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Research Article

Assessment of Nutritional composition and antioxidant activity of edible herbs *Solanum nigrum* and *Phyllanthus niruri*.

Balaguru Sakthivel and Syad Arif Nisha
PG & Research Department of Biotechnology, Srimad Andavan Arts & Science College (Autonomous), Trichy
nisha@andavancollege.ac.in

ABSTRACT

Several natural compounds have been exploited for the treatment of diseases and disorders. Unlike the synthetic compounds, these natural entities possess less or no side effects and treat the ailments effectively. It will be more advantageous if the source of natural therapeutic compounds are edible and holds high nutritive value (nutraceutical). Therefore the present study aims at evaluating the nutritional composition and antioxidant activity of two edible plants, *Solanum nigrum and Phyllanthus niruri*. The proximate composition, proline content, chlorophyll content, swelling capacity, water holding capacity, oil holding capacity and Ash content of the plants were assessed. In order to evaluate the antioxidant activity, the hydrogen peroxide radical scavenging activity and reducing power potential of the plant extracts were assessed. The results of the study suggest that the plant extracts had high nutritive value and they hold high antioxidant activity. Upon identifying the active principles of the extract, the plants can be used for various therapeutic applications.

Keywords

Edible plants, nutraceuticals, antioxidant activity, nutritional composition, therapeutic compounds

1. INTRODUCTION

Plant derived natural products have been found to possess diverse pharmacological properties including antioxidant activity, antimicrobial activity, anti-inflammatory activity and anticancer activities. Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals etc, thereby preventing or delaying the damage to the cells and tissues. As antioxidants play an

important role in inhibiting and scavenging radicals, they act against infection and degenerative diseases. However, these synthetic antioxidants cause side effects such as liver damage and carcinogenesis. Therefore, there is a need for isolation and characterization of natural antioxidants having less or no side effects from the medicinal plants to replace synthetic antioxidant. Plants are endowed with free radical scavenging molecules in the form of secondary metabolites, which includes terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains etc.[1]

Phyllanthus niruri is a weed plant belongs to Euphorbiaceae family, found in coastal areas. It's also known as gale of the wind or stonebreaker. Its leaves and fruit are used as herbal medicine from ancient period. It is extensively used to treat inflammation, diarrhea, eye sore, burns, suppurations and chafing of the skin.[2] *Solanum nigrum* is a dicot plant belongs to the family of Solanaceae, annual branched herb, the flowers are very small and five petals in the leaves, fruits are small. It's mainly found in waste lands and cultivated lands which are a common plant found in most parts of the world. Several reports demonstrated the therapeutic potential of these plants including antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, antipyretic activities.[1] Since both the herbs possess excellent medicinal value, the nutritional profile and antioxidant activity of the plants were assessed in the present study.

2. MATERIALS AND METHODS

2.1. Sample Collection:

Fresh leaves of *Phyllanthus niruri* and *solanum nigrum* L. were collected from the Gandhi Market, Trichy, Tamilnadu, India, and it was Taxonomically identified and authenticated by Botanist, Department of Botany, St. Joseph college, Trichy, Tamilnadu.

Fresh *Phyllanthus niruri* and *solanum nigrum* Plant was washed under the running tap water and dried under shade at room temperature. The *Phyllanthus niruri* and *solanum nigrum* Plant was cut into small pieces, powdered in a mixer grinder and stored in sterile containers for further use.

2.2. Preparation of extract:

Fresh leaves of *Phyllanthus niruri* and *Solanum nigrum* were washed under the running tap water and dried under shade at room temperature. The dried leaves were powdered and stored in air-tight container. 5 g of powdered leaf was placed in the beaker and required amount of sterile distilled water was added to it. The extraction was carried out for 72 h at 37°C. The extract was filtered using filter paper and were dried using desiccators.

2.3. Physico-chemical properties of *Phyllanthus niruri* and S*olanum nigrum* [3]

2.3.1. Swelling capacity (SWC)

SWC was analyzed by bed volume technique after equilibrating in excess solvent. 200mg of fresh plant materials was placed in a container with 20 mL of distilled water and vigorously stirred. To measure the effect of temperature on SWC, the sample was left to stand for 24 h in two different temperatures (25^0C and 37^0C). The swelling volume was measured and expressed as mL of swollen sample per g of sample dry weight (DW).

2.3.2. Water holding capacity (WHC)

WHC was analyzed by modified centrifugation method. 200 mg of sample was placed in 20 mL of distilled water in a centrifugation tube and were kept in a shaker for 24 h. To determine the effect of temperature on WHC, the samples were kept at 25^0C and 37^0C. WHC was expressed as weight of gram of water held by 1 g of dry weight of sample.

2.3.3. Oil holding capacity (OHC)

3g of sample was taken in 10.5 g of corn oil in a centrifugation tube. The tubes were left for 30 minutes at room temperature with constant agitation. The mixture was centrifuged at 2500 g for 30 minutes at room temperature. The oil supernatant was removed and used for measurement. The OHC of plant sample was measured as the number of grams of oil held by 1g of dry weight of sample.

2.4. Proximate composition and nutritional analysis of *Phyllanthus niruri* and S*olanum nigrum*

2.4.1. Ash content [3]

2g of freeze dried plant sample was kept at 525^0C for 5 h in muffle furnace, the ash content was expressed as g of ash obtained per 100 g of sample dry weight.

2.4.2. Carbohydrate estimation (phenol sulphuric acid method):

Total carbohydrate estimation was done by phenol-sulphuric acid method.200 mg of sample was added to 5 mL of 2.5 N HCL and the sample was hydrolyzed by keeping in boiling water bath for 3 hours. The solution was neutralized by adding solid Na_2CO_3 until effervescence ceases. The volume was then made to 50 mL and centrifuged at 8000 rpm for 10 min. The sample was made to 1 mL with and 1 mL of phenol solution was added to the sample along with 5 mL of 96% sulphuric acid. The solution was mixed well and placed in water bath for 20 min at 25^0C. The absorbance was measured at 490 nm using UV-Vis spectrophotometer.

2.4.3. Protein extraction:

1g powdered *Phyllanthus niruri* and *Solanum nigrum* plant was introduced into centrifuge tubes containing 50 ML of Diethyl ether and water (1:4). The tubes were kept in a shaker for 3 hours. The supernatant was discarded and 1N NaOH was added to the sample and kept in a shaker for 3 hours. The mixture was centrifuged at 7000 rpm for 10 min and the supernatant was collected and precipitated with 10 % solution of TCA at Ph 4.0. The samples were kept in ice for 30 mins or until visible precipitate appears. The samples were then centrifuged at 7000 rpm for 20 mins. The precipitated protein was washed and dried.

2.4.4. Protein estimation [4]

0.5 mL of standard and test sample was taken in a test and made to 2 mL by adding water. BSA was added used as standard solution (50 mg/50 L). 5 mL of solution C (2% Na_2CO_3 in 1N NaOH and 0.5% CuSO4 in 1% sodium potassium tartarate) was added to the tubes and incubated at room temperature for 10 mins. Then, 0.5 mL of solution D (Folin reagent 1:2) was added and incubated in dark for 45 mins. The samples were then read at 660 nm in UV-Vis spectrophotometer.

2.4.5. Lipid content [3]

Lipids were extracted from the powdered coriander plant sample using Soxhlet apparatus. The solvent mixture used for extraction is chloroform and methanol in the ratio of 2:1 (v/v). The contents of the lipids were determined gravimetrically after oven- drying (80^0C) the extract overnight.

2.4.6. Extraction of chlorophyll [5]

One gram finely cut fresh leaves were taken and ground with 20-40mL of 80% acetone. It was then centrifuged at 5000-7000 rpm for 5 mins. The supernatant was transferred and the procedure was repeated till the residue becomes colorless. The absorbance of the solution was red at 645 nm and 663 nm against the solvent (acetone) blank.

2.4.7. Estimation of chlorophyll content:

The concentration of chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation:

Total chlorophyll: $20.2(A_{645}) + 8.02(A_{663})$

Chlorophyll a: $12.7(A_{663}) - 2.69(A_{645})$

Chlorophyll b: $22.9(A_{645)} - 4.68(A_{663})$.

2.5. Phytochemical analysis of *Phyllanthus niruri* and S*olanum nigrum*

2.5.1. Steroids:

1mL of plant extract and add equal volume of chloroform. Add few drops of conc. H_2SO_4, And observe the formation of brown or bluish brown indicates the presence of steroids.

2.5.2. Terpenoides:

2mL of extract is dissolved with 2mL of chloroform and concentrated sulphuric acid is carefully added to form layer. A reddish brown colour indicates the presence of terpenoids.

2.5.3. Reducing sugar:

The extract was shaken with distilled water and filtered. The was boiled with fehlings solution A and B for few minutes an orange red indicates the presence of reducing sugar.

2.5.4. Alkaloids:

2mL of extract is taken and added 2mL of wagner"s reagent a brownish precipitate indicate the presence of alkaloids.

2.5.5. Flavonoids:

2mL of extract is treated with 2mL of 10% lead acetate. Yellowish green colour indicates the presence of Flavonoids.

2.5.6. Saponins:

2mL of distilled water were added to the plant extract. It was shaked well for 15 minutes at lengthwise. The formation of 1cm foam layer indicates the presence of the saponins.

2.5.7. Tannins:

A few drops of 0.1% ferric chloride were added to the sample and observed for the formation of brownish green or a blue –black coloration.

2.5.8. Anthraquinones:

1mL of extract is boiled with 10% HCl for few minutes in a water bath. It is filtered and allowed to cool. Equal volume of $CHCl_3$ is added to the filtrate few drops of 10% Ammonia is added to the mixture and heat. Formation of rose pink colour indicates the presence of anthraquinones.

2.6. Assessment of Antioxidant Activity:

2.6.1. Hydrogen peroxide scavenging capacity:

The hydrogen peroxide scavenging was determined according to the method of Gucin et al.[6] A solution of hydrogen peroxide 40mm was prepared in phosphate buffer (pH 7.4), extracts

100µg/mL in distilled water were added to a hydrogen peroxide (0.6 mL , 40mm). absorbance of hydrogen peroxide at 230 nm was determined. 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The hydrogen peroxide scavenging activity was calculated using the equation: (1- absorbance of sample/ absorbance of control)×100. Each experiment was carried out in triplicate and result averaged expressed as mean± SD.

2.6.2. Total Reducing Power:

Reducing power activity was performed according to **Oyaizu.**[7] About 1 mL of different concentration of the extract were prepared. Add 1mL of phosphate buffer (200Mm, ph6.6). Add 1 mL of potassium ferricyanide (1% v/v). mixture was incubated at 50°C for 20 min. The reaction was stopped by adding 1 mL of 10% Trichloro acetic acid. Centrifuged at 3000 rpm for min. 1.5mL of supernatant was mixed with 2mL of distilled water & 0.5mL of ferric chloride(0.1%). Incubated at room temperature for 10 min. absorbance was measured at 700nm. Higher absorbance of the reaction mixture indicates the greater reducing power. All the test were performed in triplicates ascorbic acid was used as a reference standard.

$$\text{Inhibition (\%)} = \frac{(A0 - A1)}{A0} \times 100$$

Where A0 is the absorbance of control and A1 is the absorbance of test.

3. Results and Discussion

3.1. Assessment of Physico-chemical properties of *Solanum nigrum and Phyllanthus niruri*

The physico-chemical properties determine the physiological effects of the dietary fibres. These dietary fibres are resistant to digestion, which provides bulk to faeces, holds water, acts as a site for ion-exchange, and binds organic molecules. [8] In the present study, the SWC, WHC and OHC were evaluated. The centrifugation method was employed to determine the physicochemical properties and the results were illustrated in Table 1. The effect of temperature on SWC and WHC were investigated. At 25°C, SWC of *Solanum nigrum* was 0.85±0.01mL/g and *Phyllanthus niruri* was 0.3±0.01mL/g of dry weight (DW) and at 37°C the values of 0.4±0.01mL/g (for *Sollanum nigrum*) and 0.9±0.76 mL/g (for *Phyllanthus niruri*) were obtained. At 25°C, WHC was 0.4±0.5 ml/g and 0.3±0.5 ml/g of dry weight (DW) for *S. nigrum* and *P. niruri* respectively. Interestingly, a

slight increase in the value of 0.5±0.01 ml/g and 0.4±0.01 ml/g was observed after incubating at 37°C for *S. nigrum* and *P. niruri* respectively. At 37°C, OHC of *Solanum nigrum* was 5.23±0.015 ml/g, and *Phyllanthus niruri* was 4.32±0.015ml/g of dry weight (DW) (Table 1).

Table 1: Physicochemical properties of *Sollanum nigrum* and *Phyllanthus niruri*

	SWC (mL/g DW)		WHC(ml/g DW)		OHC(ml/g DW)
Temperature	25C	37°C	25°C	37°C	37°C
S. nigrum	0.85±0.01	0.75±0.01	0.4±0.01	0.5±0.01	5.23±0.015
P. niruri	0.73±0.01	0.79±0.0.01	0.3±0.01	0.4±0.01	4.32±0.015

3.2. Evaluation of proximate composition and nutritional profiling of *S. nigrum* and *P. niruri*

The proximate composition of *S. nigrum* and *P. niruri* was evaluated and the results were tabulated in Table 2. The ash content represents the total mineral content of *S. nigrum* and *P. niruri*. The ash content of *S. nigrum* was 0.246 g/g of DW and *P. niruri* was 0.275 g/g of DW. The amount of carbohydrate present in *S. nigrum* and *P. niruri* was 0.060±0.006mg/g and 0.065±0.005mg/g of DW respectively. The amount of protein content observed was 0.078±0.007 mg/g and 0.282±0.009mg/g of DW respectively for *S. nigrum* and *P. niruri*. The amount of lipid present in *S. nigrum* and *P. niruri* was found to be 0.032±0.21mg/g of DW and 0.028±0.21mg/g of DW respectively. Recent evidences show that proline elicits stress-stimulated phenolic biosynthesis and stimulates antioxidant enzyme response pathways.[9] The proline content of *Solanum nigrum* and *Phyllanthus niruri* was observed as 0.194±0.013µ/g and 0.187±0.013µ/g of DW respectively. Apart from the major nutrient elements, the determination of chlorophyll content has become a crucial aspect of nutritional assessment. Moreover, chlorophyll possesses excellent antioxidant activity, when present in higher concentrations.[10] Determination of chlorophyll content by spectrophotometric method revealed that about 2.3 mg/g FW of chlorophyll A was present in *Solanum nigrum* .and 2.1 mg/g FW of chlorophyll was found to be present in *Phyllanthus niruri*. The amount of chlorophyll b observed in *Solanum nigrum* and *Phyllanthus niruri* was 1.8 mg/g FW and 2.9 mg/g FW respectively (Table 2).

Table 2: Proximate composition of *Solanum nigrum* and *Phyllanthus niruri*

S.No.	Composition	*Solanum nigrum*	*Phyllanthus niruri*
1.	Ash content	0.246 g/g of DW	0.275 g/g of DW
2.	Carbohydrate content	0.060±0.006mg/g of DW	0.065±0.005mg/g of DW
3.	Protein	0.078±0.007 mg/g of DW	0.282±0.009mg/g of DW
4.	Lipid content	0.032±0.21mg/g of DW	0.028±0.21 mg/g of DW
5.	Proline content	0.194±0.013µ/g of DW	0.187±0.013µ/g of DW
6.	Chlorophyll A	2.3 g/g FW	2.1 g/g FW
7.	Chlorophyll B	1.8 g/g FW	2.9 g/g FW

3.3. Evaluation of phytochemical constituents of *S. nigrum* and *P. niruri*.

Solanum nigrum and *Phyllanthus niruri* was examined for the presence of various phytochemicals. The result suggests that *Solanum nigrum* and Phy*llanthus niruri* has high amount of alkaloids, terpenoids and flavonoids. The results were tabulated in Table 3.

Table 3: Phytochmical Screening of *S. nigrum* and *P. Niruri*

TEST	*S. nigrum*	*P. niruri*
STEROIDS	+	+
TERPENOIDS	+	+
REDUCING SUGAR	+	+
SUGAR	+	+
ALKALOIDS	+	+
PHENOLIC COMPOUNDS	+	+
CATACHINS	–	–
FLAVONOIDS	+	+
SAPONINS	+	+
TANNINS	–	–
ANTHROQUINONES	–	–
AMINOACIDS	+	+

3.4. Assessment of antioxidant potential of aqueous extract of *S. nigrum* and *P. niruri*:

3.4.1. Reducing power assay:

The ability of the aqueous extract of *S. nigrum and P. niruri* to reduce Fe^{3}+ to Fe^{2}+ was measured through reducing power assay. The reducing ability of compound is determined by the presence of reductones which generally impacts the antioxidant quality of the extract by breaking the free radical chain by donating a hydrogen atom.[11] The reducing power ability of various concentration (100-500 µg/ml) of aqueous extract of *S. nigrum* and *P. niruri* was analysed and compared with standard L-ascorbic acid. The results suggested that at the concentration of 100 µg/ml of the aqueous extract of *S. nigrum* exhibited the reducing power capability of 0.019±0.014. When concentration was increased to 500 µg/ml, the absorbance also increased with the absorbance of 0.153±0.146 (Figure 1).

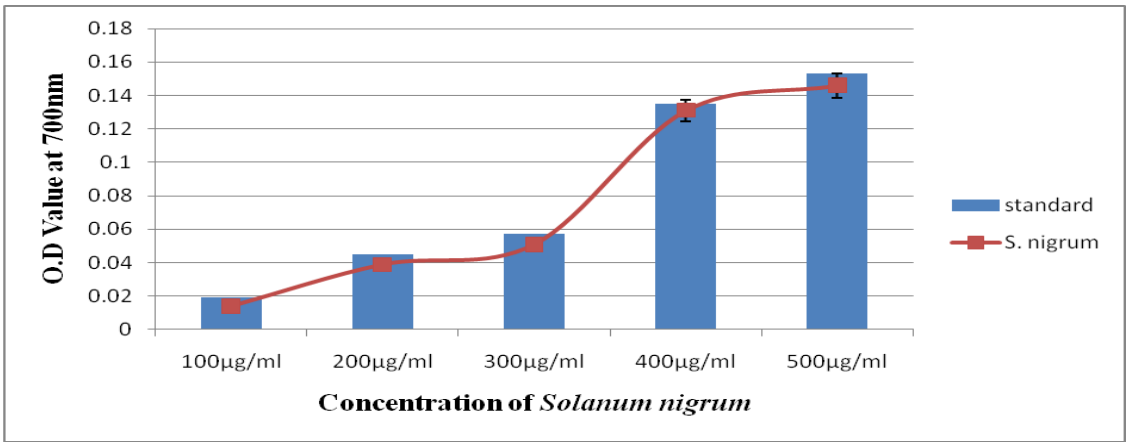

Figure 1: Reducing Power assay of aqueous extracts of S. nigrum (100-500 g/ml) in comparison with standard Ascorbic acid. Results are expressed as Mean±SD of three parallel measurements.

In the case of *P. niruri*, the results suggested that at the concentration of 100 µg/ml of the aqueous extract, the reducing power capability was 0.017±0.008. When concentration was increased to 500 µg/ml, the absorbance also increased to 0.143±0.141 (Figure 2).

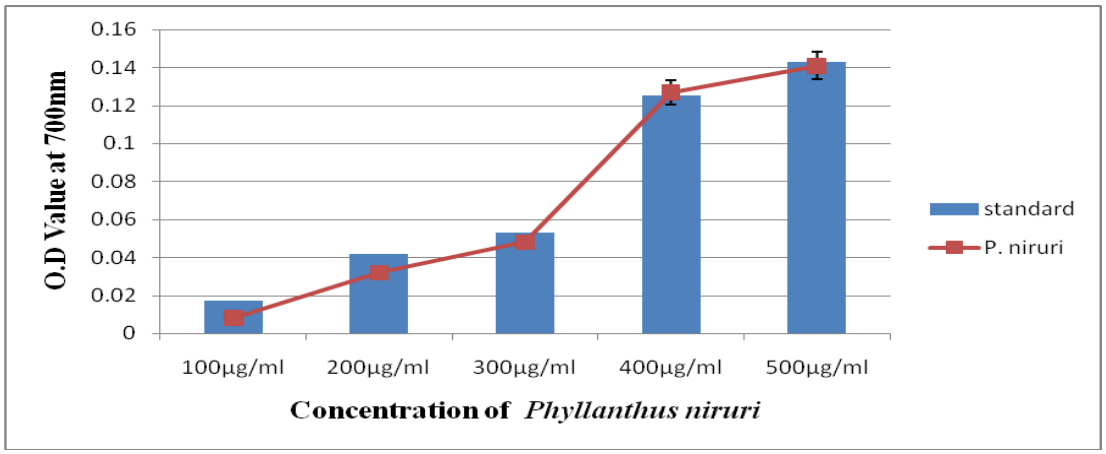

Figure 2: Reducing Power assay of aqueous extracts of *P. niruri* (100-500 g/ml) in comparison with standard Ascorbic acid. Results are expressed as Mean±SD of three parallel measurements.

3.4.2. Hydrogen Peroxide (H_2O_2) Scavenging Assay:

Hydrogen peroxide, a toxic component to the cells has the capability to form hydroxyl radical. H_2O_2 can react with Fe^{2}+ and cu ^{2}+ to form hydroxyl radicals. To examine the antioxidant potential of *S. nigrum* and *P. niruri*, the aqueous extract was assessed for its hydrogen peroxide scavenging activity. At the concentration of 100 µg/ml of aqueous extract of *S. nigrum*, the absorbance was 0.012±0.008. When the concentration was increased to 500 µg/ml the scavenging ability was increased to 0.164±0.152. The above result suggests that at lower concentration the aqueous extract of *S. nigrum* showed very low H_2O_2 scavenging capability. When extract concentration was increased, the H_2O_2 scavenging capability was also increased noticeably (Figure 3).

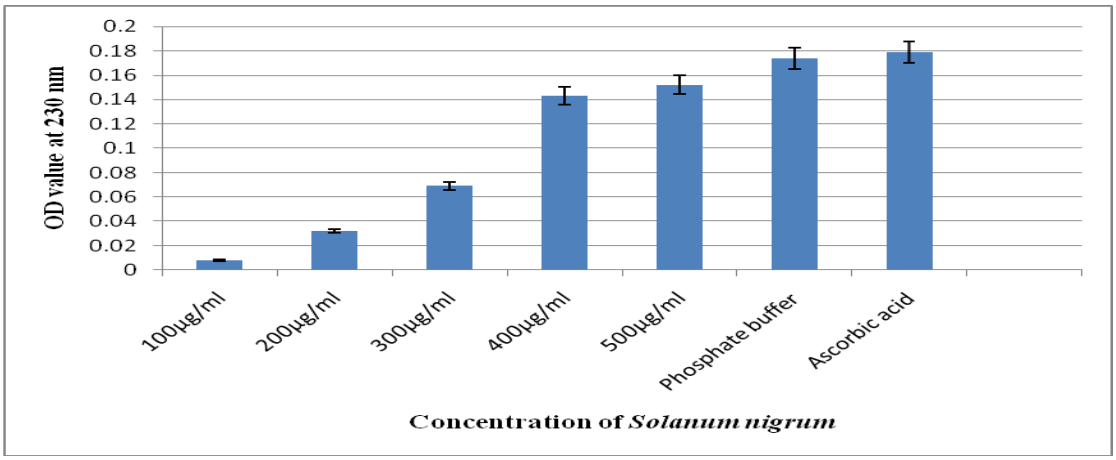

Figure 3: Hydrogen peroxide scavenging activities of Aqueous extracts of *Solanum nigrum* (100-500g/ml) in comparison with standard Ascorbic acid. Results are expressed as mean ± SD of three parallel measurements.

In the case of *P. niruri*, at the concentration of 100 µg/ml of extract, the H_2O_2 scavenging ability was 0.018±0.27. When concentration was increased to 500 µg/ml the scavenging ability was increased to 0.164±0.152. The above results suggest that at lower concentration the aqueous extract of *S. nigrum* showed very low H_2O_2 scavenging capability. When extract concentration was increased, the H_2O_2 scavenging capability was also increased noticeably. (Figure 4).

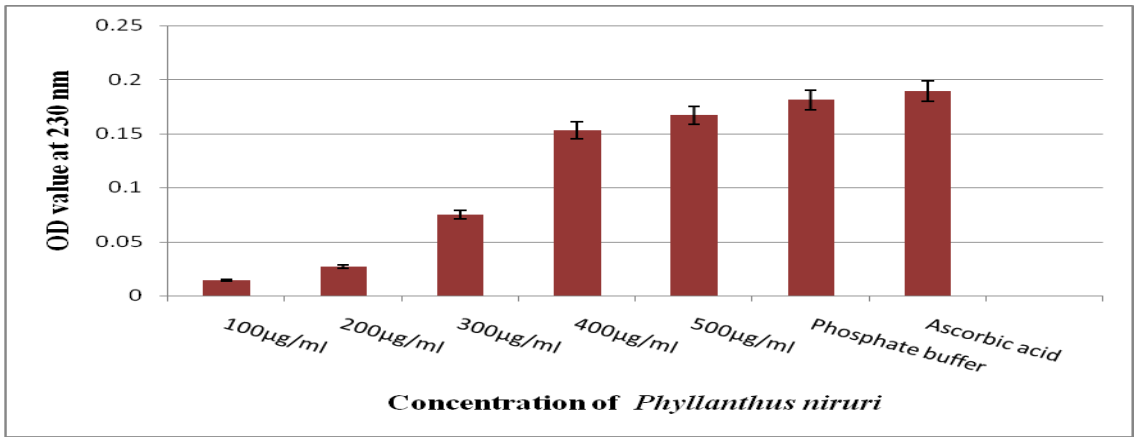

Figure 4 : Hydrogen peroxide scavenging activities of Aqueous extracts of *Phyllanthus niruri* (100-500g/ml) in comparison with standard Ascorbic acid. Results are expressed as mean ± SD of three parallel measurements.

CONCLUSION

The results of the present study suggest that the edible plants *S. nigrum* and *P. niruri* possess excellent antioxidant potential. In addition to that these plants hold high nutritive value and high fibre content with it. The results of phytochemical screening showed that the plants has high amount of flavonoids and terpenoids. Altogether, these two plants could be taken as the potential candidate for the assessment of various therapeutic potentials.

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