

# Phytochemical Screening and Antioxidant Profile of *Syngonium podophyllum* Schott Stems: A Fecund Phytopharmakon

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**Abstract:** *Background:* Oxidative stress has been shown to play an imperative role in copious disease pathology. Plants are acquaintances of mankind and mainstay for the treatment of oxidative stress linked disorders. Therefore, the objective of the existing study was to assess the phytochemical contents and antioxidant activity of crude methanol extract (CME), n-hexane (NHF), chloroform (CLF), ethyl acetate (EAF) and aqueous (AQF) fractions of *Syngonium podophyllum* (*S. podophyllum*) Schott stems.

*Methods:* The *S. podophyllum* Schott stems extract and its fractions were subjected to phytochemical analysis to detect the presence of alkaloids, carbohydrates, saponins, tannins, resins, flavonoids and steroids. The antioxidant profile was determined by total antioxidant activity (TAA), reducing power activity (RPA) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity tests and correlated with the estimation of total flavonoid content (TFC).

*Results:* In CME and its fractions of *S. podophyllum* Schott stems all of the tested phytoconstituents (alkaloids, tannins, resins, flavonoids and steroids) were detected at various concentration except carbohydrates and saponins. In TAA test, highest absorbance (2.18 nm) which is a measure of high antioxidant activity was reported in CME compared to remaining fractions. Likely in RPA test with respect to all fractions similar denouements were found for CME (3.29 nm). In case of DPPH scavenging test, the CME showed highest scavenging activity (77.89 %) having IC<sub>50</sub> of 41.02 µg/ml (P < 0.05) compared to remaining fractions. The antioxidant activity is possibly due to the highest TFC (7.45 mg of GAE/g of dried extract) reported in CME compared to existing fractions.

*Conclusion:* The aforementioned outcomes recommend that CME of *S. podophyllum* Schott stems can be a possible cradle of plant-derived natural antioxidant and can be used to avert diseases linked with free radicals.

**Keywords:** *Syngonium podophyllum*, Phytochemical Screening, Antioxidant Profile, Phenols, Oxidative Stress.

## INTRODUCTION

For a long time, herbal medications are being used to alleviate a range of symptoms of various diseases. Despite having prodigious advances in modern medicine, medicinal plants still make noteworthy contribution to health care [1]. Interests regarding these plants are from their traditional uses in folk medicine and such interests are noticeable in developing countries. In addition, these plants are less expensive than modern medicines and have huge value in global market [2]. In 2012, this export value was more than US\$2.2 billion. Although, in many countries traditional medicine is not strictly regulated, world health organization (WHO) is encouraging safer and rational use of these medications by synchronizing a network [3]. Based on the usage of medicinal plants by indigenous people for potential therapeutic purposes, numerous of compounds have been recognized using

ethnobotany [4]. However, important phytochemicals including epigallocatechin, resveratrol, curcumin, gallate and genistein are known as pan-assay interference compounds, since the data regarding their activity obtained from their *in vitro* studies are frequently unpredictable. Due to this reason, in drug discovery, phytochemicals are not often suitable as lead compounds. But the pharmaceutical industry has persistently fascinated in mining folk medicine in drug discovery [5]. A significant number of drugs have been discovered based on the traditional uses of medicinal plants. In between 1981 to 2010, out of 1073 approved small-molecule drugs, about more than half of them either directly derived from or motivated by natural substances [6,7].

An enormous amount of medicinal plants have been screened to evaluate their antioxidant assets. Imbalance in free radical generation in the body can cause many degenerative diseases including arthritis, cardiovascular disease, neurodegenerative disorder and cancer through damaged proteins, lipids, nucleic acids and other cellular components [8]. Antioxidants

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are efficient at neutralizing free radical-mediated harmful effects. In fact, antioxidants from natural sources are effective both in the form of chemical constituent and in the form of raw extracts to prevent the damaging processes triggered by oxidative stress [9]. On the other hand, recently synthetic antioxidants including butylated hydroxyanisole and butylated hydroxytoluene are found to have adverse side effects in human body [10]. Nonetheless, the toxicity profiles of most of the medicinal plants are yet to be thoroughly identified, it is quite well known that drugs derived from natural products are safer than their synthetic counterparts.

The plant, *Syngonium podophyllum* (*S. podophyllum*) Schott is an evergreen climbing plant that belongs to the family Araceae [11]. Their stems can be 10 to 20 meters long that support themselves on tree trunks by means of adventitious roots. The species is native to and commonly found in diverse region of Latin America from Mexico to Bolivia and naturalized in the Florida, West Indies, Texas, Hawaii and many other places including Bangladesh [12]. Leaf stalks and younger stems of *S. podophyllum* are green or bluish-green in color. Adult leaves of this plant have leaflets that are typical to a certain extent unequal in size, with significantly larger central leaflet than other options. Stomachache can be treated by using the decoction of the crushed and boiled leaf of this plant [13]. Instead, milky-white sap obtained from a broken stem can be used topically to treat the bite of *Paraponera* ants [13]. Furthermore, the roots and bark of this plant have anti-inflammatory and antibacterial properties; whereas, the sap is swabbed into the cavity of an aching tooth to alleviate pain [13]. *S. podophyllum* is also used to treat superficial to deep wounds, and a range of skin disorders [14]. Dose-reliant effects have been observed when extracts of the leaves and bark used to treat edema. These effects suggest the high anti-inflammatory activity of *S. podophyllum* [14].

In our previous study, we had inspected the antioxidant activities of *S. podophyllum* leaves [15] and the encouraging results of the study lead us to further explore the antioxidant potential of other parts of the plant. Therefore, the intention of this study was to examine the phytochemical contents and antioxidant profile of *S. podophyllum* Schott stems.

## MATERIALS AND METHODS

### Chemicals

Methanol, ammonium molybdate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxy-2-ribose, ascorbic acid

(ACA), ethylenediaminetetraacetic acid (EDTA), trichloro acetic acid (TCA) and thiobarbituric acid (TBA) were procured from Sigma-Aldrich, USA. Butylated hydroxy toluene (BHT) and gallic acid (GA) was purchased from Merck, Germany and Wako pure chemicals Ltd., Japan respectively. Unless else stated, all remaining used chemicals were of analytical status and procured from Active Fine Chemicals Ltd., Bangladesh and other local sources.

### Collection and Identification of Plant Materials

The *S. podophyllum* Schott stems were collected from Dhaka, Bangladesh in July 2016 and were taxonomically identified by an experienced taxonomist at the Bangladesh National Herbarium, Dhaka, Bangladesh. For this plant a voucher specimen (DACB-39385) was also deposited for future reference.

### Drying and Grinding of Plant Materials

The collected plant parts were washed with fresh water, cut into small fragments, and shed dried for two weeks. The desiccated plant material was grounded into fine powdered form and deposited for further use.

### Extraction and Fractionation of Plant Materials

The powder plant material (stems) was extracted using cold maceration method by taking 500 mg powder in 1.5 L methanol in a container composed of glass for 7 days. The extract was detached using Whatman filter paper (No.1) from other plant debris. The extract was concentrated by using rotary evaporator at 50°C temperature. The amount of yield in the extract was 8.77 g. The concerted methanolic extract was then partitioned successively by using the modified method of Kupchan [16] and the resultant partitions i.e., n-hexane (NHF) chloroform (CLF), ethyl acetate (EAT) and water (AQF) soluble fractions were evaporated to dryness and engendered NHF, 1.92 g; CLF, 1.58 g; EAF, 1.84 g and AQF 2.05 g extract respectively.

### Qualitative Phytochemical Analysis

The preliminary phytochemical group test was carried out by following standard procedure [17]. The extract was screened for the presence of alkaloids, carbohydrates, saponins, tannins, resins, flavonoids and steroids

### Total Flavonoid Content

The aluminum chloride ( $AlCl_3$ ) colorimetric method as stated by Chang *et al.* with slight alterations was

used to detect the total flavonoid content (TFC) [18]. To begin with, 0.5 mL of several extracts (i.e., 1 mg/mL) were mixed with methanol (i.e., 1.5 mL), after that the adding of 10%  $\text{AlCl}_3$  (i.e., 0.1 mL), 1M of potassium acetate (i.e., 0.1 mL) and finally 2.8 mL of distilled water. At 25 °C, the aforesaid reaction mixture was saved for 30 min. Subsequently, the absorbance of the reaction mixture was measured at 415 nm. GA was used as a reference standard and the content TFC was estimated from the calibration curve. The TFC was represented as mg of GA equivalent (GAE)/g of dried sample.

### Antioxidant Activity

In order to start antioxidant activity a solution of each obtained plant extract/fractions was made by using 98% methanol at 1 mg/mL concentration. The following tests were performed:

#### Total Antioxidant Activity

The method prescribed by Prieto *et al.* was followed to carry out the phosphomolybdenum assay of total antioxidant activity (TAA) [19]. A volume of 0.1 mL of test solution of diverse concentrations (50–500  $\mu\text{g/mL}$ ) mixed with the 1 mL of reagent solution (i.e., 28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulfuric acid). Then, the tubes were allowed to incubate at 95°C in a hot water bath for 90 min. Subsequently, the absorbance of the solution was estimated at 695 nm after cooling to 25 °C. (+)-catechin (CTC) was used as a reference standard. Total antioxidant activity was calculated by using the following equation:

$$\text{Total antioxidant activity (\%)} = \left[ \frac{Ac - As}{Ac} \right] \times 100$$

where, Ac is the absorbance of control and As is the absorbance of sample/standard solution.

#### Reducing Power Activity

The method of Oyaizu *et al.* with few adjustments was followed to determine the reducing power activity (RPA) [20]. Briefly, 0.2 mL of diverse concentrations of the test sample extracts (i.e., 50–500  $\mu\text{g/mL}$ ) were added distinctly with 0.2 M of phosphate buffer with pH 6.6 (i.e., 0.5 mL) and 1% of potassium ferricyanide (i.e., 0.5 mL). Afterward, the resultant mixture was permitted to incubate in a boiling water bath for 20 min at 50°C. After cooling at 25 °C, 10% of trichloroacetic acid (i.e., 0.5 mL) was added to it which was then centrifuged at 3,000 rpm for 10 min. Finally, obtained supernatant (i.e., 0.5 mL) was collected and 0.5 mL of distilled

water was added to it. Later a 0.1% solution of ferric chloride (i.e., 0.1 mL) was mixed and finally the blend was left at room temperature for 10 min. The absorbance of the solution was determined at 700 nm. ACA was used as a reference standard.

### DPPH Radical Scavenging Activity

The procedure of Kulisic *et al.* with few adjustments was used to resolve the DPPH radical scavenging activity [21]. A volume of 50  $\mu\text{L}$  of tested sample solution of numerous concentrations (i.e., 25–400  $\mu\text{g/mL}$ ) was added with 950  $\mu\text{L}$  of methanolic solution of DPPH (i.e., 3.4 mg/100 mL). Then the resultant mixture was allowed to incubate for 1 h in the dark at 37 °C. The invisibility of the original purple color was the marker of the free radical scavenging activity of the extracts. The absorbance of the solution was measured at 517 nm. BHT was used as a reference standard. The DPPH radical scavenging capacity was assessed by using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[ 1 - \left( \frac{As}{Ac} \right) \right] \times 100$$

where, Ac is the absorbance of control and As is the absorbance of sample/standard solution.

### Statistical Analysis

All analyses were performed in triplicates and results were represented as mean  $\pm$  SD. The data in all the tests were examined by Student's t test. The data was entered into a Microsoft Excel 2007 (Roselle, IL, USA) database and analyzed using SPSS (version 15.0) for the statistical and graphical evaluations. The denouements were calculated as statistically significant at the value of  $P < 0.05$ .

## RESULTS

### Determination of Phytochemical Content

The phytochemical analysis of *S. podophyllum* stems extract and its fractions exposed the occurrence or absenteeism of numerous bioactive components for example alkaloids, carbohydrates, saponins, tannins, resins, flavonoids and steroids presented in Table 1. In case of crude extract and its fractions, carbohydrates and saponin were entirely absent. However remaining phytoconstituents were existing at innumerable concentration (i.e., largely, moderately, mildly).

### Determination of TFC

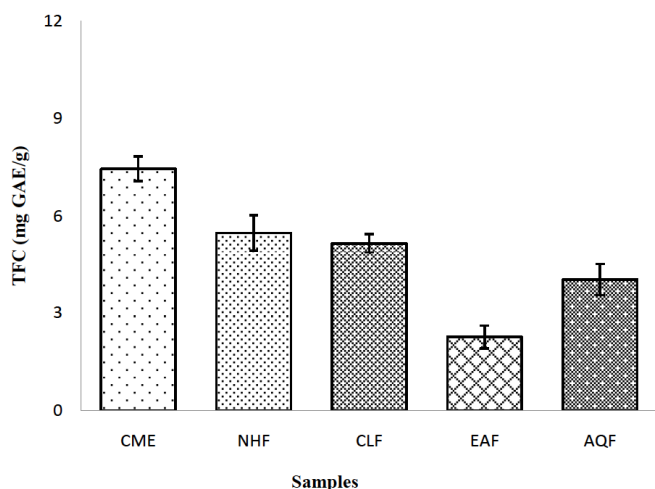
The standard curve for GA ( $y = 0.014x + 0.033$ ;  $R^2 = 0.998$ ) was used to analyze the TPC of *S.*

**Table 1: Phytochemical Screening of *S. podophyllum* Stems Extract and its Fractions**

| Phytochemicals | CME | NHF | CLF | EAF | AQF |
|----------------|-----|-----|-----|-----|-----|
| Alkaloids      | -   | -   | +   | ++  | ++  |
| Carbohydrates  | -   | -   | -   | -   | -   |
| Saponins       | -   | -   | -   | -   | -   |
| Tannins        | -   | ++  | ++  | -   | -   |
| Resins         | ++  | +   | +++ | -   | -   |
| Flavonoids     | +++ | +   | +   | ++  | +   |
| Steroids       | -   | ++  | +++ | -   | -   |

Where, CME: Crude methanol extract; NHF: n-hexane fraction; CLF: Chloroform fraction; EAF: Ethyl acetate fraction; AQF: Aqueous fraction. In view of power of the color reaction here, + = Present in mild amount, ++ = Present in moderate amount, +++ = Present in large amount, - = Not present.

*podophyllum* stems extract and its fractions. In this test, highest TFC was reported in CME ( $7.45 \pm 0.38$  mg of GAE/g of dried extract) then NHF, CLF, AQF and EAF which were  $5.48 \pm 0.55$ ,  $5.16 \pm 0.29$ ,  $4.02 \pm 0.48$  and  $2.26 \pm 0.35$  mg of GAE/g of dried sample, respectively (Figure 1).

**Figure 1: TFC of *S. podophyllum* stems extract and its fractions.**

Values were represented as mean  $\pm$  SD ( $n = 3$ ). Where, CME: Crude methanol extract; NHF: n-hexane fraction; CLF: Chloroform fraction; EAF: Ethyl acetate fraction; AQF: Aqueous fraction.

#### Determination of TAA

In Figure 2, the TAA of *S. podophyllum* stems extract and its fractions are mentioned. At highest concentration (500  $\mu$ g/mL) the following sequence was reported CTC > CME > NHF > CLF > EAF > AQF.

#### Determination of RPA

The RPA of *S. podophyllum* stems extract and its fractions are showed in Figure 3. In this test at maximum concentration (500  $\mu$ g/mL), CME exerted

highest ( $3.29 \pm 0.73$  nm) activity followed by NHF, CLF, EAF and AQF.

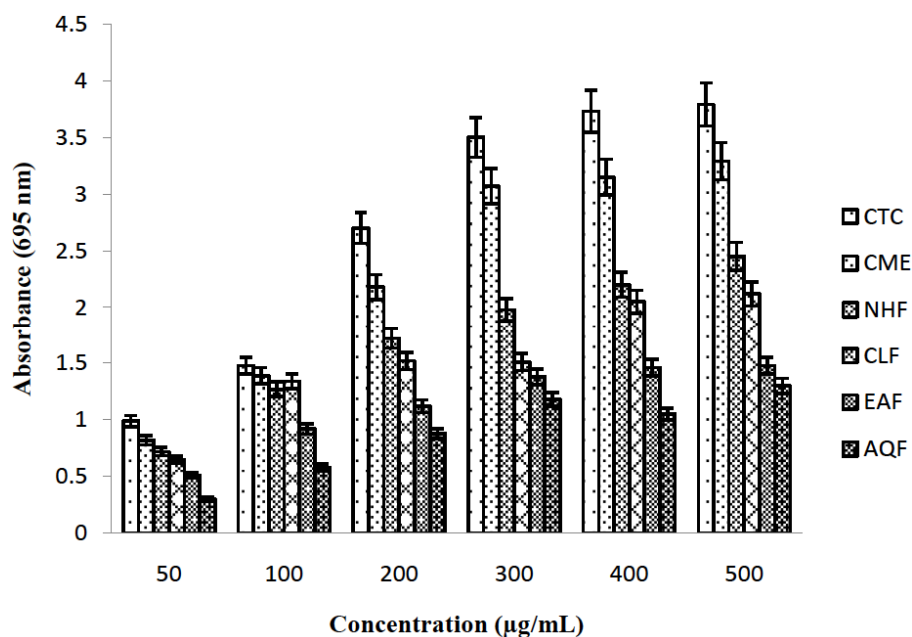
#### Determination of DPPH Radical Scavenging Activity

In DPPH radical scavenging test, amid CME and its fractions (NHF, CLF, EAF and AQF) highest radical scavenging was reported in CME ( $77.89 \pm 1.05\%$ ) given in Figure 4. The  $IC_{50}$  values of the extract and its fractions are presented in Figure 5.

#### DISCUSSION

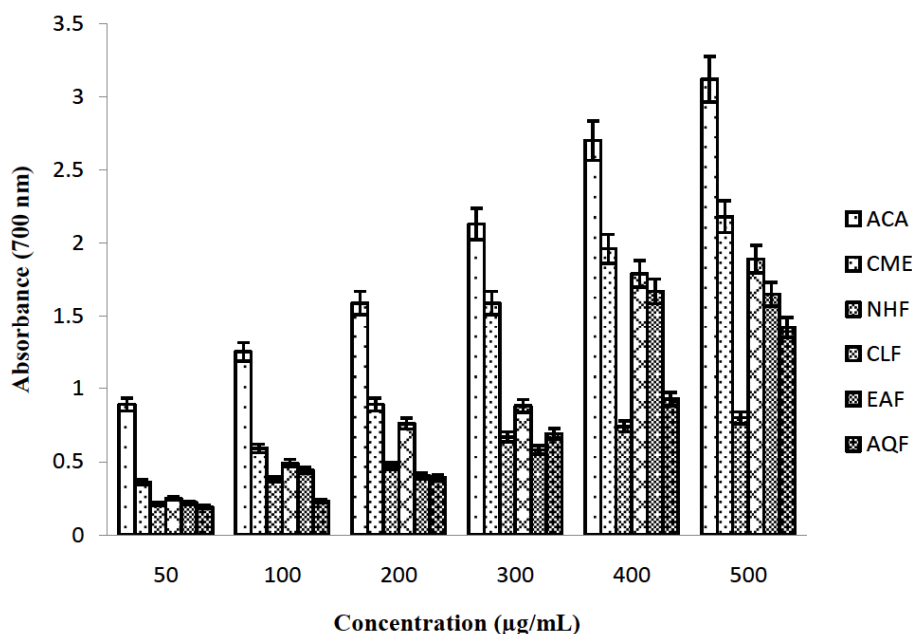
Medicinal plants are considered as the richest bio-resources. Use of these plants by the humans has a long recorded history [22]. Medicinal plants contain a range of phytochemical constituents and they are particularly useful to heal and cure various human diseases. Furthermore, research on medicinal plants nowadays is not limited to new drug discovery only, rather the research areas are expanding in association with diverse subjects [23]. In this study, phytochemical analysis and antioxidant activities of *S. podophyllum* Schott stems were carried out by using several tests.

Plants generate multiple secondary metabolite compounds including steroids, flavonoids, saponins, glycosides, alkaloids, terpenoids and glucosinolates to defend themselves from the continuous attack of environmental stresses and naturally occurring pathogens [24]. It has been found that plants that contain molecules including terpenoids, tannins, vitamins, phenolic acids, coumarins, alkaloids, quinones, flavonoids and other metabolites are responsible for the free radical scavenging and antioxidant properties [8,10]. In this study, at various concentration, copious phytoconstituents for example alkaloids, tannins, resins, flavonoids and steroids were reported in crude extract and its fraction. Almost similar



**Figure 2: TAA of *S. podophyllum* stems extract and its fractions at various concentrations.**

Values were represented as mean  $\pm$  SD (n = 3). Where, CTC: (+)-catechin; CME: Crude methanol extract; NHF: n-hexane fraction; CLF: Chloroform fraction; EAF: Ethyl acetate fraction; AQF: Aqueous fraction.



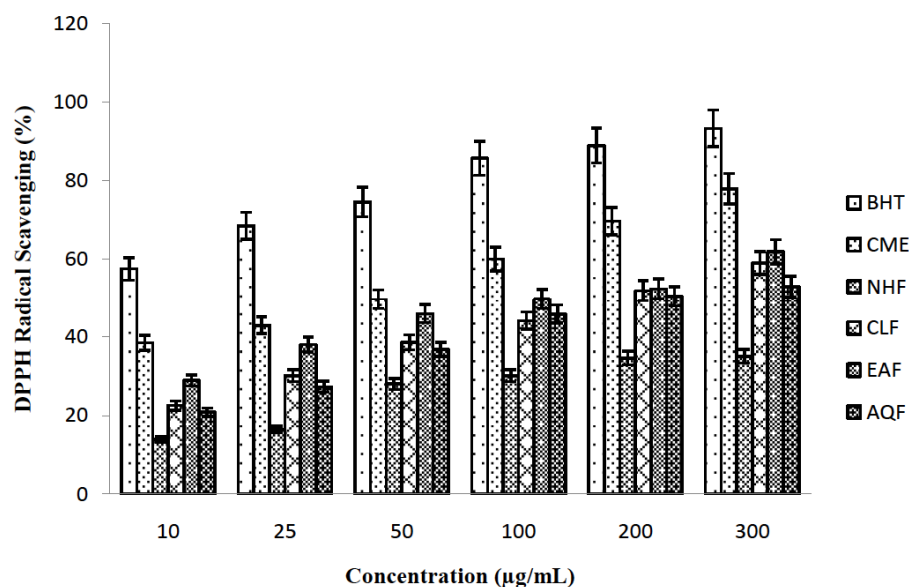
**Figure 3: RPA of *S. podophyllum* stems extract and its fractions at various concentrations.**

Values were represented as mean  $\pm$  SD (n = 3). Where, ACA: Ascorbic acid; CME: Crude methanol extract; NHF: n-hexane fraction; CLF: Chloroform fraction; EAF: Ethyl acetate fraction; AQF: Aqueous fraction.

denouements were reported in a previous study of *S. podophyllum* leaves [25].

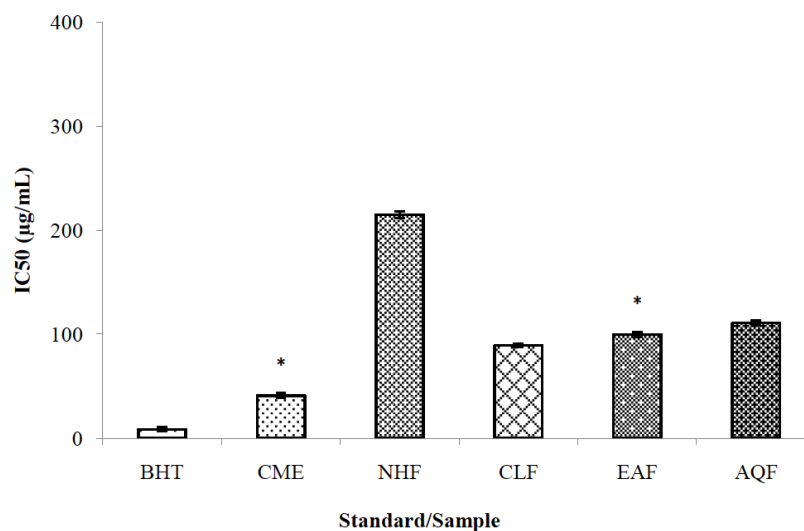
Scavenging ability of plants is mainly due to the phenolic compounds and their hydroxyl groups [25]. Instead, antioxidant activities exhibited by the plants are due the phenolic compounds present in plant parts [26]. In food industry, plant materials that have higher

phenolic compounds are being used increasingly since they can slow down oxidative damage of lipids and can improve the nutritional value and quality of food [27]. Extracts of the plants also show antioxidant activity but their activities vary with the solvent because of the copious antioxidant activity of compounds with diverse polarity [28]. In this study amid crude extract and its fraction, highest TFC was reported in CME. The results



**Figure 4: DPPH radical scavenging activity of *S. podophyllum* stems extract and its fractions at various concentrations.**

Values were represented as mean  $\pm$  SD (n = 3). Where, BHT: Butylated hydroxy toluene; CME: Crude methanol extract; NHF: n-hexane fraction; CLF: Chloroform fraction; EAF: Ethyl acetate fraction; AQF: Aqueous fraction.



**Figure 5: IC<sub>50</sub> values of *S. podophyllum* stems extract and its fractions.**

Values were expressed as mean  $\pm$  SD (n = 3). Where, BHT: Butylated hydroxy toluene; CME: Crude methanol extract; NHF: n-hexane fraction; CLF: Chloroform fraction; EAF: Ethyl acetate fraction; AQF: Aqueous fraction.

\*P < 0.05 significant difference from the standard.

of this study are analogous in a preceding study of Hossain *et al.* [15] and numerous studies reported that plant phenols are promising natural antioxidant [29].

The total antioxidant capacity of different fractions was determined spectrophotometrically following phosphomolybdenum method, which is mainly based on the reduction of molybdenum VI (Mo) to molybdenum V by the test sample and the following creation of green phosphate/Mo (V) mixes with an

acme absorption at 695 nm [30]. In this study, for phosphomolybdate reduction, it was found that CME exerted the maximum TAA. Studies have revealed that a lot of flavonoids as well as associated polyphenols pay considerably to the phosphomolybdate scavenging action of the plants containing medicinal values [31].

In terms of reducing power assay, color of the test solution changes from yellow to green depending on the test specimen's reducing power. In this assay, the

reduction of the ferricyanide complex ( $\text{Fe}^{3+}$ ) to the ferrous form ( $\text{Fe}^{2+}$ ) indicates the occurrence of the reductants in the test solution and this  $\text{Fe}^{2+}$  can be determined by measuring the absorbance at 700 nm [32]. Previous studies have revealed that the reducing possessions have been exposed to exert antioxidant act owing to the donation of a hydrogen atom in order to halt the free radical chain [33]. In this study among crude extract and fractions, CME exerted an Increment of absorbance at 700 nm specifies a surge in reducing aptitude. Hossain *et al.* reported auspicious action for the study of antioxidant activities of *S. podophyllum* leaves [15]. Plant secondary metabolites play imperative roles in reactive oxygen species metabolism [34].

The firm DPPH were reduced by all of the plant extracts, thus the invisibility of purple color to varying extent depends on the existence of antioxidant compounds [35]. Furthermore, the degree of discoloration designates the scavenging potential of the extract [36]. In this study, maximum ability to counteract DPPH radicals was observed for the CME amid all the extracts tested, and a reasonable activity was found for other extracts. In a previous study, same authors stated alike outcomes [15]. Plants produce numerous low molecular weight antioxidants within the chloroplast stroma and cytosol using nicotinamide adenine dinucleotide phosphate as the final electron donor to combats against oxidative stress [37].

The existing study exposed a potent antioxidant activity of the *S. podophyllum* Schott stems, which may be an operative source of antioxidant defense. Moreover, the study also demonstrated a clear correlation between the antioxidant effect of the plant with the presence of polyphenols in general and flavonoids in particular.

## CONCLUSION

The denouements of this study reported that, CME of *S. podophyllum* Schott stems exerted highest significant antioxidant and free radical scavenging activities as well as contained massive amounts of phenolic compounds with respect to remaining fractions. So CME can be considered as a basis of natural antioxidant. However, further studies are obligatory to isolate the bioactive compounds and detect the specific molecular mechanisms vital for antioxidant activity.

## AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors MSU and MSH designed the study,

wrote the protocol, managed the analyses of the study. Authors MSU, MSH, MTK, IR and AAM performed the laboratory tests and prepared the draft of the manuscript. Author MRJ participated in literature review and statistical analysis. Author DT revised and improved the manuscript. All the authors read and approved the final manuscript.

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## COMPETING INTERESTS

The authors proclaim that they have no competing interests.

## ABBREVIATIONS

|                       |                                 |
|-----------------------|---------------------------------|
| ACA                   | = Ascorbic acid                 |
| AQF                   | = Aqueous fraction              |
| BHT                   | = Butylated hydroxy toluene     |
| CLF                   | = Chloroform fraction           |
| CME                   | = Crude methanol extract        |
| CTC                   | = (+)-catechin                  |
| DPPH                  | = 2,2-diphenyl-1-picrylhydrazyl |
| EAF                   | = Ethyl acetate fraction        |
| GA                    | = Gallic acid                   |
| GAE                   | = Gallic acid equivalents       |
| NHF                   | = n-hexane fraction             |
| RPA                   | = Reducing power activity       |
| <i>S. podophyllum</i> | = <i>Syngonium podophyllum</i>  |
| TAA                   | = Total antioxidant activity    |
| TFC                   | = Total flavonoid content       |
| TPC                   | = Total phenolic content        |

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