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RESEARCH ARTICLE

Antioxidant and anticancer activities of *Solanum nigrum* Linn leaves

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ABSTRACT

Solanum nigrum Linn belonging to the family Solanaceae is a flowering species native to Australia, South Africa, and Asia. In the present study, the dried *S. nigrum* leaves were extracted with benzene, diethyl ether, and ethanol by Soxhlet's extraction and screened qualitatively and quantitatively to determine their total phytoconstituent content and their potential antioxidant evaluation, followed by the GC/MS analysis to predict the hit compounds. A higher amount of phenols (120.50 mg GAE/g), alkaloids (90.50±2.25 mg of AE/g), and flavonoids (108.75±2.50mg QE/g) were present in the diethyl ether extract of the Solanum nigrum leaves. In contrast, the saponins (42.50±1.50µg ginsenoside Rb1 equivalent/mg) are highly present in ethanol extract. The GCMS analysis of diethyl ether extract reported the top compounds with area %, height %, and specific retention times. The major compound in the GC/MS analysis is 2-AMINO-9-(3,4-DIHYDROXY-5-HYDROX, FARNESOL ISOMER A, alpha -D-Glucopyranoside, methyl (CAS) Me, METHYL ESTER OF 3-HYDROXY-4-MET, ISOPROPYL MYRISTATE, OCTADECANEDIOIC ACID, and HEXADECANEDIOIC ACID. Antioxidant assays such as the DPPH assay and reducing power assay revealed that the diethyl ether extract has more antioxidant activity than the standard itself. The two compounds from the GC/MS study were then docked against an oncogene BRAF V600E and a mutated tumour suppressor gene p53 Y220C mutant. The results showed that the interaction was good and had higher negative binding energies. We report that *S. nigrum* leaves have potent antioxidant and anticancer properties.

Keywords: ADME, Antioxidant, Cancer, Docking, ROS, *Solanum nigrum* Linn

INTRODUCTION

The reactive oxygen species are involved in the induction of oxidative stress in the cells, and these molecules are scavenged by natural antioxidants inside the body (Kurutas, 2016). The failure of the body's antioxidant system and the reactive oxygen species (ROS) elevation leads to various life-threatening pathogenesis, including heart diseases and cancer (Pham-Huyet al., 2008). Molecules from natural sources such as plants and marine sources are reported to have antioxidant properties against the ROS inside the body by scavenging it (Sethuraman et al., 2017; Purushothaman et al., 2020). Many plants and their phytoconstituents were reported to have strong medicinal properties and pharmacological activities at molecular target levels and resulted in successful manifestations (Nagarasan et al., 2016; Saranya et al., 2017; Purushothaman et al., 2018; Rathnasamy et al., 2018; Balakrishnan et al., 2018; Ashokkumar et al., 2020; Ashokkumar et al., 2021a & 2021b).

Solanum nigrum Linn belonging to the family Solanaceae is a flowering species highly native to Australia, South Africa, and Asia. *Solanum nigrum*Linn possesses medicinal properties and is used as a traditional medicine to treat various ailments such as headaches and infections (Leporatti and Ghedira 2009). It has various phytochemicals in different parts, especially in leaves which have several pharmacological activities like antimicrobial, anticancer antioxidant, anti-inflammatory, antimetastatic, antiproliferative, and antitumor properties (Campisiet al., 2019). The phytochemical constituents like luteolin, apigenin, and kaempferol from *S. nigrum* leaves are reported to have strong anticancer activity by inducing autophagy in AU565 breast cancer cells (Huang et al., 2010). Some of these compounds and other phytochemicals were reported to inhibit the oncogenes such as RAS, RAF, and mutated tumour suppressor genes such as p53 by targeting their active site and strong affinities (Purushothaman et al., 2019).

Extraction and isolation of active pharmaceutical ingredients from the plant is a necessary and tedious process in drug discovery but helps complement herbal medicine for treating different diseases. The antioxidant assays such as DPPH and reducing power assays ensure the antioxidant potential of the plant extracts. The present study aims to anlyse the qualitative and quantitative determination of phytochemical constituents present in *S. nigrum* leaves along with their potential antioxidant evaluation, followed by the GC/MS analysis to predict the hit compounds. Then the *In silico* molecular modelling studies were also performed along with the drug-likeness and ADME properties evaluation of the top 2 hit compounds from the GC/MS studies.

MATERIALS AND METHODS

Plant collection and extraction

The disease-free *S. nigrum* leaves were collected in and around PRIST University, Thanjavur, Tamilnadu, India, during January 2022 and washed twice before use. Botanical Survey of India, Coimbatore, identified and authenticated the plant. The leaf samples were then dried and stored correctly until further use. About 30g of the leaf samples were extracted with 150 mL of solvents such as benzene, diethyl ether, and ethanol in Soxhlet's apparatus. Then it was filtered through Whatman No.1 filter paper, and the filtrate was stored for further study (Harnafi et al., 2008).

Qualitative phytochemical screening

The phytochemicals from the different extracts of *S. nigrum* leaves were screened by the procedures prescribed (Ramalingam et al., 2017).

Estimation of total phenols

The total phenols were estimated by adding 0.5 mL of the *S. nigrum* leaves extracts to 5 mL of $D.H_2O$ and 0.5 mL of Folin-Ciocalteu reagent. Then the mixture was kept undisturbed for a few minutes, followed by the addition of 5 mL of 5% Na_2CO_3 and incubated for 1 hr at room temperature. Then the absorbance was measured at 550 nm and compared with the standard gallic acid, and the total phenols were estimated (Singleton et al., 1999*)*.

Estimation of total flavonoids

The total flavonoids were estimated by adding 0.5 mL of the *S nigrum* leaves extracts to 2 mL $D.H_2O$ and 0.5 mL of 4% $NaNO_3$ and incubated for 10 min at room temperature. To the mixture, 0.5 mL of 5% $AlCl_3$ and 0.5M NaOH solutions were added and mixed properly. Then the absorbance was measured at 510 nm and compared with the standard quercetin and the total flavonoids were estimated (Zhishenet al., 1999).

Estimation of total alkaloids

The total flavonoids were estimated by adding 2 mg/mL of the *S. nigrum* leaves extracts to 2 mL of 1 N HCL and DMSO respectively. The filtrate was separated, to which bromo-cresol green, PBS and HCL of each 5 mL added and mixed properly. Then the absorbance was measured at 470 nm and

compared with the standard atropine and the total alkaloids were estimated (Ramalingam et al., 2017).

Estimation of total saponins

The total flavonoids were estimated by adding 0.5 mL of the *S. nigrum* leaves extract to 5 mL of 30 % ethanol and heated at 60° C for 45 min. The filtrate was filtered and the same procedure was followed once again. Then 3 mL of $(C_2H_5)_2O$ mixed and aqueous layer was collected by addition of butanol. Then the absorbance was measured at 550 nm and compared with the standard Ginsenoside Rb1 and the total saponins were estimated (Son et al., 2018).

DPPH radical scavenging assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed to evaluate the antioxidant potential of the *S. nigrum* leaves extract (Aarland et al., 2017). The various concentrations (20, 40, 60, 80, 100 µg/mL) of extracts and the standard ascorbic acid was mixed with 2 mL of 0.2 mM DPPH solution and kept incubated at room temperature in dark for 45 min. Then the absorbance of the samples was measured in 517 nm and calculated as follows,

$$\% \text{ DPPH scavenging} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Reducing power assay

The reducing power assay of the *S. nigrum* leaves extract was evaluated the standard ascorbic acid was added with 2 mL of 150 mM PBS buffer solution and 10 mL of 0.8% $K_3[Fe(CN)_6]$ solution and heated at 50°C followed by spinning at 2500 rpm. The top layer was mixed with $D.H_2O$ and 0.2% $FeCl_3$. Finally, the absorbance was measured at 700 nm.Thehigher absorbance of the reaction mixture suggested that the higher reducing power.

GC/MS analysis

The GC/MS analysis was performedusing a Shimadzu GC-MS (Japan, QP2010SE) having a mass range of 1.5-1000 m/z, to predict the phytoconstituents present in the diethyl ether extract of *S. nigrum* leaves (Andrew et al., 2019). RTX-5 column was used with 30 m length coated with silica inside the walls of the columns. The mobile phase was passed by 1 mL/min flow rate and the column oven temperature was set at 120ºC, the overall time for analysis was 50 minutes, 0.4 µLof the sample was loaded into the column. Finally, the hit compounds were compared with the NIST library and hit compounds were predicted.

Molecular modelling studies

The 3D structures of proteins such as BRAF V600E (PDB ID: 5JT2) and p53 Y220C mutant (PDB ID: 6GGA), and the 3D structures of Hexadecanedioic acid (PubChem ID: 10459), and Octadecanedioic acid (PubChem ID: 70095) were retrieved from PDB and PubChem respectively. The ligand's energy was minimised by Avogadro software and the docking was performed by AutoDock 4.2 version and the interactions were analysed. Also, the drug likeness and ADME properties of the compounds were also evaluated (Hanwell et al., 2012; Daina et al., 2017).

RESULTS

The qualitative screening of the phytoconstituents in the benzene, diethyl ether, and ethanol extracts of *S. nigrum* leaves was performed, and the data is given in **Table 1**. Interestingly, diethyl ether extract showed relatively high amounts of alkaloids, saponins, tannins, phenolics and flavonoids. The total phenols, flavonoids, alkaloids, and saponins of the benzene, diethyl ether, and ethanol extracts of *S. nigrum* leaves were quantified and compared with standards gallic acid, quercetin, atropine, and ginsenoside Rb1 respectively as shown in **Table 2**. A higher amount of phenols (120.50 mg GAE/g), alkaloids (90.50±2.25 mg of AE/g), and flavonoids (108.75±2.50mg QE/g) were present in the diethyl ether extract of the *Solanum nigrum* leaves. In contrast, the saponins (42.50±1.50µg ginsenoside Rb1 equivalent/mg) are highly present in ethanol extract followed by the benzene (40.25±1.75µg ginsenoside Rb1 equivalent/mg) and diethyl ether extract (38.50±0.75µg ginsenoside Rb1 equivalent/mg). Overall, the diethyl ether extract of *S. nigrum* leaves has higher phytoconstituents and is used further for GC/MS study. The antioxidant activity of the different concentrations (20-100 µg/ml) of the extracts of the *S. nigrum* leaves was evaluated by DPPH and reducing power assays, and the data are shown in **Table 3** and **Table 4**. In both assays, the antioxidant activity of the extracts was determined by combining them with ascorbic acid as a standard. IC_{50} has been used to determine the DPPH radical scavenging activity. The greatest IC_{50} value were found in diethyl acetate extract (0.112 mg. mL^{-1}), after by benzene extract (0. 286mg.mL^{-1}), as well as subsequently the least value (0. 322mg.mL^{-1}) has been found in ether extract. Moreover, ascorbic acid (0.012mg/mL^{-1}) demonstrated greater IC_{50} value compared to three different extracts of *S. nigrum*.

Table 1. Qualitative determination of *S. nigrum* leaves extracts

S.No	Chemical constituents	Test name	Benzene extract	Diethyl ether extract	Ethanol extract
1.	Alkaloids	Wagner's test	++	++	+
		Mayer's test	+	++	+
2.	Flavonoids	Sodium hydroxide test	+	++	+
3.	Terpenoids	Copper acetate test	+	++	+
4.	Carbohydrates	Molisch's test	+	+	++
5.	Proteins	Biuret's test	+	+	+
		Millon's test	+	-	+
6.	Amino acids	Ninhydrin test	+	+	+
7.	Fats and oils (Fixed)	Spot test	-	+	-
		Saponification	+	+	-
8.	Steroids	Salkowski Tests	-	+	+
9.	Cardiac glycosides	KellarKillani's test	-	+	+
10.	Tannins and Phenolics	Ferric chloride test	+	++	+
		Lead acetate test	+	++	-
11.	Saponins	Foam test	++	++	+

Note: (++) = Highly present ;(+) = Moderately present; (-) = Absent

Table 2. Quantitative determination of *S. nigrum* leaves extract

Total composition	Benzene extract	Diethyl ether extract	Ethanol extract
Total phenolics (mg GAE/g)	90.50±2.25	120.50±1.75	98.50±2.50
Total flavonoids (mg QE/g)	92.50±1.75	108.75±2.50	88.50±1.50
Total alkaloids (mg of AE/g)	78.55±2.50	90.50±2.25	80.25±2.25
Total saponins (µg ginsenoside Rb1 equivalent/mg)	40.25±1.75	38.50±0.75	42.50±1.50

Table 3. DPPH assay of *Solanum nigrum* Linn leaves extract

Concentration (µg/ml)	Ascorbic acid	Benzene extract	Diethyl ether extract	Ethanol extract
20	43.25±1.50	38.75±2.25	45.75±1.50	36.50±1.25
40	51.75±1.25	47.50±0.75	55.50±1.50	49.75±2.75
60	68.50±2.25	64.50±1.75	70.75±1.75	61.50±2.25
80	84.50±0.75	80.75±0.25	86.75±1.25	82.50±0.50
100	92.25±1.50	87.50±1.50	93.50±0.50	86.50±1.75

One of the most well-known, reliable, and widely used techniques for assessing antioxidant activity is DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The ability of the DPPH radical to be reduced was evaluated by looking at how different antioxidants affected the DPPH radical's ability to absorb less light at 517 nm. *S. nigrum* extract showed antioxidant activity similar to ascorbic acid standard at five different concentrations 20, 40, 60, 80, 100 µg/ml.

Diethyl ether extract at a concentration 100 µg/ml and 80 µg/ml showed a inhibition of 93.50±0.50, and 86.75±1.25, respectively. For the DPPH method of measuring antioxidant activity, ascorbic acid was employed as the reference standard. Ascorbic acid standard at a concentration of 20 µg/ml and 100 µg/ml, showed a percentage inhibition of 43.25±1.50 and 92.25±1.50, respectively. In the DPPH assay, it is apparent that the diethyl ether extract has higher scavenging activity at all concentrations when compared to the standard. Benzene and ethanol extracts also showed significant scavenging activity but were lower than ascorbic acid.

Table 4. Reducing power assay of *S. nigrum* leaves extract

Concentration (µg/ml)	Ascorbic acid	Benzene extract	Diethyl ether extract	Ethanol extract
20	40.50±1.75	37.25±1.50	43.25±1.50	35.75±1.50
40	52.75±1.50	44.75±1.75	53.50±0.75	47.25±1.50
60	66.75±0.75	62.50±0.75	69.25±1.50	60.75±1.75
80	78.50±2.25	79.25±1.75	84.50±2.25	80.25±0.75
100	89.25±2.50	85.75±0.75	91.75±0.75	84.75±2.75

The reducing power experiment is commonly used to measure an antioxidant's capacity to give an electron.The capacity of extracts to decrease Fe^{3+}(potassium ferricyanide) to Fe^{2+}(potassium ferrocyanide) was assessed in this test. Based on the reducing strength of the extract, this causes the solution to change colours from green to blue. Table 4 compared the reducing abilities of different *S. nigrum* extracts to standard ascorbic acid. Diethyl ether extract indicates some degree of electron donation. The different concentrations of the extract enhanced the reducing capacity of various extracts. Ethanolic extract indicates low degree of potassium ferricyanide reduction than the diethyl ether extract. The various extracts were shown to have lower reducing powers than the standard ascorbic acid. Therefore, diethyl ether extract could have a higher amount of reductone than benzene and ethanol extract. In a reducing power assay, it was observed that the diethyl ether extract of the *S. nigrum* leaves showed higher reducing power than the other extracts and the standard. This suggests that the phytoconstituents of the extracts of the *S. nigrum* leaves have high antioxidant potential, and their phytoconstituents were evaluated in GC/MS.

The GC/MS analysis of the diethyl ether extract of *S. nigrum* leaves shows the hit compounds with respective retention times, as shown in **Figure 1** and **Table 5**. Esters and carboxylic acids were mostly predicted, and their total area % (Composition %) was determined. In GC /MS analysis, nearly 20 compounds were identified from diethyl ether extract of *S. Nigrum* leaves. The primary compound is 2-AMINO-9-(3,4-DIHYDROXY-5-HYDROX, FARNESOL ISOMER A, alpha-D-Glucopyranoside, methyl (CAS) Me, METHYL ESTER OF 3-HYDROXY-4-MET, ISOPROPYL MYRISTATEOCTADECANEDIOIC ACID and HEXADECANEDIOIC ACID. The top two higher compositions hit compounds were octadecanedioic acid and hexadecanoic acid, with retention times of 17.510 and 18.541, respectively. These two compounds were further taken for molecular docking studies.

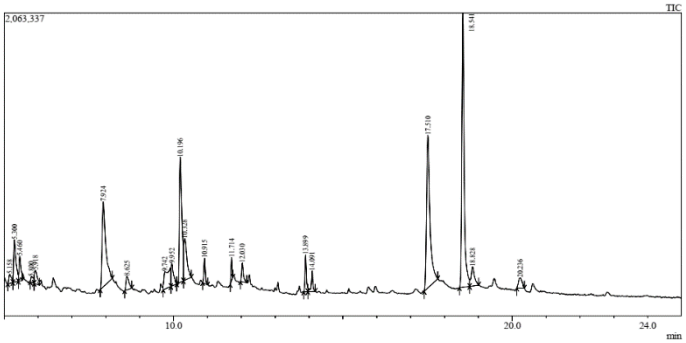

Figure 1. GC/MS chromatogram of diethyl ether extract of *S. nigrum* leaves

Table 5. Peak details of diethyl ether extract of *S. nigrum* leaves

Peak	Retention time	Area%	Height%	A/H ratio	Chemical compound name
1	5.158	0.93	0.92	5.53	BENZOFURAN, 2,3-DIHYDRO-
2	5.300	3.80	4.33	4.82	4-Hepten-3-one, 4-methyl- (CAS) 4-METHY
3	5.460	1.83	2.53	3.98	1,2,3-Propanetriol, diacetate (CAS) Diacetin
4	5.800	0.63	0.55	6.29	METHYL ESTER OF 3-HYDROXY-4-MET
5	5.918	1.45	1.36	5.83	propyl heptanoate
6	7.924	12.07	8.65	7.67	2-AMINO-9-(3,4-DIHYDROXY-5-HYDROX
7	8.625	1.47	1.40	5.76	1,6-ANHYDRO-BETA-D-GLUCOPYRANO
8	9.742	4.06	1.78	12.54	HEXADECANEDIOIC ACID
9	9.952	1.93	2.29	4.64	1,2-BENZOLDICARBONSAEURE, DI-(HE
10	10.196	11.91	12.73	5.14	1,3,4,5-TETRAHYDROXY-CYCLOHEXAN
11	10.328	5.06	4.24	6.55	alpha.-D-Glucopyranoside, methyl (CAS) Me
12	10.915	1.37	2.57	2.93	Diazene, bis(3-methylcyclohexyl)-, 1,2-dioxid
13	11.714	0.93	2.35	2.16	Pentadecanoic acid (CAS) Pentadecylic acid
14	12.030	1.29	1.94	3.64	2-PYRAZINOL
15	13.899	1.92	3.76	2.82	Palmitic acid
16	14.091	1.32	2.11	3.43	1,2-BENZENEDICARBOXYLIC ACID, DIBUTYL ESTER
17	17.510	20.01	15.56	7.07	OCTADECANEDIOIC ACID
18	18.541	24.09	27.89	4.75	HEXADECANOIC ACID
19	18.828	2.50	1.97	6.99	ISOPROPYL MYRISTATE
20	20.236	1.43	1.06	7.42	FARNESOL ISOMER A

The top 2 hit compounds from the GC/MS report, such as Octadecanedioic acid and Hexadecanedioic acid, were docked against an oncogene BRAF V600E and a mutated tumour suppressor gene p53 Y220C mutant, which revealed that these compounds have good affinities and the interactions were shown in **Figure. 2 & 3**. The binding energies in Kcal/mol and the interacting amino acid residues around 4Å were given in Table 6. The drug-likeness by lipinski's rule of five and ADME properties of Octadecanedioic acid and Hexadecanoic acid were evaluated by SWISSADME. The data are shown in **Table 7**, which was used to predict the drug-likeness properties. These two compounds displayed good physicochemical ADMET properties. It can be used as BRAF-V600E inhibitors. The major criteria employed in evaluating drugs at the initial stages of the drug development process include drug-likeness characteristics. Lipinski's rule of 5 is among the most prominent and useful rules during the beginning stages of drug discovery, predicting that if any

compounds fail to meet more than 2 of these parameters (MW.500, HBD5, HBA10, Log p5, and TPSA140 2), it will fail It is considered that the chemical is weakly absorbed. According to Lipinski's criteria, the identified compounds are judged to pass because none violate greater than two and can be classified as drug-like compounds.

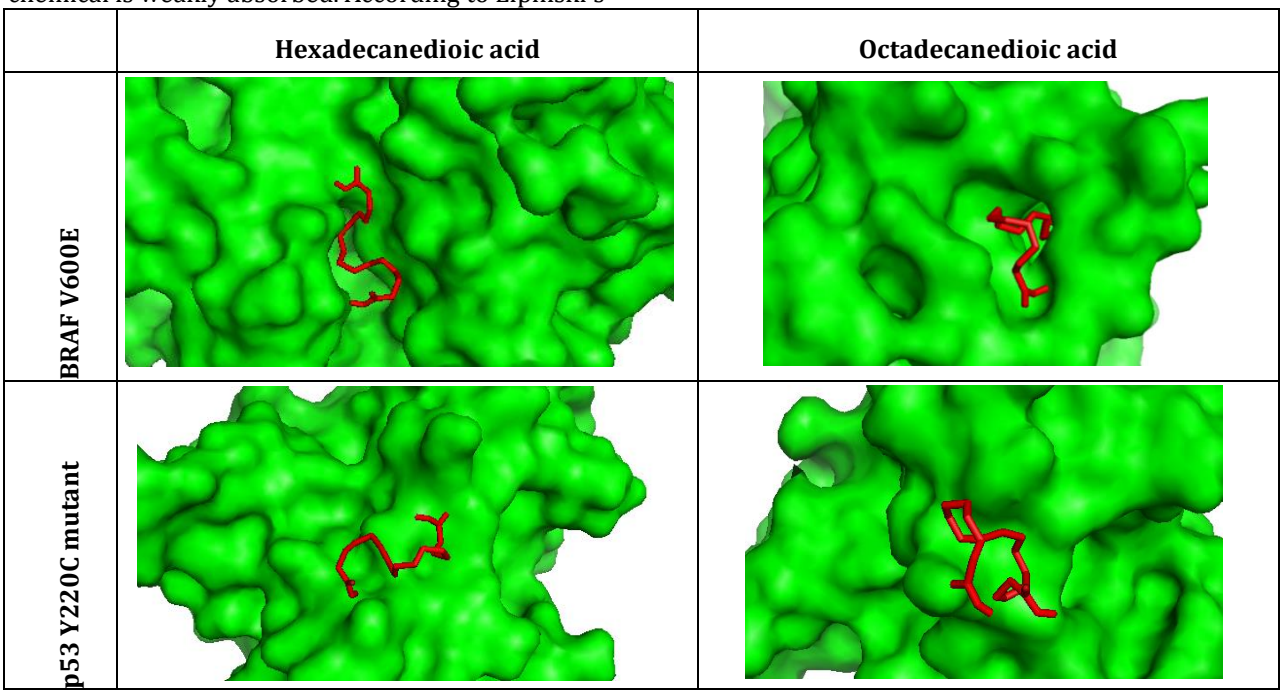

Figure 2. Surface model interactions of protein-ligand complex

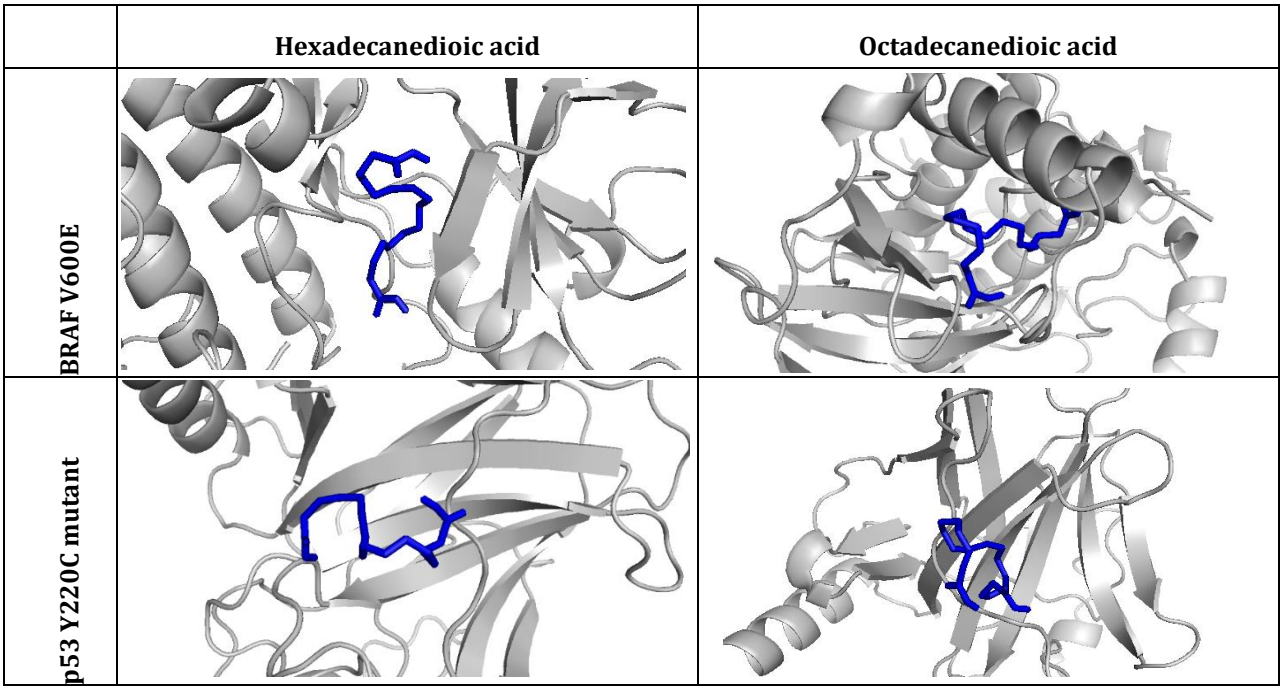

Figure 3. Ribbon model interactions of protein-ligand complex

Table 6. Binding energy, surrounding and interacting residues of protein-ligand complexes

Protein name	Compound name	Binding energy (Kcal/mol)
BRAF V600E	Hexadecanedioic acid	-5.3
	Octadecanedioic acid	-6.1
p53 Y220C mutant	Hexadecanedioic acid	-4.5
	Octadecanedioic acid	-4.3

Table 7. Physiochemical, drug likeness and ADME properties of the compounds

Compounds	MF	MW	HBD	HBA	Log P	TPSA	Log S	GIA	BBBP	BA	Lipinski	Log K_p
Octadecanedioic acid	$C_{18}H_{34}O_4$	314.4	2	4	4.84	74.60	-4.72	High	Yes	0.55	Yes	-3.65
Hexadecanedioic acid	$C_{16}H_{30}O_4$	286.4	2	4	4.08	74.60	-4.00	High	No	0.55	Yes	-4.24

DISCUSSION

Nowadays, plant-based phytoconstituents such as polyphenols and flavonoids are attracting attention due to their versatile pharmacological activities such as antioxidants, anticancer, anti-inflammatory, etc (Kumar et al., 2013; Mishra et al., 2013; Ashokkumar, 2015; Tungmunnithum et al., 2018; Ashokkumar et al., 2022). The plant-derived compounds, individually or in combination with other drugs/compounds, are reported to work as HDAC inhibitors, HAT inhibitors, topoisomerase inhibitors, and other molecular target inhibitors of cancer and disease pathogenesis (Amin et al., 2009; Mekala et al., 2022). These compounds can scavenge various free radicals in the cells, reducing the cells' oxidative stress and metabolic burden and enhancing the production of antioxidant enzymes (Ramaiah et al., 2021). High intracellular reactive oxygen species levels can cause mitochondrial dysfunction and apoptosis in cells via intrinsic and extrinsic apoptotic pathways (Venkatesh et al., 2015; Patel et al., 2016; Srinivas et al., 2016).

S. nigrum belonging to the family Solanaceae, is a flowering species native to Australia, South Africa, and Asia. *S. nigrum* possesses various medicinal properties and is used as a traditional medicine to treat various ailments such as headaches and infections (Leporatti et al.,2009). It has various phytochemicals in different parts, especially in leaves, which possess various pharmacological activities such as antimicrobial, anticancer antioxidant, anti-inflammatory, antimetastatic, antiproliferative, and antitumor properties (Campisi et al., 2019).

The present study shows that the diethyl ether (moderately polar/non-polar) is a better suitable solvent than the benzene (non-polar) and ethanol (polar) solvents to extract the polyphenol-rich extract of the. *S. nigrum* leaves. The dried plant samples were extracted with ethanol, benzene, and diethyl ether solvents by Soxhlet's apparatus, and their phytoconstituents were profiled by qualitative and quantitative determination. The body naturally produces free radicals when cells usually function under specific environmental circumstances. Since these substances are missing an electron, they have an electric charge. A popular method of evaluating an antioxidant's capacity to scavenge free radicals is to employ the stable free radical known as the DPPH. The antioxidant potential of the extracts was also determined by DPPH and reducing power assays. Combinedly, these data showed that the diethyl ether extract had more polyphenols and flavonoids than the standard ascorbic acid and showed significant antioxidant activity by outcompeting the standard ascorbic acid. The GC/MS analysis was also performed, a total of 20 compounds were identified two prominent compounds octadecanedioic acid (20.01 area %, 15.56 height %, 7.07 A/H ratio) and hexadecanoic acid (4.06 area%, 1.78 height %, 12.54 A/H ratio) with the highest peak are percentage and height percentage were identified. Compared to other compounds, the top 2 hit compounds, octadecanedioic acid, and hexadecanoic acid, were docked against an oncogene BRAF V600E and a

mutated tumour suppressor gene p53 Y220C mutant, which revealed that these compounds have good affinities and may possess anticancer activity. The drug-likeness by Lipinski's rule of five and ADME properties evaluation revealed that these compounds have passed the drug-likeness properties and other ADME properties and followed the criteria (Mekala et al., 2021; Mekala et al., 2022).

CONCLUSION

The present study found that the *S. nigrum* extracts are rich in polyphenols and flavonoids, which were evaluated by the qualitative and quantitative screening of phytochemicals. Also, it confirmed that the diethyl ether extract has more antioxidant potential than the benzene and ethanol extracts. GC/MS results revealed the presence of hit compounds with their RT, which were further docked against an oncogene BRAF V600E and a mutated tumour suppressor gene p53 Y220C mutant. The results revealed that these two hit compounds from the GC/MS study have good binding affinities against these proteins and showed perfect protein-ligand interactions. Finally, we conclude that *S. nigrum* leaves have potent antioxidant and anticancer properties.

CONFLICT OF INTEREST

We declare that we don't have interest of conflict.

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