

STUDIES ON THE PHYTOCHEMICAL SCREENING AND FREE RADICAL SCAVENGING POTENTIALS OF *SOLANUM NIGRUM* LEAVES EXTRACTSRIRAM PRASATH G^{1*}, ARCHANA PRAKASH¹, SRINIVASAN PT¹, SUBRAMANIAN S²

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ABSTRACT

Objective: *Solanum nigrum* is paramount in medicinal perspective and belongs to family Solanaceae. From different parts of the plant, significant pharmacological and biological activities have been reported previously. This study was aimed to analyze the presence of various phytoconstituents and to determine the antioxidant potential, *in vitro*.

Methods: The ethanolic extract of leaves was investigated for phytochemical analysis, vitamin, and mineral content. The antioxidant (free radical scavenging) activity of the extract was determined against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), nitric oxide (NO), and superoxide scavenging assays.

Results: Phytochemical analysis of the leaves revealed the presence of phenols, alkaloids, flavonoids, glycosides, saponins, tannins, phytosterols, and triterpenoides. The leaves extract was found to contain appreciable amounts of flavonoids and phenols. The extract showed the presence of vitamins such as ascorbic acid, folic acid, and niacinamide. It has been found that the leaves of *S. nigrum* are rich in minerals such as copper, magnesium, manganese, vanadium, chromium, calcium, zinc, sodium, and potassium. *S. nigrum* leaves extract was found to be antioxidant in nature which is evident from DPPH, ABTS, NO, and superoxide radical scavenging assays.

Conclusion: The results of the present findings suggest that *S. nigrum* contains biologically important phytoconstituents, significant amounts of vitamins and minerals. In addition, *S. nigrum* leaves extract exert free radical scavenging potential *in vitro*.

Keywords: Medicinal plants, *Solanum nigrum* leaves, Phytoconstituents, Antioxidant.

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INTRODUCTION

Plants continue to be an important therapeutic aid for alleviating ailments of humankind. Search for eternal health, longevity of life, remedy to relieve pain and discomfort prompted, the early man to explore his immediate surroundings to search for therapeutic agents. Medicinal plants since times immemorial have been used in virtually all cultures as a rich source of medicine. The World Health Organization estimated that more than 80% of the population in developing countries relies on traditional medicine, mostly herbal medicines, for their primary health-care needs due to their availability, accessibility, and affordability. India is the largest producer of medicinal herbs and is called as "botanical garden of the world" [1].

Plants have the ability to synthesize a wide array of chemical compounds that protect them against the attack from a wide variety of predators such as microbes, insects, and herbivorous mammals. Some of these compounds, while being toxic to plant predators, turn out to have beneficial effects when used to treat human diseases. Such secondary metabolites are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives.

Natural products such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. The therapeutic effects of herbs/herbal extract cannot be determined unless its active ingredient or cofactors are identified. One-way to indicate strength is standardization to one or several marker compounds that are believed to be mainly responsible for the biological effects. Although phytotherapy continues to be used in several countries, most of the traditional medicinal plants have not received scientific or medical scrutiny. One such medicinal plant, which lacks scientific evidence for its wide folklore use, is *S. nigrum*.

S. nigrum

S. nigrum (European Black Nightshade or locally known as "black nightshade," Duscle, Garden Nightshade, Hound's Berry, Petty Morel, Wonder Berry, Small-fruited black nightshade or popolo) is a species in the *Solanum* genus which belongs to the family Solanaceae. The flowers have petals greenish to whitish, recurved when aged and surround prominent bright yellow anthers. In Tamil, *S. nigrum* is called as manathakkali. *S. nigrum* has been used traditionally to treat various ailments such as pain, inflammation fever, and enteric diseases [2-4]. It possesses many activities such as antitumorigenic, antioxidant [5], hepatoprotective [6], and diuretic [7]. The methanolic extract of berries of the plant *S. nigrum* possessed antioxidant and cardioprotective activity [8,9]. The ethanolic extract of *S. nigrum* was found to possess the antidiabetic property [10].

S. nigrum contains many active components are glycoalkaloids, glycoproteins, and polysaccharides, polyphenolic compounds such as gallic acid, catechin, protocatechuic acid, caffeic acid, epicatechin, rutin, and naringenin [11]. In this study, an attempt has been made to assess the nutritive value as well as the antioxidant potential of *S. nigrum* leaves extract.

METHODS

Plant material

The leaves of *S. nigrum* were collected from the local market in Chennai. The leaves were identified and authenticated by a taxonomist at the center for Advanced Studies in Botany, University of Madras.

Preparation of extract

The leaves of the plant were collected and washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then, these leaves (2 kg) were shadow dried without any contamination for about 3-4 weeks.

Delipidation and extraction

The leaves of *S. nigrum* were dried at room temperature and powdered in an electrical grinder, which was then stored in an airtight container at 5°C until further use. The powdered leaves were delipidated with petroleum ether (60-80°C) for overnight. Almost all the chlorophyll and lipid are deposited on the side of the flask and were removed carefully. The extraction was done with ethanol. The dried powder was subjected to Soxhlet using ethanol. The ethanolic extract was filtered, dried, and weighed. The extract obtained was evaporated in a rotary evaporator to get a powdery mass. The extract was dried under reduced pressure using rotator evaporator to get the crude. It was stored below 4°C until further use.

Preparation of ash

The *S. nigrum* leaves were shadow dried, finely powdered using the electrical grinder. 100 g of properly powdered seeds was taken in a vitreous crucible and placed in an electrical muffle furnace overnight maintaining its temperature between 430°C and 450°C because the loss of zinc may occur at >450°C and loss of potassium occur if the temperature is too high (>480°C). The ash was then removed and dried in a vacuum desiccator. The yield of ash in the powdered leaves was found to be 6.03 g/100 g.

Trace element analysis

About 2 g of ash was digested with a triple acid mixture comprising of nitric acid, sulfuric acid, and perchloric acid in the ratio of 11:6:3, respectively, for the complete removal of organic content. The digested sample was made up to 100 mL using deionized water, and this sample is used for the assay of trace elements through atomic absorption spectroscopy using hollow cathode lamps.

Instrumentation and analytical procedures

The determination of the trace element content of *S. nigrum* was carried out using an atomic absorption spectrometer (GBC-Avanta, Australia).

Preliminary phytochemical screening

The ethanolic extract of *S. nigrum* leaves was subjected to preliminary phytochemical screening of various plant constituents [12,13].

Determination of total phenolic content

Total polyphenol content in the ethanol extract of *S. nigrum* was determined according to the Folin-Ciocalteu colorimetric method [14,15]. A standard curve was built with gallic acid reference solutions. Aliquots ranging from 2 to 10 mL of standard aqueous gallic acid solution (100 µg/mL) were pipetted into 100 mL volumetric flasks containing 70 mL of distilled water. Folin-Ciocalteu reagent (5 mL) and 10 mL of saturated sodium bicarbonate solution were added, and the volume was made up to 100 mL with distilled water. The solution was thoroughly mixed. The blank was prepared in the same manner but without gallic acid. After 1 hr of incubation at room temperature, the absorbance was measured at 760 nm. The samples were prepared in triplicates for each analysis, and the mean value was calculated. For the determination of the total phenolic content of *S. nigrum* leaves extract, aqueous solutions at the final concentration of 20 µg/mL were used; proceeding in the same manner described for the reference solutions, and the total polyphenolic content was expressed as mg/g of gallic acid equivalents.

Determination of total flavonoid content

Total flavonoid content in the ethanolic extract of *S. nigrum* leaves was determined according to the method of Quettier-Deleu *et al.*, 2000, with minor modifications [16]. A standard curve was built with quercetin reference solutions. Aliquots ranging from 2 to 8 mL of standard quercetin ethanol extract solution (50 µg/mL) were pipetted into 25 mL volumetric flasks containing 1 mL of 2% aluminum chloride dissolved in ethanol, and the volume was made up with ethanol. The blank was prepared by diluting 1 mL of 2% aluminum chloride dissolved in ethanol in a 25 mL volumetric flask with ethanol. After 1 hr at room temperature, the absorbance was measured at 420 nm. *S. nigrum* extract was evaluated at a final concentration of 20 µg/mL, proceeding

in the same manner described for the reference solutions and the total flavonoid content was calculated as quercetin equivalents (mg/g) from a calibration curve. The samples were prepared in triplicate for each analysis, and the mean value of absorbance was recorded.

In vitro antioxidant assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging capacity of the ethanolic extract of *S. nigrum* was determined using DPPH [17]. DPPH (200 µM) solution was prepared in 95% methanol. From the stock plant extract solution 200, 400, 800, and 1000 µg/mL were taken in five test tubes. 0.5 mL of freshly prepared DPPH solution was incubated with test drug and after 10 minutes, absorbance was taken as 517 nm using a spectrophotometer. Standard ascorbic acid was used as a reference.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

ABTS radical scavenging activity of ethanolic extract of *S. nigrum* was determined [18]. Briefly, ABTS radical cation (ABTS^{•+}) was produced by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 hrs before use. Then, ABTS^{•+} solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. To 3.0 mL of diluted ABTS^{•+} solution, different concentrations (200, 400, 800, and 1000 µg/mL) of leaves extract in ethanol were added and after 1 minute, the decrease in absorbance was measured at 734 nm spectrophotometrically.

Assay for nitric oxide (NO) scavenging activity

Sodium nitroprusside (5 mM) in phosphate buffer pH 7.7 was incubated with 200, 400, 600, and 800 µg/mL concentrations of drug dissolved in a suitable solvent (alcohol), and tubes were incubated at 25°C for 120 minutes. At intervals, 0.5 mL of incubation solution was removed and diluted with 0.5 mL of Griess reagent. The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent N-naphthyl ethylene diamine was measured at 546 nm [19].

Superoxide anion (SO) radical scavenging assay

The superoxide radical scavenging activity of *S. nigrum* was measured [20]. In this method, the activity is measured by reduction of riboflavin/light/nitro blue tetrazolium (NBT). The 1 mL of reaction mixture contained phosphate buffer, NADH, NBT, and various concentrations (200, 400, 800, and 1000 µg/mL) of the sample solution. The method is based on the generation of superoxide radical by auto-oxidation of riboflavin in the presence of light. The Superoxide radical reduces NBT to a blue-colored formazan that can be measured at 560 nm.

RESULTS

The phytochemical analysis of *S. nigrum* leaves extract is presented in Table 1. The leaves extract is found to contain alkaloids, flavonoids, saponins, tannins, phytosterol, triterpenoids, glycosides, and Phenols.

The total phenolic and flavonoid contents were found to be 322±2.66 mg/g equivalents of gallic acid and 226±2.81 mg/g equivalents of quercetin, respectively.

Table 2 represents the mineral content of *S. nigrum* leaves extract. It has been found that the leaves of *S. nigrum* are rich in minerals such as copper, magnesium, manganese, vanadium, chromium, calcium, zinc, sodium, and potassium.

Figs. 1 and 2 represent the retention time for vitamin (standard) and the vitamin content of *S. nigrum* leaves. The leaves content contains ascorbic acid, folic acid, and niacinamide in appreciable amounts.

In vitro antioxidant potential of *S. nigrum* leaves

Figs. 3 and 4 show the dose-dependent effect of *S. nigrum* on the percentage inhibition of DPPH and ABTS radicals present in the reaction

mixtures. The ethanolic extract of *S. nigrum* scavenges DPPH and ABTS radical in a concentration-dependent manner. The leaves extract of *S. nigrum* significantly and concentration-dependently reduced DPPH and ABTS radicals. However, at a concentration of 1000 µg/mL, the extract significantly scavenged 84.45% of DPPH radicals and 85.45% ABTS radicals.

The NO and superoxide scavenging potential of the leaves extract are depicted in Figs. 5 and 6, respectively. The extract exhibited a maximum of 74.15% NO scavenging potential (Fig. 5) and 86.35% superoxide scavenging activity (Fig. 6).

DISCUSSION

Plants contain bioactive chemical substances that produce remarkable physiological and biochemical actions in the human body. These

bioactive constituents include alkaloids, tannin, flavonoids, and phenolic compounds. Plant-derived natural products have received considerable attention in recent years due to diverse pharmacological properties including antioxidant and antitumor activity. Natural products/dietary phytochemical have aroused considerable interest in recent years as potential therapeutic agents to counteract free radical mediated diseases [21].

The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds. Phytochemicals include compounds with various biological properties (i.e., antioxidant, antiproliferative, and DNA repair) which allow plants to cope up with environmental challenges including exposure to radiation and toxins [22]. They are bioactive compounds (secondary metabolites) found in plants that work with nutrients and dietary fibers to protect against diseases. Certain phytochemicals are almost structurally identical to insulin and act as an "insulin-like substances" that helps in the remedy of Type I and Type II diabetes. Most plants with antidiabetic properties have been found to contain secondary metabolites such as glycosides, alkaloids, and flavonoids [23]. It has been shown that many plants exhibit efficient antioxidant properties owing to their phenolic constituents. Earlier report indicated that phytochemical screening of this species indicates the presence of alkaloids, flavonols, flavones, flavanols, saponin, flavonoids, and steroids [24]. Alkaloids such as soladunalinidine, solasonine, and solamargine have been isolated from leaf of *Solanum* species [25].

The inorganic elements have been investigated as a potential preventive and treatment agents for both Type 1 and Type 2 diabetes mellitus. The differences in the concentration of these elements are attributed to the soil composition and climate, in which a plant grows. It has been found that the leaves of *S. nigrum* are rich in minerals such as copper, magnesium, vanadium, calcium, zinc, sodium, phosphorus and potassium which do play a pivotal role in insulin metabolism.

The leaves content contains ascorbic acid, folic acid, and niacinamide in appreciable amounts. Metal as micronutrient is important for the normal functioning of vital organs and is present in many enzymes which activate them, thereby influence the biochemical processes that are required in our diet.

The present study has shown that the *S. nigrum* leaves examined have an appreciable content of contain Ascorbic acid, folic acid, and niacinamide. The leaves contain minerals with an abundance of them in calcium, zinc, iron, manganese, and magnesium while they were least in

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

Phytoconstituents	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Phytosterol	+
Diterpenes	-
Triterpenoids	+
Glycosides	+
Anthraquinones	-
Phenols	+

Solanum nigrum: *S. nigrum*

Table 2: Mineral composition of *S. nigrum* leaves

Element	Concentration (µg/g)
Calcium (Ca)	210
Magnesium (Mg)	103
Sodium (Na)	110
Potassium (K)	50
Zinc (Zn)	10
Iron (Fe)	130
Copper (Cu)	2.3
Vanadium (V)	0.8
Phosphorus (P)	750

Solanum nigrum: *S. nigrum*

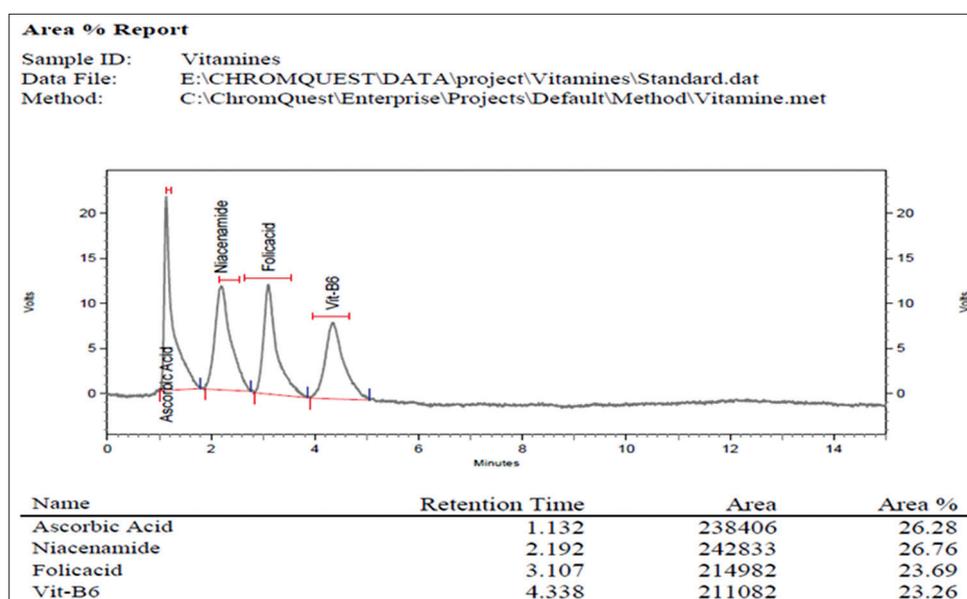


Fig. 1: Retention time for vitamins (standard)

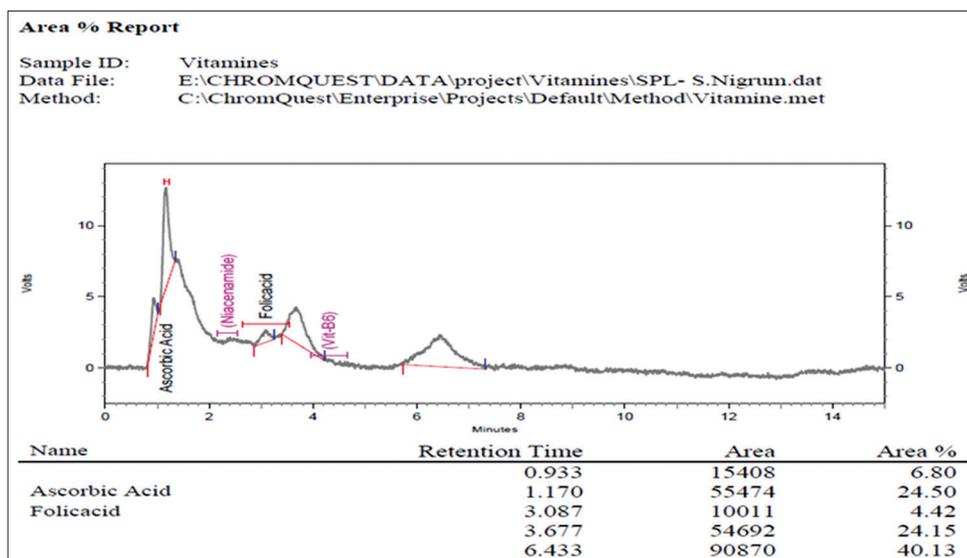


Fig. 2: Vitamin content of *Solanum nigrum* leaves extract

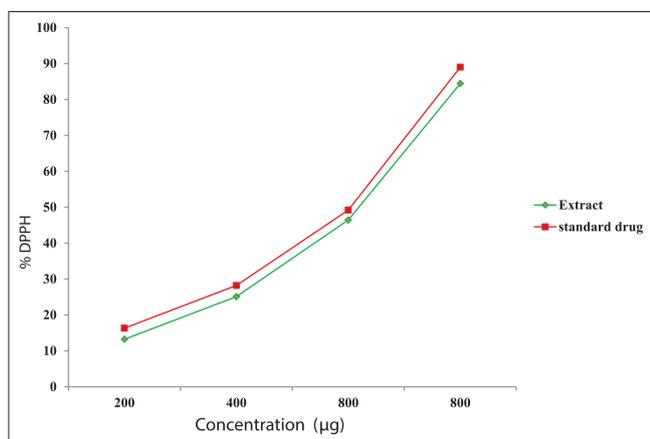


Fig. 3: 2,2-diphenyl-1-picrylhydrazyl radical scavenging potential of *Solanum nigrum* leaves extract

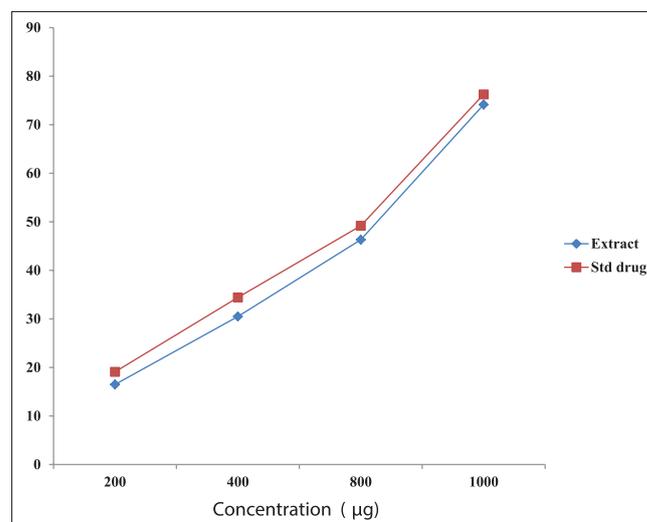


Fig. 5: Nitric oxide scavenging potential of *Solanum nigrum* leaves extract

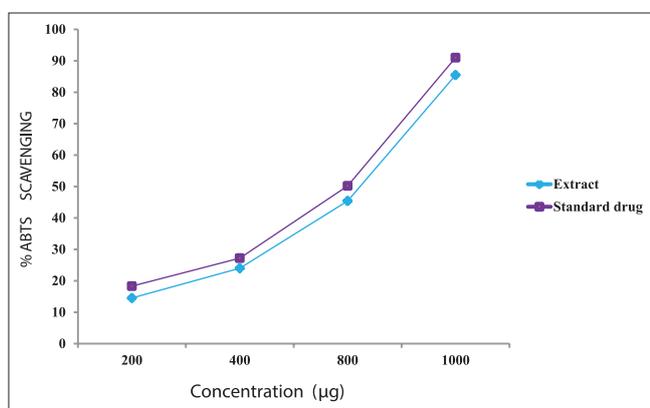


Fig. 4: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging potential of *Solanum nigrum* leaves extract

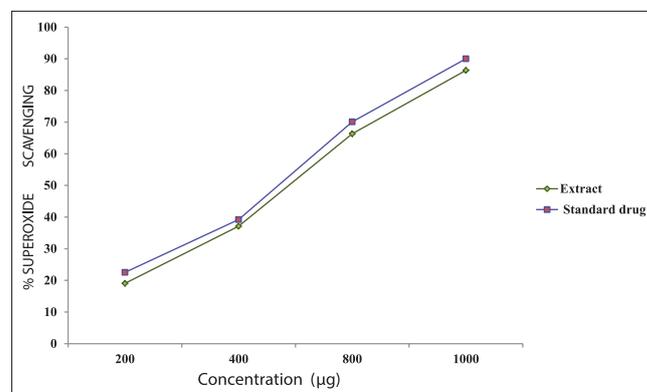


Fig. 6: Superoxide radical scavenging potential of *Solanum nigrum* leaves extract

potassium. The results suggest that the leaves if consumed in sufficient amount would contribute greatly toward meeting human nutritional requirement for normal growth and adequate protection against free radical mediated diseases [26].

Phenolic compounds are known to be the most important antioxidants of plant materials. They contribute one of the major groups and

compounds acting as primary antioxidants or free radical terminators. Antioxidant activity of a phenolic compound is based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties. Flavonoids

are a widespread group of natural compounds which possess a broad spectrum of chemical and biological activities including radical scavenging activity [27]. The presence of these compounds such as total phenolics and flavonoids in *S. nigrum* leaves extract may account for the antioxidant potential.

The free radical scavenging potential of natural products can be assessed by several assays. Among them, DPPH, ABTS, NO, and superoxide radical scavenging assays are routinely practiced for the assessment of antioxidant properties of different natural compounds, as they are easy, affordable, and reliable. In the present study, the antioxidant potential of *S. nigrum* leaves extract is examined by DPPH, ABTS, NO, and superoxide radical scavenging assays.

The ability of natural compounds to scavenge the DPPH radical can be expressed as its magnitude of antioxidative ability. DPPH radical in alcoholic solution is deep purple with an absorption peak at 515 nm. DPPH assay is based on the principle that DPPH radical on accepting a hydrogen atom from the scavenger molecule, i.e., antioxidant, results in reduction of unpaired valence electron at one atom of nitrogen bridge in DPPH leading to the change of purple to yellow with concomitant decrease in absorbance at 515 nm. The change in color from deep purple to yellow or the decrease in intensity signifies the antioxidant potential of the test compound.

ABTS assay is used for the screening of antioxidant activity of both water and lipid soluble compounds. The assay involves reduction of the color intensity of ethanolic solution containing pre-formed radical monocation of ABTS, generated by oxidation of ABTS with potassium persulfate due to the radical scavenging activity of antioxidants. The change in color intensity is proportional to the antioxidant efficiency of compounds.

The ethanolic extract of *S. nigrum* scavenges DPPH and ABTS radical in a concentration-dependent manner. The antioxidants react with DPPH, a purple-colored stable free radical, and convert it into a colorless α -diphenyl- β -picryl hydrazine. The amount of DPPH reduced could be quantified by measuring a decrease in absorbance at 517 nm. ABTS assay is used for the screening of antioxidant activity of both water and lipid soluble compounds. The assay involves reduction of the color intensity of ethanolic solution containing pre-formed radical monocation of ABTS, generated by oxidation of ABTS with potassium persulfate due to the radical scavenging activity of antioxidants. The change in color intensity is proportional to the antioxidant efficiency of the compound. The leaves extract of *S. nigrum* significantly and concentration-dependently reduced DPPH and ABTS radicals. However, at a concentration of 1000 μ g/mL, the extract significantly scavenged 84.45% of DPPH radicals and 85.45% ABTS radicals.

The primary free radical in most biological systems is superoxide ($O_2^{\cdot-}$). Although $O_2^{\cdot-}$ itself is quite unreactive compared to the other radicals, it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals. From the investigations, it was found that the *S. nigrum* leaf extract scavenged $O_2^{\cdot-}$ significantly and in a concentration-dependent manner. The $O_2^{\cdot-}$ scavenging activity was determined by phenazine methosulfate/NADH-NBT system wherein $O_2^{\cdot-}$ derived from dissolved oxygen by phenazine methosulfate/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of SOs in the reaction mixture. The leaves extract exhibited a maximum of 86.35% superoxide scavenging activity with a significant extent at a concentration of 1000 μ g/mL. *S. nigrum* leaf extract at a concentration of 1000 μ g/mL also quenched 74.15% NO radical.

NO acts as neurotransmitter through exerting their effect on different body operations such as neurotransmission, synaptic plasticity, vasodilation, and central nervous system memory [28,29]. Besides the key role of NO in facilitating normal function, it has been observed that NO has been associated with pathophysiologic states such as neurodegenerative and Alzheimer's disease. Excessive release of NO in

the body can cause DNA fragmentation, cell damage, and neuronal cell death [30,31]. Plants can play key role in reducing the amount of NO through their efficient NO scavenging activity.

S. nigrum exerts potent antioxidant activity. The antioxidants act as defense mechanism that protects against oxidative damage and includes compounds to remove or repair damaged molecules, and sufficient intake of antioxidants is supposed to protect against diseases. The phytochemical antioxidants have significant potential to neutralize free radicals or oxidants responsible for the cell damage. The present study thus scientifically validates and strengthens the candidature of *S. nigrum* in the preparation of medicinal aids to combat the wide spectrum of myriad diseases arising due to oxidative stress.

CONCLUSION

The results of the present study showed that *S. nigrum* leaves extract contains biologically active ingredients such as alkaloids, flavonoids, glycosides, saponins, triterpenoids, and phenols which possess a wide array of pharmacological properties. *S. nigrum* extract was found to contain appreciable amounts of vitamins and minerals. The leaves extract was found to be antioxidant in nature which is evident from DPPH, ABTS, NO, and superoxide radical scavenging assays. These findings suggest that *S. nigrum* can be used in the treatment of free radical mediated diseases such as diabetes and cancer.

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