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Antimicrobial activity, docking and ADMET profiling of *Salvia rosmarinus* compounds on a targeting enzymes in cervical cancer

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Abstract

Salvia rosmarinus is an ornamental garden plant with leaves that have fragrance and medicinal uses. The aim of the present study is to investigate the chemical composition, antimicrobial effects of *Salvia rosmarinus* leaf extracts, and evaluate the anticancer effects of its compounds against targeting enzymes called DNMT1 and HPV type 16 E6 in human cervical cancer through docking and ADMET profiling. *In-vitro* and *in-silico* methods were used to assess antimicrobial activity and drug-likeness, utilizing agar-discs and Biovia-2021 software for testing, and docking and ADMET profiling for analysis. Statistical analysis was performed using Microsoft Windows with GraphPad Prism version 8.0.1 (244). The plant leaves extract had indicated vastly present phytochemicals and high antimicrobial activity, with varying efficacy in inhibiting pathogens in a dose-dependent-manner (50–100 $\mu g \cdot mL^{-1}$). Petroleum ether extracts showed high antibacterial properties against *S. aureus* and *S. epidermidis* with inhibition zones of 21.37 ± 0.78 and 17.50 ± 0.50 mm at 100 $\mu g \cdot mL^{-1}$, respectively. However, this extract exhibited a comparatively lower inhibition zone against gram-negative bacteria such as *E. coli, P. aeruginosa,* and *K. pneumoniae*, respectively, and stronger antifungal activity 20.83 ± 0.76 mm inhibition-zone against *C. albicans* fungi. The methanol/chloroform (1:1) extract of the plant leaves yielded micromeric (**1**) and benzocaine (**2**) and both compounds showed good binding-affinity with DNMT1 (PDB ID: 4WXX) with minimum binding energy of −8.4 $kcal \cdot mol^{-1}$ and −5.3 $kcal \cdot mol^{-1}$ respectively similar to those of standard. HPV type 16 E6 (PDB ID: 4XR8) interacts similarly with compound **1** (−10.1 $kcal \cdot mol^{-1}$) and **2** (−6.5 $kcal \cdot mol^{-1}$) as Jaceosidin (−8.8 $kcal \cdot mol^{-1}$). The plant studied shows antimicrobial activities, with the potential for treating cervical cancer but required further study.

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Introduction

Salvia rosmarinus L. (old name *Rosmarinus officinalis*), common name Rosemary thrives well in dry regions, hills and low mountains, calcareous, shale, clay, and rocky substrates[1]. *Salvia rosmarinus* used since ancient times in traditional medicine is justified by its antiseptic, antimicrobial, anti-inflammatory, antioxidant, and antitumorigenic activity[1,2]. The main objective of the study is to evaluate the antimicrobial activity of different extracts of *Salvia rosmarinus in vitro*, and its compounds related to *in silico* targeting of enzymes involved in cervical cancer. Since the start of the 20th century, some studies have shown that microbial infections can cause cervical cancers worldwide, infections are linked to about 15% to 20% of cancers[3]. More recently, infections with certain viruses like *Human papillomaviruses* (HPV) and *Human immunodeficiency virus* (HIV), bacteria like *Chlamydia trachomatis*, and parasites like *schistosomiasis* have been recognized as risk factors for cancer in humans[3]. Then again, cancer cells are a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Many things are known to increase the risk of cancer, including dietary factors, certain infections, lack of physical activity, obesity, and environmental pollutants[4]. Some studies have found that unbalanced common flora *Lactobacillus bacteria* around the reproductive organ of females increases the growth of yeast species (like *Candida albicans*) and some studies have found that women whose blood tests showed past or current *Chlamydia trachomatis* infection may be at greater risk of cervical cancer. It could therefore be that human papillomavirus (HPV) promotes cervical cancer growth[3]. *Salvia rosmarinus* is traditionally a healer chosen as a muscle relaxant and treatment for cutaneous allergy, tumors, increases digestion, and the ability to treat depressive behavior; mothers wash their bodies to remove bacterial and fungal infections, promote hair growth, and fight bad smells[5] .

The study of plant-based chemicals, known as phytochemicals, in medicinal plants is gaining popularity due to their numerous pharmacological effects[6] against drug resistance pathogens and cancers. The causes of drug resistance to bacteria, fungi, and cancer are diverse, complex, and only partially understood. The factors may act together to initiate or promote infections and carcinogenesis in the human body is the leading cause of death[7]. Antimicrobial medicines are the cornerstone of modern medicine. The emergence and spread of drug-resistant pathogens like bacteria and fungi threaten our ability to treat common infections and to perform life-saving procedures including cancer chemotherapy and cesarean sections, hip replacements, organ transplantation, and other surgeries[7]. On

the other hand, information about the current magnitude of the burden of bacterial and fungal drug resistance, trends in different parts of the world, and the leading pathogen–drug combinations contributing to the microbial burden is crucial. If left unchecked, the spread of drug resistance could make many microbial pathogens much more lethal in the future than they are today. In addition to these, cancers can affect almost any part of the body and have many anatomies and molecular subtypes that each require specific management strategies to avoid or inhibit them. There are more than 200 different types of cancer that have been detected. The world's most common cancers affecting men are lung, prostate, colorectal, stomach, and liver cancers[8]. While breast, cervix, colorectal, lung, and stomach cancers are the most commonly diagnosed among women[8]. Although some cancers said to be preventable they seem to still be one of the causes of death to humans, for example cervical cancer. The need to fill the gap to overcome the problem of searching for antimicrobials and anticancers from one source of *Salvia rosmarinus* is of importance.

Cervical cancer is a common cancer in women and a prominent cause of death[9]. In Ethiopia, cervical cancer is a big deal for women aged 15 to 44, coming in as the second most common cancer[9]. Globally, it's the fourth most common prevalent disease for women[10]. Aberrant methylation of tumor-suppressor genes' promoters can shut down their important functions and play a big role in causing cervical tumors[10]. There are various cervical cancer repressor genes (proteins turn off or reduce gene expression from the affected gene), such as CCNA1, CHF, HIT, PAX1, PTEN, SFRP4, and TSC1. The genes play a crucial role in causing cervical cancer by regulating transcription and expression through promoter hypermethylation, leading to precursor lesions during cervical development and malignant transformation[11]. The process of DNA methylation is primarily carried out by a group of enzymes known as DNA methyltransferases (DNMT1). It has been reported that DNMT1 (PDB ID: 4WXX), a protein responsible for DNA methylation can contribute to the development of cervical cancer. DNMT1 inhibits the transcription of tumor suppressor genes, facilitating tumorigenesis, which finally develops into cervical cancer. Tumor suppressor gene transcription is inhibited by DNMT1, which helps cancer grow and eventually leads to cervical cancer. Repressive genes' hypermethylation may be decreased, their expression can be increased, and the phenotype of malignant tumors can be reversed by inhibiting the DNMT1 enzyme.

On the other hand, infection by the human papilloma virus (HPV) phenotype 16, enzyme 6 (PDB ID: 4XR8) has been correlated with a greatly increased risk of cervical cancer worldwide[12]. Based on variations in the nucleotide sequences of the virus genome, over 100 distinct varieties of the human papilloma virus (HPV) have been identified (e.g. type 1, 2 etc.). Genital warts can result from certain types 6 and 11 of sexually transmitted HPVs. Other HPV strains, still, that can infect the genitalia, do not show any symptoms of infection[8]. Persistent infection with a subset of approximately 13 so-called 'high-risk' sexually transmitted HPVs, including such as types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 different from the ones that cause warts may lead to the development of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), penile intraepithelial neoplasia (PIN), and/or anal intraepithelial neoplasia (AIN). These are precancerous lesions and can progress to invasive cancer. Almost all occurrences of cervical cancer have HPV infection as a required component[13]. Superfluous infection by HPV type 16 E6 (PDB ID: 4XR8) has been correlated with a greatly increased genital risk of precursor cervical cancer worldwide[11]. Scholars more defined in major biochemical and biological activities of HPV type 16 E6 (PDB ID: 4XR8) in high-risk HPV oncogenes and how they may work together in the development of cervical disease and cancer[13].

One potential approach to treat cervical cancer is to inhibit the activity of the DNMT1 and HPV type 16 E6 enzymes specifically[13–16]. Over 50% of clinical drug forms worldwide originate from plant compounds[17]. In the past, developing new drugs was a lengthy and costly process. However, with the emergence of bioinformatics, the use of computer-based tools and methods have become increasingly important in drug discovery. One such method is molecular docking and ADMET profiling which involves using the structure of a drug to screen for potential candidates. This approach is known as structure-based drug design and can save both time and resources during the research process[15]. Structural-based drug designing addresses ligand binding sites with a known protein structure[15]. Using free binding energies, a computational method known as docking examines a large number of molecules and suggests structural theories for impeding the target molecule[17]. Nowadays, due to increasing antibiotic resistance like bacteria, fungi, and cancer cells, natural products remain an important source for discovering antimicrobial compounds and novel drugs for anti-cancers like cervical cancers. Therefore, the purpose of this research is to assess the antimicrobial activity of extracts, molecular docking, ADMET profiling in anticancer properties of compounds isolated from *Salvia rosmarinus*, on a targeting DNMT1 and HPV type 16 E6 in human cervical cancer. In the present study, various solvent crude extracts obtained from *Salvia rosmarinus* were used for antimicrobial activity and the isolated compounds **1** and **2** were submitted for *in silico* study to target the DNMT1 and HPV type 16 E6 enzymes to inhibit the growth of human cervical cancer cells.

Materials and methods

Plant material collection and identification

Healthy *Salvia rosmarinus* leaves were collected in Bacho district, Southwest Showa, Oromia, Ethiopia, during the dry season of November 2022. The plant materials were authenticated by Melaku Wondafrish, Natural Science Department, Addis Ababa University and deposited with a voucher number 3/2-2/MD003-80/8060/15 in Addis Ababa University's National Herbarium.

Materials, reagents, and instrumentation

The most common organic solvent used in extractions of medicinal plants is 2.5 L of petroleum ether, chloroform/methanol (1:1), and methanol. The test culture medium for microbes was used and performed in sterile Petri dishes (100 mm diameter) containing sterile Muller–Hinton Agar medium (25 mL, pH 7) and Sabouraud Dextrose Agar (SDA) for bacteria and fungi, respectively. A sterile Whatman filter paper (No. 1) disc of 6 mm diameter was used to determine which antibiotics an infective organism is sensitive to prescribed by a minimum zone of inhibition (MZI). Ciprofloxacin antibiotic reference (manufactured by Wellona Pharma Ciprofloxacin tablet made in India) and Ketoconazole 2% (made in Bangladesh) were used as a positive controls for antibacterial and

antifungal, respectively and Dimethyl sulfoxide (DMSO) 98.9% was used as a negative control for antimicrobial tests. In the present study, the height of the column was 650 mm and the width was 80 mm. Several studies by previous researchers showed the acceptable efficiency of column chromatography (up to 43.0% w/w recovery) in the fractionation and separation of phenolic compounds from plant samples[18]. In column chromatography, the ideal stationary phase used silica gel 60 (0.200 mm) particles. The ^{1}H-NMR spectrums of the compounds were analyzed using a 600 MHz NMR machine and 150 MHz for ^{13}C NMR. The compounds were dissolved in MeOD for compound **1** and in DMSO for compound **2** for NMR analysis. On the other hand, UV spectroscopy (made in China) used 570 nm ultraviolet light to determine the absorbency of flavonoids ($mg \cdot g^{-1}$) phytochemicals.

Qualitative determination of the phytochemical constituents

The samples (extracts) were analyzed to detect the presence of certain chemical compounds such as alkaloids (tested using Wagner's reagents), saponins (tested using the froth test), steroids (tested with Liebermann Burchard's tests), terpenoids (tested with Lidaebermann Burchard's tests), quinones, and flavonoids (tested using Shinoda tests)[19].

Extraction and isolation

The leaves of *Salvia rosmarinus* (500 g) were successively extracted using maceration using petroleum ether, chloroform/methanol (1:1), and methanol, every one 2.5 L for 72 h to afford 3.6, 6, and 53 g crude extracts, respectively. The methanol/chloroform (1:1) extract (6 g) was loaded to silica gel (150 g) column chromatography using the increasing polarity of petroleum ether, methanol/chloroform (1:1) solvent system to afford 80 fractions (100 mL each). The fraction obtained from chloroform/methanol 1:1 (3:2) after repeated column chromatography yielded compound **1** (18 mg). Fractions 56-65, eluted with chloroform/methanol (1:1) were combined and purified with column chromatography to give compound **2** (10 mg).

Anti-bacterial and anti-fungal assays

The microorganisms were obtained from the Ethiopia Biodiversity Institution (EBI). Two gram-positive bacteria namely *Staphylococcus aureus* serotype (ATCC 25923) and *Streptococcus epidermidis* (ATCC14990); and three gram-negative bacteria, namely *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 5702), and *Klebsiella pneumonia* (ATCC e13883) were inoculated overnight at 37 °C in Muller–Hinton Agar/MHA culture medium and two fungus strains of *Candida albicans* (ATCC 16404) and *Aspergillus niger* (ATCC 11414) were inoculated overnight at 27–30 °C in Sabouraud Dextrose Agar/SDA culture medium[20].

The antibacterial and antifungal activities of different crude extracts obtained from *Salvia rosmarinus* plant leaves were evaluated by the disk diffusion method (in accordance with the 13th edition of the CLSI M02 document on hardydiagnostics.com/disk-diffusion). Briefly, the test was performed in sterile Petri dishes (100 mm diameter) containing solid and sterile Muller–Hinton Agar medium (25 mL, pH 7) and Sabouraud Dextrose Agar (SDA) for bacteria and fungi, respectively. The extracts were placed on the surface of the media that had previously been injected with a sterile microbial suspension (one microbe per petri dish) after being adsorbed on sterile paper discs (5 µL per Whatman disc of 6 mm diameter). To prevent test samples from eventually evaporating, all Petri dishes were sealed with sterile laboratory films. They were then incubated at 37 °C for 24 h, and the zone diameter of the inhibition was measured and represented in millimeters. Ciprofloxacin antibiotic reference (manufactured by Wellona Pharma Ciprofloxacin tablet, India) was used as a positive control and DMSO was used as a negative control for antibacterial activity test while Ketoconazole 2% (Bangladesh) was used as a positive control and 10 µL of 0.2% agar as a negative control for antifungal activity tests[20]. The term 'inhibitory concentration' refers to the minimum sample concentration required to kill 99.9% of the microorganisms present[21]. Three repetitions of the crude extract sample were used to precisely measure the inhibitory halo diameter (in mm), which was then expressed as mean ± standard deviation to assess the anti-microbial activity.

Identification and selection of target protein

Cervical cancer-causing protein was identified through relevant literature. The protein molecule structure of DNA (cytosine-5)-methyltransferase 1 (DNMT1) (PDB ID: 4WXX)[21] and HPV type 16 E6 (PDB ID: 4XR8)[21] - a protein known to cause cervical cancer - were downloaded from the Protein Data Bank[22]. The stability of the protein molecule was assessed using Rampage[23].

Selection of ligand molecules

Phytochemical constituents of *Salvia rosmarinus* plant leaves were used to select a source of secondary metabolites (ligands). Ligand molecules were obtained through plant extraction, and isolation, and realized with PubChem (https://pubchem.ncbi.nlm.nih.gov/). The ligands were downloaded in Silver diamine fluoride format (SDF) and then converted to PDB format using an online SMILES translator (https://cactus.nci.nih.gov/translate/). The downloaded files were in PDB format, which was utilized for running various tools and software[24].

Preparation of protein molecule

The Biovia Discovery Studio Visualizer software was used to analyze the protein molecule. The protein molecule was converted into PDB format and its hierarchy was analyzed by selecting ligands and water molecules. Both the protein molecule and the water molecules lost their attached ligands during the analysis. Finally, the protein's crystal structure was saved in a PDB file[25].

Virtual screening of ligands

PyRx software was utilized to screen secondary metabolites and identify those ligands with the lowest binding energy to the protein target. The ligands with the lowest binding energy were further screened for their drug-likeliness property through analysis. It is worth noting that PyRx runs on PDBQT format. To begin using PyRx, it needs to load a protein molecule. This molecule should be converted from PDB to the protein data bank, partial charge (Q), and Atom Type (PDBQT) format. Once the protein molecule is loaded, it can import ligands from a specific folder in Silver diamine fluoride format. The ligand energy was minimized and changed to PDBQT format. The protein was docked with the ligand and screened based on minimum binding energy (https://cactus.nci.nih.gov/translate/).

Docking

The optimal ligand was selected for final docking using AutoDock Vina and Biovia by modifying the reference of

Discovery Studio Client 2021 (https://cactus.nci.nih.gov/translate/).

AutoDock Vina

The protein target from the Protein Data Bank (PDB) was loaded onto the graphical interface of AutoDock Vina. To prepare the protein for docking, water molecules were removed, hydrogen polar atoms were added, and Kollman charges were assigned to the protein molecule. Ultimately, PDBQT format was used to store the protein. After being imported in PDB format, the Ligand molecule was transformed to PDBQT format. Next, a grid box was chosen to represent the docked region. The command prompt was used to run AutoDock Vina and the outcomes were examined (https://cactus.nci.nih.gov/translate/).

Biovia Discovery Studio Client 2021

Docking the ligand with the protein target DNMT1(PDB ID: 4WXX)[22] and HPV type 16 E6 (PDB ID: 4XR8)[21] enzymes were performed using Biovia Discovery Studio Client 2021 by loading the protein target first followed by the ligand in PDB format. The charges were attached to the protein molecule, and the energy was minimized for the ligands. Both the protein and ligand molecules were prepared for docking. Once the docking process was complete, the results were analyzed based on several parameters, including absolute energy, clean energy, conf number, mol number, relative energy, and pose number. The interaction between the protein and ligand was analyzed using structure visualization tools, such as Biovia Discovery Studio Visualizer and PyMol (https://cactus.nci.nih.gov/translate/).

Structure visualization through PyMol

The process of visualizing the structure was carried out using the PyMol tool. PyMol is a freely available software. Firstly, the protein molecule in PDBQT form was loaded on the PyMol graphical screen. Then, the output PDBQT file was added. The docked structure was visualized and the 'molecule' option was changed to 'molecular surface' under the 'shown as' menu (https://cactus.nci.nih.gov/translate/).

Drug likeliness property analysis

Drug likeliness properties of the screened ligands were evaluated using the SwissADME online server. SMILE notations were obtained from PubChem and submitted to the SwissADME web server for analysis. The drugs were subjected to Lipinski's rule of five[20] for analysis. Lipinski's rules of five were selected for final docking through AutoDock Vina and Biovia Discovery Studio Client 2021. Ligands **1** and **2** were analyzed using Lipinski's rule of five for docking with AutoDock Vina and Biovia Discovery Studio Client 2021.

Statistical data analysis

The antimicrobial analysis data generated by triplicate measurements reported as mean ± standard deviation, and a bar graph also generated by GraphPad Prism version 8.0.1 (244) for Windows were used to perform the analysis. GraphPad Prism was used and combined with scientific graphing, comprehensive bar graph fitting (nonlinear regression), understandable statistics, and data organization. Prism allows the performance and modification of basic statistical tests commonly used and determined through the statistical applications in microbiology labs (https://graphpad-prism.software.informer.com/8.0/).

Results

Phytochemical test

Phytochemical screening of the different extracts for the presence (+) and absence (–) of alkaloids, steroids, glycosides, coumarins, terpenoids, flavonoids, carbohydrates, tannins, and saponins were done. The present study showed that alkaloids, terpenoids, flavonoids, and tannins tests in *S. rosmarinus* leaves of petroleum ether, chloroform/methanol (1:1), and methanol extracts were high whereas glycoside, coumarins, and carbohydrates had a moderate presence. The extract of *S. rosmarinus* leaves contain commonly bioactive constituents such as alkaloids, steroids, terpenoids, flavonoids, tannins, and saponins. These bioactive chemicals have active medicinal properties. Phytochemical compounds found in *S. rosmarinus* leaves have the potential to treat cancer cells and pathogens. The study also found that these flavonoids are related to natural phenolic compounds with anticancer and antimicrobial properties in the human diet (Table 1).

Characterization of isolated compounds

Two compounds were isolated and characterized using NMR spectroscopic methods (Fig. 1 & Supplementary Fig. S1a–c). Compound **1** (10 mg) was isolated as yellow crystals from the methanol/chloroform (1:1) leaf extract of *Salvia rosmarinus*. The TLC profile showed a spot at Rf 0.42 with methanol/chloroform (3:2) as a mobile phase. The ^{1}H-NMR spectrum (600 MHz, MeOD, Table 2, Supplementary Fig. S1a) of compound **1** showed the presence of one olefinic proton signal at δ 5.3 (t, J = 3.7 Hz, 1H), two deshielded protons at δ 4.7 (m, 1H), and 4.1 (m, 1H) associated with the C-30 *exocyclic* methylene group, and one O-bearing methine proton at δH 3.2 (m, 1H), and six methyl

Table 1. Phytochemical screening tests result of petroleum ether, chloroform/methanol (1:1) and methanol extracts of *Salvia rosmarinus* leaves.

Botanical name	Phytochemicals	Phytochemical screening tests	Different extracts		
			Petroleum ether	Chloroform/methanol (1:1)	Mehanol
Salvia rosmarinus	Alkaloids	Wagner's test	++	++	++
	Steroids	Libermann Burchard test	++	+	++
	Glycoside	Keller-Killiani test	–	+	–
	Coumarins	Appirade test	–	+	+
	Terpenoids	Libermann Burchard test	++	++	++
	Flavonoids	Shinoda test	++	++	++
	Carbohydrate	Fehling's test	–	++	++
	Tannins	Lead acetate test	++	++	++
	Saponins	Foam test	+	+	+

+ indicates moderate presence, ++ indicates highly present, – indicates absence.

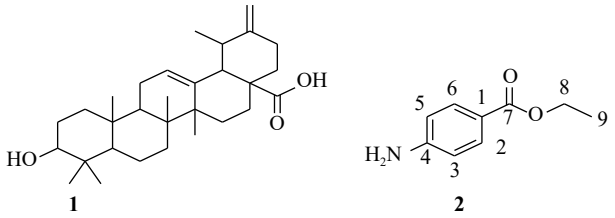

Fig. 1 Structure of isolated compounds from the leaves of *Salvia rosmarinus*.

Table 2. Comparison of the ^{13}C-NMR spectral data of compound **1** and micromeric acid (MeOD, δ in ppm).

Position	NMR data of compound **1**		Abdel-Monem et al.[26]
	^{1}H-NMR	^{13}C-NMR	^{13}C-NMR
1		38.60	39.9
2		27.8	28.5
3	3.2 (m, 1H)	78.3	80.3
4	–	39.4	39.9
5		55.3	56.7
6		18.1	18.3
7		36.7	34.2
8	–	41.9	40.7
9		53	48.8
10	–	38.4	38.2
11		23.9	24.6
12	5.3 (t, J = 3.7 Hz, 1H)	125.5	127.7
13	–	138.2	138
14	–	41.8	43.3
15		30.4	29.1
16		26.5	25.6
17	–	47.8	48
18	δ 2.22 (d, J = 13.5 Hz, 1H)	55.2	56.1
19		37.1	38.7
20	–	153.1	152.8
21		32.9	33.5
22		39.0	40.1
23		27.4	29.4
24		16.3	16.9
25		15.0	16.6
26		20.2	18.3
27		22.7	24.6
28	–	180.2	177.8
29		16.4	17.3
30	4.7 (m, 1H), and 4.1 (m, 1H)	103.9	106.5

protons at δ 1.14 (s, 3H), 1.03 (d, J = 6.3 Hz, 3H), 1.00 (s, 3H), 0.98 (s, 3H), 0.87 (s, 3H), and 0.80 (s, 3H). A proton signal at δ 2.22 (d, J = 13.5 Hz, 1H) was attributed to methine proton for H-18. Other proton signals integrate for 20 protons were observed in the range δ 2.2 to 1.2. The proton decoupled ^{13}C-NMR and DEPT-135 spectra (151 MHz, MeOD, Supplementary Fig. S1b & c) of compound 1 revealed the presence of 30 well-resolved carbon signals, suggesting a triterpene skeleton. The analysis of the ^{13}C NMR spectrum displayed signals corresponding to six methyl, nine methylene, seven methine, and eight quaternary carbons. Among them, the signal observed at δ 125.5 (C-12) belongs to olefinic carbons. The methylene carbon showed signals at δ_C 39.9, 28.5, 18.1, 36.7, 23.9, 30.4, 26.5, 32.9, and 38.6. The quaternary carbons showed a signal at δ_C 39.4, 41.9, 38.4, 138.2, 41.8, and 47.8. The signals of exocyclic methylene carbon signals appeared at δ 153.1 and 103.9. The spectrum also showed sp^3 oxygenated methine carbon at δ 78.3 and carboxyl carbon at δ 180.2. The spectrum revealed signals due to methyl groups at δ_C 27.4, 16.3, 15.0, 20.2, 22.7, and 16.4. The remaining carbon signals for aliphatic methines were shown at δ_C 55.3, 55.2, 53.0, and 37.1. The NMR spectral data of compound **1** is in good agreement with data reported for micromeric acid, previously reported from the same species by Abdel-Monem et al.[26]. (Fig. 1, Table 2).

Table 3. Comparison of the ^{1}H-NMR, and ^{13}C-NMR spectral data of compound **2** and benzocaine (DMSO, δ in ppm).

Position	NMR data of compound **2**		Alotaibi et al.[27]	
	^{1}H-NMR	^{13}C-NMR	^{1}H-NMR	^{13}C-NMR
1	–	120.9	–	119
2	7.79 (d, J = 8.7 Hz, 2H)	131.4	7.86 (d, J = 7.6 Hz)	132
3	6.90 (d, J = 8.7 Hz, 2H)	116.8	6.83 (d, J = 7.6 Hz)	114
4	–	148.2	–	151
5	6.90 (d, J = 8.7 Hz, 2H)	116.8	6.83 (d, J = 7.6 Hz)	114
6	7.79 (d, J = 8.7 Hz, 2H)	131.4	7.86 (d, J = 7.6 Hz)	132
7	–	166.0	–	169
8	4.3 (q, J = 7.1 Hz, 2H)	60.4	4.3 (q, J = 7.0 Hz)	61
9	1.3 (t, J = 7.1 Hz, 3H)	14.7	1.36 (t, J = 7.0 Hz)	15

Compound **2** (18 mg) was obtained as a white amorphous isolated from 40% methanol/chloroform (1:1) in petroleum ether fraction with an Rf value of 0.49. The ^{1}H NMR (600 MHz, DMSO, Supplementary Fig. S2a) spectral-data showed two doublets at 7.79 (d, J = 8.7 Hz, 2H), and 6.90 (d, J = 8.7 Hz, 2H) which are evident for the presence of 1,4-disubstituted aromatic group. The oxygenated methylene and terminal methyl protons were shown at δ 4.25 (q, J = 7.1 Hz, 2H) and 1.29 (t, J = 7.1 Hz, 3H), respectively. The^{13}C-NMR spectrum, with the aid of DEPT-135 (151 MHz, DMSO, Table 3, Supplementary Fig. S2b & c) spectra of compound 2 confirmed the presence of well-resolved seven carbon peaks corresponding to nine carbons including threee quaternary carbons, one oxygenated methylene carbon, one terminal methyl carbon, and two symmetrical aromatic methine carbons. The presence of quaternary carbon signals was shown at δ 120.9 (C-1), 148.2 (C-4), and ester carbonyl at δ 166.0 (C-7). The symmetry aromatic carbons signal was observed at δ 131.4 (C-2, 6), and 116.8 (C-3, 5). The oxygenated methylene and terminal methyl carbons appeared at δ_C 60.4 (C-8) and 14.7 (C-9), respectively. The spectral results provided above were in good agreement with those for benzocaine in the study by Alotaibi et al.[27]. Accordingly, compound **2** was elucidated to be benzocaine (4-Aminobenzoic acid-ethyl ester) (Table 3, Fig. 1, Supplementary Fig. S2a–c), this compound has never been reported before from the leaves of *Salvia rosmarinus*.

Antimicrobial activities assay

The extracts and isolated compounds from *Salvia rosmarinus* were evaluated *in vitro* against microbes from gram-positive bacteria (*S. aureus* and *S. epidermidis*), gram-negative bacteria (*E. coli*, *P. aeruginosa*, and *K. pneumoniae*) and fungi (*C. albicans* and *A. Niger*) (Table 4). The petroleum ether extracts exhibited significant activity against all the present study-tested microbes at 100 μg·mL^{-1}, resulting in an inhibition zone ranging from 7 to 21 mm. Chloroform/methanol (1:1) and methanol extracts demonstrated significant activity against all the present study-tested microbes at 100 μg·mL^{-1} exhibiting inhibition zones from 6 to 14 mm and 6 to 13 mm, respectively (Table 4). The

Table 4. Comparison of mean zone of inhibition (MZI) leaf extracts of *Salvia rosmarinus*.

Type of specimen, and standard antibiotics for each sample	Concentration (μg·mL^{-1}) of extract in 99.8% DMSO	Average values of the zone of inhibition (mm)						
		Gram-positive (+) bacteria		Gram-negative (–) bacteria			Fungai	
		S. aurous	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*	*A. niger*
Petroleum ether extracts								
S. rosmarinus	50	18.50 ± 0.50	15.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00	15.93 ± 0.12	4.47 ± 0.50
	75	19.87 ± 0.06	17.00 ± 0.00	9.33 ± 0.29	10.53 ± 0.50	10.93 ± 0.12	18.87 ± 0.23	5.47 ± 0.50
	100	21.37 ± 0.78	17.50 ± 0.50	11.47 ± 0.50	13.17 ± 0.29	12.43 ± 0.51	20.83 ± 0.76	6.70 ± 0.10
Standard antibiotics	Cipro.	21.33 ± 1.15	18.33 ± 0.58	9.33 ± 0.58	12.30 ± 0.52	15.00 ± 0.00		
	Ketocon.						22.00 ± 1.00	10.67 ± 0.58
Chloroform/methanol (1:1) extracts								
	50	5.47 ± 0.42	0.00 ± 0.00	10.33 ± 0.00	0.00 ± 0.00	9.70 ± 0.00	0.00 ± 0.12	8.47 ± 0.50
S. rosmarinus	75	5.93 ± 0.06	0.00 ± 0.00	11.33 ± 0.29	0.00 ± 0.50	12.50 ± 0.12	0.00 ± 0.23	10.67 ± 0.50
	100	6.47 ± 0.06	0.00 ± 0.00	14.17 ± 0.50	7.33 ± 0.29	14.17 ± 0.51	0.00 ± 0.76	12.67 ± 0.10
Standard antibiotics	Cipro.	15.00 ± 0.00	11.00 ± 1.00	11.33 ± 0.58	10.00 ± 0.52	12.67 ± 0.00		
	Ketocon.						7.00 ± 1.00	13.67 ± 0.58
Methanol extracts								
	50	9.17 ± 0.29	5.50 ± 0.50	0.00 ± 0.00	7.50 ± 0.00	0.00 ± 0.00	6.57 ± 0.12	0.00 ± 0.50
S. rosmarinus	75	9.90 ± 0.10	6.93 ± 0.12	9.33 ± 0.29	8.50 ± 0.50	0.00 ± 0.00	8.70 ± 0.23	0.00 ± 0.50
	100	11.63 ± 0.55	7.97 ± 0.06	11.47 ± 0.50	9.90 ± 0.10	0.00 ± 0.00	10.83 ± 0.76	13.13 ± 0.10
Standard antibiotics	Cipro.	13.00 ± 0.00	11.50 ± 0.50	14.20 ± 0.58	13.33 ± 0.29	10.00 ± 0.00		
	Ketocon.						12.00 ± 1.00	15.00 ± 0.58
Mean values of flavonoids (mg·g^{-1}) by 570 nm								
S. rosmarinus		Petroleum ether extracts		Chloroform/methanol (1:1) extracts		Methanol extracts		
	50	0.736		0.797		0.862		
	75	0.902		0.881		0.890		
	100	0.922		0.904		0.940		

Samples: Antibiotics: Cipro., Ciprofloxacin; Ketocon., ketoconazole (Nizoral); DMSO 99.8%, Dimethyl sulfoxide.

chloroform/methanol (1:1) extracts were significantly active against bacteria of *E. coli* and *K. pneumonia*, and *A. Niger* fungi at 100 μg·mL^{-1}. On the other hand, chloroform/methanol (1:1) extracts were significantly inactive against the *S. rosmarinus* and *P. aeruginosa* of bacteria and *C. albicans* of fungi, and again chloroform/methanol (1:1) extracts overall significantly active produced an inhibition zone of 12 to 14 mm (Table 4). Methanol extracts exhibited significant activity against *S. aureus, E. coli* bacteria, and *A. Niger* fungi at 100 μg·mL^{-1}. The inhibition zone was recorded to be 11 to 13 mm. However, methanol extracts exhibited significant inactivity against *K. pneumoniae* (Table 4). The overall result of our studies shows that Salvia rosmarinus was extracted and evaluated in vitro, exhibiting significant antibacterial and antifungal activity, with inhibition zones recorded between 6 to 21 mm for bacteria and 5 to 21 mm for fungi. In our study, the positive control for ciprofloxacin exhibited antibacterial activity measured at 21.33 ± 1.15 mm, 15.00 ± 0.00 mm, and 14.20 ± 0.50 mm for petroleum ether, chloroform/methanol (1:1), and methanol extracts, respectively. Similarly, the positive control for ketoconazole demonstrated antifungal activity of 22.00 ± 1.00 mm, 13.67 ± 0.58 mm, and 15.00 ± 0.58 mm for petroleum ether, chloroform/methanol (1:1), and methanol extracts, respectively. Additionally, our findings indicated that the mean values of flavonoids (mg/g) tested were 92.2%, 90.4%, and 94.0% for petroleum ether, chloroform/methanol (1:1), and methanol extracts, respectively. This suggests that the groups of phenolic compounds evaluated play a significant role in antimicrobial activities, particularly against antibiotic-resistant strains.

Determining the three solvent extracts in *S. rosmarinus* plants resulted in relatively high comparable with positive (+) control. Especially, the *S. rosmarinus* petroleum ether leaf extracts against drug resistance human pathogenic bacteria S. *aureus, S. epidermidis, E. coli, P. aeruginosa*, and *K. pneumoniae* were minimum zone of inhibition (MZI) recorded that 21.37 ± 0.78, 17.50 ± 0.50, 11.47 ± 0.50, 13.17 ± 0.29, and 12.43 ± 0.51 mm, respectively and against human pathogenic fungi *C. albicans* and *A. niger* were minimum zone of inhibition (MZI) recorded that 20.83 ± 0.76 and 6.70 ± 0.10 mm, respectively which was used from bacteria against *S. aureus* MZI recorded that 21.37 ± 0.78 mm higher than the positive control (21.33 ± 1.15 mm). The *S. rosmarinus* of chloroform/methanol (1:1) extracts were found to be against *E. coli* (14.17 ± 0.50 mm) and *K. pneumoniae* (14.17 ± 0.51 mm) higher than the positive control 11.33 ± 0.58 and 12.67 ± 0.00 mm, respectively. The methanol extracts of leaves in the present study plants were found to have overall MZI recorded less than the positive control. The *Salvia rosmarinus* crude extracts showed better antifungal activities than the gram-negative (–) bacteria (Table 4, Fig 2, Supplementary Fig. S3). Therefore, the three extracts, using various solvents of different polarity indexes, have been attributed to specific biological activities. For example, the antimicrobial activities of *Salvia rosmarinus* extracts may be due to the presence of alkaloids, terpenoids, flavonoids, tannins, and saponins in natural products (Table 1).

Compounds **1** and **2** were isolated from chloroform/methanol (1:1) extract of *Salvia rosmarinus* (Fig. 1, Tables 2 & 3). The plant extract exhibited highest antibacterial results recorded a mean inhibition with diameters of 21 and 14 mm at a concentration of 100 mg·mL^{-1} against *S. aureus* and *E. coli/K. pneumoniae*, respectively. After testing, overall it was found that the highly active petroleum ether extract of *Salvia*

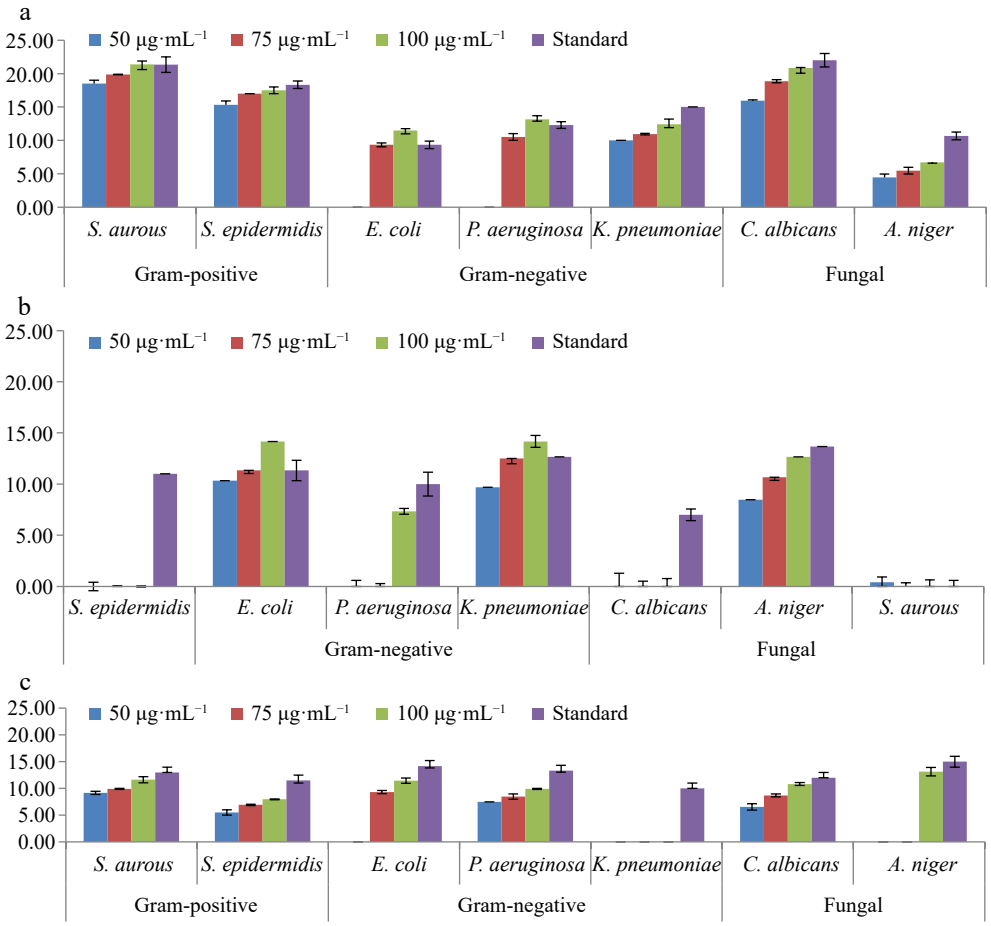

Fig. 2 Microbes' resistance with drugs relative to standard antibiotics in extracts of *Salvia rosmarinus*. The figures represent understudy of three extracts derived from *Salvia rosmarinus*. (a) Petroleum ether, (b) chloroform/methanol (1:1), and (c) methanol extracts tested in *Salvia rosmarinus*.

rosmarinus was able to inhibit the growth of *S. aureus* and *C. albicans*, with inhibition zones of 21 and 20 mm, respectively. The petroleum ether extracts showed good efficacy against all tested microbes, particularly gram-positive bacteria and fungi (Table 4). This is noteworthy because gram-negative bacteria generally exhibit greater resistance to antimicrobial agents. Petroleum ether and chloroform/methanol (1:1) extracts of the leaves were used at a concentration of 100 mg·mL^{-1}, resulting in impressive inhibition zone diameters of 11 and 14 mm for *E. coli*, 13 and 7 mm for *P. aeruginosa,* and 12 and 14 mm for *K. pneumoniae*, respectively.

The present study found that at a concentration of 50 µg·mL^{-1}, petroleum ether, chloroform/methanol (1:1), and MeOH extracts did not display any significant inhibition zone effects against the tested microbes. This implies that the samples have a dose-dependent inhibitory effect on the pathogens. The leaves of *Salvia rosmarinus* have been found to possess remarkable antimicrobial properties against gram-negative bacteria in different extracts such as *E. coli, P. aeruginosa*, and *K. pneumoniae* with 14.17 ± 0.50 in chloroform/methanol (1:1), 13.17 ± 0.29 in petroleum ether and 14.17 ± 0.51 in chloroform/methanol (1:1), respectively. However, in the present study, *Salvia rosmarinus* was found to possess remarkable high zones of inhibition with diameters of 21.37 ± 0.78 and 17.50 ± 0.50 mm antimicrobial properties against *S. aureus,* and *S. epidermidis* of gram-positive bacteria, respectively (Supplementary Fig. S3). The results are summarized in Fig. 2a–c.

Binding mode of isolated compounds docked against DNMT1 enzyme

The crystal structure of human DNMT1 (351-1600), classification transferase, resolution: 2.62 Å, PDB ID: 4WXX. Active site dimensions were set as grid size of center X = –12.800500 Å, center Y = 34.654981 Å, center Z = –24.870231 Å (XYZ axis) and radius 59.081291. A study was conducted to investigate the binding interaction of the isolated compounds **1** and **2** of the leaves of *Salvia rosmarinus* with the binding sites of the DNMT1 enzyme in human cervical cancer (PDB ID: 4WXX), using molecular docking analysis.

The study also compared the results with those of standard anti-cancer agents Jaceosidin (Table 5 & Fig. 3). The compounds isolated had a final fixing energy extending from –5.3 to –8.4 kcal·mol^{-1}, as shown in Table 4. It was compared to jaceosidin (–7.8 kcal·mol^{-1}). The results of the molecular docking analysis showed that, compound **1** (–8.4 kcal·mol^{-1}) showed the highest binding energy values compared with the standard drugs jaceosidin (–7.8 kcal·mol^{-1}). Compound **2** has shown lower docking affinity (–5.3 kcal·mol^{-1}) but good matching amino acid residue interactions compared to jaceosidin. After analyzing the results, it was found that the isolated

Table 5. Molecular docking results of ligand compounds **1** and **2** against DNMT1 enzyme (PDB ID: 4WXX).

Ligands	Binding affinity (kcal·mol^{-1})	H-bond	Residual interactions	
			Hydrophobic/electrostatic	Van der Waals
1	−8.4	ARG778 (2.85249), ARG778 (2.97417), VAL894 (2.42832)	–	Lys-889, Pro-879, Tyr-865, His-795, Cys-893, Gly-760, Val-759, Phe-892, Phe-890, Pro-884, Lys-749
2	−5.3	ARG596 (2.73996), ALA597 (1.84126), ILE422 (2.99493), THR424 (2.1965), ILE422 (2.93653)	Electrostatic Pi-Cation-ARG595 (3.56619), Hydrophobic Alkyl-ARG595 (4.15839), Hydrophobic Pi-Alkyl-ARG595 (5.14967)	Asp-423, Glu-428, Gly-425, Ile-427, Trp-464, Phe-556, Gln-560, Gln-594, Glu-559, Gln-598, Ser-563
Jaceosidin	−7.8	ASP571 (2.93566), GLN573 (2.02126), GLU562 (2.42376), GLN573 (3.49555), GLU562 (3.46629)	Hydrophobic Alkyl-PRO574 (4.59409), Hydrophobic Alkyl-ARG690 (5.09748), Hydrophobic Pi-Alkyl-PHE576 (5.1314), Hydrophobic Pi-Alkyl-PRO574 (4.97072), Hydrophobic Pi-Alkyl-ARG690 (5.07356)	Glu-698, Cys-691, Ala-695, Pro-692, Val-658, Glu-566, Asp-565

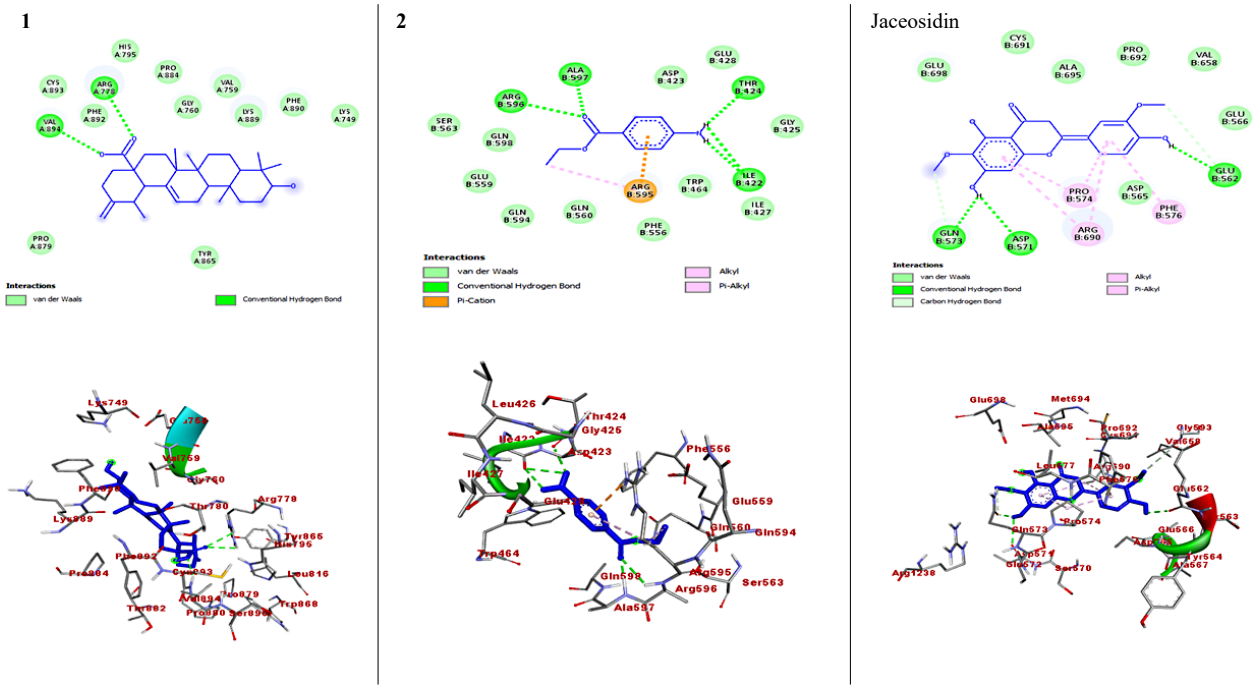

Fig. 3 The 2D and 3D binding interactions of compounds against DNMT1 enzyme (PDB ID: 4WXX). The 2D and 3D binding interactions of compound **1** and **2** represent against DNMT1 enzyme, and jaceosidin (standard) against DNMT1 enzyme.

compounds had similar residual interactions and docking scores with jaceosidin.

Hence, compound **1** might have potential anti-cancer agents. However, anti-cancer *in vitro* analysis has not yet been performed. Promising *in silico* results indicate that further research could be beneficial. The 2D and 3D binding interactions of compounds **1** and **2** against human cervical cancer of DNMT1 enzyme (PDB ID: 4WXX) are presented in Fig. 3. The binding interactions between the DNMT1 enzyme (PDB ID: 4WXX), and compound **1** (Fig. 3) and compound **2** (Fig. 3) were displayed in 3D. Compounds and amino acids are connected by hydrogen bonds (green dash lines) and hydrophobic interactions (non-green lines).

Binding mode of isolated compounds docked against HPV type 16 E6

Crystal structure of the HPV16 E6/E6AP/p53 ternary complex at 2.25 Å resolution, classification viral protein, PDB ID: 4XR8. Active site dimensions were set as grid size of center X = −43.202782 Å, center Y = −39.085513 Å, center Z = −29.194115 Å (XYZ axis), R-value observed 0.196, and Radius 65.584122. A study was conducted to investigate the binding interaction of the isolated compounds **1** and **2** of the leaves of *Salvia rosmarinus* with the binding sites of the enzyme of human papilloma virus (HPV) type 16 E6 (PDB ID: 4XR8), using molecular docking analysis software. The study also compared the results with those of standard anti-cancer agents jaceosidin (Table 6 & Fig. 4). The compounds isolated had a bottom most fixing energy extending from −6.3 to −10.1 kcal·mol^{-1}, as shown in Table 6. It was compared to jaceosidin (−8.8 kcal·mol^{-1}). The results of the molecular docking analysis showed that, compound **1** (−10.1 kcal·mol^{-1}) showed the highest binding energy values compared with the standard drugs jaceosidin (−8.8 kcal·mol^{-1}). Compound **2** has shown lower docking affinity (−6.3 kcal·mol^{-1}) but good matching amino acid residue interactions compared to jaceosidin. After analyzing the results, it was found that the isolated compounds had similar residual interactions and docking scores with jaceosidin.

Hence, compounds **1** and **2** might have potential anti-cancer agents of HPV as good inhibitors. However, anti-cancer *in vitro* analysis has not been performed yet on HPV that causes

Table 6. Molecular docking results of ligand compounds **1** and **2** against HPV type 16 E6 (PDB ID: 4XR8).

Ligands	Binding affinity (kcal·mol^{-1})	H-bond	Residual interactions	
			Hydrophobic/electrostatic	Van der Waals
1	−10.1	ASN101 (2.25622), ASP228 (2.88341)	–	Asp-148, Lys-176, Lys-180, Asp-178, Ile-179, Tyr-177, Ile-334, Glu-382, Gln-336, Pro-335, Gln-73, Arg-383, Tyr-100
2	−6.5	TRP63 (1.90011), ARG67 (2.16075), ARG67 (2.8181)	Hydrophobic Pi-Sigma-TRP341 (3.76182), Hydrophobic Pi-Pi Stacked-TYR156 (4.36581), Hydrophobic Pi-Pi T-shaped-TRP63 (5.16561), Hydrophobic Pi-Pi T-shaped-TRP63 (5.44632), Hydrophobic Alkyl-PRO155 (4.34691), Hydrophobic Pi-Alkyl-TRP341 (4.11391), Hydrophobic Pi-Alkyl-ALA64 (4.61525)	Glu-154, Arg-345, Asp-66, Met-331, Glu-112, Lys-16, Trp-231
Jaceosidin	−8.8	ARG146 (2.06941), GLY70 (3.49991), GLN73 (3.38801)	Electrostatic Pi-Cation-ARG67 (3.93442), Hydrophobic Pi-Alkyl-PRO49 (5.40012)	Tyr-342, Tyr-79, Ser-338, Arg-129, Pro-335, Leu-76, Tyr-81, Ser-74, Tyr-71, Ser-80, Glu-46

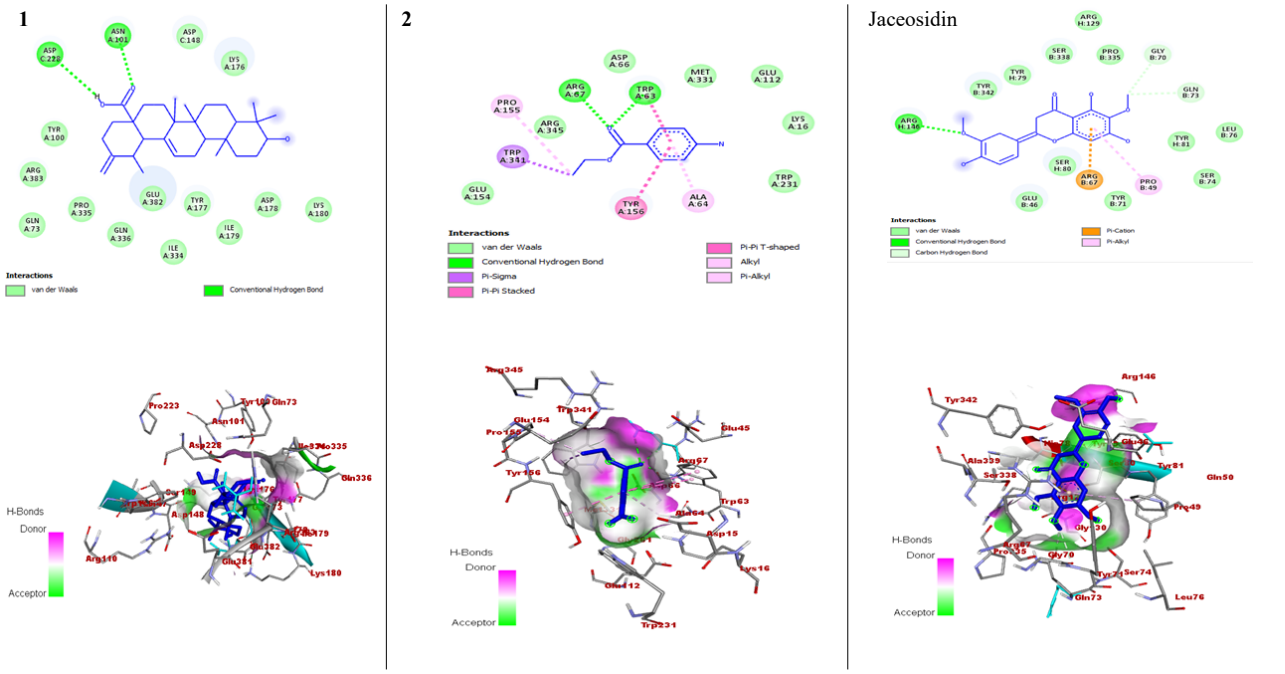

Fig. 4 The 2D and 3D binding interactions of compounds against HPV type 16 E6 (PDB ID: 4XR8). The 2D and 3D binding interactions of compound **1** and **2** represent against HPV type 16 E6 enzyme, and jaceosidin (standard) against HPV type 16 E6 enzyme.

cervical cancer agents. Promising *in silico* results indicate that further research could be beneficial. The 2D and 3D binding interactions of compounds **1** and **2** against human papilloma virus (HPV) type 16 E6 enzyme (PDB ID: 4XR8) are presented in Fig. 4. The binding interactions between the HPV type 16 E6 enzyme (PDB ID: 4XR8) and compound **1** (Fig. 4) and compound **2** (Fig. 4) were displayed in 3D. Compounds and amino acids are connected by hydrogen bonds (magenta lines) and hydrophobic interactions (non-green lines).

ADMET profiling in grug-likeness and toxicity analysis

In silico bioactivities of a drug, including drug-likeness and toxicity, predict its oral activity based on the document of Lipinski's Rule[25] was stated and the results of the current study showed that the compounds displayed conform to Lipinski's rule of five (Table 7). Therefore, both compounds **1** and **2** should undergo further investigation as potential anti-cancer agents. Table 8 shows the acute toxicity predictions, such as LD_{50} values and toxicity class classification (ranging from 1 for toxic, to 6 for non-toxic), for each ligand, revealing that none of them were acutely toxic. Furthermore, they were found to be similar to standard drugs. Isolated compound **1** has shown toxicity class classification 4 (harmful if swallowed), while **2** showed even better toxicity prediction giving results of endpoints such as hepatotoxicity, mutagenicity, cytotoxicity, and irritant (Table 8). All the isolated compounds were predicted to be non-hepatotoxic, non-irritant, and non-cytotoxic. However, compound **1** has shown carcinogenicity and immunotoxicity (Table 9). Hence, based on ADMET prediction analysis, none of the compounds have shown acute toxicity, so they might be proven as good drug candidates.

Discussion

Rosemary is an evergreen perennial plant that belongs to the family *Lamiaceae*, previously known as *Rosmarinus officinalis*. Recently, the genus *Rosmarinus* was combined with the genus *Salvia* in a phylogenetic study and became known as *Salvia rosmarinus*[28,29] and it has been used since ancient times for

Table 7. Drug-likeness predictions of compounds computed by Swiss ADME.

Ligands	Formula	Mol. Wt. (g·mol^{-1})	NRB	NHA	NHD	TPSA (A°^{2})	Log P (iLOGP)	Log S (ESOL)	Lipinski's rule of five
1	$C_{30}H_{46}O_3$	454.68	1	3	2	57.53	3.56	−6.21	1
2	$C_9H_{11}NO_2$	165.19	3	2	1	52.32	1.89	−2.21	0
Jaceosidin	$C_{17}H_{14}O_7$	330.3	3	7	3	105	1.7	1	0

NHD, number of hydrogen donors; NHA, number of hydrogen acceptors; NRB, number of rotatable bonds; TPSA, total polar surface area; and log P, octanol-water partition coefficients; Log S, turbid metric of solubility.

Table 8. Pre ADMET predictions of compounds, computed by Swiss ADME.

Ligands	Formula	Skin permeation value (logKp - cm·s^{-1})	GI absorption	Inhibitor interaction					
				BBB permeability	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor
1	$C_{30}H46O_3$	−4.44	Low	No	No	No	No	No	No
2	$C_9H_{11}NO_2$	−5.99	High	Yes	No	No	No	No	No
Jaceosidin	$C_{17}H_{14}O_7$	−6.13	High	No	No	Yes	No	Yes	Yes

GI, gastrointestinal; BBB, blood brain barrier; Pgp, P-glycoprotein; and CYP, cytochrome-P.

Table 9. Toxicity prediction of compounds, computed by ProTox-II and OSIRIS property explorer.

Ligands	Formula	LD_{50} (mg·kg^{-1})	Toxicity class	Organ toxicity					
				Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	Irritant
1	$C_{30}H_{46}O_3$	2,000	4	Inactive	Active	Active	Inactive	Inactive	Inactive
2	$C_9H_{11}NO_2$	NA	NA	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Jaceosidin	$C_{17}H_{14}O_7$	69	3	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

NA, not available.

various medicinal, culinary, and ornamental purposes. In the field of food science, rosemary is well known as its essential oil is used as a food preservative, thanks to its antimicrobial and antioxidant properties, rosemary has many other food applications such as cooking, medicinal, and pharmacology uses[30]. According to the study, certain phytochemical compounds found in *Salvia rosmarinus* leaves have the potential to halt the growth of cancer cells, and pathogens or even kill them[31]. In literature, alkaloids are found mostly in fungi and are known for their strong antimicrobial properties, which make them valuable in traditional medicine[32,33]. However, in the present study, *S. rosmarinus* species have been shown to possess alkaloids. Most alkaloids have a bitter taste and are used to protect against antimalarial, antiasthma, anticancer, antiarrhythmic, analgesic, and antibacterial[33] also some alkaloids containing nitrogen such as vincristine, are used to treat cancer.

Steroids occur naturally in the human body. They are hormones that help regulate our body's reaction to infection or injury, the speed of metabolism, and more. On the other hand, steroids are reported to have various biological activities such as chronic obstructive pulmonary disease (COPD), multiple sclerosis, and imitate male sex hormones[34]. It is a natural steroid compound occurring both in plants and animals[35]. Thus, were found in the present study. Terpenoids are derived from mevalonic acid (MVA) which is composed of a plurality of isoprene (C_5) structural units. Terpenoids, like mono-terpenes and sesquiterpenes, are widely found in nature and more than 50,000 have been found in plants that reduce tumors and cancers. Many volatile terpenoids, such as menthol and perillyl alcohol, are used as raw materials for spices, flavorings, and cosmetics[36]. In the present study, high levels of these compounds were found in *Salvia rosmarinus* leaves.

Flavonoids are a class of phenolic compounds commonly found in fruits and vegetables and are considered excellent antioxidants[37]. Similarly, the results of this study revealed that *S. rosmarinus* contain flavonoids. According to the literature, these flavonoids, terpenoids, and steroid activities include anti-diabetic, anti-inflammatory, anti-cancer, anti-bacterial, hepatic-protective, and antioxidant effects[36]. Tannins are commonly found in most terrestrial plants[38] and have the potential to treat cancer, and HIV/AIDS as well as to treat inflamed or ulcerated tissues. Similarly, in the present study, tannins were highly found in the presented plant. On the other hand, 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[30] and add to these cancer is a complex multi-gene disease[39] as in various cervical cancer repressor genes[11] that by proteins turn off or reduce gene expression from the affected gene to cause cervical cancer by regulating transcription and expression through promoter hypermethylation (DNMT1), leading to precursor lesions during cervical development and malignant transformation.

In a previous study[40], a good antibacterial result was recorded at a median concentration (65 μg·mL^{-1}). Methanol extract showed a maximum and minimum zone antibacterial result against negative bacteria *E. coli* 14 + 0.71 and most of the petroleum ether tests show null zone of inhibition. However, in the present study at a concentration of 100 μg·mL^{-1}, the methanol extract demonstrated both maximum and minimum antibacterial zones against *E. coli* 11.47 ± 0.50. Conversely, the test conducted with petroleum ether exhibited a good zone of inhibition by increasing concentration. Further research may be necessary to determine the optimal concentration for this extract to maximize its efficacy. The results obtained in gram-negative bacteria such as *E. coli*, *P. aeruginosa*, and *K. pneumoniae* are consistent with previous research findings[41]. However, in the present study, *Salvia rosmarinus* has been

found to possess high zones of inhibition with diameters of 21.37 ± 0.78 and 17.50 ± 0.50 mm antimicrobial properties against *S. aureus*, and *S.epidermidis* of gram-positive bacteria, respectively (Table 4 & Fig. 2, Supplementary Fig. S3). According to a previous study[42], the ethanolic leaf extract of *Salvia rosmarinus* did exhibit activity against *C. albicans* strains. In the present study, the antifungal activity of petroleum ether extracts from *Salvia rosmarinus* were evaluated against two human pathogenic fungi, namely *C. albicans* and *A. niger*. The findings showed that at a concentration of 100 μg·mL^{-1}, the extracts were able to inhibit the growth of *C. albicans* 20.83 ± 0.76 resulting in a minimum zone of inhibition.

Antimicrobial agents can be divided into groups based on the mechanism of antimicrobial activity. The main groups are: agents that inhibit cell wall synthesis, depolarize the cell membrane, inhibit protein synthesis, inhibit nucleic acid synthesis, and inhibit metabolic pathways in bacteria. On the other hand, antimicrobial resistance mechanisms fall into four main categories: limiting the uptake of a drug; modifying a drug target; inactivating a drug; and active drug efflux. Because of differences in structure, etc., there is a variation in the types of mechanisms used by gram-negative bacteria vs gram-positive bacteria. Gram-negative bacteria make use of all four main mechanisms, whereas gram-positive bacteria less commonly use limiting the uptake of a drug[43]. The present findings showed similar activity in chloroform/methanol (1:1) and methanol extracts of leaves of *Salvia rosmarinus* than gram-negative bacteria like *P. aeruginosa* and *Klebsiella pneumoniae*. However, *Staphylococcus epidermidis* of gram-positive bacteria under chloroform/methanol (1:1) extracts have similarly shown antimicrobial résistance. This occurred due to intrinsic resistance that may make use of limiting uptake, drug inactivation, and drug efflux that need further study. The structure of the cell wall thickness and thinners of gram-negative and gram-positive bacteria cells, respectively when exposed to an antimicrobial agent, there happen two main scenarios may occur regarding resistance and persistence. In the first scenario, resistant cells survive after non-resistant ones are killed. When these resistant cells regrow, the culture consists entirely of resistant bacteria. In the second scenario, dormant persistent cells survive. While the non-persistent cells are killed, the persistent cells remain. When regrown, any active cells from this group will still be susceptible to the antimicrobial agent.

Ferreira et al.[44] explained that with molecular docking, the interaction energy of small molecular weight compounds with macromolecules such as target protein (enzymes), and hydrophobic interactions and hydrogen bonds at the atomic level can be calculated as energy. Several studies have been conducted showing natural products such as epigallocatechin-3-gallate-3-gallate (EGCG), curcumin, and genistein can be used as an inhibitor of DNMT1[45–47] . In the literature micromenic (**1**) is used for antimicrobial activities and for antibiotic-resistance like methicillin-resistant *Staphylococcus aureus* (MRSA)[48], and benzocaine (**2**) is used to relieve pain and itching caused by conditions such as sunburn or other minor burns, insect bites or stings, poison ivy, poison oak, poison sumac, minor cuts, or scratches[49]. However, in the present study, *Salvia rosmarinus* was used as a source of secondary metabolites (ligands) by using chloroform/methanol (1:1) extract of the plant leaves yielded to isolate micromeric (**1**) and benzocaine (**2**) in design structure as a candidate for drugs as inhibitors of the DNMT1 enzyme by inhibiting the activity of DNMT1 that prevent the formation of cervical cancer cells.

Cervical cancer is one of the most dangerous and deadly cancers in women caused by *Human papillomaviruses* (HPV). Some sexually transmitted HPVs (type 6 owner of E6) may cause genital warts. There are several options for the treatment of early-stage cervical cancer such as surgery, nonspecific chemotherapy, radiation therapy, laser therapy, hormonal therapy, targeted therapy, and immunotherapy, but there is no effective cure for an ongoing HPV infection. In the present study, *Salvia rosmarinus* leaves extracted and isolated compounds **1** and **2** are one of the therapeutic drugs design structure as a candidate drug for inhibiting HPV type 16 E6 enzyme. Similarly, numerous researchers have conducted studies on the impact of plant metabolites on the treatment of cervical cancer. Their research has demonstrated that several compounds such as jaceosidin, resveratrol, berberin, gingerol, and silymarin may be active in treating the growth of cells[47].

Small-molecule drugs are still most commonly used in the treatment of cancer[50]. Molecular docking in *in silico* looks for novel small-molecule (ligands) interacting with genes or DNA or protein structure agents which are still in demand, newly designed compounds are required to have a specific even multi-targeted mechanism of action to anticancer and good selectivity over normal cells. In addition to these, in the literature, anti-cancer drugs are not easily classified into different groups[51]. Thus, drugs have been grouped according to their chemical structure, presumed mechanism of action, and cytotoxic activity related to cell cycle arrest, transcription regulation, modulating autophagy, inhibition of signaling pathways, suppression of metabolic enzymes, and membrane disruption[52]. Another problem for grouping anticancers often encountered is the resistance that may emerge after a brief period of a positive reaction to the therapy or may even occur in drug-naïve patients[50]. In recent years, many studies have investigated the molecular mechanism of compounds affecting cancer cells and results suggest that compounds exert their anticancer effects by providing free electron charge inhibiting some of the signaling pathways that are effective in the progression of cancer cells[53] and numerous studies have shown that plant-based compounds such as phenolic acids and sesquiterpene act as anticancer agents by affecting a wide range of molecular mechanisms related to cancer[53]. The present investigations may similarly support molecular mechanisms provided for the suppression of metabolic enzymes of cervical cancer.

Conclusions

The main aim of the study was to evaluate the antimicrobial activity of different extracts of *Salvia rosmarinus in vitro*, and its compounds related to *in silico* targeting of enzymes involved in cervical cancer. The phytochemical screening tests indicated the presence of phytochemicals such as alkaloids, terpenoids, flavonoids, and tannins in its extracts. The plant also exhibited high antimicrobial activity, with varying efficacy in inhibiting pathogens in a dose-dependent manner (50–100 μg·mL^{-1}). However, this extract exhibited a comparatively high inhibition zone in gram-positive and gram-negative bacteria had lower inhibition zones against *E. coli, P. aeruginosa,* and *K. pneumoniae,* respectively, and stronger antifungal activity 20.83 ±

0.76 mm inhibition zone against *C. albicans* fungi. Molecular docking is a promising approach to developing effective drugs through a structure-based drug design process. Based on the docking results, the *in silico* study predicts the best interaction between the ligand molecule and the protein target DNMT1 and HPV type 16 E6. Compound **1** (–8.3 kcal·mol^{-1}) and **2** (–5.3 kcal·mol^{-1}) interacted with DNMT1 (PDB ID: 4WXX) and the same compound **1** (–10.1 kcal·mol^{-1}) and **2** (–6.5 kcal·mol^{-1}) interacted with HPV type 16 E6 (PDB ID: 4XR8). Compounds **1** and **2** may have potential as a medicine for treating agents of cancer by inhibiting enzymes DNMT1 and HPV type 16 E6 sites, as well as for antimicrobial activities. None of the compounds exhibited acute toxicity in ADMET prediction analysis, indicating their potential as drug candidates. Further studies are required using the *in silico* approach to generate a potential drug through a structure-based drug-designing approach.

Author contributions

The authors confirm contribution to the paper as follows: all authors designed and comprehended the research work; plant materials collection, experiments performing, data evaluation and manuscript draft: Dejene M; research supervision and manuscript revision: Dekebo A, Jemal K; NMR results generation: Tufa LT; NMR data analysis: Dekebo A, Tegegn G; molecular docking analysis: Aliye M. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article.

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Conflict of interest

The authors declare that they have no conflict of interest.

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