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Research Article
Antioxidant Activities of Leaves and Fruits of *Piper nigrum* and *Piper longum*

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Abstract

Background and Objectives: Herbs and spices have been used to enhance flavors of food, as well as for their medicinal purposes. Herbs usually contain antioxidant properties. The present study was focused on the importance of the antioxidants present in *Piper nigrum* and *Piper longum* widely used by the people of India in their food. **Materials and Methods:** The methanolic extracts of the leaves and fruits of both *Piper nigrum* and *Piper longum* were prepared using soxhlet extraction method. The total phenolic content (TPC) of the plant samples were determined by the Folin-Ciocalteu method. The total flavonoid content (TFC) of the plant was determined. The inhibitory effect of the plant against oxidation by peroxides was evaluated by ferric thiocyanate assay. **Results:** The highest concentration of phenol was obtained from *Piper nigrum* leaves. The highest flavonoid content was observed in the *Piper nigrum* leaves (0.15 mg). The higher reducing potency of the antioxidants was present in the leaves and fruit of *Piper nigrum* and *Piper longum* exhibiting their antioxidant properties. The ability of the plant extracts of *Piper nigrum* and *Piper longum* against lipid peroxidation was revealed through the efficiency of inhibiting the radicals at a percentage of 58.33, 77.77, 66.66 and 22.22, respectively. **Conclusion:** From the study it was concluded that leaves and fruits of *Piper nigrum* and *Piper longum* have shown high antioxidant properties. So, they are considered to be rich sources of natural antioxidants for food, cosmetic and pharmaceutical industries.

Key words: Antioxidant, lipid peroxidation, flavonoid content, free radicals, cosmetics, pharmaceutical industries

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants have been the source of medicines since thousands of years. In ancient times, spices, spice extracts and herbs have been thought to cure diseases. Herbs and spices have been used to enhance flavors and fragrances of food, as well as for their medicinal purposes[1]. Many spices have antioxidant properties and also prevent food rancidity.

Piper nigrum and *Piper longum*, belonging to the family Piperaceae, are considered to be potent for their antioxidant source[2]. The plant and its active component piperine can stimulate the digestive enzymes of pancreas and intestines and also increases biliary bile acid secretion when orally administrated[3-4]. *Piper nigrum* has several uses such as they help in pain relief, rheumatism, chills, flu, colds, muscular aches and fever. *Piper longum* have strong aromatic and medicinal properties. Indian long pepper, or pippali (*Piper longum*), indigenous to North-eastern and Southern India and Sri Lanka, is a powerful stimulant for both the digestive and the respiratory systems. It has been shown to have a rejuvenating effect on the lungs. The plant plays an important role in aiding the thermogenic response. They release metabolic energy. This effect is the increased thyroid hormone level in the body and makes Pippali a typical Ayurvedic complementary component whose benefit is to increase the bioavailability and enhance absorption of the other active ingredients[5-6].

The objective of the present study was to determine the antioxidant properties of two important spices i.e., *Piper nigrum* and *Piper longum*. The antioxidants properties of leaf and fruit extracts of these spices were compared to understand the efficacy of these extracts to be used as a new source of natural antioxidant for food, cosmetic and pharmaceutical industries.

MATERIALS AND METHODS

Study area: The present study was carried out in the life science laboratory of Department of Life Sciences, CHRIST (Deemed to be University), Bangalore. The total duration of this study was from 15 June, 2017-20 March, 2018.

Preparation of methanolic extract: The methanolic extracts of the leaves and fruits of both *Piper nigrum* and *Piper longum* were collected from CHRIST University central campus garden, Christ University Kengeri campus, and St. Mary's farm, Kengeri, Bengaluru. They were dried under shade or using an oven. The dried sample then powdered and keep it for further use.

The dry powdered plant samples were mixed with methanol at a ratio of 1:10 and maintained temperature controlled shaker for 48 h at 30 (±2)°C. The crude extracts obtained upon filtering and concentrating, was recognized with solvent for further analysis.

Determination of total phenolic content: The total phenolic content (TPC) of the plant samples of the leaves and fruits of both *Piper nigrum* and *Piper longum* were determined by the Folin-Ciocalteu method[7]. One hundred microliter of the plant extracts, 500 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added and incubated for 6 min at room temperature. The final volume of the solution was made up to 3 mL after addition of 1.25 mL of 7% sodium carbonate. The mixture was incubated for 90 min, followed by measuring the absorbance at 760 nm using Colorimeter. The total phenolic content was expressed as mg AE (Gallic acid equivalents) per g of the dry weight of the plant, using a standard plot of Gallic acid.

Determination of total flavonoid content: The total flavonoid content (TFC) of the plant was determined by the method adopted by Moussa *et al.*[8] Two hundred microliter of the plant extracts were taken in a test tube and the solvent was allowed to evaporate by showing the boiling tube on the flame of the spirit lamp. The residue was mixed and shaken well with 5 mL of 0.1 M aluminum chloride. Upon incubation of the solution for 40 min at room temperature, the absorbance value was measured at 415 nm. A standard plot of quercetin at varying concentrations was used to evaluate the total flavonoid content, expressed as µg QE (Quercetin Equivalent) g^{-1} dry weight of the plant material.

Determination of total antioxidants: Phosphomolybdenum method was employed for the estimation of total antioxidant activity[9]. A reagent solution of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was used for the experiment.

The plant extracts (0.5 mL) were mixed with 4.5 mL of the phosphomolybdenum reagent solution and maintained in a boiling water bath at 95°C for 90 min. the absorbance value was measured at 695 nm upon cooling the solution at room temperature. Ascorbic acid was used as positive reference standard.

Ferric reducing antioxidant power (FRAP) assay: A mixture of plant extract (1 mL), Phosphate buffer -2.5 mL (of 0.2 M, pH 7) and 1% potassium ferricyanide (2.5 mL) was incubated at 50°C for 30 min. To the solution, 2.5 mL of 10%

Trichloroacetic acid was added, mixed and centrifuged for 10 min at 6500 rpm. Distilled water of 2.5, 0.5 mL of 0.1% $FeCl_3$ was added to 2.5 mL of the supernatant. The absorbance of the solution was measured at 700 nm. The results were expressed as Ascorbic acid equivalent antioxidant capacity (AEAC)[10].

Thiobarbituric acid assay: Two milliliters each of 20% trichloroacetic acid and 0.67% Thiobarbituric acid were mixed with 1 mL of 2.51% linoleic acid and 1 mL of plant extract. The solution was maintained in boiling water bath for 10 min. Upon cooling, the solution was centrifuged at 3000 rpm. The supernatant was passed through UV-visible spectrophotometer at 532 nm to measure the absorbance[11]. The percentage inhibition of the plant against the secondary products of lipid peroxidation was evaluated with reference to the standard solution of Butylated hydroxyl toluene (BHT):

$$\text{Inhibition } (\%) = \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Statistical analysis: The statistical analysis was carried out by evaluating the lack of fit, coefficient of regression (R^2) and the Fisher test value (f-value) obtained from the analysis of variance (ANOVA) considering $p<0.05$ as significant using the software SPSS. Three-dimensional response plots were generated by keeping antioxidant properties as dependent variable and plotting it against two factors, i.e., plant species and different parts of the plants as independent variables.

RESULTS

The total phenolic content at 760 nm absorbance reading showed that *Piper nigrum* leaves had the highest value of absorbance (1.87). *Piper nigrum* fruits showed the absorbance of 1.43, *Piper longum* leaves had the absorbance of 1.27 and the lowest value was that of *Piper longum* fruits 1.15. From the standard phenol graph, the concentrations of the plant samples were obtained. The highest concentration of phenol was obtained from *Piper nigrum* leaves (1.50 mg g^{-1}). *Piper nigrum* fruits, *Piper longum* leaves and *Piper longum* fruits have obtained little lower concentrations 1.15, 1.03 and 0.93 mg g^{-1}, respectively (Table 1). The highest concentration of phenol in *Piper nigrum* in comparison to other plant samples tested proved its high antioxidant properties.

The total flavonoid content of the plant samples was given in Table 2. Standard values of flavonoid content using quercetin at 765 nm were given. The highest flavonoid content was observed in the *Piper nigrum* leaves (0.15 mg). While the other values were observed were *Piper nigrum* fruits (0.14 mg), *Piper longum* leaves (0.03 mg) and *Piper longum* fruits (0.07 mg) (Table 3).

Table 1: Total phenolic content of the leaf and fruit extracts of *P. nigrum* and *P. longum*

Plant samples	Concentration (mg g^{-1})
Piper nigrum leaves	1.50±0.002
Piper nigrum fruits	1.15±0.005
Piper longum leaves	1.03±0.003
Piper longum fruits	0.93±0.003

Table 2: Standard absorbance readings of quercetin at 765 nm

Volume (mL)	Quercetin
Blank	0.00
0.2	0.29
0.4	0.53
0.6	0.62
0.8	0.71
1.0	0.94

Table 3: Total flavonoid content of the leaf and fruit extracts of *P. nigrum* and *P. longum*

Plant samples	Concentration (mg g^{-1})
Piper nigrum leaves	0.15±0.05
Piper nigrum fruits	0.14±0.03
Piper longum leaves	0.03±0.04
Piper longum fruits	0.07±0.02

Table 4: Absorbance reading of plant sample at 700 nm of FRAP assay

Plant samples	Concentration (mg g^{-1})
Piper nigrum leaves	0.92±0.001
Piper nigrum fruits	0.89±0.003
Piper longum leaves	0.47±0.003
Piper longum fruits	0.70±0.001

The results obtained of the different samples using the FRAP assay were given in the Table 4. FRAP measures the reducing potency of extract and standard antioxidant. By comparing with the standard values using ascorbic acid as standard at 700 nm the plant sample extracts had higher absorbance. This higher absorbance indicated the higher reducing potency of the antioxidants in the leaves and fruit of *Piper nigrum* and *Piper longum*.

Phosphomolybdenum assay was based on the reduction Phosphate- Mo (VI) to Phosphate Mo (V) by the sample and subsequent formation of bluish green colored phosphate/Mo (V) complex at acid pH. This Phosphomolybdenum method was mainly used in the laboratory to evaluate the total antioxidant capacity of plant extracts. Comparing the absorbance reading of the plant extracts and the standard using ascorbic acid (Table 5) it was noted that the plant extract absorbance was higher. The higher colour intensity proved that plant extracts had more antioxidant capacity than

Table 5: Absorbance reading of standard using ascorbic acid at 695 nm

Volume (µL)	Absorbance reading at 695 nm
100	0.143±0.01
200	0.333±0.04
300	0.529±0.02
400	0.871±0.02
500	1.135±0.01

Table 6: Absorbance reading of plant samples at 695 nm of PM assay

Plant samples	Concentration (mg g^{-1})
Piper nigrum leaves	0.470±0.01
Piper nigrum fruits	0.632±0.03
Piper longum leaves	0.204±0.04
Piper longum fruits	0.212±0.02

Table 7: Absorbance reading of plant samples at 700 nm of total antioxidant content

Plant samples	Absorbance reading at 700 nm
Piper nigrum leaves	1.27
Piper nigrum fruits	1.15
Piper longum leaves	1.48
Piper longum fruits	1.72

Table 8: Absorbance reading of plant samples at 532 nm of thiobarbituric acid assay

Samples	Absorbance at 532 nm	Inhibition (%)
Blank	0.00	
Control	0.36	
Piper nigrum leaves	0.15	58.33
Piper nigrum fruits	0.08	77.77
Piper longum leaves	0.12	66.66
Piper longum fruits	0.28	22.22

Table 9: ANOVA table showing the statistical analysis for the antioxidant properties of plants and their different parts used

Source	Type III sum of squares	df	Mean square	F-value	Significance
Corrected model	00290.481[a]	3	0096.827	0.165	0.918
Intercept	02401.840	1	2401.840	4.101	0.056
Plant samples	00098.626	1	0098.626	0.168	0.686[b]
Parts used	00025.892	1	0025.892	0.044	0.836[b]
Plant samples* Parts used	00165.964	1	0165.964	0.283	0.600[b]
Error	11712.209	20	0585.610		
Total	14404.530	24			
Corrected total	12002.690	23			

[a]R squared: 0.024 (Adjusted R squared = -0.122), [b]Not statistically significant difference is observed, *p>0.05

the synthetic antioxidants. The least absorbance was obtained from *Piper longum* leaves. The leaf and fruits extracts of *Piper nigrum* and *Piper longum* contained 0.470, 0.632, 0.204, 0.212 mg g^{-1}, respectively as presented in Table 5 and 6.

The obtained results of total antioxidant content of the plant samples were given in Table 7. The values obtained of the plant samples such as *Piper nigrum* leaves, *Piper nigrum* fruits, *Piper longum* leaves and *Piper longum* fruits were at the absorbance reading at 700 nm are 1.27, 1.15, 1.48 and 1.72, respectively. The highest value of the total antioxidant observed was *Piper longum* fruits (1.72). The lowest value of the total antioxidant observed was *Piper nigrum* fruits (1.15).

The ability of the leaf and fruit extracts of *Piper nigrum* and *Piper longum* against lipid peroxidation was evaluated using 2-thio Barbituric acid (TBA) and it revealed the efficiency of inhibiting the radicals at a percentage of 58.33, 77.77, 66.66 and 22.22, respectively as presented in Table 8.

STATISTICAL ANALYSIS

Statistical significance study conducted using ANOVA revealed that there was no very significant difference in the antioxidant activities between parts used and between the plants studied, i.e., *Piper nigrum* and *Piper longum* as presented in Table 9. Leaf and fruits of both the plants showed the good antioxidant properties.

DISCUSSION

The present study has revealed that leaf and fruit extracts of *P. nigrum* and *P. longum* are very good sources of antioxidant properties. Both the plants contained the high concentrations of phenolic compounds. Various studies have reported that the presence of phytochemical like flavonoids and phenols might be responsible for the free radical scavenging activity of the plants pointing to their use as a potential source of natural antioxidant[12]. In the present scenario, the study of antioxidant has got very significant role because of its health benefits. The antioxidants belong to a diverse group of chemical compounds which act as a protection to the body from oxidative damage induced by free radicals and reactive oxygen species by suppressing their formation acting as scavengers and acting as their substrate[13]. A positive correlation between total phenol and antioxidant activity of plant species have been reported in many of the plant species and it is attributed to the scavenging ability of their phenolic hydroxyl groups[14]. Medicinal plants are important sources of antioxidants. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases[15]. The major plant compounds with antioxidant activity are polyphenols. It is reported that the phenolic compounds are responsible for the antioxidant activities of the plants. They exhibit antioxidant activity by inactivation of lipid free radicals or they prevent decomposition of hydro peroxides into free radicals[14]. In another study it was confirmed that natural antioxidants present in herbs and spices were responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contained free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the

present study, it was evaluated the free radical scavenger activity of methanolic leaves and fruits extract of *Piper nigrum* and *Piper longum.* Among the four samples extracted, the study revealed the existence of difference in total phenolic content, flavonoid content and antioxidant activities. *Piper nigrum* leaves showed higher phenolic content. The antioxidants protect against the damage induced by free radicals acting at various levels. The relation between free radicals, antioxidants and functioning of various organs and organ systems are highly complex[15].

As we know oxygen is very essential in the human body for our very life. But the oxygen in excess in the body is very harmful. The reactive oxygen species (ROS) are generated by many redox processes that normally occur in the metabolism of aerobic cells in the human body. These reactive species of oxygen are highly reactive and harmful to the cells. If we do not eliminate it causes damage to important molecules such as proteins, DNA and lipids. Our human body has the capacity of producing antioxidants which help in the recovery of the cells. But as we become old the capacity of body to produce antioxidant will get reduced and there is a chance of getting affected by the free radical activities in the body. Hence there is a need of survival. Herbs and spices are producing the antioxidants which help in the survival of our body to protest against the activity of free radicals.

CONCLUSION

The study of fruits and leaves extracts of *Piper nigrum* and *Piper longum* on total phenolic content, total flavonoid content and antioxidant content revealed the existence of differences among them. The total phenolic and flavonoid contents were found to be high in *Piper nigrum* leaves. Better reduction of ferrous ions to ferric ions was recorded for the plant samples comparing with the standards. A better reduction occurred in Phosphomolybdenum assay in fruits of both plant species than the leaves of the same. A better inhibition percentage of radicals in TBA assay were found to be in *Piper nigrum* fruits.

SIGNIFICANCE STATEMENT

This study discovered that the leaf and fruit extracts of *P. nigrum* and *P. longum* are rich of antioxidants that can be beneficial for eradication of many diseases. So, this study will help the researchers to uncover the critical areas of pharmaceutical analysis and develop new drugs based on the herbal products from these plants.

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