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Antioxidant, antimicrobial activities and GCMS analysis of fruit extract of *Solanum nigrum* L.

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Abstract
Solanum nigrum is an important plant in traditional Indian medicines. Infusions are used in dysentery, stomach complaints, and fever. In India, the berries are casually grown and eaten for maintaining good health. The study aims to evaluate antioxidant, antibacterial activities and GC-MS analysis of ethanol fruit extract of *Solanum nigrum.* The antioxidant assays such as DPPH˙ radical, superoxide radical, $ABTS^{\cdot+}$ radical cation, phoshomolybdenum reduction and Fe^{3+} reducing power assays were carried out for fruit extract. The maximum DPPH˙ radical scavenging activity was 73.16±5.12% at 120 µg/mL concentrations and the IC_{50} was 81.02 µg/mL concentrations. The maximum superoxide radical scavenging activity was 60.06±4.20% at 120 µg/mL concentrations and the IC_{50} was 57.82 µg/mL. The maximum $ABTS^{\cdot+}$ radical cation scavenging activity was 67.70± 4.73% at 60 µg/mL concentrations and the IC_{50} was 35.56 µg/mL concentrations. The maximum phosphomolybdenum reduction was 96.77± 6.77% at 120 µg/mL concentrations and the RC_{50} was 21.25 µg/mL concentrations. The maximum Fe^{3+} reduction was 72.10± 5.04% at 120 µg/mL concentrations and the RC_{50} was 63.74 µg/mL concentrations. The antibacterial activity showed maximum zone of inhibition of 25 mm for *Escherichia coli* at 625 µg/mL concentration. GC-MS analysis showed presence of antioxidant compounds such as flavone and oleic acid in the fruit extract of *Solanum nigrum.*

Keywords: *Solanum nigrum,* DPPH˙ radical, $ABTS^{\cdot+}$ radical cation, GC-MS analysis

Introduction
Solanum nigrum (black nightshade) is a medicinal plant member of the Solanaceae family comprises many genera, well known for their therapeutic properties [1]. It is medicinally used in the management of several ailments, such as pneumonia aching teeth, stomache ache, tonsilitis, wing worms, pain, inflammation, fever, tumor, as tonic, as antioxidant, as anti-inflammatory, as hepaprotective, as diuretic, and as antipyretic [2]. The plant is frequently used as an elemental ingredient for clinical traditional Chinese medicine cancer therapy. Black nightshade is a common herb or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats. It reaches a height of 30 to 120 cm (12 to 47 in), leaves 4.0 to 7.5 cm (1.6 to 3.0 in) long and 2 to 5 cm (1 to 2 in) wide; ovate to heart-shaped, with wavy or large-toothed edges; both surfaces hairy or hairless; petiole 1 to 3 cm (0.5 to 1 in) long with a winged upper portion. The flowers have petals greenish to whitish recurved when aged and surround prominent bright yellow anthers. The berry is mostly 6 to 8 mm (0.24 to 0.31 in) in diam., dull black or purple-black. In India, another strain is found with berries that turn red when ripe [3]. Traditionally the plant was used to treat tuberculosis. It is known as peddakashapandlakoora in the Telangana region. This plant's leaves are used to treat mouth ulcers that happen during winter periods of Tamil Nadu, India. It is known as manathakkali keerai in Tamil Nadu and kaagesoppu in Karnataka, and apart from its use as a home remedy for mouth ulcers, is used in cooking like spinach. In North India, the boiled extracts of leaves and berries are also used to alleviate liver-related ailments, including jaundice. In Assam, the juice from its roots is used against asthma and whooping cough [4, 5].

Taxonomic classification
Kingdom: Plantae
Division: Angiosperms
Class: Eudicots
Sub class: Asterids
Order: Solanales
Family: Solanaceae
Genus: *Solanum*
Species: *S.nigrum*

Fig 1: Habitat of *S. nigrum*

Materials and methods

Collection of plant material and preparation of the extract

S. nigrum fruits were collected from the home garden, Kundrathur, Chennai, Tamilnadu, India. The fruits were washed in distilled water and soaked in ethanol for 72 h. The supernatant was filtered by filter paper and condensed at 50ºC in a rotor evaporator, which yields brown gummy extract.

Qualitative phytochemical analysis

The fruit extract of S. *nigrum* was subjected to preliminary phytochemical analysis for different classes of phytoconstituents using specific reagents [6, 7].

Estimation of total phenols

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds with slight modifications [8]. One hundred µL of ethanol fruit extract (1mg/mL) of *S. nigrum* was mixed with 900 µL of ethanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). Then, 1 mL of 20% (w/v) Na_2CO_3 solution was added and shaken well. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

Estimation of total flavonoids

The total flavonoid content of ethanol fruit extract of *S. nigrum* was determined using aluminium chloride reagent method with slight modifications [9]. Five hundred µL of extract (1mg/mL) was mixed with 500 µL of ethanol and 0.5 mL of 5% (w/v) sodium nitrite solution. Then, 0.5 mL 10% (w/v) aluminium chloride solution was added followed by 50 µL of 1 M NaOH solution was added and shaken well. Absorbance was measured at 510 nm using spectrophotometer and the result was expressed as quercetin equivalent (µg/mg of extract), which is a common reference compound.

In vitro antioxidant activity

DPPH˙ radical scavenging activity

The antioxidant activity of ethanol fruit extract of *S.nigrum* was measured on the basis of stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) radical scavenging activity [10]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (10-60 µg/mL) of fruit extract. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. One mL methanol mixed with 1 mL DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \frac{\text{Control} - \text{Sample} \times 100}{\text{Control}}$$

Superoxide radical scavenging assay

Assay for superoxide radical scavenging activity was based on the capacity of the fruit extract to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system [11]. The reaction mixture contained various concentrations of (20-120 µg/mL) of fruit extract, 1.5 mM of riboflavin (200 µL), 12 mM of EDTA (100 µL) and 50 mM of NBT (50 µL) and added in that sequence. The reagents should be prepared in 50 mM of phosphate buffer (pH 7.6) solution. The reaction was started by illuminating the reaction mixture for 5 min. Immediately after illumination, the absorbance was measured at 590 nm. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated as:

$$\% \text{ of superoxide radical inhibition} = \frac{\text{Control-Sample} \times 100}{\text{Control}}$$

$ABTS^{\cdot+}$ radical cation scavenging activity

The antioxidant capacity of ethanol fruit extract of *S. nigrum* was evaluated in terms of the $ABTS^{\bullet+}$ radical cation scavenging activity with slight modifications [12]. $ABTS^{\bullet+}$ was obtained by reaction between 7 mM of ABTS solution and 2.45 mM of potassium persulfate solution and the mixture was left to stand in dark at room temperature for 12-16 h, before use. The $ABTS^{\bullet+}$ solution (stable for 2 days) was diluted with phosphate buffer (pH 7.4) and set an absorbance of 0.70±0.02 at 734 nm. Fresh diluted $ABTS^{\cdot+}$ solution was prepared every day. Ethanol fruit extract of various concentrations (5-30 µg/mL) was mixed with 500 µL of diluted $ABTS^{\bullet+}$ solution and the absorbance was measured at 734 nm after 5 min. Ascorbic acid was used as the standard reference. The $ABTS^{\bullet+}$ radical cation scavenging activity was expressed as:

$$\% \text{ of ABTS}^{\cdot+} \text{ radical cation inhibition} = \frac{\text{Control-Sample} \times 100}{\text{Control}}$$

Phosphomolybdenum reduction assay

The antioxidant capacity of ethanol fruit extract of *S. nigrum* was assessed by the method of Prieto *et al*. [13] The fruit extract with different concentrations (20-120 µg/mL) was combined with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in a water bath at 95ºC for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as standard reference. The percentage of reduction was calculated as:

$$\% \text{ of } Mo^{6+} \text{ reduction} = \frac{\text{Sample} - \text{Control} \times 100}{\text{Sample}}$$

Ferric (Fe^{3+}) reducing power assay

The reducing power of ethanol fruit extract of *S. nigrum* was determined by slightly modified potassium ferricyanide

method [14]. One mL of fruit extract of different concentrations (20 - 120 µg/mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v) solution. The mixture was then incubated at 50ºC in a water bath for 20 min. Five hundred µL of trichloroacetic acid (10% w/v) was added to each mixture. Then 100 µL of freshly prepared $FeCl_3$ (0.1%, w/v) solution was added, shaken well and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated as:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \frac{\text{Sample - Control}}{\text{Sample}} \times 100$$

Antibacterial activity

Microbial strains

The microorganisms of Gram-positive strains such as *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus* as well as Gram-negative strains such as *Escherichia coli*, *Proteus vulgaris* and *Shigella flexneri* was taken for antibacterial activity.

Nutrient broth agar medium preparation

Nutrient broth agar medium was prepared according to the standard methods (peptone-5 g, yeast-3 g, NaCl-5 g, distilled water- 1000 mL, agar-20 g). Depending upon the availability of strains, the medium was calculated and suspended in 150 mL of distilled water in a conical flask, stirred, boiled to dissolve and then autoclaved at 15 lbs and at 121ºC for 15 min. The hot medium was poured in sterile petri plates which were kept in the aseptic laminar air flow chamber and allowed to solidify for 15 min.

Agar well Diffusion method

Antibacterial activity of ethanol fruit extract of *S. nigrum* was carried out using agar well diffusion method. The solidified nutrient agar in the petri plates was inoculated by dispensing the inoculum using sterilized cotton swabs which is previously immersed in the inoculum containing test tube and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The root extract was then poured into each well containing 250, 375, 500 and 625 µg/mL concentrations. All the plates with extract loaded wells were incubated at 37ºC for 24 h and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well [15]. Tetracycline (25 µg) was used as positive control.

Gas chromatography–Mass Spectrometry (GC–MS)

The fruit extract of *S. nigrum* was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model for GCMS analysis. The chromatographic conditions were fixed as helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. The mass spectrum conditions were fixed as ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C and mass range of 50-600 mass units[16].

Results and discussion

Qualitative phytochemical analysis

The phytochemical analysis of ethanol fruit extract of *S. nigrum* showed the presence of alkaloids, terpenoids, steroids phenolic compounds, flavonoids, tannins, glycosides and saponins.

Table 1: Phytochemical analysis of fruit extract of S. *nigrum*

Phytochemicals	Test	Inference	Result
Alkaloids	**Hager's test:** To the extract, saturated aqueous solution of picric acid was added and shaken well.	No yellow precipitate	+
Terpenoids	**Salkowski test:** To the extract, chloroform was added and mixed well. Then, few drops of Conc.H_2SO_4 were added along the sides of the test tube.	Red ring appears.	+
Steroids	**Libermann-Burchard's test:** To the extract, 1 mL of acetic anhydride was added and shaken well. To this, few drops of Conc.H_2SO_4 were added along the sides of the test tube.	Dark violet colour appears	+
Flavonoids	**Alkaline Reagent test:** To the extract, few drops of 2% NaOH solution was added and shaken well.	Yellow colour appears	+
Tannins	**Lead acetate test:** To the extract, few drops of 5% $Pb(CH_3COO)_2$ solution was added and shaken well.	White colour appears	
Glycosides	**Legal's test:** To the extract, few drops of pyridine and few drops of alkaline sodium nitroprusside solution was added and shaken well.	Blood red color appears.	+
Carbohydrate	**Molisch test:** To the extract, two drops of alcoholic α-naphthol solution was added and shaken well. To this, few drops of Conc.H_2SO_4 was added.	Violet ring appears	+
Saponins	**Foam test:** To the extract, 3 mL of distilled water was added and shaken vigorously.	Foam appears	+

Total Phenol and Flavonoid content

Flavonoids, and phenolic compounds are known to play major roles in the antioxidant and prooxidant capacities exhibited by plant extracts. The antioxidant effect conferred by these compounds are due to the phenolic hydroxyl groups attached to their respective ring structures that can act as reducing agents, hydrogen donors, singlet oxygen quenchers, super oxide radical scavengers, and as metal chelators. They are also said to reduce α–tocopherol radicals or tocopheroxyls, activate antioxidant enzymes, mitigate nitrosative stress, and inhibit oxidases [17]. After proton donation, these compounds are oxidized to resonance-stabilized radicals that can further act as prooxidants at high concentrations, high pH, and in the presence of metal ions [18]. Flavonoids and phenolic acids present in food such as quercetin, myricetin, caffeic acid, gallic acid, chlorogenic acid, coumaric acid, ferulic acid, and ellagic acid have been proven to exhibit dual character, which are antioxidant and prooxidant behaviour [19]. The total phenol and flavonoid content of ethanol fruit extract of *S. nigrum* were 421.54±3.29 µg/mg and 15.54±0.28 µg/mg respectively.

Table 2: Quantitative estimation of ethanol fruit extract of *S. nigrum*

Phytochemicals	Amount (µg/mg)
Phenols	421.54±3.29 GAE
Flavonoids	15.54±0.28 QE

DPPH· radical scavenging activity

The ability of ethanol extract of *S. nigrum* to scavenge DPPH· (1,1-diphenyl-2-picrylhydrazyl) radical was evaluated by reducing the purple coloured stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increases with increasing concentration of the extract[20]. The maximum DPPH· radical scavenging activity was 73.16±5.12% at 120 µg/mL concentration. Ethanol fruit extract of *S. nigrum* has good capacity to scavenge DPPH free radicals and the IC_{50} was 81.02 µg/mL concentration (Table 3; Figure 2). It was compared with the standard ascorbic acid (IC_{50} = 11.98 µg/mL concentration).

Table 3: DPPH· radical and superoxide radical scavenging activity of ethanol fruit extract of S. *nigrum*

Concentration (µg/mL)	% of inhibition	
	DPPH radical	Superoxide radical
20	25.32±1.77	6.6±0.46
40	33.67±2.36	33.33±2.33
60	43.04±3.01	51.88±3.63
80	49.37±3.45	56.6±3.96
100	52.41±3.66	59.43±4.16
120	73.16±5.12	60.06±4.20

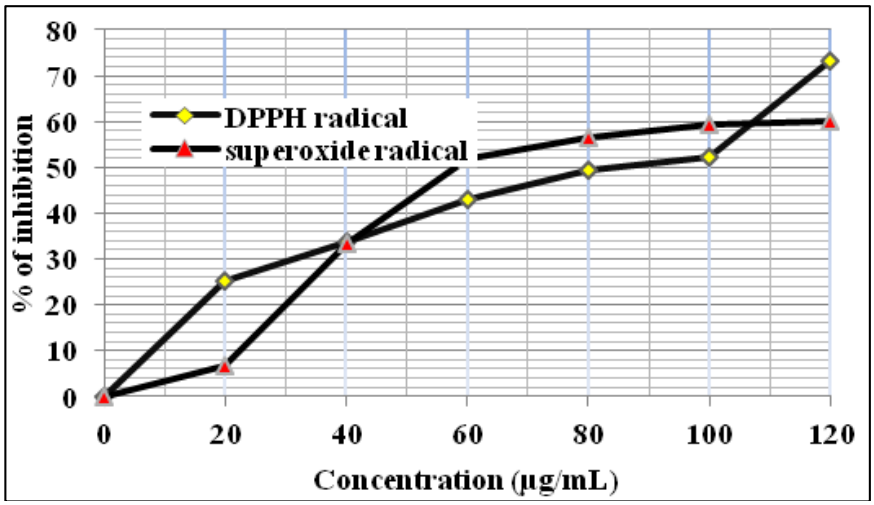


Fig 2: DPPH· radical and superoxide radical scavenging activity of ethanol fruit extract of S. *nigrum*

Superoxide radical scavenging activity

Superoxide anion is also very harmful to cells and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents [21]. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen from the riboflavin-light system and the evolving superoxide anions will reduce NBT. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to blue formazan, which was measured at 590 nm in UV-Vis spectrophotometer. Antioxidants have the ability to inhibit the blue NBT formation and the decreasing absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The maximum superoxide radical scavenging activity of *S. nigrum* was 60.06 ± 4.20% at 120 µg/mL concentration (Table 3; Figure 2) and the IC_{50} was 57.82 µg/mL concentration. It was compared with the standard of ascorbic acid (IC_{50} = 9.65 µg/mL concentration).

$ABTS^{\cdot+}$ radical cation scavenging activity

$ABTS^{\bullet+}$ radical cation is a stable blue-green coloured chromophore produced by the reaction between ABTS and potassium persulfate and has absorption maxima at 734 nm. The addition of antioxidants to the pre-formed $ABTS^{\bullet+}$ radical cation, reduces it to ABTS depending on the antioxidant activity and the concentration of the antioxidant. Antioxidant compounds quench the blue-green colour and changed decolouration of the solution which is proportional to amount of antioxidants present in the fruit extract [22]. This reaction is rapid and the end point, which is stable, is taken as a measure of the antioxidative efficiency. The maximum $ABTS^{\bullet+}$ radical cation scavenging activity was 67.70±4.73% at 30 µg/mL concentration and the IC_{50} was 17.78 µg/mL concentration (Table 4; Figure 3). It was compared with the standard ascorbic acid (IC_{50} = 4.21 µg/mL concentration).

Table 4: $ABTS^{\cdot+}$ radical cation scavenging activity of ethanol fruit extract of *S. nigrum*

Concentration (µg/mL)	% of inhibition
	$ABTS^{\cdot+}$ radical cation
5	15.78±1.04
10	26.19±1.83
15	42.86±3.0
20	56.25±3.93
25	63.69±4.45
30	67.70±4.73

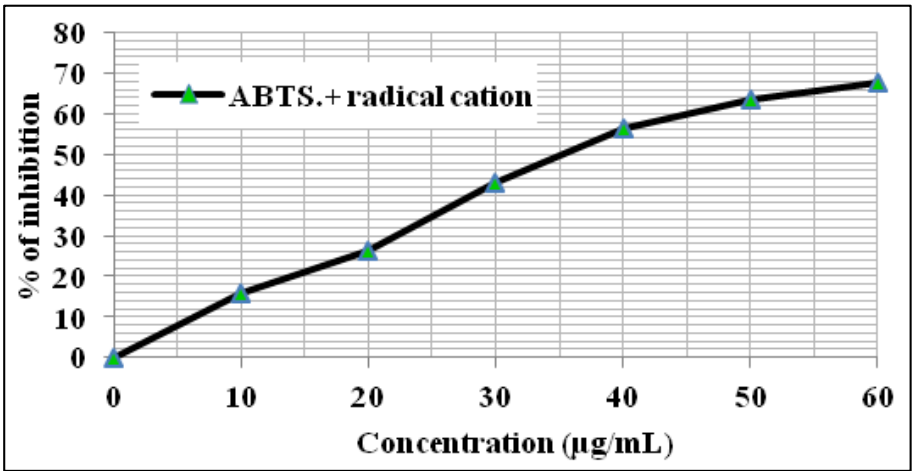


Fig 3: $ABTS^{\cdot+}$ radical cation scavenging activity of ethanol fruit extract of *S. nigrum*

Phosphomolybdenum reduction activity

The total antioxidant activity of ethanol fruit extract of *S. nigrum* was measured by phosphomolybdenum reduction assay method which is based on the reduction of Mo (VI) to Mo(V) and the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm [23]. The maximum phosphomolybdenum reduction was 96.77±6.77% at 120 µg/mL concentration and the RC_{50} was 21.25 µg/mL concentration (Table 5; Figure 4). It was compared with the standard ascorbic acid (RC_{50} = 6.34 µg/mL concentration).

Table 5: Phosphomolybdenum reduction and Fe^{3+} reducing power activity of ethanol fruit extract of *S. nigrum*

Concentration (µg/mL)	% of reduction	
	Phosphomolybdenum reduction	Fe^{3+} reduction
20	47.05±3.29	33.72±2.36
40	65.9±4.613	34.73±2.43
60	68.53±4.79	47.06±3.29
80	76.56±5.35	65.87±4.61
100	82.82±5.79	70.77±4.95
120	96.77±6.77	72.10±5.04

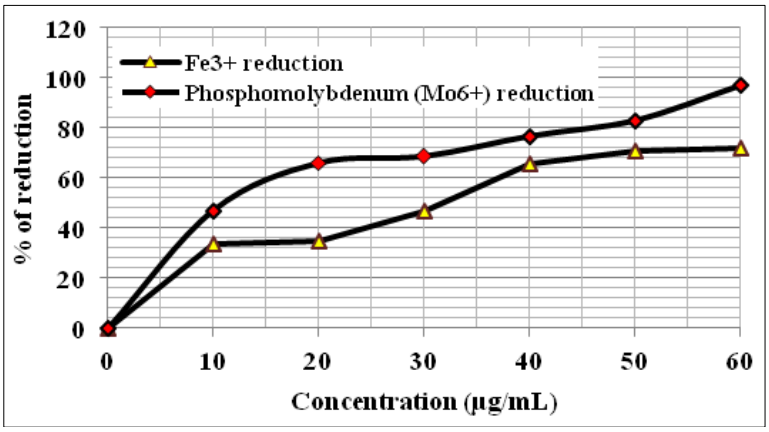


Fig 4: Phosphomolybdenum reduction and Fe^{3+} reducing power activity of ethanol fruit extract of *S. nigrum*

Ferric (Fe^{3+}) reducing power assay

The reducing power of Fe^{3+} to Fe^{2+} by the ethanol fruit extract of *S. nigrum* was measured and the reduction ability increases with increasing concentration of the extract (Table 5; Figure 4) due to the formation of ferro-ferric complex [24]. Ferric ion reducing power activity determines the electron donating ability of an antioxidant. The flavonoids and phenolic compounds present in the fruit extract have the ability to donate electrons, which reflects strong antioxidant activity. The maximum Fe^{3+} reduction was 72.10±5.04% at 120 µg/mL concentration and the RC_{50} was 63.74 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50}= 7.72 µg/mL concentration).

Antibacterial activity

The antibacterial activity of ethanol fruit extract of *S. nigrum* was studied against microorganism including Gram-positive bacteria such as *Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus* as well as Gram-negative bacteria such as *Escherichia coli*, *Shigella flexneri*. The maximum zone of inhibition showed for *Staphylococcus aureus*, which was 23 mm at 625 µg/mL concentration (Table 6; Figure 5). An important quality for an antimicrobial drug is selective toxicity, meaning that it selectively kills or inhibits the growth of microbial targets while causing minimal or no harm to the host. Different classes of antibacterial compound block steps in the biosynthesis of peptidoglycan, making cells more susceptible to osmotic lysis. Therefore, antibacterial compounds that target cell wall biosynthesis and act as bactericidal due to the presence of phytocompounds such as polyphenols, terpenoids, and alkaloids that inhibits the growth of microbes [25].

Table 6: Antibacterial activity of ethanol fruit extract of *S. nigrum*

Organism	Zone of inhibition				
	250 µg	375 µg	500 µg	625 µg	Standard (Tetracycline)
Bacillus subtilis	11	12	14	15	15
Micrococcus luteus	17	19	20	22	18
Staphylococcus aureus	14	16	17	20	16
Escherichia coli	15	17	20	21	22
Proteus vulgaris	12	14	15	16	15
Shigella flexneri	14	16	18	20	24

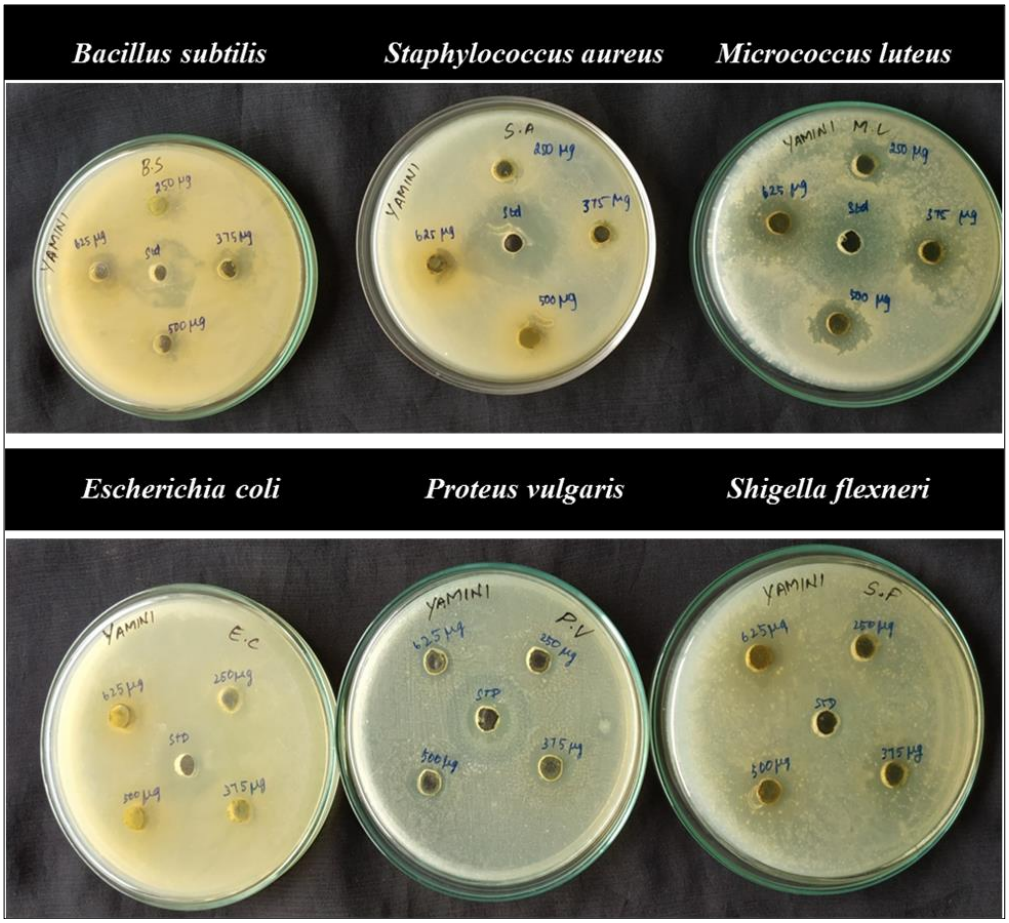
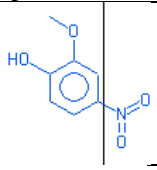
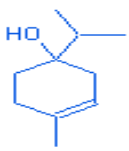


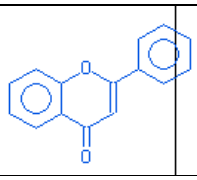
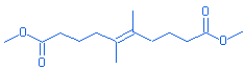
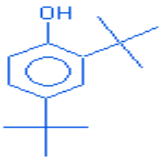
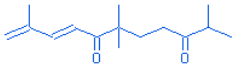
Fig 5: Antibacterial activity of ethanol fruit extract of *S. nigrum*

GC-MS analysis

GC-MS analysis was carried out for the ethanol extract of *S. nigrum* and the eluted compounds were showed in Table 7. Two compounds namely Flavone and Oleic acid were reported and they posses antioxidant activity and could be one of the reasons for the antioxidant property of the extract.

Table 7: Active compounds identified in ethanol fruit extract of *S.nigrum* by GC-MS analysis.

RT	Compound Name	Compound Structure	Mol. Formula	Mol. Weight g/mol
16.08	4-nitroguaiacol		$C_7H_7NO_4$	169.13
16.73	3-cyclohexen-1-ol,4-methyl-1-(methylethyl)		$C_{10}H_{18}O$	154.00
17.52	Nonanoic acid,1-methyl ethyl ester		$C_{12}H_{24}O_2$	200.18

18.03	Flavone		$C_{15}H_{10}O_2$	222.00
20.67	5-Decenedioic acid,5,6-dimethyl,dimethyl ester		$C_{14}H_{24}O_4$	256.17
21.4	Oleic acid		$C_{18}H_{34}O_2$	282.26
19.65	Z,E-2methyl-3,13-octadecadien-1-ol		$C_{19}H_{36}O$	280.28
17.82	2,4-di-tert-butylphenol		$C_{14}H_{22}O$	206.17
18.47	2,6,6,10-tetramethyl-undeca-8,10diene-3,7-dione		$C_{15}H_{24}$	236.18

Conclusion

Oxidative stress is one of the reasons to cause human diseases, such as atherosclerosis, ischemic reperfusion injury, inflammation, carcinogenesis, aging, and neurodegenerative diseases. Polyphenols are the secondary metabolites in plants have defence mechanism against stress factors. Antioxidants have been used in the food industry to prolong the shelf life of foods, to avoid food deterioration, decoloration, and nutritional losses, especially those rich in polyunsaturated fats, due to the lipids peroxidation. In order to stop these deterioration processes the synthetic antioxidants namely butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone, and propyl-gallate has been widely used industrially but has been questioned due to their potential health risks and toxicity. The present study provides the useful information about phytochemicals, antioxidant properties and polyphenolic contents of ethanol fruit extract of *S. nigrum* which is used for the therapeutic purposes.

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