

PHYTOCHEMICAL, ANTIBACTERIAL AND ANTI-OXIDANT ACTIVITY OF PIPER LONGUM LEAVES

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Abstract

Piper longum L. (Piperaceae) commonly known as “long pepper” is a well-known medicinal plant in ayurveda. Different parts of this plant, such as root, seed, fruit, whole plant etc. are traditionally used in various ailments. The methanol extract of leaves of *Piper longum* was subjected to evaluate phytochemical, antibacterial and antioxidant activity. In order to investigate the antioxidant activity, different complementary test systems, namely, 2,2-diphenyl-1-picryl-hydrazil (DPPH) free radical scavenging, total phenolic, total tannin and flavonoid contents were used. Total flavonoids content was spectrophotometrically determined using aluminum chloride colorimetric assay while total phenolic and tannin content by Folin Chiocalteu’s reagent. Antibacterial activity was evaluated by disc diffusion assay. Phytochemical analysis of the extract indicated the presence of saponins, tannins, glycosides, alkaloids, and flavonoids. Extract contains greater amount of total phenolic (93.94 mg GAE/g dry extract), flavonoids (44.15 mg QE/g dry extract) and tannin content (58.06 mg QE/g dry extract). Extract showed DPPH scavenging activity (149.92 µg/mL) that was comparable to standard ascorbic acid (25.42 µg/mL). In disc diffusion assay experimental extract showed potent antibacterial activity.

Keywords: *Piper longum*, Total phenolic content, Total flavonoids content, Total tannin content, Antioxidant activity, Antibacterial activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Introduction

Plants, as the source of medicine, have been playing an important role in the health services around the globe [1]. The medicinal value of plants lies in some chemical substances that they contain. The most important of these bioactive compounds of plants are alkaloids, tannins and phenolic compounds [2]. Phenolic compounds are the largest group of phytochemicals that account for the antioxidant and antimicrobial activities in plants or plant products. [3]. Antioxidants decrease oxidative stress and minimize the incidence of pathological conditions caused by the oxidants. The generation of oxidative stress is harmful to the body and may cause peroxidation of membrane lipids leading to loss of membrane integrity and cell death, denaturation of proteins including enzymes, ion channels and strand breakage in DNA. [4] Thus antioxidant based drug formulations are instrumental in the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer. [5] The important benefits in using medicinal plants are that they are easily available, yield profound therapeutic benefits without any adverse effect and are inexpensive treatment in comparison to their synthetic alternatives. [6]

Piper longum Linn, sometimes called Indian Long Pepper, is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. It is a close relative of the black pepper plant. The fruits have a bitter, hot, sharp taste, tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive. *Piper longum* contains piperine, piper longamine, volatile oil, resin, gums and fatty oil. The fruits of *Piper longum* are useful in spleen disorders, bronchitis, tuberculosis and jaundices. They have a pungent pepper-like taste and produce salivation and numbness of the mouth [7]. The fruits and roots are attributed with numerous medicinal uses, and may be used for diseases of respiratory tract, viz. cough, bronchitis, asthma etc; as counter-irritant and analgesic when applied locally for muscular pains and inflammation; as snuff in coma and drowsiness and internally as carminative. Besides fruits, the roots and thicker parts of stem are cut

and dried and used as an important drug in the Ayurvedic and Unani systems [8].

The present study is, therefore, designed to assess the antioxidant activities and antibacterial activity of leaves extracts of *Piper longum* on some selected microorganisms.

Methods

Chemicals and reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH.), Folin-Ciocalteu reagent, Gallic acid, Quercetin, Ascorbic acid and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, ethanol were obtained from Merck (Darmstadt, Germany).

Microorganisms

Six species of both Gram positive and Gram negative bacteria were used for antibacterial assay. The bacterial strains used for the investigation were *Shigella dysenteriae*, *Escherichia coli*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*. The bacterial strains were collected from the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B).

Plant materials and extraction

The leaves of *Piper longum*, were collected in January, 2018 from Botanical garden, Dhaka, Bangladesh and identified by experts at Bangladesh National Herbarium, Bangladesh. A voucher specimen (DACB 53271) has been submitted there for future reference.

Preparation of crude extract

The collected plant leaves were separated from undesirable materials and shade-dried and finally ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). About 500 gm of leaf were macerated in 1.5 liters methanol (98%), for a period of 14 days accompanying routine shaking and stirring. After filtration and evaporation, using rotary vacuum evaporator (Bibby RE200, Sterilin Ltd., UK), the weight of the obtained crude extract was 6.3gm. Then the crude extract was stored in a refrigerator at 4 °C.

Phytochemical screening

Different phytochemical groups such as carbohydrates, alkaloids, glycosides, flavonoids, tannins, phenols, gums and saponins were identified by characteristic color change using standard chemical tests [9]. Lead acetate, alkaline reagent, ferric chloride and ammonia tests were used for detection of flavonoids. Alkaloids were detected using the Dragendorff's, Mayer's, Hager's and Wagner's tests. For identification of tannin potassium dichromate test, ferric chloride, potassium hydroxide and lead acetate tests were followed. Legal's test, Keller-Kiliani and Borntrager's tests were performed to identify glycosides. Salkowski test and froth test were used to detect the presence of saponins. Molisch test was performed for detecting the existence of gums in the samples. Carbohydrates are detected by Molisch test, Benedict's test and Fehling's test.

Antioxidant activity

The antioxidant potential of the extracts was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical [10]. At first different concentrations (400-1.57 µg/ml) of crude and fractionated extracts were prepared. In 2mL of each of the different concentration 6mL of freshly prepared DPPH solution (0.004%) was added. They were kept for 30 minutes in dark at room temperature to complete the reaction. Then absorbance was measured at 517 nm against suitable blank. In this assay well known free radical scavenger, ascorbic acid was used as standard. IC₅₀ value, the concentration of sample required to scavenge 50% of the DPPH free radicals was determined by using the following formula:

$$\% \text{ inhibition} = \{(\text{Blank absorbance} - \text{Sample absorbance}) / \text{Blank absorbance}\} \times 100$$

Total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu's method [11]. Methanol solution of the extract (1 mg/mL) was mixed with 5 mL of 10% (v/v) Folin-Ciocalteu reagent. Then 4 mL sodium carbonate (75 g/L) was added to the mixture. It was kept at 40 °C for 30 min. Absorbance of the reaction mixture was measured at 765 nm. Different concentrations (0.1–

0.5 mg/ mL) of gallic acid were used to prepare the standard calibration curve from where total phenol content was determined and expressed as mg gallic acid equivalent (GAE) per gram of dry extract.

Total flavonoids content

Total flavonoid content was estimated using aluminum chloride colorimetric assay [12]. In 1 mL of the extract solution (1 mg/mL), 0.2 mL aluminium chloride (1% w/v), 0.2 mL potassium acetate (1 M) and 5.4 mL distilled water were added and mixed well. Then absorbance was measured at 415 nm against blank solution. For this assay, quercetin (0.1–0.5 mg/mL) was used to prepare standard calibration curve and total flavonoid content of the extract was expressed in terms of mg quercetin equivalent (QE)/g of dried extract.

Total tannin content

The tannin content of the extracts was determined by Folin Ciocalteu method [13]. In 0.1 mL of the extract solution, 7.5 mL of distilled water and 0.5 mL of FolinCiocalteu phenol reagent, 1 mL of 35% (w/v) Na₂CO₃ solution were added and dilute to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20-100 µg/mL) were prepared for standard calibration curve. Absorbance for test and standard solutions were measured against suitable blank at 725 nm. The tannin content was expressed in terms of mg of GAE /g of the dry extract.

Antibacterial activity by Disc diffusion method

Antibacterial activity of was tested by using the disc diffusion method [14]. In this method-measured amount of the test samples were dissolved in definite volumes of solvent to prepare solutions of desired concentration (µg/ml). The sterile Matricel (BBL, Cocksville, USA) filter paper discs were impregnated with known amount of test substances using micropipette and dried. Kanamycin (5µg/disc) was used as standard. Disk of sample, standard and control were then placed in petridishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop. The plates were then kept at 40°C for facilitating maximum diffusion and then in an incubator for 12-18 hour to allow the growth of the microorganisms.

After the incubation period the diameter of the zone of inhibition was measured in term of millimeter. The experiments are carried out duplicate manner.

Results

Phytochemical screening

Phytochemical studies showed that alkaloids, glycosides, flavonoids, tannins, and saponins were present, while steroids and gums were absent in the extract.

Antioxidant activity

Extract was subjected to free radical scavenging activity. In this investigation, the leaves extract showed free radical scavenging activity ($IC_{50}=149.42 \mu\text{g/ml}$) while ascorbic acid was $25.42 \mu\text{g/ml}$.

Total phenolic content

In case of total phenolic content, the absorbance values obtained in the test using different concentrations of gallic acid were plotted against respective concentrations. A standard calibration curve was obtained with the equation $y = 4.033X + 1.8251$ ($R^2 = 0.8784$) (Fig.2). Total phenolic content of the *Piper longum* leaves extract was calculated using the equation and found to be 93.94 mg GAE/g dry extract.

Total flavonoid content

The absorbance values obtained in the test using different concentrations of quercetin were plotted against respective concentrations. A standard calibration curve was obtained with the equation $y = 0.106X + 0.0922$ ($R^2 = 0.9522$) (Fig.3). Total Flavonoid content of the leaves extract was calculated using the equation and found to be 44.15 mg QE/g dry extract.

Total tannin content

The absorbance values obtained in the total tannin content test using different concentrations of quercetin were plotted against respective concentrations. A standard calibration curve was obtained with the equation $y = 1.822X - 0.1208$ ($R^2 = 0.9803$). Total tannin content of the leaves extract was calculated using the equation and found to be 58.06 QE/g dry extract respectively.

Antibacterial activity

The extract showed good activity against *Staphylococcus aureus* and *Salmonella typhi* at different doses but reduced activity against *Bacillus subtilis*, *Salmonella paratyphi* and *Shigella dysenteriae* and mild activity against *Escherichia coli* (Table 3).

Discussion

Experimental investigation revealed that the methanol leaves extract of *Piper longum* showed different type indication for the presence of chemical compound in phytochemical screening which is correlated with pharmacological investigation. In the present study, phytochemical tests revealed the existence of saponins, alkaloid, tannin, flavonoid and glycosides. These phytochemical compounds presence can be correlated to the biological activities of *Piper longum*. Phenolic and flavonoid compounds are considered as very important secondary metabolites for biological activities [15]. Phenolic compounds have therapeutic potential against different diseases because of their antioxidant property. Flavonoids are a group of polyphenolic substances present in most plants and are responsible for various bio-chemical and antimicrobial activities. Antioxidants and anti-microbial properties of flavonoids from plant extracts have been reported in different studies [16][17]. It exerts the antioxidant activity via radical scavenging, metal ion chelation, and membrane protective efficacy. Moreover, some plant components like tannins can be responsible for antioxidant property [18]. The DPPH method was evidently introduced nearly 50 years ago by Blois [19] and it is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. The parameter IC_{50} (—efficient concentration value), is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) [20]. The experimental extract possess good amount of phenol, flavonoids and tannin contents. In the quantitative DPPH radical scavenging assay, *Piper longum* leaves extract explored free radical scavenging activity ($IC_{50} 149.92 \mu\text{g/ml}$). In the

present investigation, the leaves of *Piper longum* was evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria and revealed potent activity. Therefore, considering the potential bioactivity, the plant materials can further be studied extensively to find out their unexplored efficacy and to rationalize their uses as traditional medicines

Conclusion

Piper longum was proved to contain medicinal properties in various studies but the leaf of the plant also possess antibacterial activity against the multi-drug resistant organism is been reported by this study. It also has been determined that it possesses potent antioxidant activity. It needs to be further analyzed to know their beneficial effect in medical and pharmaceutical industries.

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References

1. Thomson GE. Further consideration of Asian Medicinal plants in treating common chronic disease in West. *Journal of Medicinal Plants Research* 2010; 4(2): 125.
2. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 2005; 4: 685–688.
3. Miller AL. Antioxidant flavonoids: structure, function, and clinical usages. *Alternat Med rev* 1996; 1:103-11.
4. Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. *Phytochem* 1987; 26: 2489-91.
5. Devasagayam TPA, Tilak JC, Boloor KK, Ketaki S Sane, Saroj S Ghaskadbi, et al. Review: Free radical and antioxidants in human health. *Curr Stat Fut Pros* 2004; 53:794-804.
6. Borokini TI, Omotayo FO. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *J Med Plants Res* 2012; 6:1106-118.
7. Kirtikar, K.R. and B.D. Basu. Indian medicinal plants, International book distributors, Dehradun, India, 1987; 3: 2128-2129.
8. CSIR. The Wealth of India: A dictionary of Indian Raw Materials and Industrial Products. Council of Scientific and Industrial Research, New Delhi, 1989; 8: 96-99.
9. Ghani A. Practical phytochemistry. Parash Publishers, Dhaka, 2005; 8–20.
10. Zilani MNH, Sultana T, Rahman SMA, Anisuzzman M, Islam MA, Shilpi JA, Hossain MG. Chemical composition and pharmacological activities of *Pisum sativum*. *BMC Complementary and Alternative Medicine* 2017; 17:171.
11. Zilani MNH, Islam MA, Khushi SS, Shilpi JA, Rahman MM, Hossain MG. Analgesic and antioxidant activities of *Colocasia fallax*. *Orient Pharm Exp Med*. 2016; 16 (2):131-37.
12. Imtiaz M, Zilani MNH, Biswas NN, Bokshi B. Bioactivities of *Bruguiera Gymnorhiza* and profiling of its bioactive polyphenols by HPLC-DAD. *Clinical Phytoscience* 2017; 3:11.
13. Salma AS, Siraj MA, Hossain A, Mia MS, Afrin S, Rahman MM. Investigation of the key pharmacological activities of *Ficus racemosa* and analysis of its major bioactive polyphenols by HPLC-DAD. *Evid Based Complement Alternat Med* 2016.
14. Zilani, M.N.H., Sultana, N.A., Bakshi, M.K. et al. Bioactivities of leaf and root extract of *Ceriscoids turgida* (Roxb.). *Orient Pharm Exp Med* 2018; 18: 159.
15. Dixit A, Singh H, Sharma RA, Sharma A. Estimation of antioxidant and antibacterial activity of crude extracts of *Thevetia peruviana* (pers.) K. Schum. *Int J Pharm Pharm Sci* 2015; 7:55–59.
16. Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. *Curr Cancer Drug Targets* 2008; 8: 634–646.
17. Lopez-Lazaro M. Distribution and biological activities of the flavonoid luteolin. *Mini Rev Med Chem* 2009; 9(1):31–59.
18. Zhang LL, Lin YM. Tannins from *Canarium album* with potent antioxidant activity. *J Zhejiang Univ Sci B* 2008; 9(5):407–415.

18. Blois, M.S. Antioxidant determinations by the use of a stable free radical. *Nature* 1958; 181: 1199–1200.
19. Charalampos P., et al. Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils, *Antioxidants* 2013; 2: 11-22.

Table 1: Phytochemical screening of extract

Test Sample	Steroids	Alkaloids	Glycosides	Flavonoids	Tannins	Gums	Saponins
Extract	-	+	+	+	+	-	+

Table 2: IC₅₀ values of standard and extract in antioxidant assay

Test Sample	IC ₅₀ (µg/ml)
Ascorbic acid	25.42
Extract	149.92

Table 3: Average zone of inhibition of extract against different bacterial strains

Kanamycin (30*)	Control	Extract (250*)	Extract (500*)	Bacterial Strains
18	0	7	8	<i>Escherichia coli</i>
19	0	7	8	<i>Shigella dysenteriae</i>
19	0	9	10	<i>Salmonella typhi</i>
18	0	5.5	7	<i>Salmonella paratyphi</i>
18	0	8	9	<i>Staphylococcus aureus</i>
19	0	6	8	<i>Bacillus subtilis</i>

* = µg/Disc

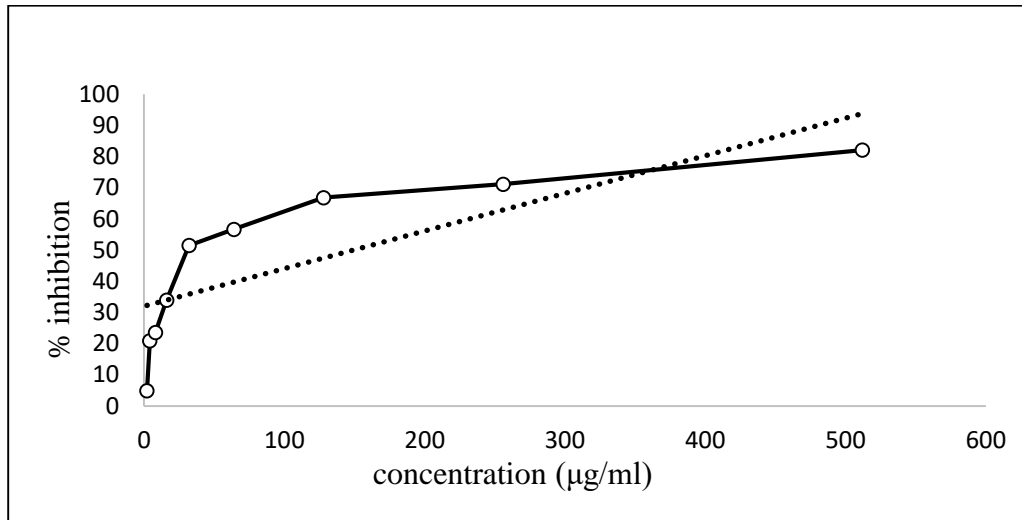
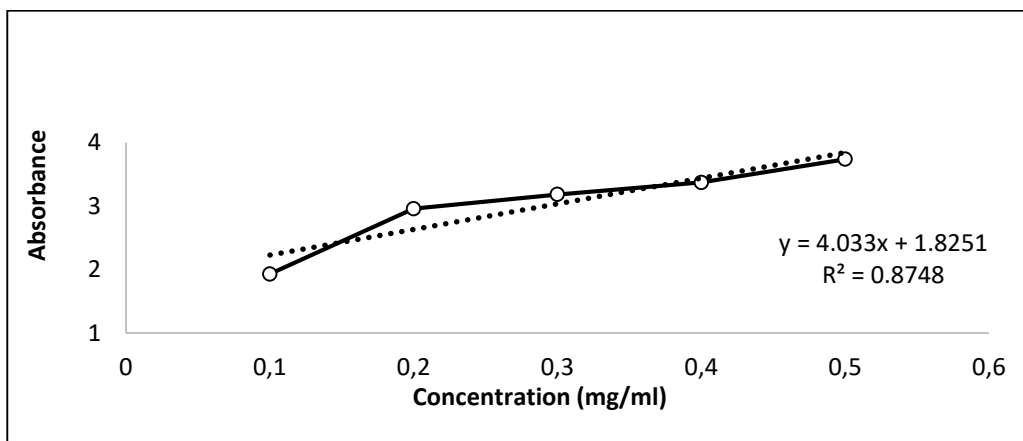
Fig. 1: DPPH radical scavenging activity of leaves of *Piper longum***Fig. 2:** Standard gallic acid calibration curve

Fig. 3: Standard quercetin calibration curve

