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In vitro antioxidant properties of methanolic extract of *Solanum nigrum* L. fruit

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Abstract
Solanum nigrum L. (Solanaceae) has been extensively used in traditional medicine in India and other parts of the world to cure liver disorders, chronic skin ailments, inflammatory conditions, painful periods, fevers, diarrhea, eye diseases etc. In the present study crude methanolic extract of fruits of *Solanum nigrum* L. were prepared and evaluated for the presence of total phenol content, total flavonoid content and antioxidant activity by DPPH assay and H_2O_2 radical scavenging assay. The total phenol content (TPC), total flavonoid content (TFC) and flavonoid/ Phenol (F/P) of methanolic extract of *S.nigrum* L. fruits were 4.57 ± 0.57 GAE/g, 3.61± 0.07 mg QAE/g and 0.78. The fruit extract shown marked antioxidant activity with an IC_{50} value of 70.73 µg/ml for DPPH radical scavenging and IC_{50} 59.72 µg/ml for Hydrogen peroxide scavenging activity. Based on the above results it was concluded that the methanolic extract of *S.nigrum* fruits exhibited significant antioxidant activity and it could be a good source of natural antioxidants for the treatment of metabolic diseases

Keywords: *S. nigrum*. flavonoid, phenol, antioxidant, DPPH assay, metabolic disease

1. Introduction
Herbal medicines have been used since the dawn of civilization to maintain health and to treat diseases. The use of herbs for medical benefit has played an important role in nearly every culture on earth [1]. Most of the herbal drugs are a mixture of a number of plant ingredients whose cumulative effect increases their efficacy in treating diseases [2]. Plant based antioxidant rich foods traditionally formed a major part of the human diet, and are hypothesized to have an important role in maintaining human health [3]. Now-a-days plants with antioxidant properties are attractive sources of new drugs [4]. Thousands of herbal and traditional compounds are being screened worldwide to validate their use as antioxidants [5]. This involves the isolation and identification of secondary metabolites from the plants and their use as active principle in medicinal preparations. During recent years, active principles with diverse chemical structures have been isolated form plants possessing both the hepatoprotective and antioxidant effects [6].
Solanum nigrum L. (Solanaceae) commonly known as Black Berried Nightshade is a common herb distributed throughout India. The plant has a great medicinal value. The plant has been traditionally used as hepatoprotective agent in India. Fruits make a delightful Jam [7]. The fruit of *S.nigrum* L. is also used as a nervous tonic in the Mexican medicine. Chemically, solasodine, solasonine and solanidine have been identified from plant [8]. Fruits of plant have also been used as an antioxidant, antiulcer and anticancer agent [9]. In this study an attempt has been taken to investigate the *in vitro* antioxidant property of the methanolic extracts of *S. nigrum* L. fruit

2. Materials and methods
2.1 Plant material collection
The fruits of *Solanum nigrum* L. were procured from local Uzhavar Sandhai, Perambalur, Tamilnadu. The plant material was identified with the help of different floras [10-12] and documented properly.

2.2 Preparation of extract
The fruits were dried under shade. After complete drying the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder and the powder was stored in a suitable container. The dried fruit powder 100g was soaked in 500ml of methanol at room temperature in glass stoppered bottle container for two days. The extracts were filtered through a Whatmann No. 1

filter paper and then through cotton wool. The extracts were concentrated using a rotary evaporator with the hot water bath set at 40°C. The crude extract was investigated for total phenol content (TPC), total flavonoid content (TFC) and *in vitro* antioxidant activity.

2.3 Determination of total phenol content

Total phenol content was determined by using Folin Ciocalteu reagent method [13]. *S. nigrum* L. fruit methanolic extract 1ml (1mg/ml) was mixed with 5ml of Foiln Ciocalteu's reagent (diluted with distilled water 1:10) and 4ml of sodium carbonate(1M). The mixture was allowed to stand for 30 mins at 40ºC for development of colour. The absorbance was read at 765nm in a UV-Vis Spectrophotometer. The standard curve was prepared using 20, 40,60, 80 and 100 mg /l solution of gallic acid. The total phenol content were expressed as mg/g of gallic acid equivalents per gram of extract.

2.4 Determination of total flavonoid content

Total flavonoid content of *S. nigrum* L. fruit methanolic extract was measured by the aluminium chloride colorimetric assay [15]. An aliquot (1ml) of extract was added to 10 ml volumetric flask containing 4 ml of distilled water and 0.3 ml 5% $NaNO_2$ was added to the flask and allowed to stand for five minutes. Then 0.3 ml 10 % $AlCl_3$was added. After five minutes, 2 ml 1M Na OH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The standard curve was prepared using 20, 40, 60, 80 and 100 mg /l solution of quercetin. The total flavonoid content was expressed as mg quercetin equivalents per gram of extracts.

2.5 *In* vitro antioxidant activity

2.5.1 DPPH assay

The antioxidant activity of *S. nigrum* L. fruit methanolic extract was assessed by the DPPH assay. The DPPH radical scavenging activity was estimated based on the method Bidchol *et al*. [15]. The stock solution of standard and methanolic fruit extracts were prepared to achieve a concentration of 1 mg/ml. Five different concentrations were prepared from a stock solution (25, 50,100, 150 and 200 µg/ml). Then 1.0 ml of 0.1 mM DPPH solution was mixed with 2.0 ml of sample solution of different concentrations. The reaction mixture was incubated in room temperature in the dark for 30 min and the absorbance was read at 517 nm. The radical scavenging activity of ascorbic acid was also determined, which served as standard. The decrease in absorbance on addition of test samples was used to calculate the antiradical activity, as expressed by the inhibition percentage (1%) of DPPH radical by the following

$$\% \text{ scavenged [DPPH]} = [(Ac-As)/Ac] \times 100$$

where Ac was the absorbance of the control, and As was the absorbance of sample or standards.

2.5.2 Hydrogen peroxide Scavenging Activity

The hydrogen peroxide scavenging ability of extracts was determined according to the method of used by Bozin *et al*. [16]. 40 mM H_2O_2 and crude extracts/standard (Gallic acid) in different concentrations (25, 50,100, 150 and 200 µg/ml) were prepared in phosphate buffer (pH 7.4). An aliquot (3.4 ml) sample solution was added to 0.6 ml of H_2O_2 solution. The absorbance of resulting solutions was read at 230 nm after 10 mins. The percentage of scavenging of hydrogen peroxide of sample and standard compounds were calculated using the following equation

$$\% \text{ scavenged } [H_2O_2] = [(Ac-As)/Ac] \times 100$$

where Ac was the absorbance of the control, and As was the absorbance of sample or standards.

3. Results and discussion

The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant in taxonomically distinct[17]. In the present study *Solanum nigrum* L. fruit was extracted with methanol and investigated for the presence of total phenol content, total flavonoid content and *in vitro* antioxidant activity.

3.1 Total Flavonoid and Phenol content

Flavonoid content was calculated from the regression equation ($Y = 0.0084X + 0.011$, $R^2 = 0.9993$) of the calibration curve (Table 1) and is expressed as Quercetin equivalents (QE). The total flavonoid content of *S. nigrum* fruit methanolic extract was 3.61± 0.07mg QAE/g (Table 2). Similarly Gbadamosi and Afolayan [13] reported that the flavonoid content of ethanolic extract of *S. nigrum fruit* contain 2.11mg/g.

Flavonoid compounds are naturally occurring compounds having a polyphenolic structure. They are mostly soluble in water and are ubiquitous in nature. However, they mainly occur in a plant as sugar derivatives known as glycosides. Nearest all pigments that colour most flowers, fruits, and seeds are due to the presence of flavonoids [18]. High flavonoid contents might be related to the high chlorophyll content [19] and different phytochemical compounds present in plants. Flavonoids are concentrated in fruits, vegetables, wine tea and cocoa, their antioxidant and cardioprotective effects are attributed to the ability to inhibit lipid peroxidation, chelate redox active metals and attenuate other processes involving reactive oxygen species [20-21].

Total phenol content (TPC) content in the extract was calculated from the regression equation ($Y = 0.0035X+0.031$, $R^2 = 0.9985$) of the calibration curve (Table 1) and is expressed as gallic acid equivalents (GAE). The total phenolic content of *S.nigrum* fruit methanolic extract was 4.57± 0.57 mg GAE/g (Table 2). The F/P ratio of *S.nigrum* fruit methanolic extract was 0.78. The Flavonoids / Phenolics (F/P) ratio indicates the specificity of flavonoids among the phenolic compounds [22].

3.2 *In vitro* antioxidant assay

3.2.1 DPPH Radical Scavenging activity

Antioxidants are able to reduce free radicals by donating an electron or hydrogen atom to the free radical. The hydrogen atom transfer (HAT) activity of plant extracts was studied using the DPPH free radical and its reaction with a phenolic antioxidant. Antioxidants quench dreaded free radicals produced due to environmental and physiological stress which leads to aging, atherosclerosis and cancer [23]. Selection of appropriate phytoextracts to compare the antioxidant potential of experimental plant samples is important. There are different antioxidant components in plants which cannot measure each antioxidant component, separately, due to

complexity of the oxidation and anti-oxidation processes. Therefore various methods are used to determine the antioxidant potentiality of phytoextracts. This diversity in methods of analysis is due to the complexity of analyzed substrate, where often a mixture of various compounds with different functional groups, polarity and chemical behavior react differently [24]. In the present study methanolic fruit extracts of S. *nigrum* L. showed significant reduction of DPPH radical. At a concentration of 200µg/ml the extract significantly scavenged 88.23 % of DPPH radicals and an IC_{50} value of 70.73µg/ml (Table 3& Fig. 1) and the ascorbic acid standard IC_{50} value of 39.11µg/ml. Similar result was reported by Alam *et al*. [9] in which the DPPH scavenging activity of ethanolic extract of *S. nigrum* fruit was IC_{50}= 194.98µg/ml. Maharana *et al*. [25] also evaluated antioxidant activity of *Solanum nigrum* L. and found that the percentage of inhibition was 54.16% and the IC_{50} value was 165µg/ml for DPPH radical.

Many researchers have reported positive correlation between free radical scavenging activity and total phenolic compound. DPPH radical scavenging activity increased with the increase of phenolic compound content [26-28]. The DPPH scavenging ability of the extract may be attributed to its hydrogen donating ability.

3.2.2 Hydrogen peroxide Radical Scavenging activity

Scavenging of H2O2 by extracts may be attributed to their phenolics, which can donate electrons to H2O2, thus neutralizing it to water 29, 30. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. IC50 value of methanolic fruit extracts of S. nigrum L. and standard gallic acid for scavenging of H2O2 were 59.72 µg/ml and 43.17µg/ml (Table 3 & Fig. 2).

Thangaraj *et al* 31 similarly reported that hydrogen peroxide scavenging activity of aqueous and hydro alcoholic (methanol-water) extracts of S. nigrum fruit was found to be 77.39

Table 1: Linear equations and their R^2 values obtained from the standard calibration curves.

Assays	Calibration curve	R^2
TFC	Y= 0.0084X+0.011	0.9993
TPC	Y= 0.0035X+0.031	0.9985

Table 2: Total flavonoid, Phenol content and F/ P ratio of *S.nigrum* fruit methanolic extract

TFC (mg QAE /g)	TPC (mg GAE / g)	F/P ratio
3.61± 0.07	4.57 ± 0.57	0.78

Values were expressed as mean ± S.D. (n= 3)

Table 3: Antioxidant activity of *S. nigrum* fruit methanolic extract

Antioxidant activity	Plant extract	Standard
DPPH Scavenging activity IC_{50} (µg/ml)	70.73	39.11
Hydrogen peroxide scavenging activity IC_{50} (µg/ml)	59.72	43.17

DPPPH activity standard – Ascorbic acid, H_2O_2 scavenging activity standard – Gallic acid

and 78.25% of inhibition and IC_{50} value of 94.33 and 90.33 µg/ml respectively. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly. It probably reacts with Fe^{2+} and possibly Cu $^{2+}$ ions to form hydroxyl radical which may be the origin of many of its toxic effects[32]. Thus it is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate and removing H_2O_2 is very important throughout food systems [33, 34].

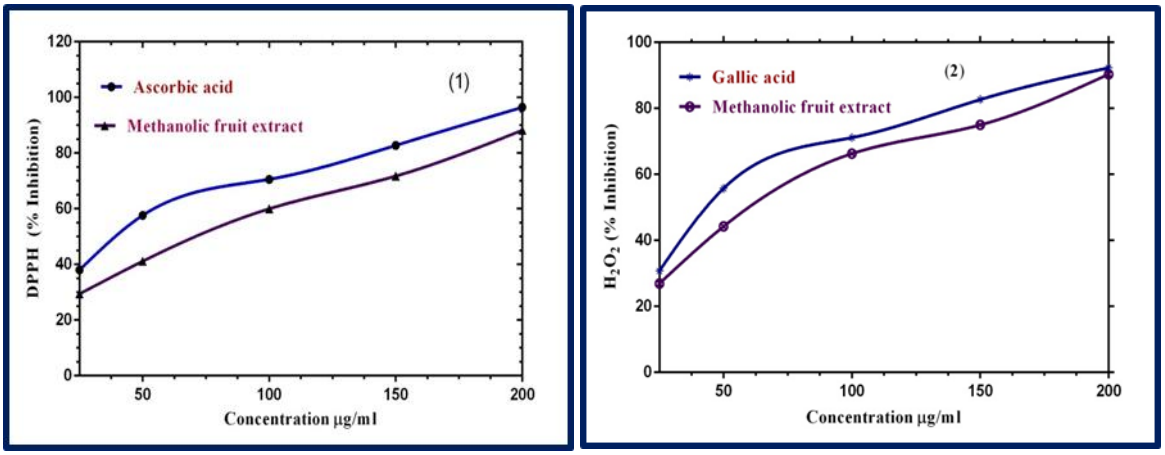

Fig 1: DPPH Scavenging activity. Figure 2. H_2O_2 Scavenging activity of *S. nigrum* fruit methanolic extract

4. Conclusion

In conclusion the results of the present study indicated the total flavonoid content (TFC) and total phenol content (TPC) in the *S. nigrum* fruit extracted with methanol and also it exhibited significant DPPH and Hydrogen peroxide free radical scavenging activity. The findings of the present study suggest that *Solanum nigrum* L**.** fruit could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases such as hypertension, arthritis, atherosclerosis and hepatic toxicity. The information from the above studies may be useful for standardization of herbal drugs and having an essential role in medicine.

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