



Bioactivity of *Peperomia Pellucida* Leaves from Bangladesh

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Abstract

Peperomia pellucida (L.) belonging to family Piperaceae mainly grows in rainy season in Bangladesh. n-Hexane, dichloromethane and methanol extracts of leaves of *P. pellucida* were tested for antioxidant activity, antioxidant capacity, total phenolic content, total flavonoid content and cytotoxicity. Dichloromethane extract of the plant showed significant phenolic, flavonoid and antioxidant capacity while the methanol extract possessed potent antioxidant activity. None of the extract exhibited any cytotoxicity against HeLa cell lines.

Keywords: *Peperomia Pellucida*; Piperaceae; Antioxidant Activity; Cytotoxicity

Introduction

Peperomia pellucida (L.) belongs to Piperaceae family. There are about 1500-1700 species of the genus *Peperomia* [1]. It is an herbaceous plant found in many south American, Asian and African countries and also known as shiny bush or silver bush [2]. The species usually develops during rainy seasons and blooms in loose, humid soils under the shade of trees [3-6]. It was reported that the plant contained various secondary metabolites including alkaloids, saponins, tannins, cardenolides, flavonoid, essential oils and carotol [7,8]. *P. pellucida* has been used for treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain [8,9]. Bangladesh is a tropical country with six seasons and May-June are rainy seasons with heavy monsoon rain. *P. pellucida* usually grows abundant during monsoon time. The present study was aimed to study biological activities *i.e.*, antioxidant activity, antioxidant capacity, total phenolic content and total flavonoid content and cytotoxicity assay of different extracts of *P. pellucida* leaves.

Materials and Methods

Sample collection and extraction: Leaves of *P. pellucida* were collected from different places of Dhaka city in 2017. The plant was identified and confirmed by the Botanist of the Department of Botany, University of Dhaka. The fresh plants were washed with clean running water to remove all the soils and dirt and then air dried followed by grinding into powder (172.0 g). The finely ground

powder of *P. pellucida* leaves (172.0 g) were extracted successively with n-hexane, dichloromethane and methanol at room temperature for 72 hours with 3 replications. All filtrates were dried separately by rotary vacuum evaporator at 40 °C and n-hexane (3.50 g), dichloromethane (3.80 g) and methanol (16.0 g) were obtained.

Total Phenolic Content

The total phenolic contents were determined by modified Folin-Ciocalteu method and expressed as mg gallic acid equivalents per gram of dry extract using the equation obtained from gallic acid calibration curve, $y = 0.0036x - 0.0013$, $r^2 = 0.9979$ [10].

Total Flavonoid Content

Aluminium chloride colorimetric method was used for the determination of total flavonoid content of the *P. pellucida* extracts which was determined as mg quercetin equivalent per gram of dry extract using the equation obtained from quercetin calibration curve $y = 0.0422x + 0.0029$; $r^2 = 0.9984$ [11].

Total Antioxidant Capacity

The total antioxidant capacity of the *P. pellucida* extracts were evaluated by the Phosphomolybdenum assay method [12]. The total antioxidant capacity was determined and expressed as mg ascorbic acid equivalents per gram of dry extract using the equation obtained from ascorbic acid calibration curve, $y = 0.0021x - 0.0044$, $r^2 = 0.9924$.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The stable DPPH radical-scavenging activity was measured using the modified method described by Gupta [13].

$$\text{DPPH radical-scavenging activity (I \%)}, \\ = [(A_0 - A) / A_0] \times 100$$

where A_0 is the absorbance of the control solution; A is the absorbance of the DPPH solution containing plant extract.

Cytotoxicity assay on cancer cell line

Cytotoxicity assay was examined against HeLa, a human cervical carcinoma cell line and was maintained in DMEM (Dulbecco's modified Eagles medium) containing 1% penicillin-streptomycin (1:1) and 0.2% gentamycin and 10% fetal bovine serum (FBS). Cells ($4 \times 10^4 / 200 \mu\text{l}$) were seeded onto 48-well plate and incubated at $37^\circ\text{C} + 5\% \text{CO}_2$. Next day, 50 μl sample (filtered) was added each well. Cytotoxicity was examined under an inverted light microscope after 48 hours of incubation. n-hexane, dichloromethane and methanol extracts of *P. pellucida* were tested against HeLa cell line (Figure 2). Duplicate wells were used for each sample.

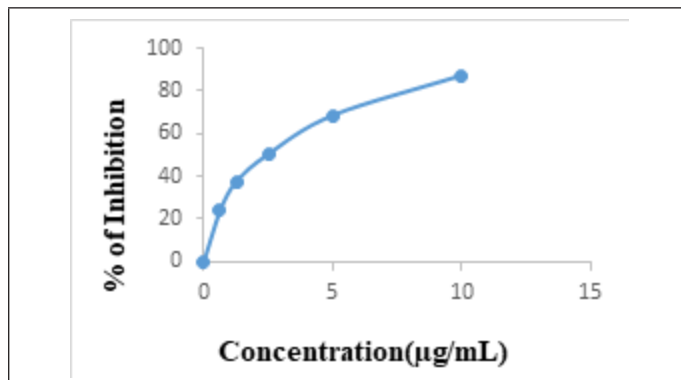


Figure 1: DPPH radical scavenging activity of ascorbic acid.

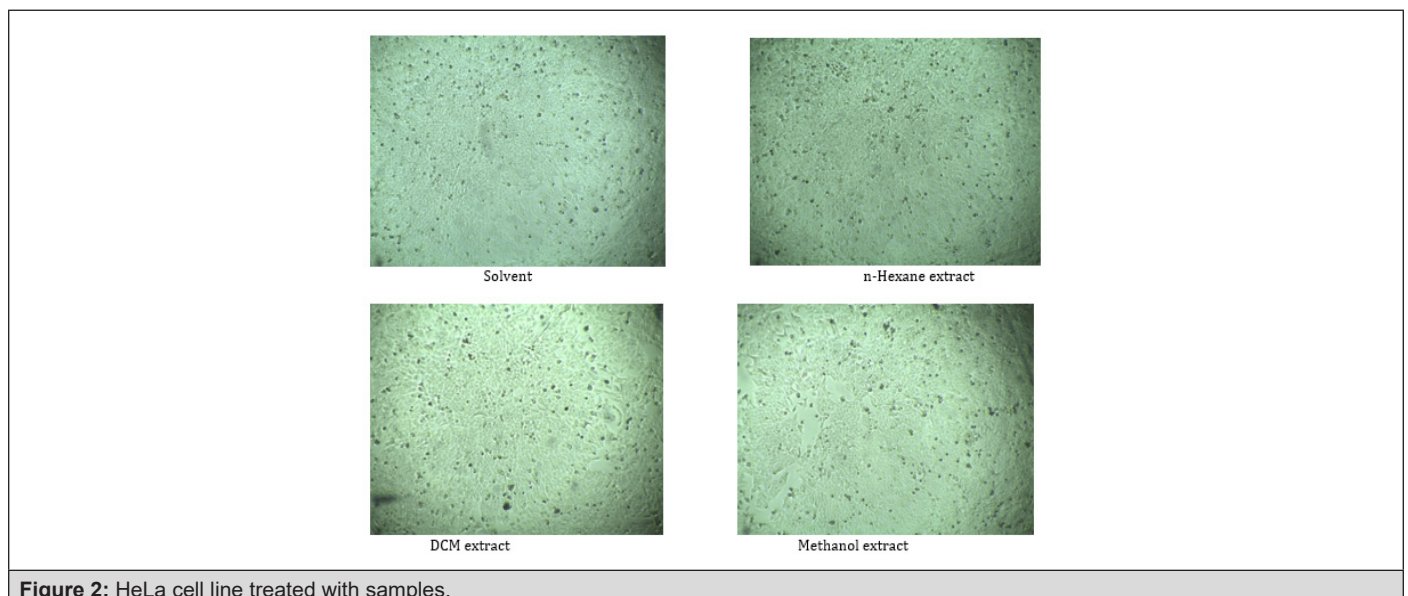


Figure 2: HeLa cell line treated with samples.

Results and Discussion

Total Phenolic Content

The total phenolic content of the n-hexane, dichloromethane and methanol extracts were 43.528 ± 0.0016 , 82.417 ± 0.0016 and 9.316 ± 0.0037 mg GAE/ g of dry extract, respectively.

Total Flavonoid Content

The total flavonoid content of the n-hexane, dichloromethane and methanol extracts were 64.65 ± 0.012 , 94.72 ± 0.021 and 9.316 ± 0.0037 mg QE/ g of dry extract, respectively.

Total Antioxidant Capacity

The total antioxidant capacity was based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of green phosphate complex at acidic pH. It was observed that the DCM extract possessed significant total antioxidant capacity equivalent to 338 mg ascorbic acid/g of dry extract. However, MeOH and n-hexane extracts were found to be 9.429 ± 2.190 and 326.571 ± 2.810 mg ascorbic acid equivalent/g of dry extract, respectively.

DPPH Free Radical Scavenging Activity

Ascorbic acid was used as positive control (Figure 1). The IC_{50} value of n-hexane, DCM and MeOH extracts were $1041 \pm 0.140 \mu\text{g/mL}$, $527.594 \pm 0.0263 \mu\text{g/mL}$ and $135.831 \pm 0.0086 \mu\text{g/mL}$, respectively while that of ascorbic acid was $2.157 \pm 0.0004 \mu\text{g/mL}$. A lower IC_{50} value is associated with a higher radical scavenging activity.

Cytotoxicity Assay

Cytotoxicity assay of n-hexane, DCM and MeOH extracts of *P. pellucida* were tested against HeLa cell line. Samples were dissolved in 5% DMSO solvent. None of extracts of *P. pellucida* showed any activity against HeLa cell lines.

Conclusion

Different extracts of *P. pellucida* exhibited different phenolic content, flavonoid content, antioxidant capacity and DPPH Free radical scavenging activity. The methanol extract possessed potent antioxidant activity. However, none of the extracts showed activity against HeLa cell line.

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