PHYTOCHEMICAL INVESTIGATION & QUANTITATIVE ESTIMATION OF BARK EXTRACTS OF *SOLANUM NIGRUM*

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Abstract
In current study, we accepted out a systematic record of the relative antioxidant activity in bark of *Solanum nigrum* of different extracts. Phytochemical screening shows the presence of carbohydrates, glycosides and alkaloids in the maximum quantity. The total phenol of methanolic extract of *Solanum nigrum* and ethyl extract of *Solanum nigrum* varied from 0.311 to 0.195 mg/gm respectively in the extracts. Flavonoid contents of methanolic extract of *Solanum nigrum* and ethyl extract of *Solanum nigrum* were between 0.275 to 0.139 mg/gm respectively. The superior amount of phenolic compounds leads to further research work on radical scavenging of *Solanum nigrum* bark extract.
Keywords: *Solanum nigrum;* DPPH; ABTS; Hydrogen peroxide

Introduction

Free radicals donate to more than one hundred disorders in humans counting atherosclerosis, arthritis, ischemia and reperfusion damage of numerous tissues, central nervous system injury, gastritis, cancer and AIDS [1, 2]. Free radicals due to ecological pollutants, radiation, chemicals, toxins, profound fried and spicy foods as well as corporeal stress, cause exhaustion of immune system antioxidants, modify in gene expression and persuade abnormal proteins. Oxidation development is one of the most imperative routs for producing free radicals in food, drugs and still living systems. Catalase and hydroperoxidase enzymes change hydrogen peroxide and hydroperoxides to non-radical forms and purpose as natural antioxidants in human body. Owing to depletion of immune system natural antioxidants in dissimilar maladies, overwhelming antioxidants as free radical scavengers may be essential [3-6]. At present available synthetic antioxidants similar to butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been supposed to cause or punctual negative health effects. Consequently, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants also show low solubility and reasonable antioxidant activity [7-8]. Recently there has been an increase of interest in the therapeutic potentials of medicinal plants as antioxidants in dropping such free radical induced tissue injury. Polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [9]. A number of confirmations suggest that the biological actions of these compounds are related to their antioxidant activity [10]. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the occurrence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases [11]. In particular, despite extensive use of wild plants as medicines in Iran, the prose contains few reports of antioxidant activity and chemical composition of these plants. In current study, we carried out a systematic record of the relative free radical scavenging activity in selected medicinal plant species, which are being used traditionally *Solanum nigrum* also known as Black Nightshade or Makoi (h) Kakamachi is a perennial shrub found in wooded areas. The plant height is 40 cm 130 cm and its leaves are 4-8 cm wide. The berry is used as medicine for fruit, root, leaves, flowers, and the entire plant. It grows usually in nitrogen rich soil. Since Vedic era it has relation to being used. It is an essential Ayurvedic shrub. Its white flower and purple- black berries are characteristic of this [12].

Materials and Methods

Selection and collection of plant

The Bark Extract of *Solanum Nigrum* was selected on the basis of Ethano botanical survey. Various considerations were involved in the plant selection especially for its cytoprotective and anti- bacterial activity. The barks of *Solanum Nigrum* are collected from the Pinnacle Biomedical Research Institute, Bhopal Campus.

Authentication of plant

The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science and Bhopal. A voucher specimen number 189/Saif. /Sci./Clg/Bpl. was kept in Department of Botany, Saifia College of Science, Bhopal forfuture reference.

Chemicals

All the chemicals used were of analytical grade and were obtained from Merck or Rankem or S. D. Fine Chemicals Mumbai.

Solvent extraction

Plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of Bark Extract of *Solanum Nigrum* was placed in thimble of soxhlet apparatus. Soxhlation was performed at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethyl acetate and methanol solvent. For each solvent, soxhlation was continued till no visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporated using rotary vacuum evaporator (Buchi type) at 40°C.

Qualitative phytochemical analysis of plant extract

Bark Extract of *Solanum Nigrum* was subjected to the preliminary phytochemical analysis [13, 14]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Quantitative phytochemical estimation

Determination of total phenolic contents in the plant extracts [15]

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Galic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1 mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5 ml of a 10- fold dilute folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue color upon reaction. This blue color was measured spectrophotometrically. Line of regression from Gallic acid was used for estimation of unknown phenol content. From standard curve of gallic acid line of regression was found to be.

$y = 0.005x + 2.569$ and $R^2 = 0.991$

Thus, the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above-mentioned GA.

Determination of total flavonoid concentrations in the plant extracts

Total flavonoids were measured by a colorimetric assay [16]. An aliquot of diluted sample or standard solution of rutin was added to a 75 µl of $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with

distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutine/g DW), through the calibration curve of Rutin. All samples were analysed in three replications.

Line of regression from rutin was used for estimation of unknown flavonoid content. From standard curve of rutin, line of regression was found to be

$y = 0.001x - 0.020$ and $R^2 = 0.994$

Thus, the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned rutin.

Result & Discussion

Qualitative evaluation of different extracts

Table 1: Phytochemical evaluation of different extract of *S. Nigrum*

S. No	Chemical tests	Pet Ether Extract of *S.nigrum* bark	Ethyl acetate Extract of *S. nigrum* bark	Methanolic Extract of *S. nigrum* bark
	Test for Carbohydrates			
1	Molisch's Test	-ve	+ve	+ve
2	Fehling's Test	-ve	+ve	+ve
3	Benedict's Test	-ve	+ve	+ve
	Test for Protein & Amino acids			
4	Biuret's Test	-ve	-ve	-ve
5	Ninhydrin Test	-ve	-ve	-ve
	Test for Glycosides			
6	BorntragerTest	+ve	+ve	+ve
7	Killer killaniTest	+ve	+ve	+ve
	Test for Alkaloids			
8	Mayer's Test	-ve	+ve	+ve
9	Hager's Test	-ve	+ve	+ve
10	Wagner's Test	-ve	+ve	+ve
	Test for Saponins			
11	Froth Test	-ve	-ve	-ve
	Test for Flavonoids			
12	Lead acetate	-ve	+ve	+ve
13	Alkaline reagent test	-ve	+ve	+ve
	Test for Triterpenoids and Steroids			
14	Libermann-Burchard Test	-ve	+ve	+ve
15	Salkowski Test	-ve	+ve	+ve
	Test for Tannin and Phenolic compounds			
16	Ferric Chloride Test	-ve	+ve	+ve
17	Gelatin Test	-ve	+ve	+ve

Quantitative phytochemical estimation

Determination of total phenolic contents in the plant extracts

By using the UV spectrophotometer the total phenolic concentration of *Solanum nigrum* was calculated with a regression equation based on a standard curve and as a result TPC was found to be 124 mg/gm of methanolic extract of *S. nigrum* (MSN) and 66.167 mg/gm of ethyl acetate extract of *S. nigrum* (EASN) which were expressed in terms equivalent to gallic acid. In total phenolic concentration the absorbance of gallic acid was observed at the λ_{max} = 765 nm.

Table 2: Ethyl acetate (EASN) and methanolic (MSN) extract with IC_{50} (inhibitoryconcentration)

Sample	IC_{50}
EASN (ethyl acetate *S. nigrum* extract)	09.00
MSN (methanolic *S. nigrum* extract)	21.80

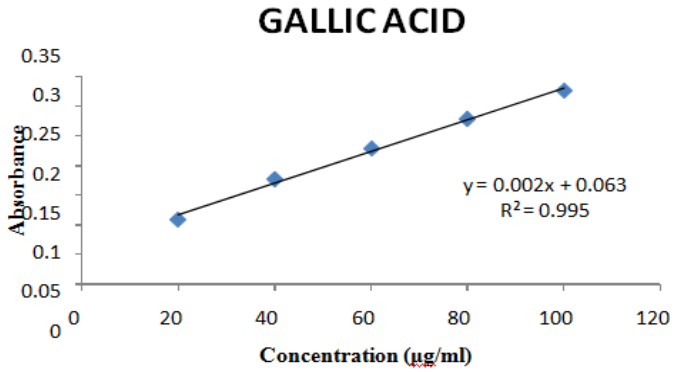

Figure: 1 Standard Curve of Gallic Acid

Table 3: Standard Curve Absorbance of Gallic Acid for Total Phenolic ContentDetermination

Standard curve of Gallic acid		
S. No.	**Concentration (µg/ml)**	**Absorbance**
1	20	0.1098
2	40	0.1763
3	60	0.2290
4	80	0.2783
5	100	0.3258

Table 4: Total Phenolic Concentration (TPC) of different extracts of *S. nigrum* and mean absorbance of Gallic Acid

TPC Expressed as mg/gm Gallic Acid Equivalent		
S. No.	**MSN (methanolic *S. nigrum* extract)**	**EASN (ethyl acetate *S. nigrum* extract)**
1	0.311	0.197
2	0.309	0.195
3	0.313	0.194
Mean Absorbance	0.311	0.195
TPC value	124.000	66.167

Determination of total flavonoid concentrations in the plant extracts

By using the UV spectrophotometer the flavonoid concentration extracts of *S. nigrum* was calculated with a regression equation based on a standard curve and as a result TFC was found tobe 183.3 mg/gm of methanolic extract of *S. nigrum* (MSN) and 46.667 mg/gm of ethyl acetate extract of *S. nigrum* (EASN) which were expressed in terms equivalent to Rutin. In total Flavonoid concentration the absorbance of Rutin was observed at the λmax = 415 nm.

Table 5: Ethyl acetate (EASN) and methanolic (MSN) extract with IC_{50}

Sample	IC 50
EASN (ethyl acetate *S. nigrum* extract)	188.20
MSN (methanolic *S. nigrum* extract)	230.40

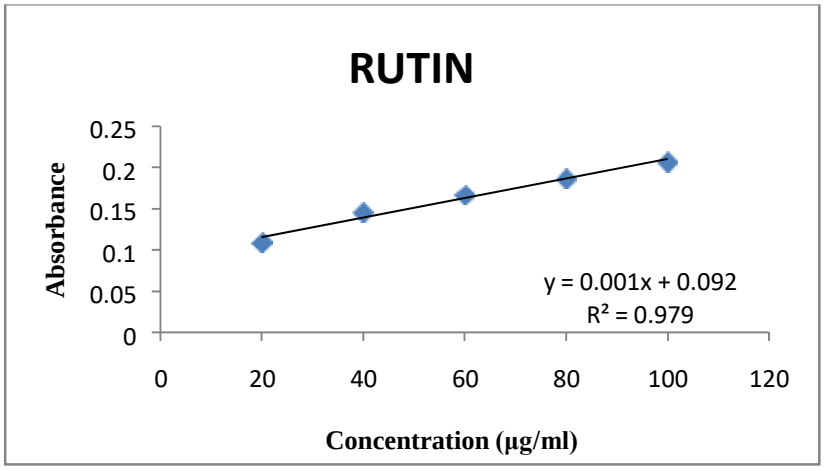

Figure: 2 Standard Calibration curve of Rutin for Total Flavonoid Content

Table 6: Standard Curve Absorbance of Rutin for Total Flavonoid Content Determination

Standard curve of Rutin		
S. No	**Concentration (µg/ml)**	**Absorbance**
1	20	0.109
2	40	0.146
3	60	0.167
4	80	0.187
5	100	0.207

Table 7: Total Flavonoid Concentration (TFC) of extractsof *S. nigrum* and meanabsorbance of Rutin

TFC Expressed as mg/gm Rutin Equivalent		
S. No.	**MSN (methanolic *S. nigrum* extract)**	**EASN (ethyl acetate *S. nigrum* extract)**
1	0.274	0.138
2	0.278	0.142
3	0.274	0.136
Mean Absorbance	0.275	0.139
TFC value	183.333	46.667

Hydrogen peroxide assay was performed in order to found out the antioxidant activity of the extract by scavenging the free radicals. The H_2O_2 assay was firstly performed by taking ascorbic acid as a standard for extract and calibrations graph prepared with the regression of 0.957 at 230 nm absorbance was taken in spectrophotometer. Inhibitory concentration was measured and it was found to be 36.27 mcg/ml with regression 0.953. Likewise, this assay performed for methanolic and ethyl acetate extracts and their regression of calibration graph of different absorbance at different concentration was obtained and they were 0.994 nm and 0.958. These two extracts show the inhibitory concentration graph with the regression value 0.990 of MSN on Hydrogen peroxide assay and 0.972 of EASN on Hydrogen peroxide assay. The inhibitory concentration of MSN on hydrogen peroxide assay was found to be 95.57mcg/ml and EASN onhydrogen peroxide assay was found to be 145.13 mcg/ml respectively.

Conclusion

The result of the current study showed that the bark extract of *Solanum nigrum*, which hold highest amount of flavonoid and phenolic compounds, exhibited the maximum antioxidant activity. The high scavenging property of *Solanum nigrum* may be due to hydroxyl groups accessible in the phenolic compounds' chemical structure that can offer the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a probable anticipatory intervention for the diseases [19]. All of the extracts in this research exhibited dissimilar amount of antioxidant activity. *Solanum nigrum* extract showed a higher potency than ascorbic acid in scavenging of DPPH free radical. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract.

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