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Research Article

Preliminary Qualitative Analysis, Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Ethanolic Extract of Young and Mature Stems of *Piper chaba* Hunter: A Comparative Study

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Abstract

Free radicals can injure cells that cause diseases including inflammatory, cardiovascular, and cancerous diseases as well as speed up aging. Antioxidants avert free radicals-induced tissue damage by limiting the generation of free radicals, stimulating their breakdown, or scavenging them. The goal of this research was to carry out a comparative study of preliminary qualitative analysis and assess the total phenolic and flavonoid contents and the in-vitro antioxidant activities of the young stems (YS) and mature stems (MS) extracts of Piper chaba Hunter. A preliminary qualitative analysis was carried out following some standard methods; spectrophotometrically the total phenolic and flavonoid contents were computed through the Folin–Ciocalteu colorimetric method and aluminiumtrichloride ($AlCl_3$) colorimetric assay accordingly, as well as antioxidant activities were assessed by DPPH free radicals scavenging activity assay. Preliminary qualitative analysis divulged the existence of carbohydrates, phenols, tannins, alkaloids, flavonoids, glycosides, and terpenoids in both extracts but quinones and gums were found in YS and MS extracts respectively. A comparable amount of total phenolic contents (TPC), i.e. (67.37 ± 0.39 and 40.95 ± 0.23 mg GAE)/g, and total flavonoid contents (TFC), i.e. (347.51.74 ± 9.98 and 382.14 ± 10.05 mg QE/g) were noticeable in the extracts of the young stems (YS) and mature stems (MS) respectively which indicating the YS extract had greater TPC compared to the MS extract whereas the MS extract had higher TFC than the YS extract. Besides, young stems extract showed a lower IC_{50} value of 125.89 µg/ml than mature stems extract (154.53 µg/ml). Hence, both extracts exhibited comparable antioxidant activities but more free radicals were neutralized by the young stems extract, indicating the young stems extract had more significant antioxidant activities than the mature stems extract. Both young and mature stems may be valuable supplements as a regular culinary food to protect the body from free radical hazards, and their ethanolic extract may be employed as promising natural antioxidants in the nutraceuticals industry, young stems are preferable since the young stems extract significantly outperformed the mature stems extract in terms of neutralizing free radicals.

Keywords: *Piper chaba* Hunter, Antioxidant Activity, Phytochemical Screening, DPPH· scavenging assay.

1. Introduction:

Numerous serious human illnesses, including heart, renal, and liver disease, neurological disorders, arthritis, cancer, and aging may be brought on by abnormal free radical generation within the body (Martin, 2003). Herbal remedies for diseases are an ancient idea. Different phytoconstituents including alkaloids, flavonoids, tannins, phenols, and glycosides are naturally found in plants. Flavonoids and phenols are the usual phytoconstituents of various fruits, veggies, and plant parts that exhibit antioxidant properties (Scalbert *et al*., 2005). By eradicating free radicals, antioxidants slow down or prevent oxidative damage to organisms' cells, lowering the risk of degenerative illnesses (Soobrattee *et al*., 2005). Antioxidants like flavonoids and phenols from plants are becoming more widespread nowadays because of the possible toxicological consequences of artificial antioxidants (Zhong & Zhou, 2013). Numerous medicinal plants have had their antioxidant and other therapeutic properties studied (Martin, 2003).

Piper chaba Hunter is a culinary, creeping herb of the family Piperaceae. It is better branded as Morich Lota (locally) or Chui Jhal in the Khulna Division of Bangladesh. *Piper chaba* Hunter has been treating different kinds of diseases, and it has a great number of herbal uses (Haque *et al*., 2018). Different parts of the plant are enriched with different amounts of chemical constituents. In this study, the authors tried to compare the existence of phytochemical constituents and the antioxidant properties of ethanolic extract of the young stems (YS) and mature stems (MS) of *Piper chaba* Hunter.

2. Materials and Methods:

2.1. Chemicals and Reagents:

Quercetin and ascorbic acid were bought from Merck (India). FCR, gallic acid, and DPPH were bought from Sigma-Aldrich (USA). The remaining laboratory-grade chemicals were procured from reputable vendors. Water was purified by using a distillatory in the Phytochemistry and Pharmacology Research Laboratory at Khwaja Yunus Ali University.

2.2.Plant Material Collection and Processing:

In October 2021, fresh, young, and mature stems of *Piper chaba* Hunter were harvested from a commercially cultivated farmin Satkhira, Khulna, Bangladesh. It was further authenticated by a professor of the Botany Department of Rajshahi University. The collected sample was washed with tap water, followed by distilled water. After that, it was sliced into small parts, shade dried, and finally in an oven at 50°C separately. Then the dried samples were coarsely powdered through a grinding machine.

2.3. Crude Extract Preparation:

Extraction was done using the maceration technique. 800 g powdered samples of young and mature stems were soaked in 2000 ml of ethanol in a stopper amber glass bottle separately. After that, it was permitted to stand at 25–30 °C for 10 days with frequent shaking. The solvent was vaporized at 55°C via a rotary evaporator and dried at 55°C in an oven.

2.4. Qualitative Analysis of Ethanolic Extract:

Different chemical reagents were prepared for specific phytochemical tests to find out the presence or absence of the main groups of phytoconstituents in the crude extract using color reactions. The preliminary phytochemical screening (qualitative analysis) was conducted using standard procedures (Shaikh & Patil, 2020; Trease & Evans, 1989; Talukdar & Chaudhary, 2010).

2.5. *In Vitro* Quantitative Analysis of Ethanolic Crude Extract:

2.5.1. Method for Total Phenolic Content (TPC) Assessment:

The TPC of young stems (YS) extract and mature stems (MS) extract was estimated using the Folin–Ciocalteu colorimetric method with slight modifications (Singleton *et al*., 1999). Several concentrations (500-15.62 µg/mL) of gallic acid were prepared by dissolving the gallic acid in methanol by serial dilution as a standard. A 10 ml test solution was prepared by overtaxing of 1 ml of each concentration, 5 mL of diluted FCR (1:10 v/v with DW), and 4 mL of 7% Na_2CO_3. This mixture was kept in a dark area for 30 minutes. The absorbance of the standard and sample was measured through a spectrophotometer (UV-Vis) at 760 nm against the blank.

2.5.2. Method for Total Flavonoid Content (TFC) Assessment:

The TFC of young stems (YS) extract and mature stems (MS) extract was estimated using the $AlCl_3$ colorimetric method (Chandra *et al*., 2014). Several concentrations (500-31.25 µg/mL) of quercetin were prepared by dissolving in methanol by serial dilution as standard. A 10 mL test solution was prepared by overtexing of 1 mL quercetin from each concentration, 6.4 mL of distilled water, 0.3 mL of 10% $AlCl_3$, 0.3 mL of 5% $NaNO_2$, and then 2 mL of 1M NaOH was mixed after 5 minutes. Centrifuged it and took the supernatant. The absorbance of the standard and sample was measured through a spectrophotometer (UV-Vis) at 510 nm against the blank. A calibration plot was utilized to calculate the sample's TFC as mg QE/g of dried crude extract.

2.5.3. *In Vitro* Assessment of Antioxidant Activities:

In vitro, antioxidant activities of young stems (YS) extract and mature stems (MS) extract was performed using the DPPH free radical scavenging activity assay with some modifications (Phuyal *et al*., 2020). By serial dilution in methanol, various concentrations of extract solution (500 - 1 µg/mL) and ascorbic acid (as standard) were prepared. In a volumetric flask, 100 mL of methanol was taken and 0.39 mg of DPPH was dissolved. 2 mL sample solutions of each concentration and 2mL DPPH solution were mixed properly and incubated for 0.5 hours at 25–30 °C in the dark. Then absorbance of the standard and sample were measured through a spectrophotometer (UV-Vis) at 517 nm. IC_{50} values of both YS & MS extracts were computed graphically through a curve of concentration versus % of DPPH radicals scavenging activity. The equation used to compute the % of DPPH radicals scavenging activity was:

$$I\% = (AC-AO)/AC \times 100$$

Here, AC was the absorbance of a mixture of methanol (2 mL) and DPPH solution (2 mL) as control, and AO was the absorbance of the sample solution.

2.6. Statistical Analysis:

All the tests for assessment of TPC, TFC, and antioxidant properties using DPPH were performed in triplicate. To compute the regression equation, the correlation coefficient, and to visualize the data, Microsoft Excel 2013 was utilized.

3. Results:

3.1. Qualitative Estimation of Photochemical

Piper chaba Hunter was very rich in different types of phytochemical constituents shown in Table 1.

Phytochemicals	YS Extract	MS Extract
Carbohydrate	+	+
Phenol	+	+
Flavonoid	+	+
Tannin	+	+
Terpenoid	+	+
Alkaloid	+	+
Glycoside	+	+
Quinone	+	-
Gum	-	+
Saponin	-	-

Table 1: Preliminary qualitative analysisof YS and MS extracts of Piper *chaba* Hunter

3.2. Total Phenolic Content Assessment:

The TPC of the YS and MS extracts, computed from the standard calibration curve ($y = 0.0052x + 0.0476$; $R^2 = 0.9935$,Figure 1) was 67.37 ± 0.39 and 40.95 ± 0.23 mg gallic acid equivalent (GAE)/g of dry extract respectively (Figure 4).

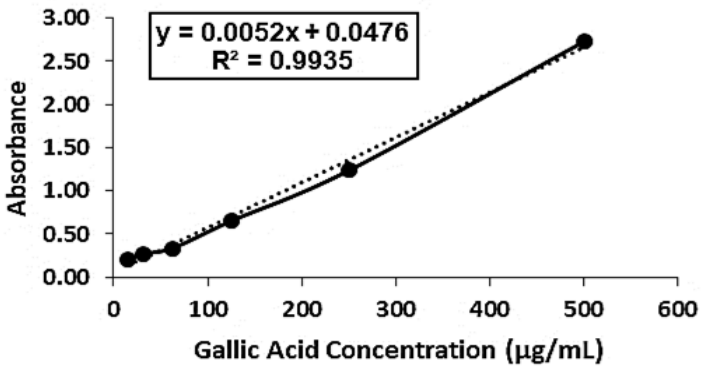

Figure 1: Calibration curve for standard gallic acid

3.3. Total Flavonoid Content Estimation:

The TFC of the YS and MS extracts, computed from the standard calibration curve ($y = 0.0006x + 0.1355$; $R^2 = 0.9954$, Figure 2) was 347.51.74 ± 9.98 and 382.14 ± 10.05 mg quercetin equivalent (QE)/g of dry extract respectively (Figure 5).

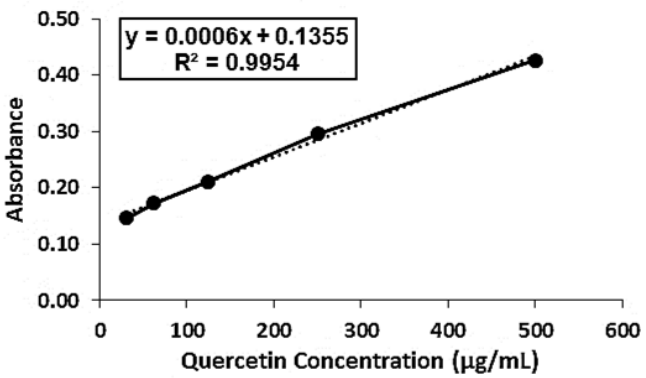

Figure 2: Calibration curve for standard quercetin

3.4. DPPH scavenging activities:

As shown in Figure 3, YS and MS extracts scavenged DPPH free radicals. The scavenging activity increased as the concentration of standard and YS and MS extract increased. IC_{50} values of ascorbic acid, young stems (YS) extract, and mature stems (MS) extract were 12.11 µg/ml, 125.89 µg/ml, and 154.53 µg/ml respectively (Figure 6).

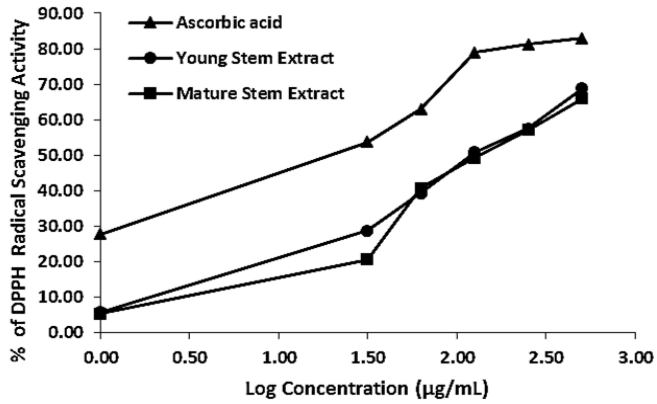

Figure 3: DPPH radicals scavenging activity of YS and MS extracts and ascorbic acid

4. Discussion:

Plants are a natural reservoir of chemical constituents (Phuyal *et al*., 2020). Several bioactive compounds, including carbohydrates, flavonoids, phenols, tannins, terpenoids, alkaloids, and glycosides, were present in both the extracts of young stems (YS) and mature stems (MS), while quinones and gums were only found in the YS and MS extracts, respectively. The TPC was higher in the YS extract (67.37 ± 0.39 mg) than MS extract (40.95 ± 0.23 mg) shown in Figure 4, and the TFC was lower in the YS extract (347.51.74 ± 9.98 mg) compared to the MS extract (382.14 ± 10.05 mg) shown in Figure 5.

Due to their redox characteristics, phenolic substances can function as antioxidants (Soobrattee *et al*., 2005).

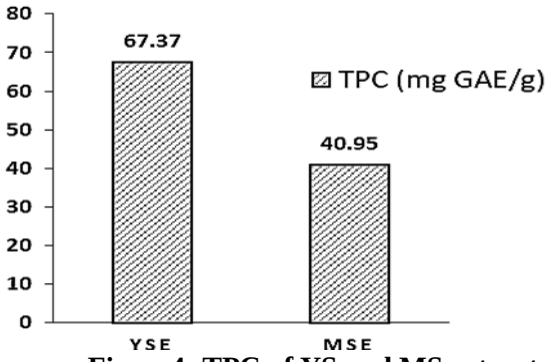

Figure4: TPC of YS and MS extract

The plant's phenolic compound might serve as a cornerstone of antioxidant activities since its hydroxyl groups facilitate its free radical scavenging ability (Paul *et al*., 2020).

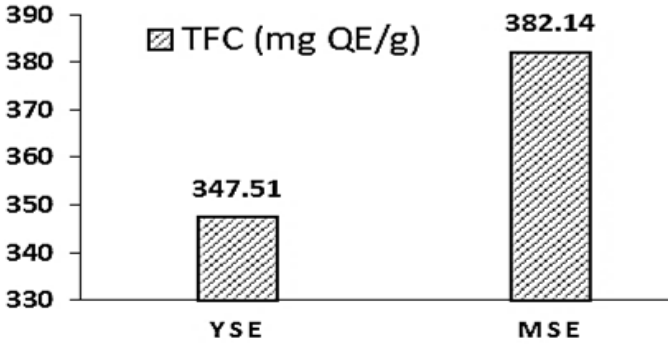

Figure5: TFC of YS and MS extract

Plant flavonoids also might serve as a foundation for antioxidant activities (Shimoi *et al*., 1996). Both ethanolic extracts of young stems (YS) and mature stems (MS) scavenged DPPH radicals (Figure 3). YS extract exhibited a higher % of free radicals scavenging activity than MS extract. A lower IC_{50} value indicates greater antioxidant activities. YS extract showed a lower IC_{50} value of 125.89 µg/ml than MS extract (154.53 µg/ml) shown in Figure 6.

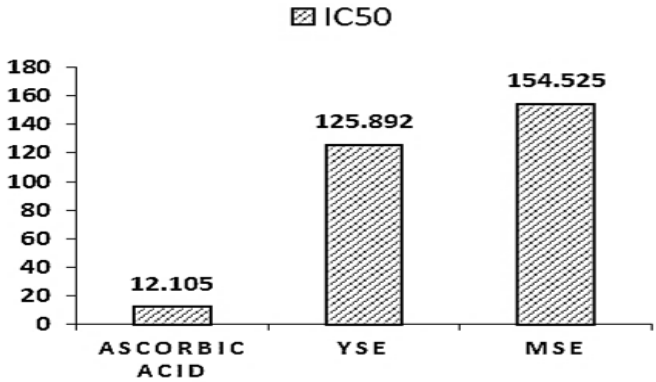

Figure 6: IC_{50} of YS and MS extracts and ascorbic acid as a standard

Consequently, the finding shows that the young stems had a greater yield and comparable total phenolics contents, and antioxidant properties as compared to mature stems. But the mature stems had greater total flavonoid contents as compared to the young stems. Young stems of *Piper chaba* had increased antioxidant activities may be for their increased phenolic contents.

5. Conclusion:

Both ethanolic extracts from young stems (YS) and mature stems (MS) exhibited comparable good antioxidant properties with higher phenolic and flavonoid contents. Therefore, both young and mature stems of *Piper chaba* may be useful supplements as a regular culinary food to shield the body against free radical hazards. Additionally, their ethanolic extract may be employed as a promising natural antioxidant in the nutraceutical industries. However, young stems are preferred since the extract from young stems significantly outperformed the extract from mature stems in terms of scavenging free radicals.

6. Abbreviations:

FCR: Folin–Ciocalteu Reagent; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; TPC: Total Phenolic Contents; TFC: Total Flavonoid Content; YSE: Young Stems Extract; MSE: Mature Stems Extract; QE: Quercetin Equivalent.

7. Author Contributions:

Research concept- Md. Shihab Uddin Sohag, Research design- Md. Shihab Uddin Sohag, Supervision- Md. Shihab Uddin Sohag, Sanjay Dutta, Materials- Quazi Istiaque Bari, Abida Sultana, Md. Boniamin, Md. Saju Ahmad, and Fazle Rabbi Shakil Ahmed. Data collection- Quazi Istiaque Bari, Abida Sultana, Data analysis and Interpretation- Md. Shihab Uddin Sohag, Sanjay Dutta and Fazle Rabbi Shakil Ahmed, Literature search- Quazi Istiaque Bari, Abida Sultana, Md. Boniamin and Md. Saju Ahmad, Writing article- Md. Shihab Uddin Sohag, Critical review- Sanjay Dutta and Fazle Rabbi Shakil Ahmed, Article editing- Md. Shihab Uddin Sohag, Quazi Istiaque Bari, Md. Boniamin, Final approval- All authors.

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9. Conflict of interests:

The authors declare no conflict of interest.

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