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Original Article

Phytochemical Screening and Antioxidant Activity of Ethanolic Extract of Leaves of *Solanum nigrum* L.

Veerapagu M [1,] *, Jeya K R 2, Sakthivel V [1]

[1] Department of Biotechnology, P.G.Extension Centre, Bharathidasan University, Kurumbalur- 621107, Perambalur, Tamilnadu, India.

[2] Department of Biotechnology, Bharathidasan University Constituent Model College (W), Veppur - 621717, Perambalur, Tamilnadu

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ABSTRACT

Solanum nigrum Linn. (Solanaceae) has been extensively used in traditional medicine in India and other parts of the world to cure liver disorders, chronic skin ailments (psoriasis and ringworm),inflammatory conditions, painful periods, fevers, diarrhea, eye diseases etc. In the present study crude ethanolic extracts of leaves of *Solanum nigrum* were prepared and evaluated for the presence of phytochemicals, total phenol content, total flavonoid content and antioxidant activity by DPPH assay. Phytochemical analysis of the leaves revealed the presence of alkaloids, flavonoids, phenols, saponins, tannins and terpenoids. The total phenol content (TPC), total flavonoid content (TFC) and flavonoid/ Phenol (F/P) of ethanolic extract of *S.nigrum* leaves were 2.5mg GAE/g, 1.8mg QAE/g and 0.72. The leaf extract shown marked antioxidant activity with an IC50 value of 83µg/ml for DPPH radical. Hence based on the above results it was concluded that the ethanol extract of leaves of S. nigrum showed significant antioxidant activity.

Keywords: *S.nigrum.* Flavonoid, Phenol, DPPH assay, antioxidant activity

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

Corresponding author *
Dr.M.Veerapagu,
Assistant Professor,
Department of Biotechnology,
P.G.Extension Centre, Bharathidaan University,
Kurumbalur- 621107, Perambalur, Tamilnadu.
Email: mveerapagu@gmail.com

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 found in disturbed habitats, distributed throughout India. The plant has a great medicinal value. The leaves are known to be used to treat headache & diseases of nose, ringworm[7], heart & liver ailments, wounds & burns[8], toothache[9]. The ethnomedical information cited that hot aqueous extract of dried leaves is used for its antidiabetic[10], antiviral[11], antipyretic, anticonvulsant, sedative, antimalarial, antispasmodic & diaphoretic[12], molluscicidal[13], anti-bronchitis & anti-gastralgia[14] activities. The leaves are reported to contain several constituents e.g. flavonols like Quercetin, Hyperoside, Steroids and alkaloids like Sitosterol, Solamargine, Stigmastrol, Campesterol, Cholesterol, Solasodine and Sapogenin like Tigogenin[15].In this study anattempt has been taken to investigate the presence of phytochemicals and *in vitro* antioxidant property of the ethanolic leaf extracts of *S. nigrum*L.

2. MATERIALS AND METHODS

Plant material

The leaves of *S. nigrum* L. were procured from local UzhavarSandhai, Perambalur. . The plant leaves were dried in shadow for 3- 4 days. Then the leaves were ground to obtain a fine powder. The fine powder is collected and used for extraction.

Extract preparation

The dried leaf powder 100g was soaked in 500ml of ethanol at room temperature in glass stoppered bottle container for two days. The extracts were filtered first 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 phytochemicals and antioxidant activity.

Phytochemical screening

Alkaloids: A fraction of extract was treated with 3-5drops of Wagner's reagent and the appearance of reddish brown precipitate (or colouration) indicates the presence of alkaloids.

Flavonoids: 2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Phenolics: To 1 ml of extracts, 2 ml of distilled water and few drops of 10% ferric chloride solution were added. Formation of blue or green colour indicates the presence of phenols.

Saponins: To 2mls of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Tannins: 2mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Terpenoids : 2 mL of extracts were treated with 2 mL of chloroform and concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colour formation at the interface confirms the presence of terpenoids.

Determination of total phenol content

Total phenol content was determined by using FolinCiocalteu reagent method[16]. *S.nigrum* leaf ethanol extract 1ml (1mg/ml) was mixed with 5ml of FoilnCiocalteu'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 50, 100, 150, 200 and 250 mg /l solution of gallic acid. The total phenol content s were expressed as mg/g of gallic acid equivalent of dry extract.

Determination of total flavonoid content

Total flavonoid content of *S.nigrum* leaf ethanolextract was measured by the aluminium chloride colorimetric assay[17]. 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 NaOH 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.

In vitro antioxidant DPPH assay

The antioxidant activity of *Solanum nigrum* L. leaf ethanol extract was assessed by the DPPH assay. The DPPH radical scavenging activity was estimated based on the method Bidchol et al. (2011).The stock solution of standard and ethanolic leaf extracts were prepared achieve a concentration of 1 mg/ml. Furthermore, five different concentrations were prepared from a stock solution (25, 50,75,100, 125 µg/ml). Briefly, 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 recorded at 517 nm. 1ml ethanol with 2.0 ml extracts solution was used as a blank, DPPH solution (1.0

ml, 0.1 mM) with ethanol (2.0 ml) was served as control. The radical scavenging activity of ascorbic acid was also determined, which served as positive control. The decrease in absorbance on addition of test samples was used to calculate the antiradical activity, as expressed by the inhibition percentage (I %) of DPPH radical by following

Inhibition % = [(Ac-As)/Ac] x 100

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[19]. In the present study *Solanum nigtum* L. leaf was extracted with ethanol and investigated for the presence of various phytochemicals and the results are presented in Table 1**.** Phytochemical screening of ethanolic leaf extract revealed the presence of alkaloid, flavonoids, phenols, Saponins, tannins and Terpenoids.

Table 1: Phytochemical analysis of *Solanum nigrum* L.

S.No	Phytochemical test	Ethanolicleaf extract
1	Alkaloid test	+
2	Flavonoids	+
3	Phenols	+
4	Saponins	+
5	Tannins	+
6	Terpenoids	+

Similar results was reported by Karunakar (2017)[20] in which the qualitative screening of phytochemical constituents on leaf extracts of *S.nigrum* revealed the presence of alkaloid, saponin, tannins, flavonoids, proteins etc. Gayathri and Karthika (2016)[21] have also reported that the leaves of *S. nigrum* showed the presence of alkaloids, flavonoids, steroids, terpenoids, quinone, phenols, starch, cellulose, oil and fat. In the present study, the observed alkaloid content in *S.nigrum* could be responsible for their much acclaimed medicinal values though the exact mode of action is poorly understood.

Saponins are a special class glycosides which have soapy characteristics Some of the characteristics of saponin include formation of foams in aqueous solution, haemolytic activity, cholesterol binding properties and bitterness[22].Tannins are also known antimicrobial agent. Tannins are water soluble plant polyphenols that precipitate proteins. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins[23].

Total phenolic content (TPC) in the extract was assessed by FolinCiocalteu reagent and result is expressed as µggallic acid equivalents per gram extract with reference to gallic acid. The total phenolic content of *S.nigrum* leaf ethanol extract was 2.5mg GAE/g. The total flavonoid content of *S.nigrum* leaf ethanol extract was 1.8mg QAE/g. The Flavonoids / Phenolics (F/P) ratio indicates the specificity of flavonoids among the phenolic compounds[24] .The F/P ratio of *S.nigrum* leaf ethanol extract was 0.72.

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[25]. High flavonoid contents might be related to the high chlorophyll content[26] and different phytochemical compounds present in leaves. Flavanoids 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[27,28].

Invitro antioxidant assay

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. Antioxidants inhibit oxidation of food also quench dreaded free radicals produced due to environmental and physiological stress which leads to aging, atherosclerosis and cancer[29]. 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 it is required to use various methods to determine AE to provide a comprehensive picture of 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[30].

In the present study ethanol leaf extracts of S. *nigrum*L. showed significant antioxidant activity.The percentage of inhibition in DPPH in different concentration of 25, 50, 75,100 and , 125 µg/ were 28.75, 37.5, 43.75, 67.5 and 87.5 for ethanol leaf extract and were 31.25, 46.25, 62.5, 75 and 92.5 for ascorbic acid used as positive control respectively (Table.2& fig 1.) . The IC_{50} value of ascorbic acid and *S.nigrum*L. ethanol leaf extract were 55.6µg/ml and 83 µg/ml.

Table 2: Antioxidant activity

Extract/ Standard	Concentration (µg/ml)	Percentage inhibition	IC_{50} (µg/ml)
Ethanol leaf extract	25	28.75	
	50	37.5	
	75	43.75	83
	100	67.5	
	125	87.5	
Ascorbic acid	25	31.25	
	50	46.25	
	75	62.5	55.6
	100	75	
	125	92.5	

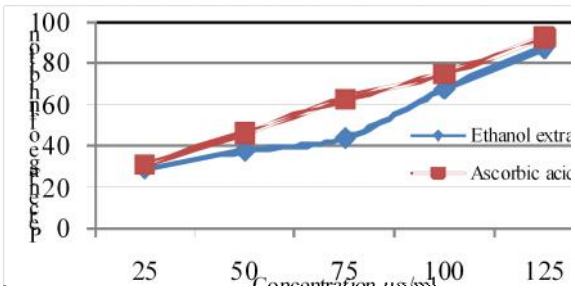

Fig 1: DPPH scavenging activity of ethanolic extracts of *S. nigrum v* leaf *Solanum nigrum* L.leaf extract

Similar results was reported by Maharana et al. (2010) [15] who 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. Abdulkadira et al. (2016) [31] evaluated the antioxidant activity of the extract of fruit, leaf, and stem of *S. torvum*. They reported that extracts of leaf showed a significantly higher percentage of inhibition (78.7%) than the stem (56.3%) and fruit (33%), whereas the extracts of stem showed a significantly higher percentage of inhibition than fruit extract.

3. CONCLUSION

In conclusion the results of the present study indicated the presence of phytochemicals alkaloids, flavonoids, phenols, saponins, tannins and terpenoids in the S.nigrum leaf extracted with ethanol and also leaf extract exhibited significant free radical scavenging activity. The findings of the present study suggest that *Solanumnigrum*L.*leaves* 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. The above studies provide information in respect of their chemical constituents and antioxidant property which may be useful for standardization of herbal drugs and having an essential role in medicine.

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