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Antioxygenic Activity of *Solanum nigrum L.* Leaves in Sunflower Oil Model System and Its Thermal Stability

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Abstract

The antioxygenic activity of *Solanum nigrum L.* leaves and its various solvent extracts were evaluated using sunflower oil model system. Leaf powder and its methanol/water (80:20) soluble fraction showed strong antioxygenic activity in refined sunflower oil. On the other hand, ethyl acetate fraction exhibited marginal antioxygenic activity, whereas the water soluble fraction was practically devoid of any activity in refined sunflower oil. Thermal stability of different extracts of *Solanum nigrum L.* leaves heated at 80˚C in refined sunflower oil also indicated the strong efficacy of methanol/water (80:20) extract to inhibit thermal oxidation. *Solanum nigrum L.* contain high levels of magnesium (239.0 mg/100g) and phosphorous (80.3 mg/100g). Fatty acid analysis of the lipid extracted from *Solanum nigrum L.* leaves indicated the presence of linoleic (59.1%) as a major fatty acid. The result of this study confirmed the presence of antioxygenic compounds in leaves, in particular its methanol/water (80:20) extracts showed great potential as a natural antioxidant to inhibit lipid peroxidation in foods.

Keywords

***Solanum nigrum L.*, Antioxygenic Activity, Solvent Extraction, Thermal Stability Phenolics, Flavonoids**

1. Introduction

Lipid peroxidation is one of the major causes of deterioration during the storage and processing of foods which

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leads to the development of off flavour as well as nutritional loss [1]-[5]. Rancidity development is an oxidative change that can be blocked by antioxidants preventing the formation of free radicals through the donation of electrons or hydrogen ions. Therefore, much attention has been paid to the antioxidants, which are expected to prevent food and living systems from peroxidative damage.

Synthetic antioxidants such as BHA, BHT, TBHQ have been widely used to prevent the oxidation of oils and fats to extend the shelf life of lipid containing foods [6]. Although these synthetic antioxidants are efficient and relatively cheap, their use in foods has suffered severe criticism as they are suspected of having some toxic properties [2] [3] [7] [8]. Therefore, there is a need for effective antioxidants from natural sources as alternatives [9]. In the past few years, several herbs, spices and cereals [10]-[12] have been expected to provide significant protection in retarding lipid peroxidation. However, among them rosemary and tocopherol (vit E) have commercial significance. *Solanum nigrum L.* is an annual herbaceous plant and may sometimes be perinneal. The leaves are alternate and sometimes ovate with irregular toothed wavy margin. Occasionally, leaves are used as vegetables in soup and as spinach in some parts of Nigeria [13]. Nutrition potential and phyto chemical contents of *Solanum nigrum L.* leaves have been reported by Akugbagwo [13] [14]. Various workers have also reported the *invitro* and *invivo* antioxygenic activities of *Solanum nigrum L.* leaves and its fruits in different solvent extracts [15]-[18]. Antioxygenic activity of *Solanum nigrum L.* is generally believed to be due to the presence of phenolics and flavonoids in them [15] [19] [20]. However, there is no systematic study on the antioxygenic activity of various fractions of *Solanum nigrum L.* in sunflower oil or food products. Also, it has been reported that antioxygenic activity of various extracts of herbs/plants varies considerably on the nature of the substrate and source of herbs/plants [21]-[23]. Therefore, the present study addresses the utilization of *Solanum nigrum L.* leaves and their solvent extractives as a source of natural antioxidant by evaluating its antioxygenic activities in sunflower oil model system and its thermal stability at elevated temperature.

2. Materials and Methods

Refined sunflower oil (Sunpure brand) without added synthetic antioxidant was supplied by sunflower oil producers (M/s MK Agrotech, Srirangapattanam, Karnataka, India). Fresh leaves of *Solanum nigrum L.* was procured from local market. Tertiary Butyl Hydro Quinone (TBHQ) and catechin were procured from Sigma chemicals, Mumbai, India. Chloroform, methanol, ethyl acetate, petroleum ether (40˚C - 60˚C) and gallic acid were procured from E-Merck, Mumbai, India. All other chemicals were of analytical reagent grade and used as such.

2.1. Preparation of *Solanum nigrum L.* Leaves Powder

Fresh *Solanum nigrum L.* leaves were washed in running tap water to remove the adhering dust and dirt. Washed leaves were dried in a fluidized bed drier at 60˚C (Model 30D, Chemec Engineering, Mumbai, India) to a moisture level of 7% - 8%. Dehydrated leaves of *Solanum nigrum L.* were ground to a fine powder in an ultra centrifugal mill (Model Restch R1, Haan, Germany) using 1 mm sieve and used for experimental studies.

2.2. Solvent Extraction Procedure

Solanum nigrum L. leaves (20 g) powder was taken in a jacket fitted with 250 ml round bottom flask and individually extracted with approximately 150 ml each of methanol, methanol/water (80:20), ethyl acetate and water in a soxhlet extraction apparatus (M/S Jain Scientific, Ambala contt, India) at 60˚C for 16 h. Each fraction was separately evaporated using a rotary vacuum evaporator (Model Superfit PBU6 Continental instruments, Mumbai, India), and preserved at 5˚C prior to use. The percentage yield of ethyl acetate, methanol, methanol/water (80:20) and water extracts were 4.82%, 18.65%, 25.90%, 25.91% respectively. A flow chart for the preparation of various fractions from *Solanum nigrum L.* is given in **Figure 1**.

2.3. Extraction and Estimation of Total Phenolics and Flavonoids

Dry powder of *Solanum nigrum L.* leaves (2 g) as well as their solvent extracts (0.1 g) were extracted with 25 ml of 70% methanol for 1 h under sonication and the extracts were filtered. The residues were re-extracted for 1 h and both the filtrates were combined and made up to 50 ml with 70% methanol. The total phenolics and flavonoids were estimated according to the method of Gerard and Roberts [24].

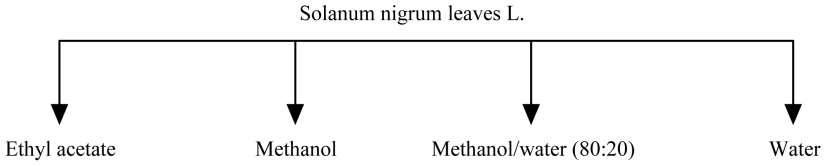

Figure 1. Flow chart for the solvent extraction of *Solanum nigrum L.* leaves.

2.4. Test for Antioxygenic Activity in Sunflower Oil

The antioxygenic activity of *Solanum nigrum L.* leaves were evaluated in refined sunflower oil. 0.5, 1.0 and 2.0 g powder of *Solanum nigrum L.* leaves and an equivalent amount (2 g of powder) of its various fractions were incorporated in to 100 g of sunflower oil, incubated in 250 ml glass beaker at 37˚C ± 1˚C. A synthetic antioxidant TBHQ (200 ppm) was also tested for its antioxygenic activity for comparison purpose. Initially and after regular intervals of 15 days, 20 g samples were removed and analysed for peroxide value (PV) as well as thiobarbituric acid (TBA) values by the methods reported earlier [25]. Antioxygenic activity was calculated as the ratio between the peroxide or thiobarbituric acid value of control and the peroxide or thiobarbituric acid value of samples.

2.5. Test for Thermal Stability in Sunflower Oil

Thermal stability of *Solanum nigrum L.* leaves (2%) and its various solvent extracts (equivalent to 2% level) were carried out by incorporating them to refined sunflower oil and heating continuously at 80˚C for 24 h. Oil was drawn at the interval of 8 h and analysed for PV and TBA values and antioxygenic activity was calculated as mentioned above.

2.6. Gas Chromatography Analysis

Fatty acid composition of lipid extracted from *Solanum nigrum L.* leaves were carried out as per the method of Uma and Sharma [26]. The methyl esters of fatty acids were separated using a gas chromatograph (1000HR, Chemito, Chennai, India) equipped with flame ionization detector and BP-X 70 column (60 m × 25 mm i.d). Injector and detector temperatures were maintained at 240˚C and 250˚C respectively. The oven temperature was programmed from 50˚C to 220˚C with an initial hold time of 2 min and a heating rate of 4˚C/min. The injection volume was 0.3 µL with a split ratio of 1:30 and hydrogen (40 cm/sec) as a carrier gas.

2.7. Statistical Analysis

All the reported values are the mean of three replicates each and statistical analysis was carried out by using statistical software (Statistica, Ver 7.0 of Stat Soft Incorporation, Tulsa OK, USA). Experimental results were subjected to two way analysis of variance (ANOVA) for significance ($p \leq 0.01$) using Duncan's multiple range tests.

3. Results and Discussion

The fresh and dehydrated powder of *Solanum nigrum L.* leaves contained 86.05% and 7.39% moisture, 4.38% and 33.39% protein, 0.52% and 3.83% fat, 1.52% and 12.02% ash, 0.56% and 6.22% crude fibre and 6.97% and 37.15% carbohydrate (calculated by difference) and provided 50.08 and 316.63 Kcal/100g energy respectively. Mineral composition of dried powder of *Solanum nigrum L.* leaves are shown in **Table 1**. It indicates that leaves contain higher levels of magnesium and phosphorus but relatively low level of zinc. Leaves also contain higher amount of potassium (43.0 mg/100g) and very low level of sodium (3.2 mg/100g). Calcium, iron and copper levels were found to be 22.2, 14.2 and 0.16 mg/100g respectively. The obtained results for mineral composition of *Solanum nigum L.* leaves are almost in conformity with the published data from Akugbugwo *et al.* [13] [14] except for the calcium content showing slightly higher than the reported value. The total carotenoids and chlorophyll contents of fresh and dehydrated leaf powders were 26.9 and 168.2 mg/100g, 218.4 and 1416.2 mg/100g respectively, whereas ascorbic acid and tocopherol were 14.1 and 88.0 mg/100g, 1.5 and 8.8 mg/100g respectively in fresh and dehydrated *Solanum nigrum L.* leaves (**Table 2**).

Table 1. Mineral composition of *Solanum nigrum L.* leaves (mg/100g).

Ca	22.2 ± 0.12
Fe	14.2 ± 0.09
Na	3.2 ± 0.02
K	43.0 ± 1.33
Zn	0.11 ± 0.002
Cu	0.16 ± 0.04
Mg	239.0 ± 1.26
P	80.3 ± 1.95

Values are mean ± standard deviation of three measurements (n = 3).

Table 2. Nutrient composition of *Solanum nigrum L.* leaves (mg/100g).

	Fresh	Dehydrated
Ascorbic acid	14.1 ± 0.46	88.0 ± 1.05
Total carotenoids	26.9 ± 1.28	168.2 ± 8
Total chlorophyll	218.4 ± 1.31	1416.2 ± 4.0
Total phenols	321.1 ± 0.68	2026.6 ± 10
Total flavonoids	273.1 ± 1.06	1698.2 ± 6
Total tocopherol	1.5 ± 0.07	8.8 ± 0.42

Values are mean ± standard deviation of three measurements (n = 3).

3.1. Total Phenol and Flavonoid Contents of *Solanum nigrum L.* Leaves and Their Extracts

The total phenol and flavonoid contents of dehydrated powder of *Solanum nigrum L.* and its extracts are shown in **Table 3**. *Solanum nigrum L.* leaves contains 2026.6 mg/100g and 1698.2 mg/100g of total phenolics and flavonoids respectively. In relation to various solvents used for extraction, leaf powder and its methanol/water (80:20) extracts showed highest amount of both phenolics and flavonoids as compared to other solvent extracts and this may be the reason for the higher antioxygenic activity of leaf powder and methanol/water (80:20) extracts.

3.2. Antioxygenic Actrivity of *Solanum nigrum L.* Leaves and Its Extracts in Sunflower Oil

The effect of *Solanum nigrum L.* leaf powder at concentrations of 0.5%, 1.0% and 2.0% on the rate of auto oxidation in refined sunflower oil is shown in **Table 4**. Addition of a leaf powder even at 0.5% level significantly ($p \leq 0.01$) retarded the rate of lipid peroxidation in sunflower oil. The catalytic action of *Solanum nigrum L.* leaves powder was dependent on its concentration and the antioxygenic activity was increased as the concentration is increased from 0.5% to 2.0%.

Table 5 shows the effect of leaves powder (2%) and its extracts (equivalent to 2.0% of leaves powder) obtained by individual extraction with ethyl acetate, methanol, methanol/water (80:20) and water on peroxide and malonaldehyde formation in sunflower oil in comparison to synthetic antioxidant TBHQ. The control gave higher peroxide and thiobarbituric acid values than the sample containing *Solanum nigrum L.* leaves powder or its extractives. *Solanum nigrum L.* leaves powder and its extracts significantly ($p \leq 0.01$) reduced peroxide and malonaldehyde formation in sunflower oil during storage at 37˚C. It may be observed that leaves powder at 2.0% level and its methanol/water (80:20) extract showed strong antioxygenic activity, whereas ethyl acetate extract exhibited only moderate activity (**Table 6**). The antioxygenic activity of *Solanum nigrum L.* leaves powder were slightly higher than its methanol/water (80:20) soluble fraction. The water soluble fraction of the leaves was practically devoid of any antioxygenic activity. The higher antioxygenic activity of methanol/water (80:20) soluble fraction may be due to the solubility of phenolic and flavonoid compounds in methanolic medium which

Table 3. Total phenolics and flavonoid contents of *Solanum nigrum L.* leaves and its extracts (mg/100g).

	Total phenolics	Total flavonoids
Solanum nigrum L.	2026.6 ± 10	1698.2 ± 6
Ethyl acetate	240.8 ± 0.46	650.6 ± 0.88
Methanol	1386.5 ± 7	1012.4 ± 3
Methanol/water (80:20)	1896.9 ± 6	1510.5 ± 4
Water	647.7 ± 1.42	542.6 ± 0.94

Values are mean ± standard deviation of three measurements (n = 3).

Table 4. Changes in peroxide (meq O_2/kg oil) and thiobarbituric acid (mg malonaldehyde (MA)/kg sample) values of *Solanum nigrum L.* leaves in sunflower oil during storage at 37˚C.

	PV			TBA		
	Storage period (Days)					
	15D	30D	45D	15D	30D	45D
Control	28.8 ± 0.52[a]	94.8 ± 0.61[a]	174.7 ± 0.81[a]	0.20 ± 0.004[a]	0.45 ± 0.005[a]	0.71 ± 0.004[a]
TBHQ	10.5 ± 0.29[b]	20.2 ± 0.23[b]	85.6 ± 0.46[b]	0.10 ± 0.003[b]	0.22 ± 0.004[b]	0.31 ± 0.003[b]
0.5%	17.1 ± 0.32[c]	72.2 ± 0.29[c]	163.0 ± 0.79[c]	0.17 ± 0.002[c]	0.36 ± 0.003[c]	0.56 ± 0.003[c]
1.0%	11.7 ± 0.18[d]	60.6 ± 0.31[d]	142.3 ± 0.87[d]	0.15 ± 0.003[d]	0.30 ± 0.002[d]	0.48 ± 0.005[d]
2.0%	9.6 ± 0.11[e]	44.6 ± 0.26[e]	120.5 ± 0.52[e]	0.14 ± 0.002[e]	0.24 ± 0.005[e]	0.34 ± 0.002[e]

Values are mean ± standard deviation of three measurements (n = 3); Means in a column with different superscripts differ significantly ($p \leq 0.01$); Initial PV & TBA values were 6.42 meq O_2/kg fat & 0.07 mg MA/kg sample respectively.

Table 5. Changes in peroxide (meq O_2/kg oil) and thiobarbituric acid (mg malonaldehyde/kg sample) values of *Solanum nigrum L.* leaves and their various solvent extracts* in sunflower oil during storage at 37˚C.

	PV			TBA		
	Storage period (Days)					
	15D	30D	45D	15D	30D	45D
Control	30.7 ± 0.66[a]	72.4 ± 0.75[a]	156.2 ± 0.89[a]	0.28 ± 0.004[a]	0.52 ± 0.004[a]	0.82 ± 0.007[a]
TBHQ	11.4 ± 0.31[b]	19.6 ± 0.19[b]	23.5 ± 0.31[b]	0.12 ± 0.004[b]	0.23 ± 0.002[b]	0.37 ± 0.005[b]
Solanum nigrum L. (2%)	13.7 ± 0.26[c]	26.2 ± 0.36[c]	86.9 ± 0.68[c]	0.14 ± 0.005[c]	0.26 ± 0.004[c]	0.41 ± 0.006[c]
Ethyl acetate	25.6 ± 0.22[d]	70.0 ± 0.42[d]	135.3 ± 0.86[d]	0.23 ± 0.004[d]	0.42 ± 0.003[d]	0.65 ± 0.004[d]
Methanol	22.4 ± 0.23[e]	50.5 ± 0.51[e]	120.2 ± 0.99[e]	0.19 ± 0.003[e]	0.38 ± 0.003[e]	0.55 ± 0.006[e]
Methanol/Water (80:20)	17.0 ± 0.16[f]	28.3 ± 0.21[f]	90.5 ± 0.77[f]	0.16 ± 0.002[f]	0.27 ± 0.002[c]	0.38 ± 0.006[b]
Water	29.1 ± 0.22[g]	71.5 ± 0.49[g]	153.9 ± 0.91[g]	0.26 ± 0.003[g]	0.50 ± 0.006[f]	0.82 ± 0.004[a]

Values are mean ± standard deviation of three measurements (n = 3); Means in a column with different superscripts differ significantly ($p \leq 0.01$); *Concentrations equivalent to 2% of the ground spice. Initial PV & TBA values were 5.63 meq O_2/kg fat & 0.10 mg MA/kg sample respectively.

are known to have antioxygenic activity in lipids [27]-[29].

3.3. Antioxygenic Activity of *Solanum nigrum L.* in Sunflower Oil at 80˚C

The stability of *Solanum nigrum L.* leaves and its various extracts heated for 24 h at an elevated temperature of 80˚C normally used for cooking of traditional Indian foods are represented in **Table 7**. The results of the study

Table 6. Antioxygenic activity of various extracts[a] of *Solanum nigrum L.* leaves.

Extracts	Antioxygenic activity[b]
TBHQ	2.3 ± 0.17
Solanum nigrum L. (2%)	2.1 ± 0.10
Ethyl acetate	1.3 ± 0.08
Methanol	1.5 ± 0.04
Methanol/water (80:20)	2.0 ± 0.16
Water	1.0 ± 0.09

Values are mean ± standard deviation of three measurements (n = 3); [a]Concentrations equivalent to 2% of the ground sample; [b]Mean of three values after 15, 30, 45 days of storage. Calculated as the ratio of TBA value of the control to the TBA value of the sample; Values >1 indicates antioxygenic activity and values <1 indicates prooxygenic activity.

Table 7. Antioxygenic activity of *Solanum nigrum L.* leaves in sunflower oil at 80˚C.

Solanum nigrum L. and its extracts	After 8 h	After 24 h
TBHQ	2.36 ± 0.05[a]	2.60 ± 0.08[a]
Solanum nigrum L. (2%)	1.86 ± 0.07[b]	1.73 ± 0.05[b]
Ethyl acetate	1.08 ± 0.06[c]	1.13 ± 0.05[c]
Methanol	1.44 ± 0.10[d]	1.33 ± 0.08[d]
Methanol/water (80:20)	1.73 ± 0.11[e]	1.63 ± 0.10[e]
Water	1.08 ± 0.02[c]	0.96 ± 0.04[f]

Values are mean ± standard deviation of three measurements (n = 3); Means in a column with different superscripts differ significantly ($p \leq 0.01$); Calculated as the ratio of TBA value of the control to the TBA value of the sample; Initial TBA value is 0.11 mg Malonaldehyde/kg sample.

clearly indicates that, among the different extracts studied, the resistance to thermal oxidation was exhibited strongly and significantly ($p \leq 0.01$) by the methanol/water (80:20) extract followed by the methanol extract. *Solanum nigrum L.* powder at 2% level exhibited slightly higher antioxygenic activity as compared to methanol/water (80:20) extract. Ethyl acetate extract has shown moderate activity, while the water extract has shown nil antioxygenic activity indicating the maximum potency of the methanol/water (80:20) extract to inhibit lipid peroxidation even after 24 h of continuous heating.

3.4. Fatty Acid Analysis of *Solanum nigrum L.*

The fatty acid analysis of the fat extracted from leaves is shown in **Table 8**. Gas chromatography analysis revealed the presence of linoleic acid (59.1%) as the major fatty acid. The leaf also contained a substantial amount of palmitic acid (13.9%) and moderate amounts of oleic (7.8%), stearic (3.6%), myristic (2.2%), and palmitoleic (1.6%) acids while other fatty acids are present at less than 1%.

4. Conclusion

During the investigation, among the different extracts studied, methanol/water (80:20) extracts of the leaf exhibited higher antioxygenic activity than other solvent extracts when evaluated for antioxygenic activity by using sunflower oil model system. The result shows that the leaf of *Solanum nigrum L.*, an underutilized and unconventional part of the plant, contains a good amount of antioxidants to counteract the damaging effect of free radicals. Further studies are warranted for the isolation and identification of individual phenolic compounds

Table 8. Fatty acid composition of *Solanum nigrum L.* leaves.

Fatty acids	Percentage
Lauric acid ($C_{12:0}$)	0.12 ± 0.01
Myristic acid ($C_{14:0}$)	2.2 ± 0.03
Palmitic acid ($C_{16:0}$)	13.9 ± 0.12
Palmitoleic acid ($C_{16:1}$)	1.6 ± 0.02
Stearic acid ($C_{18:0}$)	3.6 ± 0.03
Oleic acid ($C_{18:1}$)	7.8 ± 0.09
Linoleic acid ($C_{18:2}$)	59.1 ± 0.23
Arachidic acid ($C_{20:0}$)	0.55 ± 0.03
Linolenic acid ($C_{18:3}$)	0.41 ± 0.01
Henicosanoic acid ($C_{21:0}$)	0.43 ± 0.01
Behenic acid ($C_{22:0}$)	0.68 ± 0.04
Lignoceric acid ($C_{24:0}$)	0.15 ± 0.002
Saturated fatty acid	21.6 ± 0.07
Manounsaturated fatty acid	9.4 ± 0.08
Polyunsaturated fatty acid	59.5 ± 0.19

Values are mean ± standard deviation of three measurements (n = 3).

from the leaves and its methanol/water (80:20) extracts which have great potential as natural antioxidants.

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