially be inhibited by the extract. After incubation, 10 μ L of (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg/mL) was added to each well. The plate was incubated for an additional 2 hours to allow the MTT to be converted to formazan. After the incubation, 100 μ L of DMSO solution was added to each well to dissolve the formazan crystals. The plate was observed visually, and a color change from yellow to purple was considered positive. This indicated the presence of living cells. The MIC was defined as the lowest concentration of *R.officinals* extract that completely inhibited microbial growth. This was determined by identifying the lowest concentration of extract that did not produce any visible color change in the wells. The experiment was carried out in triplicate.

Cell culture

The human A549 lung cancer cell line was obtained from the Kings Institute of Preventive Medicine and Research, Chennai, Tamil Nadu. This cell line is commonly used in cancer research as it is derived from lung adenocarcinoma and retains some of the characteristics of the original tumor. The cells were grown in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotic-antimycotic solution. The cell culture was incubated at 37°C in a humidified atmosphere of 5% CO₂. Once the cells achieved 70-80% confluency, they were trypsinized and seeded in a 96-well plate. After 21 to 24 hours of incubation, the cells were utilized for further experimentation (Ashma *et al.*, 2022).

Preparation of extract for MTT assay

The crude methanolic leaf extract of *R. officinalis* was prepared in the DMEM at a concentration of 1 mg/mL for future use in the MTT assay.

RESULTS AND DISCUSSION

In the present study, the GC-MS investigation of the *R. officinalis* methanolic leaf extract led to the identification of twenty-six scientifically, industrially, and biologically important phytochemicals (Fig. 3). The identified phytochemicals included terpenes (mono and diterpenes), sterols, fatty acid esters, fatty alcohols, ethers, long-chain hydrocarbons, a furanocoumarin, and vitamin metabolite. The list of phytochemicals with decreasing peak area % is mentioned in Table 1.

The chemical profiling of *R. officinalis* methanolic leaf extract by the other authors has shown the presence of twenty-six important phytochemicals, including ter-

penes, sterols, fatty acid esters, fatty alcohols, ethers, long-chain hydrocarbons, a furanocoumarin, and a vitamin metabolite. The identified phytochemicals have various scientifically, industrially, and biologically important properties and many of them have therapeutic potential with significant bioactivities. Fatty acid methyl esters (FAMES) such as stearate, methyl oleate, and methyl linoleate are well-known biodiesel components with antioxidant and antifungal activities (Nomgboye and Hansen, (2008); Tariq *et al.*, 2011; Jiaqiang *et al.*, 2016; Pinto *et al.*, 2017). Moreover, compounds such as ferruginol, carnosol, and oxypeucedanin have promising anticancer activities (de Jesus *et al.*, 2008; Kang *et al.*, 2009; Vergara *et al.*, 2014; Giacomelli *et al.*, 2016; Xiong *et al.*, 2017; Luo *et al.*, 2019; Park *et al.*, 2020), while γ -sitosterol has been reported to reduce hyperglycemia in diabetic rats (Balamurugan, *et al.*, 2011). Additionally, some of the identified compounds like ferruginol, carnosol, and dotriacontane have antimicrobial activity (Attia, (2019); Surekha *et al.*, 2022; Lim *et al.*, 2022).

Antimicrobial activity of *Rosmarinus officinalis*

Regardless of the incredible progress in modern medicine, infectious diseases caused by pathogenic microbes still stand as a major public health hazard. The indiscriminate use of synthetic antibiotics has resulted in the emergence of resistant strains of microbes. Therefore, the development of new antimicrobial agents with minimal side effects is required to improve the existing therapeutic strategies (Aslam *et al.*, 2018; Sofi *et al.*, 2022 c). Organic solvents such as methanol, Ethyl acetate, ethanol, acetone, chloroform and hexane are often used to extract bioactive compounds because these solvents easily elute polar molecules (Abubakar

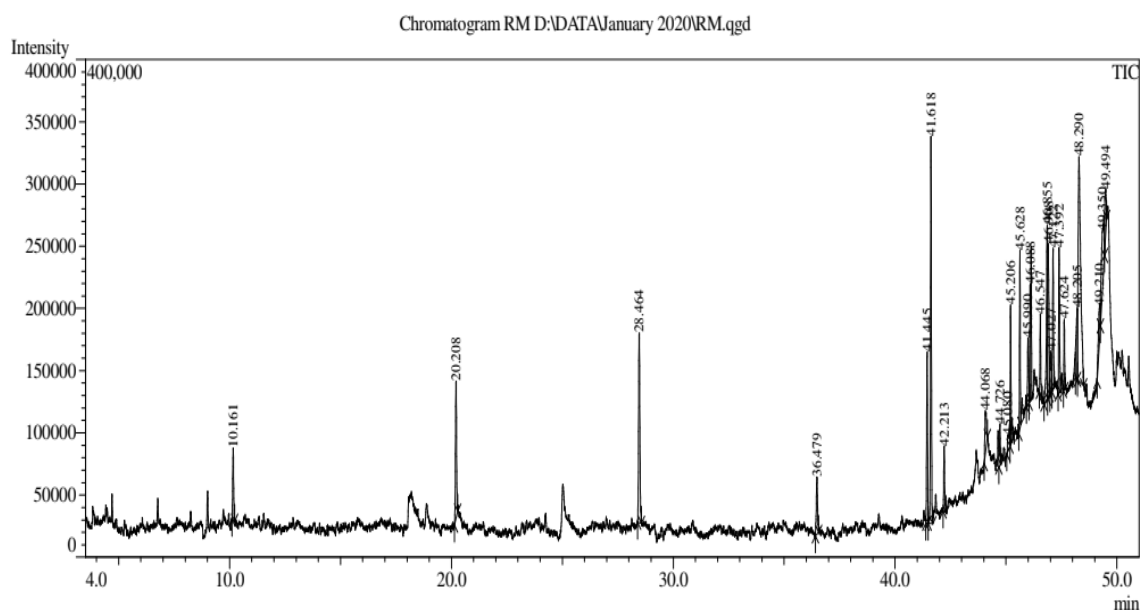


Fig. 3. Chromatogram of *Rosmarinus officinalis* methanolic leaf extract showing peaks of number of compounds

Table 1. Phytocompounds identified in methanolic leaf extracts of *Rosmarinus officinalis* L by GC-MS analysis

Sl. No.	Name Peak#	Area%	Formula
1	Bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimeth-, (1S)-	1.98	C ₁₀ H ₁₄ O
2	1-Dodecanol	4.33	C ₁₂ H ₂₆ O
3	2-Propenoic acid, pentadecyl ester	6.93	C ₁₈ H ₃₄ O ₂
4	Hexadecanoic Acid, Methyl ester	2.12	C ₁₇ H ₃₄ O ₂
5	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	4.64	C ₁₉ H ₃₄ O ₂
6	9-Octadecenoic acid, methyl ester, (E)-	10.64	C ₁₉ H ₃₆ O ₂
7	Methyl stearate	1.68	C ₁₉ H ₃₈ O ₂
8	9H-Carbazole,1,9-Dimethyl-3-Phenyl	1.86	C ₂₀ H ₁₇ N
9	9(1H)-Phenanthrenone, 2,3,4,4A,10, 10A-Hexahydro-6-Hydroxy-1,1,4A,7-Tetramethyl-, (4as-trans)	0.96	C ₁₈ H ₂₄ O ₂
10	4,6-Bis(1,1'-dimethylethyl)-2',5'-dimethoxy-1,1'-biphenyl-2-ol	1.08	C ₂₂ H ₃₀ O ₃
11	Ferruginol	2.96	C ₂₀ H ₃₀ O
12	Isocarnosol	3.79	C ₂₀ H ₂₆ O ₄
13	(-)-20-Deoxocarnosol	1.97	C ₂₀ H ₂₈ O ₃
14	Isocarnosol	2.68	C ₂₀ H ₂₆ O ₄
15	2,2-Bis(4'-methoxyphenyl)-2-ethoxyethane	1.90	C ₁₈ H ₂₂ O ₃
16	Phosphorin, 2,6-BIS(1,1-Dimethylethyl)-4-Phenyl	6.23	C ₁₉ H ₂₅ P
17	Dotriacontane	5.26	C ₃₂ H ₆₆
18	alpha.-Tocopherol-.beta.-D-mannoside	1.57	C ₃₅ H ₆₀ O ₇
19	Carnosol	3.76	C ₂₀ H ₂₆ O ₄
20	Hexadecanoic acid, 2-Hydroxy-1- (Hydroxymethyl)Ethyl ester	3.88	C ₁₉ H ₃₈ O ₄
21	13-Isopropylpodocarpene-12-ol-20-al	1.55	C ₂₀ H ₂₈ O ₂
22	beta.-Pregnane-3.alpha.,17.alpha.-diol-2-one	2.68	C ₂₁ H ₃₄ O ₃
23	gamma.-Sitosterol	16.78	C ₂₉ H ₅₀ O
24	4-(3-Methyl-2-oxobutoxy)-7H-furo[3,2-g][1]benzopyran-7-one	1.95	C ₁₆ H ₁₄ O ₅
25	Docosanoic acid, Docosyl ester	4.82	C ₄₄ H ₈₈ O
26	DicyclohexylPimelate	1.99	C ₁₉ H ₃₂ O ₄
		100%	

and Haque, 2020). Realizing the growing demand for new remedies to combat various infections caused by antibiotic-resistant microbes, Lamiaceae members can serve as the promising raw material for their development (Kozłowska *et al.*, 2015).

In the present study methanolic extract of *R. officinalis*, used by local people for different remedies, was tested to check the antimicrobial efficacy against various microbial strains, including *E. coli*, *E. faecalis*, *C. albicans*, *S. aureus*, and *P. aeruginosa*. The results showed that the extract exhibited significant antimicrobial activity against all the tested strains, with the highest zone of inhibition observed against *E. coli* (21 ± 0.7 mm), *E. faecalis* (19 ± 0.8 mm), *C. albicans* (18 ± 0.6 mm), followed by *S. aureus* (18 ± 0.8 mm) and *P. aeruginosa* (11 ± 0.5 mm). The MIC values for the extract ranged

from 128 to 256 µg/mL (Table 2, Fig. 4). Several other studies investigated the antimicrobial potential of plant extracts from the Kashmir Himalayas against *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. flexneri*, *S. aureus* and *C. albicans* (Khan *et al.*, 2013, Shameem *et al.*, 2015; Reshi *et al.*, 2017). For instance, the study by Nabi *et al.* (2022), tested the methanolic leaf extract of *Skimmia anquetilla* against *P. aeruginosa* (MTCC424), *E. coli* (MTCC739), and *S. aureus* (MTCC96) and found that the MIC values ranged from 2 to 16 mg/ml. Another study by Nawchoo *et al.*, (2012), tested the methanolic extract of *Hypericum perforatum* L. leaves against *S. aureus* and *E. coli*, with MIC values of 0.78 and 3.12 mg/mL, respectively. It is worth noting that the MIC values reported in these studies are higher than those found in our study with *R. officinalis* extract. This

could be due to differences in the extraction methods used or the specific bioactive compounds present in each plant species. A recent study conducted on the methanolic and hexane extracts of plants used in Kashmir Himalayas, including *Sisymbrium irio*, *Iris germanica*, *Geum elatum*, and *Urtica parviflora*, did not show any sensitivity to *C. albicans* (Guna, (2018)). In the present study, the *C. albicans* was significantly inhibited with MIC value 128 µg/mL. Overall, the results of the present study were comparatively better than these previous studies, which could be due to differences in the extraction methods, phytochemicals present in the plant species extracts. The findings of this research are promising since these microbes are among the most common bacteria responsible for both community and hospital-acquired illnesses. The growth of resistance against these microbes to even last-line antibiotics emphasized the need for new medicines with novel targets. The antimicrobial activity of *R. officinalis* was relevant in this perspective since the MIC values of the active extract in the present investigation were much below 1mg/mL, which may be regarded as significant in light of the discovery of potent antimicrobials from plants.

Anticancerous activity of *Rosmarinus officinalis*

Although numerous anticancer drugs have been discovered, they also have some serious side effects in addition to being costly. It is therefore essential to develop a safe, efficient and economical treatment of the disease. Many herbal medicines have received scientific attention for their therapeutic effectiveness against

various diseases, including cancer (Akhouri et al., 2020). Extracts from Lamiaceae members demonstrated significant efficacy in suppressing the proliferation of cancer cells (Makrane et al., 2018; Tundis et al., 2017). The present study examined the effects of methanolic leaf extract on human lung cancer A549 cells. The results obtained from the MTT assay demonstrated that *R. officinalis* methanolic extract significantly ($p \leq 0.05$) suppressed the growth of A549 cancer cells with an IC₅₀ of 39.7 and 33.6 µg/mL for 24 and 48 hours, respectively, indicating a promising anti-lung cancer activity of methanolic extract of *R. officinalis* leaf extract. The MTT assay showed that methanolic extract was able to inhibit the growth of A549 cancer cells in a dose-dependent manner as shown in (Table. 3). Previous studies have reported the *in vitro* anti-lung cancer activity of plant extracts of the same botanical family, named *Origanum compactum*, *Salvia officinalis*, with an IC₅₀ equal to 198 ± 12 µg/mL and 235.00±1.00 µg/mL, respectively (Chaouki et al., 2015). In separate studies, the essential oil from Lamaciae plants, *M. piperita* L (IC₅₀ 183.00 µg/mL), *M. pulegium* L (IC₅₀ 253.64 µg/mL), *L. angustifolia* Mill (IC₅₀ 88.90 of µg/mL), *S. lavandulifolia*, (IC₅₀ 140.10 µg/mL), (Miller et al., 2018; Donadu et al., 2017; Perez-Gonzalez et al., 2019) have shown higher IC₅₀ values against A549 lung cancer cell line as compared to our study (39.9 and 33.6 µg/mL for 24 and 48 hours). These differences can be due to the difference in chemotypes and the difference in climatic and geographical areas where the plants grow.

The essential oil of traditionally used medicinal plants in the Kashmir Himalayas, such as *Chenopodium ambro-*

Table 2. Showing zone of inhibition and MIC values of the *Rosmarinus officinalis* methanolic leaf extract against microbes

Microbes' names	Zone of Inhibition (mm)					
	Plant extract stock solution 10mg/mL				1mg/mL Standard Drug Ampilox/ Ciprofloxacin , fluconazole	
	25 µL	50 µL	75 µL	100 µL	2 Standard Drug 5 µL	DMSO Negative Control
<i>E. faecalis</i> ATCC 29212	-	13 ± 0.8	15 ± 0.7	19 ± 0.9	17 ± 0.7	-
<i>S. aureus</i> ATCC 25923	15 ± 0.7	16 ± 0.8	18 ± 0.7	18 ± 0.6	18 ± 0.8	-
<i>C. albicans</i> ATCC 10231	12± 0.6	15 ± 0.7	16 ± 0.7	18 ± 0.8	-	-
<i>E. coli</i> ATCC 11229	-	14 ± 0.6	18 ± 0.7	21± 0.8	36 ± 0.8	-
<i>P. aeruginosa</i> ATCC 15442	-	10 ± 0.5	11± 0.6	11 ± 0.5	33 ± 0.9	-

- Means no activity. Values are the mean ± standard deviation of three repetitions (n=3)

Minimum inhibitory concentration (MIC) µg/mL				
Gram Positive Bacteria		Gram Negative Bacteria		Fungus
<i>E. faecalis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>C. albicans</i>
128	128	256	128	128

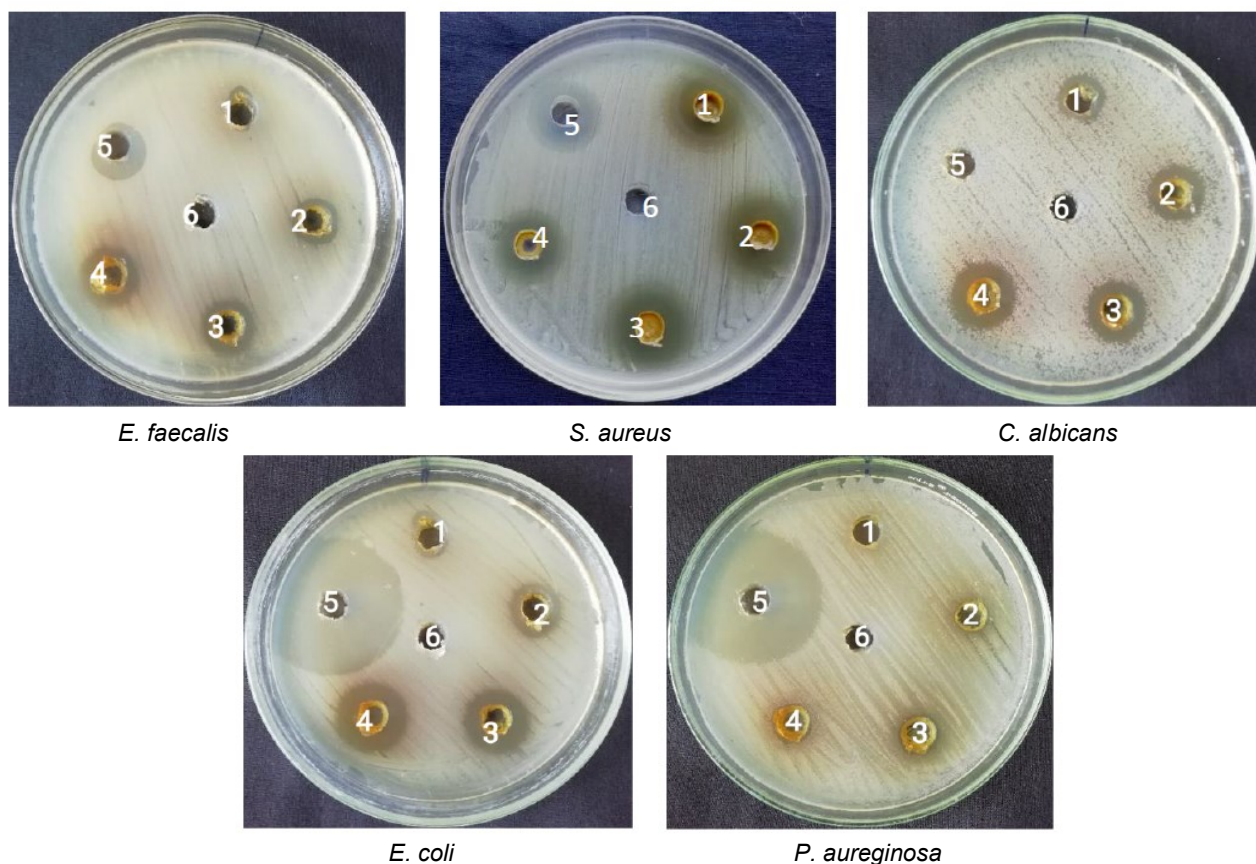


Fig. 4. Showing antimicrobial activity of *R. officinalis* methanolic leaf extract using well diffusion method. (Numerals depicted on each petridish represents the wells introduced with different micro volumes of *R officinalis* leaf extract (10 mg/mL stock solution), standard drugs 25µL (1 mg/ mL) and negative control. 1: 25 µL; 2: 50 µL; 3: 75 µL; 4: 100 µL; 5: 25µL Standard drug positive control, Ampilox for Gram-positive: *E. faecalis* and *S. aureus*, Ciprofloxacin for Gram-negative : *P. aeruginosa* and *E. coli*, Fluconazole for fungi: *C. albicans*. 6: DMSO as a negative control).

Table 3. Representing MTT assay of *R. officinalis* methanolic leaf extract on A549 cell line. % of viability is the mean of three absorbance values (n=3) and \pm SD

Concentration µg/mL	% of cell viability After 24 Hours	% of cell viability After 48 Hours
10	83.23 \pm 1.31	76.64 \pm 1.23
20	75.65 \pm 0.98	68.76 \pm 1.12
30	66.42 \pm 1.11	57.81 \pm 0.96
40	58.23 \pm 1.12	45.34 \pm 1.37
50	37.34 \pm 0.99	26.67 \pm 1.27
60	20.31 \pm 1.52	11.91 \pm 1.23

sioides L. *Chenopodium botrys* L, inhibited the growth of the A549 cancerous cell line around 40% at 125 µg/mL concentration (Shameem et al., 2019). Similarly, *Abies pindrow*, essential when treated against A549 lung cancer cells, showed around 40 % inhibition at 50 µg/mL (Yatoo et al., 2021). In another study, *Salvia moorcroftiana* Wall ethanolic extract also showed strong anticancer activity against A549 lung cancer cells with an IC₅₀ value of 129.32 µg/mL (Yasir et al., 2022). In contrast to the above-discussed plants, our

data show 50% inhibition of the same cell line (A549) at concentrations of 39.9 and 33.3 µg/mL for 24 and 48 hours, respectively indicating a promising anti-lung cancer potential of methanolic leaf extract of *R. officinalis*. Further studies are necessary to confirm the anti-cancer properties of *R. officinalis* and to investigate the underlying mechanisms of action. Additionally, the potential toxicity and side effects of the extract should also be evaluated to ensure its safety for human use.

Conclusion

The phytochemical screening of *R. officinalis* leaves by GC-MS analysis led to the identification of scientifically valuable phytochemicals being utilized in the current scientific research scenario due to their broad spectrum of applications and bioactivities. The study also revealed that the methanolic extract of *R. officinalis* significantly suppressed all the tested microbes and induced cell death in the lung cancer cell line (A549). However, further research is required to understand the mechanism of action of these phytochemicals and their efficacy *in vivo* which will help to evaluate the safety and efficacy of these compounds and determine their therapeutic potential. The discovery of novel lead molecules from *R. officinalis* will greatly contribute to developing new and effective therapies for combating antimicrobial resistance and deadly diseases like cancer.

Conflict of interest

The authors declare that they have no conflict of interest.

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