Antimicrobial, Antioxidant, Cytotoxic, DNA Protective Activities, and Molecular Docking Studies of the Methanolic Extract of *Salvia siirtica* Kahraman, Celep & Dogan sp. Nov. (*Lamiaceae*)

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Abstract

Background: Medicinal plants have long been utilized in traditional medicine in Turkey and worldwide. The flora of Turkey is so rich it needs to be discovered, documented, and explored. This plant species is the origin of many valuable medicinal chemicals. Salvia species was used in folk medicine for many years in Turkey. One of the new Salvia species is Salvia siirtica.

Methods: The antimicrobial, cytotoxic, antioxidant, DNA restriction inhibition, and DNA protective activities of methanolic extract of S. siirtica were studied.

Results: The extract has better activity than the standard antibiotics against P. aeruginosa, meanwhile, showed high DPPH scavenging activity with an IC50 value of 28.72 ± 8.02 µg/ml. The extract exhibited good cytotoxic potential with IC50 value 0.31 ± 0.07 mg/ml towards H1299 cell line. The restriction enzyme inhibition in presence of plant extract proves that extract can interact with restriction endonuclease enzymes. Apigenin and Luteolin 7- glycoside are two high components of S. siirtica were docked against potential intracellular targets to evaluate their inhibition capability. Luteolin its derivative luteolin-7-glycoside showed the highest potential in molecular docking studies. Conclusions: Determining the bioactivity of these components by isolating them from the plant may pave the way for their use as supportive agents in the fight against many diseases.

Keywords: Antioxidant, Cytotoxicity, DNA-interaction, Docking, Salvia siirtica

Significance | Bioactivity of new Salvia species to fight diseases.

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1. Introduction

Medicinal plants have been used in medicine for the treatment of many diseases since ancient times. Studies are ongoing all over the world to test the effectiveness of these plants. The cost of herbal medicines in the world market exceeds approximately 100 billion dollars per year (Sofowora et al., 2013). Turkey has the richest flora of any country in the temperate zone, with 10,000 species of vascular plants and ferns (Davis, 2019). New plant species are still being discovered in Turkey at a rate of more than one a week. This excellent source of plant species is the origin of many valuable medicinal chemicals (Ozkan et al., 2016). Medicinal plants have been used against various diseases for thousands of years, and it is known that many drugs used in modern medicine today are of plant origin (Süntar, 2020). The

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Salvia genus is economically important and has been used since ancient times in folk medicine (Uysal et al., 2023).

Salvia siirtica Kahraman, Celep & Doğan sp. nov. (Lamiaceae) is described and illustrated as a new species from the northern part of Siirt province in southeast Anatolia, Turkey, where it was found growing in open forests of Quercus (Kahraman et al., 2011). *S. siirtica* species belongs to the genus *Salvia*. It is one of the largest and the most important genus of the *Lamiaceae* family and comprises about one thousand species (Yumrutas et al., 2023). In addition to being broadly consumed for medicinal purposes, essential oils of some *Salvia* species are also used in the food flavoring industry (Cui et al., 2015). In addition, members of the genus *Salvia* have wide use in phytopreparations and dietetic products all over the world. The most popular of them, sage (*Salvia officinalis* L.) has extensive applications in the production of tea mixtures (Alaşalvar and Çam, 2019), prevention of UV radiation (Khare et al., 2021), and treatment of Alzheimer's (Uţă et al., 2021) and HIV diseases (Geuenich et al., 2008). *Salvia* species are used in medicine for the treatment of many common illnesses, like using it as an herbal tea for stomach aches, colds, and pain in the throat (Aşkun et al., 2010). They possess several biological activities, including antiseptic, antibacterial, antibiofilm, antioxidant, anti-inflammatory, antiviral, antitumoral cytotoxic, spasmolytic, anticonvulsant, antimycobacterial, and carminative activity (Küçük et al., 2019). Recently, *S. siirtica*'s some biological properties (antioxidant, cytotoxic, anticholinesterase, antiurease, antityrosinase, antielastase, and anticollagenase activities) have been identified by Fidan et al. (2021). However, phytochemical contents in any plant (within the species) vary from place to place. The phytochemical content is affected by several factors such as latitude, temperature, quality of soil, light intensity (Pant et al., 2021). Therefore, this research aimed to evaluate the antimicrobial, antioxidant, cytotoxic, and DNA protective activities of *S. siirtica* aerial extracts collected from a different place than Fidan et al.'s species. Also, Fidan et al. investigated the cytotoxic effects of ethanol, petroleum ether and chloroform extracts of *S. siirtica* on HT-29 (colon cancer) and MCF-7 (breast cancer) cell lines. They found that all samples with the exception of the one sample, had no cytotoxic effect on the colon cancer cell line HT-29, and the extracts showed a moderate cytotoxic effect on the breast cancer cell line MCF-7. In this study, the cytotoxic effect of *S. siirtica* methanol extract on another colon cancer cell line, Caco-2 and non-small cell lung cancer cell line H1299, was investigated.

The three most common compounds determined by Fidan et al. were apigenin, luteolin 7-glucoside, and rosmarinic acid. In this study, Fidan et al.'s compounds selected for molecular docking were apigenin, luteolin 7-glucoside, and rosmarinic acid.

Antimicrobial activity, cytotoxicity, antioxidant activity, DNA restriction endonuclease inhibition, and docking activity of two main constituents are studied to reveal the medicinal properties of S. *siirtica* for the first time. Determining the bioactivity of these components by isolating them from the plant may pave the way for their use as supportive agents in the fight against many diseases.

2. Materials and experimental methods

2.1. Preparation of plant crude extracts

The plant material was collected from Siirt province southeastern part of Turkey by Dr. Osman Karabacak. The aerial parts of the plant were dried at 35°C for three days. After drying, it was ground with a homogenizer, and dry sample was mixed with methanol at a ratio of 1:10 (wt:vol) and left to macerate for seven days. After maceration the plant material was filtered, and then the solvent was evaporated to complete dryness using a standard rotary evaporator (Abdelbaky, 2021).

2.2. Antimicrobial activity assay

Staphylococcus aureus ATCC 25923, *Bacillus subtilis* ATCC, *Enterococcus hirae* ATCC 9790, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Proteus vulgaris* RSKK 96029, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231, and *Candida krusei* ATCC 6258 were used for the current study. Minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and minimal fungicidal concentration (MFC) values of the plant extract was determined by the broth micro-dilution assay using sterile 96-well plates according to Clinical & Laboratory Standards Institute (CLSI) reference methods for bacteria M7-A7 (CLSI, 2018) and yeasts M27-A3 (CLSI, 2008). Standard antimicrobial agents chloramphenicol, ampicillin, and ketoconazole were used as positive controls for bacteria and fungi, respectively.

2.3. Determination of total phenolic content (TPC)

The total phenolic content of the extract was determined according to the method of Folin-Ciocalteu (Ahmed et al., 2019). Gallic acid was used as an external standard for preparing the calibration curve. The total phenolic content was expressed as mg gallic acid equivalents (GE) per g of extract.

2.4. Determination of β carotene and lycopene content

β-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was mixed with 10 ml of acetone–hexane mixture (4:6) and filtered through Whatman filter paper.

The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 ml) = −0.0458 A_{663} + 0.372 A_{505} − 0.0806 A_{453}; β-carotene (mg/100 ml) = 0.216 A_{663} − 0.304 A_{505} + 0.452 A_{453}. The results were expressed as mg of carotenoid/g of extract.

2.5. Cytotoxicity assays

The cytotoxic effect of *S. siirtica* methanol extract in mouse L929 fibroblast, H1299 non-small-cell lung carcinoma, and Caco-2 colorectal adenocarcinoma cell lines were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay where cisplatin was used as a reference of the International Organization for Standardization (ISO 10993-5, 2009). Cell lines provided by Kırıkkale University Scientific Research Application and Research Center. Briefly, the cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS), then incubated at 37°C and 5% carbon dioxide (CO_2) for 24 hours. Incubated cells were treated with the plant extract at six different concentrations (5-0.16 mg/mL) for 24 hours. Following washing and incubation with MTT solution for 2 h, isopropanol was added to the wells. The absorbance was measured using a microplate reader (Wallac 1420 Multilabel counter, PerkinElmer) at a wavelength of 570 nm. The percentage of viable cells was calculated according to the formula below:

Cell viability (%) = (Plant extract OD/Control OD) x100

OD: Optical Density at 570 nm

2.6. Determination of scavenging activity with DPPH

The ability of *S. siirtica* methanol extract to scavenge stable 1,1-diphenyl-2-picrylhydrazil (DPPH) free radicals was determined by the modified Braca method (Braca et al., 2001). 1 mL of plant extract was mixed with 1 mL of DPPH with methanol (0.04 mg/mL) then the solution was incubated in a dark place at room temperature. After 30 min, the absorbance of the sample was measured at 517 nm. Methanol and the standard butylated hydroxytoluene (BHT) were used as negative controls. The calculation of the inhibitory activity of DPPH radicals was performed with $[(A_0-A_1)/A_0] \times 100$ equation, which A_0 is the absorbance of the negative control and A_1 is the absorbance of the extracts.

2.7. Plant extract interactions with plasmid DNA

Interaction between the methanol extract of *S. siirtica* and pBR322 plasmid DNA was carried out using gel electrophoresis following a method described by Deqnah et al. (2012).

2.8. Restriction enzyme *Bam*HI *and Hind*III digestion

*Bam*HI and *Hind*III are restriction endonuclease that binds at the recognition sequences 5'-G'GATCC-3', and 5'-A'AGCTT-3' and cut these sequences just after the 5'-G and 5'-A on each strand, respectively (Obalı et al., 2020). The plasmid DNA contains a single restriction site for both enzymes that convert the supercoiled form I and singly nicked circular form II to linear form III DNA. The compounds and the DNA were incubated for 24 and 48 h in an incubator bath at 37˚C and then subjected to restriction enzyme digestion. The mixtures were left in an incubator at 37˚C for another 1 h and then electrophoresed in 1% agarose gel for 1 h. The gel was photographed using Biometra, Gel Imaging Systems after stained with ethidium bromide.

2.9. DNA damage protective activity

DNA damage was induced by hydroxyl radicals. DNA damage protective activity of *S. siirtica* extract was performed using pBR322 plasmid DNA (Lee et al., 2002). A mixture of the plant extract (100 µg/mL) and plasmid DNA was incubated for 10 min at room temperature, followed by the addition of Fenton's reagent and incubated for 30 min at 37°C. The DNA was analyzed on 1% agarose gel using ethidium bromide staining and photographed in Bio Doc. analysis.

2.10. Molecular docking

Structure files of the ligand compounds were availed from PubChem Database. Crystal structures of the receptor proteins and DNA were obtained from Protein Data Bank (PDB IDs: 1BAM, 2E52, 1BHM, 4DUH, 3SRW, 2EX6, 1K01, 2JJP). Ligand and Receptor structures were prepared and structure files in .pdbqt file format were generated using AUTODOCK TOOLS 1.5.6 software (Morris et al., 2009). To meet the requirements of the .pdbqt file format, water and other non-bound molecules that was not a part of the active site of the enzymes were removed from the structures, any missing atoms in the structure were restored, polar hydrogens and Kollman charges were added. Ligand molecules were docked to active sites of the receptor molecules using AUTODOCK VINA software (Trott and Olson, 2010). Docking results with the lowest binding energies, in which docked ligands interacted with the active site of the receptor were selected. 3D and 2D pose views were generated using Discovery Studio Visualizer (Biovia, 2021) for further analysis of docking results. Root Mean Square Deviation (RMSD) between crystal structures and docked structures of Drug controls were calculated using the online tool DOCKRMSD (Bell and Zhang, 2019).

2.11. Statistical analysis

All experimental analyzes were run in triplicate. Antioxidant, and cytotoxic activity results are shown by calculating mean and standard error values.

Table 1. Minimal inhibitory concentration (MIC) of methanol extract of *S. siirtica* (mg/mL).

Microorganisms		Methanol Extract	Positive Control		
			Amp*	C**	Keto***
Escherichia coli ATCC 35218	MIC	125	>125	>125	NS
	MBC	>500	>125	>125	NS
B. subtilis ATCC 6633	MIC	250	62.5	3.91	NS
	MBC	250	62.5	3.91	NS
Staphylococcus aureus ATCC 25923	MIC	250	62.5	125	NS
	MBC	500	62.5	>125	NS
Enterococcus faecalis ATCC 29212	MIC	>500	31.25	62.5	NS
	MBC	>500	125	>125	NS
Pseudomonas aeruginosa ATCC 27853	MIC	62.5	>125	>125	NS
	MBC	62.5	>125	>125	NS
Klebsiella pneumoniae ATCC 13883	MIC	125	125	15.63	NS
	MBC	250	125	15.63	NS
Salmonella typhimurium ATCC 14028	MIC	250	62.5	125	NS
	MBC	500	62.5	125	NS
Proteus vulgaris RSKK 96029	MIC	125	>125	125	NS
	MBC	500	>125	>125	NS
Enterococcus hirae ATCC 9790	MIC	125	62.5	62.5	NS
	MBC	500	62.5	>125	NS
Candida albicans ATCC 10231	MIC	125	NS	NS	31.25
	MFC	250	NS	NS	62.5
Candida krusei ATCC 6258	MIC	62.5	NS	NS	<0.98
	MFC	125	NS	NS	15.63

*Ampicillin, **Chloramphenicol, ***Ketoconazole

Table 2. Lycopen, β carotene, total phenol contents and IC50 value of DPPH scavenging effect of *S. siirtica* methanol extract.

Parameter	**Result**
Lycopene content (mg/100 mL)	0.688 ± 0.001
β carotene content (mg/100 mL)	1.079 ± 0.004
Total phenolic content (mg GAE/g extract)	102.83 ± 1.50
DPPH scavenging activity of the extract (IC50) (µg/mL)	28.72 ± 8.02
DPPH scavenging activity of the BHT (IC50) (µg/mL)	96.47 ± 0.32

Values are mean ± SD of 3 replicates.

Table 3. IC_{50} Values of *S. siirtica* for L929 mouse fibroblast, H1299 non-small-cell lung carcinoma and Caco-2 colorectal adenocarcinoma cells.

	IC_{50} (mg/mL)		
	L929	**H1299**	**Caco-2**
Extract	0.25 ± 0.18	0.31 ± 0.07	5.67 ± 0.34
Cisplatin	0.00717 ± 0.00175	0.006022 ± 0.0012	0.0584 ± 0.0028

Table 4. Root Mean Square Deviation (RMSD) between crystal structures and docked structures of Drug Controls

Drug Control	**RMSD between crystal structure and docked structure**
Ampicillin	4.665 Å
Chloramphenicol	2.298 Å
Ketoconazole	4.547 Å

Table 5. Binding Energies Table for Molecule-DNA and Molecule-Restriction Enzyme Interactions.

	Binding Energies (kcal/mol)			
Molecules	***Bam*HI** (PDB ID: 1BAM)	***Hind*III** (PDB ID: 2E52)	***Bam*HI recognition site DNA** (PDB ID: 1BHM)	***Hind*III Recognition site DNA** (PDB ID: 2E52)
Apigenin	-6.0	-6.8	-7.6	-7.4
Luteolin 7-glucoside	**-7.0**	**-7.8**	**-9.1**	**-8.7**
Luteolin	-6.3	-7.0	-8.3	-7.9
Rosmarinic Acid	-5.8	-6.4	-7.6	-7.4
Paclitaxel (Control)	-	-	-6.8	-6.9
Luteolin 7-glucoside	-8.9	**-10.0**	**-9.0**	**-10.5**
Luteolin	**-9.0**	-8.9	-8.1	-9.1
Rosmarinic Acid	-8.9	-8.8	-7.9	-9.1
Ampicillin (control)	-	-	-7.2	-
Chloramphenicol (control)	-	-	-	-6.8
Ketoconazole (control)	-	-	-	-

3. Results and Discussion

3.1. Antimicrobial activity assay

The plant extract was evaluated for its antimicrobial activity against Gram-positive (4 strains) and Gram-negative bacteria (5 strains) and fungi (2 strains) by assay for minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC). Table 1 shows the antimicrobial activity of *S. siirtica* methanol extract against Gram-positive and Gram-negative bacteria and fungi. The MICs and MBCs of antibiotics for microorganisms were between <0.98 to >125 mg/mL. The MIC concentrations of the methanol extract of the plant ranged from >500 to 62.5 mg/mL. The very high MIC values in many bacterial species indicate only a very limited antibacterial efficacy. However, a better activity at the extract was determined with a MIC value of 62.5 mg/mL against the pathogenic strain of *P. aeruginosa* (gram negative) than the standard antibiotics (MIC values of Ampicillin and Chloramphenicol are >125mg/mL). Although there is no report on the antimicrobial activity of *S. siirtica* in literature, other *Salvia* species have mild to strong antimicrobial activity against bacterial strains. For instance, Cui et al. (2015) stated that *Salvia sclarea* has strong inhibitory activity against a gram-negative bacterium *E. coli*. Similar to this, methanol extract of *S. siirtica* has high antimicrobial activity against the pathogenic gram-negative strain of *P. aeruginosa*. Although *P. aeruginosa* was the microorganism in which *Salvia siirtica* methanol extract showed the highest antimicrobial effect, Toplan et al. determined that extracts of *Salvia hydrangea* species did not show any antimicrobial activity against the same bacterial strain (Toplan et al., 2022).

3.2. Antioxidant activity

The antioxidant assays (DPPH scavenging activity, lycopene content, β carotene content, and total phenolic content) measure the relative antioxidant ability to scavenge the free radicals produced in the reagents. The DPPH radical scavenging activity result is shown in Figure 1 and Table 2 as compared with known antioxidant BHT. Table 2 shows antioxidant activity with IC_{50} values of *S. siirtica*, measured by DPPH radical-scavenging assays. *S. siirtica* revealed excellent antioxidant properties (IC_{50} value=28.72 ± 8.02 µg/ml) and control BHT revealed very poor antioxidant activity (IC50 values= 96.47 ± 0.32 µg/ml). *S. siirtica* extract showed a higher potency than BHT in the scavenging of DPPH free radicals. Antioxidant tests were strongly correlated with results of Fidan et al. (2021) which had very high antioxidant activity. The high antioxidant property of *S. siirtica* extract in both studies may be related to the high amount of flavonoid and phenolic compounds in this plant extract. Yilmaz et al. investigated the DPPH radical scavenging effect of *S. viridis*, *S. wiedemannii*, *S. aytachii*, *S. heldreichiana*, *S. aucheri* subsp. *canescens* species in the genus Salvia. They found that *S. viridis* and *S. wiedemannii* species had no DPPH radical scavenging effect, while the IC50 values of other species ranged between 168.4 and 1259.08 µg/mL (Yilmaz et al., 2023). Bardakci et al. (2019) investigated the DPPH radical scavenging effect of *Salvia heldreichiana* methanol extract and determined that the IC50 value was 606.56 ± 1.74 µg/mL. These values show that *S. siirtica* has a stronger free radical scavenging effect than the species in these studies.

The total phenolic content of the methanol extract from the *S. siirtica* was 102.83 ± 1.50 mg gallic acid equivalent (GAE) /g extract. Toplan et al. (2022) determined that *Salvia hydrangea* methanol extract contains 122 ± 1.1 mg gallic acid equivalent (GAE)/g extract total phenolic substance. The lycopene and β carotene content were 0.688 ± 0.001 and 1.079 ± 0.004 mg/100 mL, respectively. The lycopene and β carotene content were higher than that of Fidan et al. (2021). This variation in the lycopene and b-carotene contents of the same species could be caused by several factors such as the plant variety, genotype, season, geographic location/climate, stage of maturity and growing conditions of climate or harvesting season, harvesting stage, stage of maturity, growing location or geographic site and even by the fertilizer used (Maiani et al., 2009).

3.3. Cytotoxicity assay

The study was conducted to evaluate the cytotoxic activities of the methanol extract of *S. siirtica* at various concentrations on L929 fibroblast, H1299 non-small-cell lung carcinoma, and Caco-2 colorectal adenocarcinoma cell lines by MTT assay using reference drug cisplatin (Figure 2 a-c). The IC_{50} value is the drug concentration required for 50% cell kill. The IC_{50} values were calculated from cell viability graphs. The cytotoxicity results revealed that the extract exhibited good cytotoxic potential having an IC_{50} value of 0.31 ± 0.07 mg/mL against H1299 non-small-cell lung carcinoma cell line.

The percent viability of cells was found to reduce with an increase in the concentration of the extract. The plant extracts exhibited in-vitro cytotoxicity at 24h incubation leading to decreased viability in the treated cells compared to the untreated controls. At 0.16 mg/mL- 5mg/mL concentration of methanol extract, viability values of L929 decreased from 66.65% to 14.43%, H1299 from 77.25% to 14.89%, and Caco-2 colorectal adenocarcinoma from 105.42% to 58.94%, respectively. The results show that the methanol extract of *S. siirtica* is less active in Caco-2 cell lines than cisplatin. Moreover, the extract has similar activity with cisplatin to H1299 non-small-cell lung carcinoma cell line. Considering the efficacy of the plant extract depending on the

concentration, it was observed that it could be potentially evaluated because it showed similar activity to cisplatin on H1299 cells but had fewer toxic effects on L929 cells than cisplatin. In conclusion, the extract had shown comparable cytotoxicity to cisplatin against H1299 non-small-cell lung carcinoma cell line. Some of the *Salvia* species studied were subjected to cytotoxic bioassays by different authors. One of them is Firuzi et al. (2013) found that IC_{50} values of *Salvia eremophil* and *Salvia santolinifolia* were 10.5-75.2 µg/mL against the HL60, K562, and MCF-7 cell lines. There are some more studies where the IC_{50} values are bigger than that of the current study. Fidan et al. (2021) showed that *S. siirtica* ethanol extract showed high cytotoxic activity against the breast cancer cell line; however, it had no cytotoxic effect on the cancerous colon cell line (HT-29). In this study, the extract had very little effect on Caco-2 cell line. The reason for that, is the plant content in members of the Labiatae family even the same plant species grow in different places is affected by light ecosystems and seasons.

3.4. The interaction of plasmid DNA with methanol extract of *S. siirtica*

When pBR322 plasmid DNA interacted with decreasing concentrations of the methanol extract of *S. siirtica*, generally two DNA bands corresponding to Form I and II were observed in treated plasmid DNA (Figure 3). As the concentration of the compounds was increased, the mobility of both Form I and Form II bands decreased slightly at 24 h incubation at the highest concentration (Figure 3a). In the case of 48 h incubation, a faint linear Form III band could be seen at two high concentrations of the extract (Figure 3b). The appearance of a linear band in addition to the presence of two Form I and Form II bands at higher concentrations indicates the change in the conformation of the Form I DNA from being negatively supercoiled Form I through to linear Form III. A single but faint band smaller than the Form I band was observed at the highest concentrations of the extract.

3.5. *Bam*H1 and *Hind*III digestion

To learn more about changes in DNA conformation and extract binding to DNA extract-DNA incubation was followed by *Bam*H1 (Figure 4a) and *Hind*III digestion (Figure 4b). When untreated plasmid DNA was restricted by one of these enzymes only one band corresponding to form III was observed. Whereas plasmid DNA interacted with the methanol extract of *S. siirtica* followed by restriction enzyme digestion, two bands corresponding to Form I and Form II were observed. None of the restriction enzymes tested did cut the DNA indicating that extract is binding to the recognition site of enzymes on DNA to A/A or G/G nucleotides or the restriction enzymes *Bam*H1 and *Hind*III.

3.6. DNA damage protective activity

DNA damage was induced by hydroxyl radicals. DNA damage protective activity of *S. siirtica* extract was performed using pBR322 plasmid DNA. Form I DNA is converted to mostly single strand nicked form II DNA and linear DNA induced by hydroxyl radical was converted to mostly linear DNA by the extract (Figure 5).

3.7. Molecular docking

To further investigate the mode of action of the antibacterial and antifungal activities of the extract, molecular docking studies were performed where the three most abundant compounds (Apigenin, Luteolin 7-glucoside, Rosmarinic Acid) and Drug controls (Ampicillin, Chloramphenicol, Ketoconazole) were docked against several bacterial and fungal targets including *E. coli* DNA Gyrase B (PDB ID: 4DUH) (Brvar et al., 2012), *S. aureus* Dihydrofolate Reductase (PDB ID: 3SRW) (Li et al., 2011), *E.coli* Penicillin Binding Protein 4 (dacB) which included Ampicillin molecule in the crystal structure (PDB ID: 2EX6) (Kishida et al., 2006), *D. radiodurans* 23S rRNA which included Chloramphenicol in the crystal structure (PDB ID: 1K01) (Schlünzen et al., 2001). *S. erythraea* Cytochrome P450 EryK which included Ketoconazole in the crystal structure (PDB ID: 2JJP) (Montemiglio et al., 2010) (Figure 6). These drugs were used as controls and the RMSD between the best-docked result and crystal structure was calculated to address the accuracy of the docking method, results are given in Table 4. RMSD values were low, indicating that the docking software is very capable. Also, to address the mechanism of action of restriction enzyme digestion inhibition, the same compounds were docked against *Bam*HI R.E. (PDB ID: 1BAM) (Newman et al., 1994), *Hind*III R.E. (PDB ID: 2E52) (Watanabe et al., 2009), *Bam*HI recognition site (PDB ID: 1BHM) (Newman et al., 1995), *Hind*III recognition site (PDB ID: 2E52) (Watanabe et al., 2009). Dockings were performed using AutoDock Vina Software (Trott et al., 2010), and all the compounds were selectively docked against the active sites of molecules. The best dockings resulting from that are predicted conformations in the active sites of the molecules with the lowest binding energies for each receptor-ligand are given in Tables 5 and 6. Lower binding energy means better binding between the receptor and the ligand. For further analysis of the aspects of the interaction between the ligand and receptor molecules, 3D and 2D diagrams are shown in Figure 6 for best interactions. Among the compounds tested, Luteolin 7-glucoside was found to be the most active compound, it had the lowest binding energy for the two DNA molecules, the 23S rRNA, and 5 out of 6 proteins tested. It forms hydrogen bonds with adenine, guanosine, and cytosine bases in DNA binding to the 'GATC' site in its lowest energy binding state which is a part of

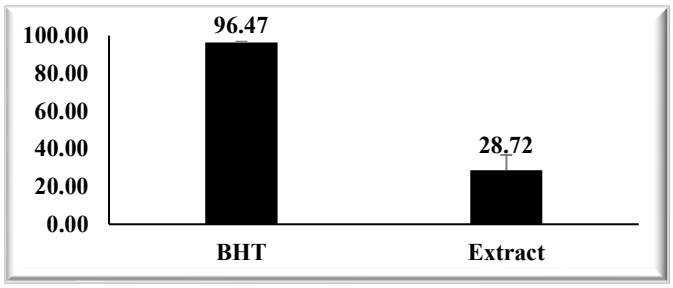

Figure 1. The DPPH radical scavenging activity of *S. siirtica* extract *and BHT.*

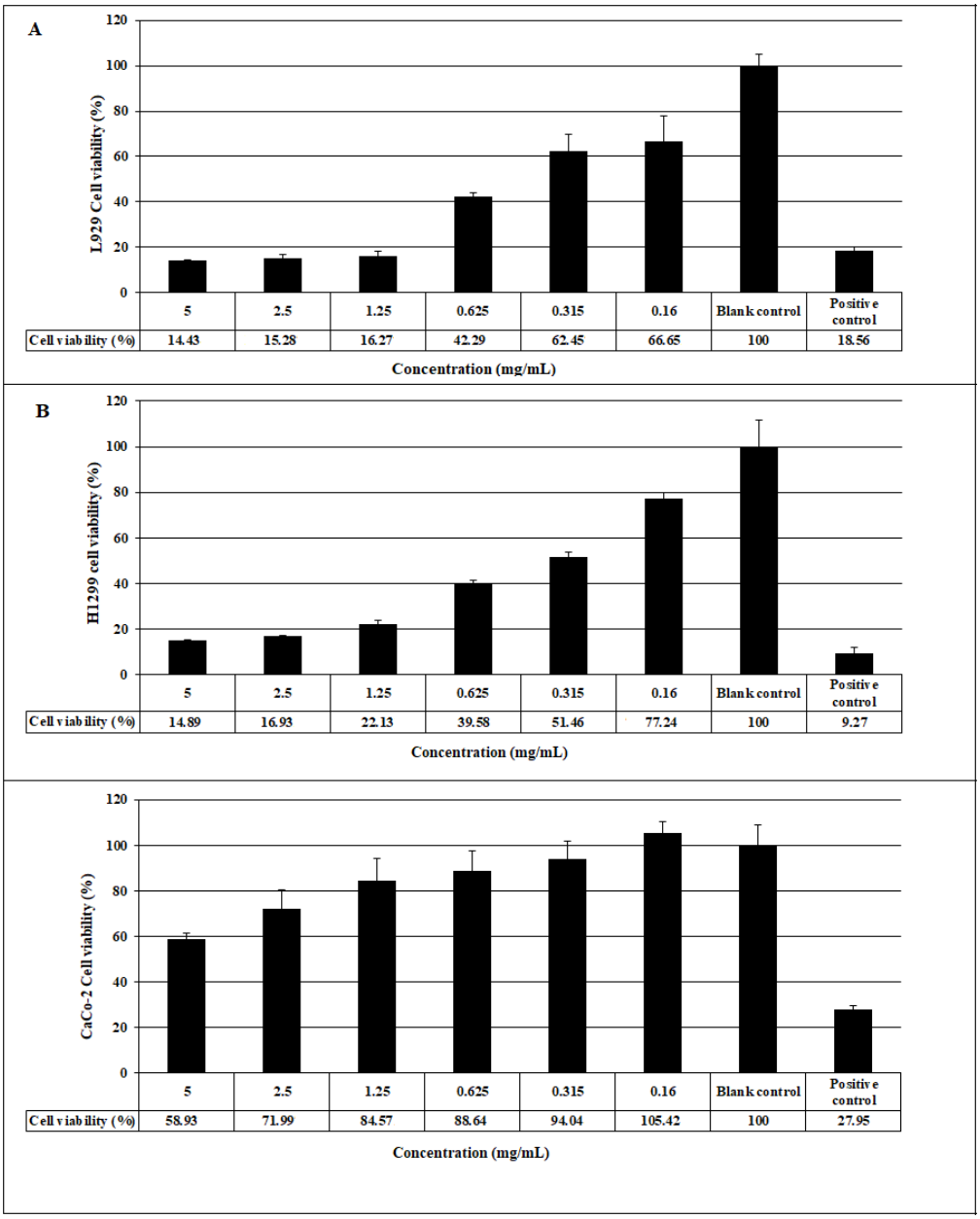

Figure 2. Cytotoxic activity (percentage of cell viability) of varying concentrations of *S. siirtica*, negative control, and positive control (cisplatin) on the L929 fibroblast cells (A), H1299 non-small-cell lung carcinoma cells (B) and Caco-2 colorectal adenocarcinoma cells (C) revealed by MTT assay.

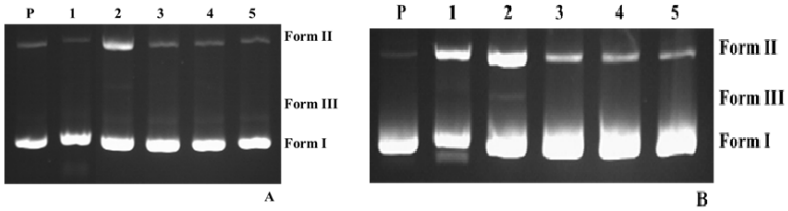

Figure 3. Electrophotograms applying to the interaction of pBR322 plasmid DNA with decreasing concentrations of the methanol extract of *S. siirtica*. Lane P applied to untreated plasmid DNA to serve as a control. Lanes 1 to 5 applied to plasmid DNA interacted with decreasing concentrations of the extract (A: 24 h; B: 48 h incubation). The extract concentrations: lane 1-5: 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625mg/mL

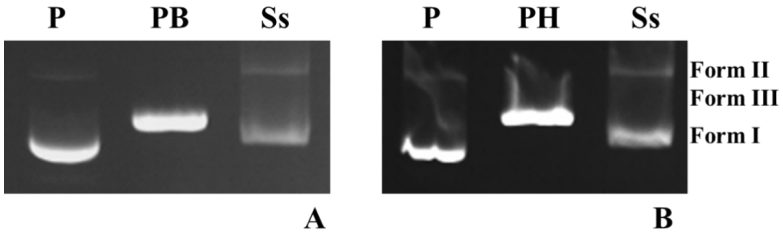

Figure 4. The restriction enzyme results indicate that *S. siirtica* methanol extract is able to bind DNA or enzyme. The plasmid DNA is doubly nicked by *Bam*H1 (4A) and *Hind*III (4B) at the specific GG site, AA site, respectively. The results indicate prevention of both enzyme digestion at the highest concentration of extract

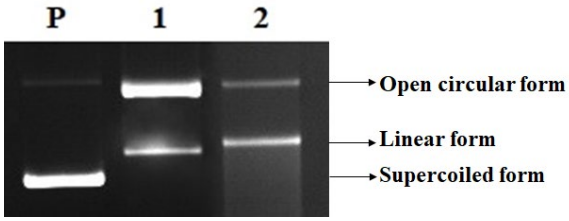

Figure 5. Effect of *S. siirtica* methanol extract on oxidative DNA nicking caused by hydroxyl radicals. Lane P: native pBR322 DNA; Lane 1: DNA + Fenton's reagent; Lane 2: DNA + Fenton's reagent + *S. siirtica* methanol extract (100 µg/mL)

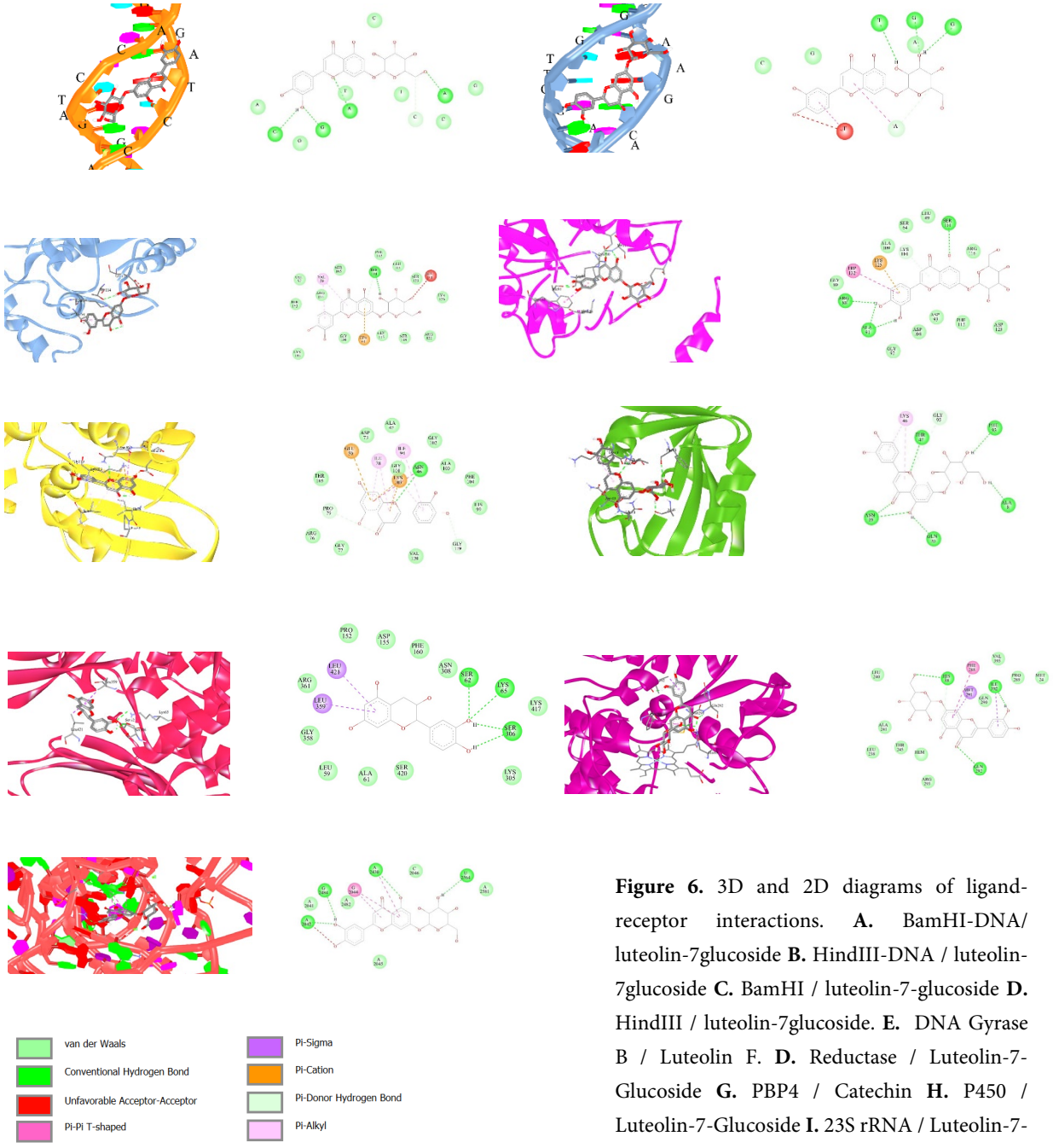

Figure 6. 3D and 2D diagrams of ligand-receptor interactions. **A.** BamHI-DNA/ luteolin-7glucoside **B.** HindIII-DNA / luteolin-7glucoside **C.** BamHI / luteolin-7-glucoside **D.** HindIII / luteolin-7glucoside. **E.** DNA Gyrase B / Luteolin F. **D.** Reductase / Luteolin-7-Glucoside **G.** PBP4 / Catechin **H.** P450 / Luteolin-7-Glucoside **I.** 23S rRNA / Luteolin-7-glucoside **J.** Legend

the *Bam*HI recognition sequence. It also binds to the 'AAGC' sequence which is a part of the *Hind*III recognition sequence. Luteolin 7-Glucoside may interfere with the Restriction endonuclease activity by binding to the recognition site in the DNA. It also can be inhibiting the activity by binding to the restriction enzymes. It can bind to the active site of *Bam*HI interacting with residues Glu 111, Phe112, and Glu 113 which forms the active site of the protein. It can also bind to the DNA binding site of the *Hind*III enzyme forming Pi-cation interaction with Lys 125, Pi-Pi stacked interaction with Trp 132, and hydrogen bond with Arg 88 which normally interacts with DNA when bound. Apigenin, Rosmarinic Acid also had binding energies close to Luteolin 7-glucoside, with a tendency to have lower binding energy for the DNA. All these molecules had lower binding energies for DNA than the control drug Paclitaxel, a molecule that has known DNA binding activity (Malonga et al., 2005). For DNA Gyrase B, Luteolin has the lowest binding energy. Luteolin showed hydrogen bond interaction with the essential residue Asn 46, Pi-cation interaction with Lys 103, and Pi-Alkyl interaction with Ile 94. For the Dihydrofolate Reductase enzyme, Luteolin 7-glucoside showed hydrogen bonding with the essential residues of Asn19, Gln20, Ala8, Phe93, and Thr47. It also interacts with Lys 46 residue with Pi-Alkyl interaction. Luteolin 7-glucoside showed its potential as an inhibitor for the 23S rRNA as well. 23S rRNA is the target of the common antibiotic chloramphenicol. RMSD value calculated between the crystal structure and the docked structure of the chloramphenicol was 2.298 Å, the lowest among drug controls in the study. Luteolin–7-Glucoside has a lower binding energy than chloramphenicol -10.5 kcal/mol compared to -6.5 kcal/mol. Luteolin 7-Glucoside forms pi-pi stacking interactions with nucleotides G 2044 and A 2430, Hydrogen bonds with nucleotides U 2564, A 2430, G 2484, A 2042. Catechin showed lower binding energy for PBP4 than the standard antibiotic Ampicillin. Though it should be noted that ampicillin normally binds to its target covalently, creating a much stronger interaction than a docking result. The closest binding energy to ketoconazole was shown by Luteolin- 7 Glucoside, which interacted with Phe 288 residue with pi-pi T-shaped interactions, with met 291 and Ile 392 residues through pi-sigma interactions, and Gln 292, Ile 392, and His 88 residues through hydrogen bonding. Hence, compounds that are dominantly found in the extract have shown their potential as antibiotic and antifungal agents. Luteolin-7-glucoside is leading; apigenin and rosmarinic acid are candidates for antibiotic and antifungal agents.

4. Conclusion

In this study, consecutive experiments were performed to deeply analyze the biological potential of a newly identified plant species, whose relatives (*Salvia* genus) have known medicinal and industrial importance. Antimicrobial activity experiments showed that the extract has a strong antimicrobial activity, especially against the pathogenic strain of *P. aeruginosa*, which showed better activity than standard antibiotics. DPPH scavenging assay also showed excellent antioxidant activity and high content of total phenolics was found for methanol extract. Further investigations of the bioactivity of the extract included cytotoxicity experiments in L929 mouse fibroblast, H1299 non-small-cell lung carcinoma, and Caco-2 colorectal adenocarcinoma cell lines. The extract showed strong cytotoxic activity comparable to positive control cisplatin for the above-mentioned cell lines, especially in the H1299 cell line. Moreover, studies exploring the extract's DNA binding and restriction enzyme digestion inhibitor activities were pursued. It was observed that extract inhibits restriction enzyme digestion by either binding to DNA or enzyme. These bioactivities raise may be from Rosmarinic acid, which is an ester of caffeic acid and 3,4-dihydroxy phenyl lactic acid, and Luteolin and its derivatives were also in high abundance. Furthermore, to evaluate the intracellular activities of these compounds, molecular docking studies were performed where the three most abundant compounds from the extract were docked to potential bacterial and fungal targets. Luteolin-7-glucoside showed the highest potential, having the lowest binding energy for most of the targets. It showed lower binding energy than positive controls paclitaxel, ampicillin, and chloramphenicol. Apigenin and rosmarinic acid also have a very high potential for being inhibitors of the studied targets. Hence, antimicrobial, antioxidant, cytotoxic, phenolic composition, and molecular docking analysis show that the plant extract has shown its potential as a lead for the future expansion of novel antimicrobial and cytotoxic drugs.

Abbreviations: ATCC, American Type Culture Collection; CLSI, Clinical & Laboratory Standards Institute; GAE, gallic acid equivalents; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ISO, International Organization for Standardization; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; OD, Optical Density; DPPH, 1,1-diphenyl-2-picrylhydrazil; BHT, butylated hydroxytoluene; PDB, from Protein Data Bank; RMSD, Root Mean Square Deviation; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; IC50, Inhibitor Concentration 50.

Author Contribution

BA, DNP, and AÇ performed the experiments. BA, LA, AÇ, and YCA prepared and analyzed the data. BA, LA, YCA, and DNP drafted

the manuscript. BA, LA, and LYG reviewed the manuscript. All authors approved the manuscript.

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Competing financial interests

The authors declare that they have no potential conflict of interest in publishing this research output.

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