

***In vitro* Screening for Phytochemicals and Antioxidant Activities of *Syngonium podophyllum* L.: An Incredible Therapeutic Plant**

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ABSTRACT

Searching for natural curative agents with a superior safety profile for the management of oxidative stress linked disorders are continuing owing to multiple unwanted effects linked with synthetic antioxidants. Medicinal plants are still considered as one of the imperative cradles of modern medicine. Therefore the drive of this study was to analyse the phytochemical constituents and antioxidant activities of the *Syngonium podophyllum* (*S. podophyllum*) L. leaves. The fresh leaves of *S. podophyllum* L. were extracted with methanol (CME) afterward fractionation using n-hexane (NHF), chloroform (CLF), ethyl acetate (EAF) and aqueous (AQF). The plant extract and its fractions were evaluated for phytochemical content by alkaloids, carbohydrates, saponins, tannins, resins, flavonoids and steroids tests. Total phenolic content (TPC) was also determined. Antioxidant activities were determined by total antioxidant activity (TAA), reducing power activity (RPA) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity tests. The phytochemical analysis of CME and its fractions of *S. podophyllum* L. leaves exposed the presence of alkaloids, tannins, resins, flavonoids and steroids. The CME of *S. podophyllum* L. leaves exhibited the highest amount (10.55 mg of GAE/g of dried extract) of TPC compared to remaining fractions. The highest absorbance, 3.791 nm was found in CME with respect to remaining fractions for TAA test. Among the crude extract and its different fractions, highest absorbance, 3.015 nm was reported for CME in RPA test. In the DPPH radical scavenging test, CME showed the highest scavenging activity (79.89%) with IC₅₀ of 46.55 µg/mL. This study indorsed that CME has marked antioxidant activities. This plant extract might be a promising source of natural antioxidant which can be effective for the treatment of oxidative stress related disorders.

Keywords: *Syngonium podophyllum*, Antioxidant, Oxidative stress, Safety profile, Synthetic antioxidant.

INTRODUCTION

Traditionally, usage of different medicinal plants to treat a number of diseases is well

established¹. Surprisingly, 80% of the total global population is primarily dependent on traditional medicines to treat various maladies². In India, since ancient times, it is well established that thousands of

species have known medicinal values and different parts of these plants are used to alleviate specific illnesses³. Medicinal plants are considered as a precious natural resource. Generally, medicinal plants are considered as much safer drugs and over the time their biological, antioxidant, hypoglycemic and antimicrobial effects have been tested and they are often found to play a vital role in the modern medicine^{4,5}. It is also often found that most of the synthetic drugs that we have today have their origin from medicinal plants⁶. Currently scientific interest has been particularly flourished due to the improved efficiency of plant derived products and their comparatively lesser side effects than modern medicine.

Free radicals including hydroxyl radical ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), superoxide radical ($\text{O}_2\cdot^-$) and singlet oxygen ($^1\text{O}_2$) when generated in great amount in human body, can exert toxic effects like oxidative stress⁷⁻⁹. These free radicals are highly reactive at larger amount and during oxidative stress they are strong enough to damage body's proteins, lipids and even deoxyribonucleic acid molecules that can ultimately lead to a range of disorders including anemia, diabetes, allergies, inflammation, ischemia, kidney failure, liver and cardiovascular diseases and various degenerative diseases of the nerve and even cancer¹⁰⁻¹². Nevertheless, at low level reactive oxygen species is essential to maintain various normal physiological and biochemical functions including cell signaling, apoptosis of defective cells and so on¹⁰⁻¹². On the other hand, compounds which are obtained from medicinal plant are found to prevent the harmful effects of oxidative stress. In addition, these compounds are cheaper to obtain and have lesser side effects than synthetic antioxidant agents¹³⁻¹⁷.

However, medicinal plants are also found to produce a great number of secondary metabolites that contain various physiological and biochemical effects¹⁸. Other than their antioxidant effects, these secondary metabolites and their derivatives also contain significant pharmacological effects such as diuretic, anti-hepatotoxic, anti-allergic and anticancer effects¹⁹⁻²¹. Strong antioxidant activity containing phytochemicals including tocopherols, ascorbates, phenols, carotenoids and tocotrienols have already well-established their imperative roles in health care

system²². Presently, there is an increased research interest in the discovery of new natural antioxidant agents from medicinal plant. Furthermore, the demand of these antioxidant agents is rising not only in pharmaceutical industry but also in food and cosmetic sector. Medicinal plant contains antioxidant compounds which show a protective role by averting the production of free radicals within the cell²³⁻²⁵.

The plant, *Syngonium podophyllum* (*S. podophyllum*) L. belongs to the family of Araceae, which is a simple yet elegant looking and commonly used as an ornamental plant²⁶. The species is mainly native to a diverse region of Latin America from Mexico to Bolivia. Furthermore, the species is naturalized in the Florida, Hawaii, Texas, West Indies and many other places²⁷. A decoction of the plant's crushed and boiled leaf is used as a remedy to treat stomachache²⁸. In addition, milky-white sap from a broken stem of the plant is applied on skin as a topical agent for the bite of *Paraponera* ants²⁸. The sap is also applied into the cavity of an aching tooth in order to relieve toothache²⁸. The roots and bark have antibacterial and anti-inflammatory activity; hence they are used to treat superficial and deep wound, and several skin disorders²⁸. Moreover, extracts of the leaves and bark have significant anti-inflammatory activity and possess a dose-dependent effect upon treating edema²⁸. In the local folk medicine of Belize, traditionally leaves and bark of the plant have been found to be used due to their wound healing effects, while the tincture of the leaf is used to treat arthritis, rheumatism, swelling and pain²⁹.

The literature is indigent about biochemical actions of *S. podophyllum* L. leaves. In our previous study we examined the analgesic and anti-inflammatory activities of this plant³⁰. Hence the present study was conducted to evaluate the phytochemical content and antioxidant activities of *S. podophyllum* L. leaves.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu (FC) reagent, ammonium molybdate, ascorbic acid (ACA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, EDTA (ethylenediaminetetraacetic acid), trichloro acetic acid

(TCA), thiobarbituric acid (TBA), methanol and quercetin (QR) were purchased from Sigma-Aldrich, USA. Sulphuric acid and butylatedhydroxy toluene (BHT) were purchased from Merck, Germany. Gallic acid (GA) was purchased from Wako pure chemicals Ltd., Japan. Unless otherwise specified, all other chemicals were of analytical grade and purchased from Active Fine Chemicals Ltd., Bangladesh.

Collection and Identification of Plant Materials

In the month of January 2016, the leaves of *S. podophyllum* L. were entirely collected from Dhaka City of Bangladesh. For the plant identification purposes, help from an expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh was taken. Additionally, for future references, the herbarium also preserved a voucher specimen (DACB-38722).

Drying and Grinding of Plant Materials

Freshly collected leaves of *S. podophyllum* L. were washed properly to eliminate any possible dirty materials. Washed leaves were then shade dried for several days with sporadic sun drying. Then the dried leaves were grinded to make powder and then labeled and hermetic for further use.

Extraction and Fractionation of Plant Materials

Powdered plant materials about 500 g were extracted with 1.5 L of 98% methanol in an amber colored glass bottle for 7 days and filtered by cotton

and then through Whatman No.1 filter paper. The extracts were then subjected to rotary evaporator at 50°C temperature to evaporate methanol and give crude extract (10.45 g). This crude methanol extract (CME) was subjected to sequential fractionated, at first with n-hexane (NHF) after that chloroform (CLF) then ethyl acetate (EAF) and lastly by using water (AQF) consistent with the method of Kupchan with minor modification³¹. The generated methanol extract and its fractions were re-filtered and then evaporated to remove extra solvent and obtained NHF, 2.95 g; CLF, 2.58 g; EAF, 1.09 g and AQF 3.38 g extract respectively.

Phytochemical Content

Phytochemical screening was carried out on CME and its four fractions (NHF, CLF, EAF, AQF) to detect the presence of secondary metabolites such as alkaloids, carbohydrates, saponins, tannins, resins, flavonoids and steroids employing standard phytochemical screening³².

Total Phenolic Content

The method as described by Singleton and Rossi with slight modification was used to determine the total phenolic content (TPC) of *S. podophyllum* L. leaves extract and its fraction(s)³³. Although previously diluted 1000-fold using distilled water, 0.1 mL of plant extract/fraction(s) with a concentration of 1000 µg/mL was mixed with 0.75 mL of Folin-ciocalteu reagent. Followed by 5 min

Table 1: Phytochemical screening of *S. podophyllum* L. leaves extract and its fractions

Phytochemicals	CME	NHF	CLF	EAF	AQF
Alkaloids	++	-	+	++	++
Carbohydrates	-	-	-	-	-
Saponins	-	-	-	-	-
Tannins	+	+	+	+	-
Resins	+++	+	+++	-	-
Flavonoids (Ammonia test)	++	+	-	+	-
Flavonoids (Aluminium chloride solution test)	+	+	-	-	-
Steroids	++	++	++	-	-

Based on intensity of the color reaction where, + = Present in mild amount, ++ = Present in moderate amount, +++ = Present in large amount, - = Not present.

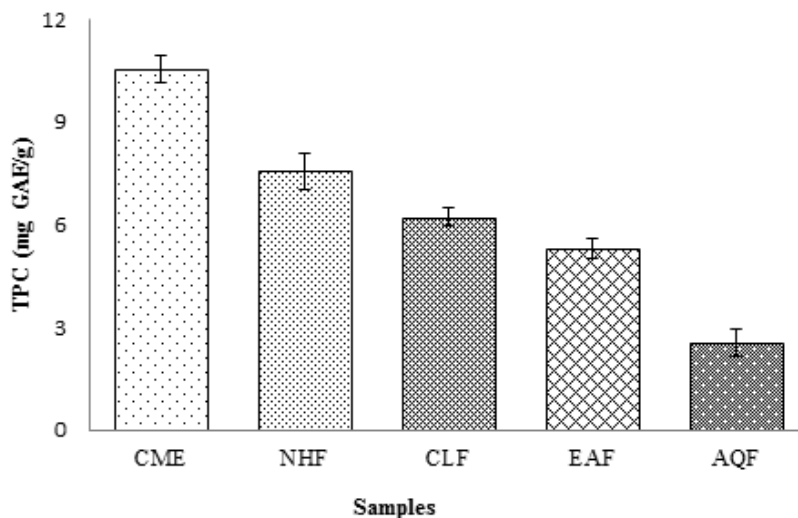
Where, CME: Crude methanol extract; NHF: n-hexane fraction; CLF: Chloroform fraction; EAF: Ethyl acetate fraction; AQF: Aqueous fraction

incubation period, 0.06% sodium carbonate solution was added; afterward again incubated for the period of 90 min at 22°C. A spectrophotometer (at 725 nm) was used to estimate the absorbance of the solution against a reagent blank that is a solution contained all the reagents excluding plant extract/fraction(s)

or standard (GA). The results of dried sample were denoted as mg of gallic acid equivalents (GAE)/g.

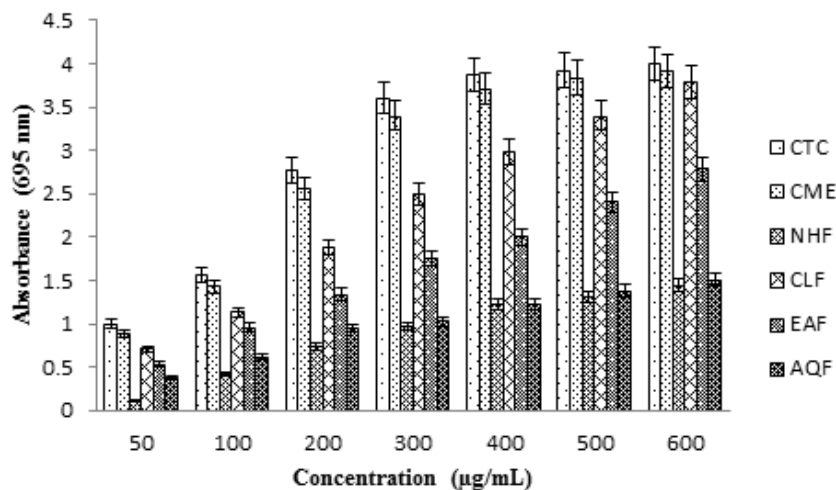
Antioxidant Activity

In order to start antioxidant activity a solution of each obtained plant extract/fractions



Values were reported 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

Fig. 1: TPC of *S. podophyllum* L. leaves extract and its fractions



Values were reported 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.

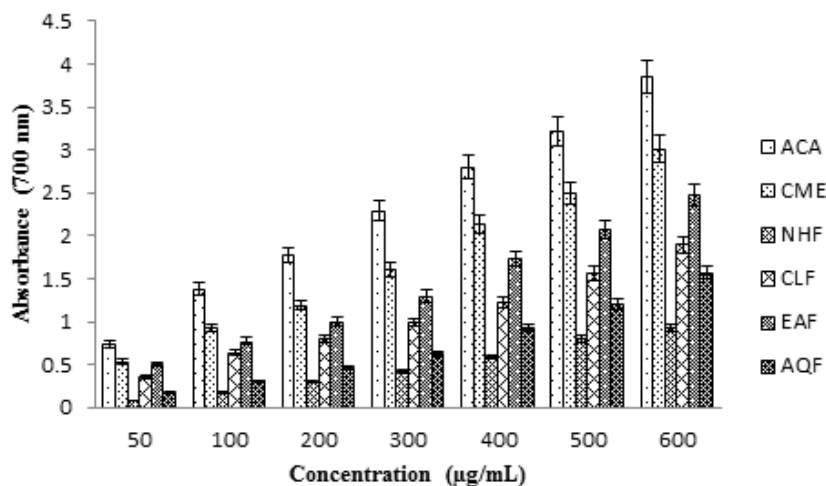
Fig. 2: TAA of *S. podophyllum* L. leaves extract and its fractions at various concentrations

was made by using 98% methanol at 1 mg/mL concentration.

Total Antioxidant Activity

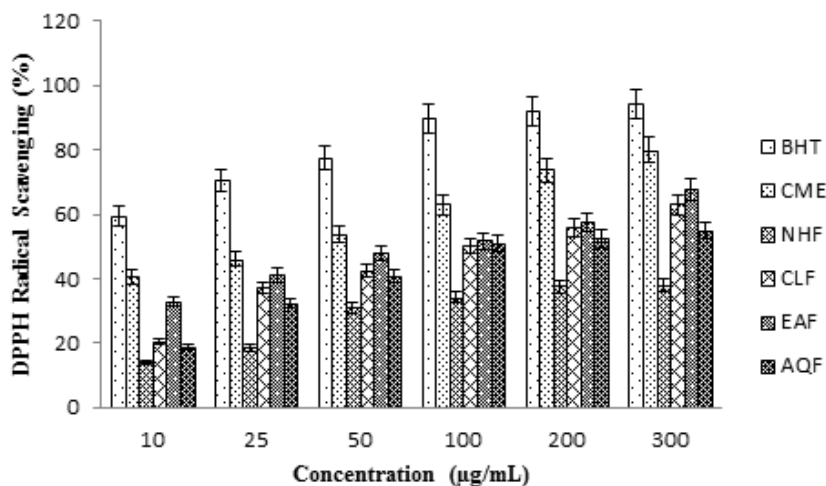
The prescribed method of Prieto *et al.*, with minor adjustments was used to determine the total antioxidant activity (TAA) of different extractives of *S. podophyllum* L. leaves extract and its fraction(s)³⁴.

0.5 mL of *S. podophyllum* L. extract/fraction(s) or standard [(+)-catechin, CTC] solution of different concentration was taken and mixed with 3 mL of reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The absorbance of the solution was determined by a spectrophotometer (at 695 nm) against a reagent blank that is a solution contained



Values were reported 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.

Fig. 3: RPA of *S. podophyllum* L. leaves extract and its fractions at various concentrations



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

Fig. 4: DPPH radical scavenging activity of *S. podophyllum* L. leaves extract and its fractions at various concentrations

all reagents excluding plant extract/fraction(s) or standard and the whole spectrophotometric analysis was followed by an incubation period of 15 min at 95°C.

Reducing Power Activity

The reducing power activity (RPA) of *S. podophyllum* L. leaves extract and its fraction(s) was evaluated by the method of Oyaizu with minor modifications³⁵. 0.2 mL of plant extract/fraction(s) or standard (ACA) solution of different concentration was taken and mixed with 0.5 mL of 200 mM/L trisodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated for 20 min at 50°C then 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 650 rpm for 10 min. From the upper layer of the solution, 0.5 mL was mixed with 0.5 mL distilled water and 0.1 mL of ferric chloride (0.1%). After that the absorbance of the solution was measured at 700 nm using a spectrophotometer against a reagent blank that is a solution contained all reagents except plant extract/fraction(s) or standard.

DPPH Radical Scavenging Activity

The method described by Choi *et al.*, with minor modifications, was used to determine the

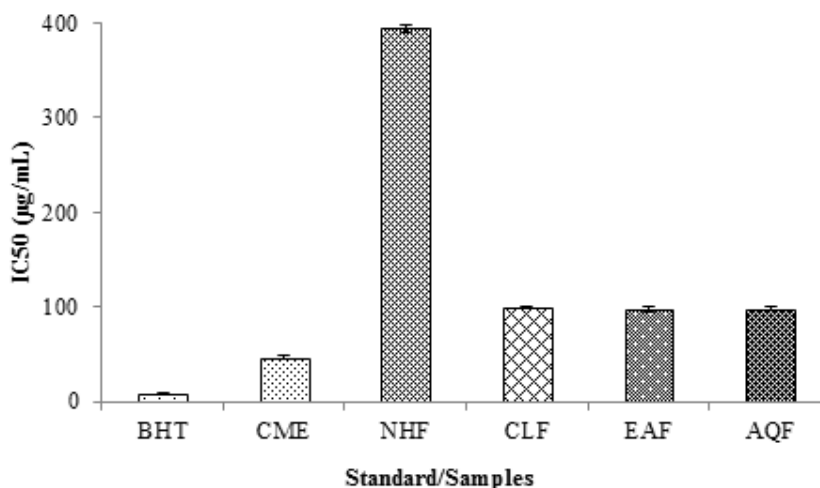
DPPH radical scavenging activity of *S. podophyllum* L. leaves extract and its fraction(s). 2 mL of plant extract/fraction(s) or standard (BHT) solution of different concentration was added to 3 mL 0.02 % of methanol solution of DPPH. Before measuring the absorbance of the solution, it was incubated in a dark place for 30 min. Subsequently, absorbance of the solution was determined at 517 nm using a spectrophotometer against a reagent blank that is a solution contained all reagents except plant extract/fraction(s) or standard. The percentage of scavenging of the DPPH free radical was calculated using the following equation:

$$\text{DPPH radical scavenging (\%)} = [1 - (A/A_0)] \times 100$$

Where, A_0 is the absorbance of control and A is the absorbance of extract/fraction(s) or standard.

Statistical Analysis

The experiments were carried out in triplicate and all the results were expressed as mean \pm SD. The data in all the experiments were analyzed by Student's *t* test. For the statistical and graphical evaluations SPSS 14.0 (Chicago, IL, USA) and Microsoft Excel 2010 (Roselle, IL, USA) were used. The level of significance was considered at $P < 0.05$.



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

Fig. 5: IC₅₀ values of *S. podophyllum* L. leaves extract and its fractions

RESULTS

Determination of Phytochemical Content

Phytochemical examination of *S. podophyllum* L. leaves extract and its fractions revealed the presence or absence of bioactive components such as alkaloids, carbohydrates, saponins, tannins, resins, flavonoids and steroids showed in Table 1. Saponin and carbohydrates are absent in crude extract and its all fractions but others are present at various concentration (i.e. largely, moderately, mildly).

Determination of TPC

The TPC of *S. podophyllum* L. leaves extract and its fractions was calculated on the basis of the standard curve for GA ($y = 0.0144x + 0.0328$; $R^2 = 0.9991$) that is expressed in mg of GAE/g of dried sample shown in Figure 1. The CME of *S. podophyllum* L. leaves exhibited the highest amount (10.55±0.41 mg of GAE/g of dried extract) of total phenolics, followed by NHF, CLF, EAF and AQF, which were 7.56±0.51, 6.22 ±0.26, 5.31±0.28 and 2.55±0.38 mg of GAE/g of dried extract, respectively.

Determination of TAA

In the TAA, at maximum concentration (600 µg/mL), the absorbance of CME and CAT were 3.916±0.006 and 3.988±0.003 nm respectively that is showed in Figure 2. Among the crude methanol extract and its different fractions, CME showed the highest TAA followed by CLF, EAF, AQF and NHF with absorbance's which are 3.791±0.005, 2.788±0.009, 1.505±0.012 and 1.440±0.006 nm respectively at maximum concentration.

Determination of RPA

The RPA of CME, NHF, CLF, EAF and AQF of leaves of *S. podophyllum* L. are showed in Figure 3. The extract/fractions increased the reducing activity with the increased concentration of the extract/fractions. Among the CME and its different fractions, CME showed the highest iron RPA with absorbance of 3.015±0.006 nm followed by EAF, CLF, AQF and NHF which are 2.477±0.004, 1.898±0.009, 1.569±0.007 and 0.929±0.006 nm respectively at maximum concentration (600 µg/mL).

Determination of DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of *S. podophyllum* L. leaves extract and its fractions is given in Figure 4. Among the crude extracts and its fractions the most potent activity was found in CME at highest concentration having IC₅₀ value of 46.55±2.25 mg/mL (Figure 5). On the other hand, NHF, CLF, EAF and AQF showed DPPH radical scavenging activity with IC₅₀ value of 394.01±4.27, 99.78±1.29, 96.58±2.88 and 98.31±2.74 mg/mL respectively.

DISCUSSION

Over the past few decades, the significance of medicinal values of various plants has been widely accepted³⁷. Medicinal plants produce a wide range of secondary metabolites that are known to have antioxidant effects which are essential to block the pathogenesis of several diseases and the process of aging caused by the free radicals^{38,39}. In the present study, phytochemical content and antioxidant activities of *S. podophyllum* L. leaves were carried out by using various tests.

In this study phytochemical analysis of crude extract and its fractions exposed a wide range of phytoconstituents such as alkaloids, tannins, resins, flavonoids and steroids. A previous study reported that plant phenols, flavonoids and tannins are accountable for antioxidant activity⁴⁰.

Phenolic compounds are the most important and abundantly found antioxidant agents in various medicinal plants. Phenolic compounds contribute to numerous essential activities in plants including cementing material linking phenolic polymers to cell wall polysaccharides, defense against herbivores and pathogens, regulation of cell growth and cell division⁴¹. In this study CME of *S. podophyllum* L. leaves reported highest TPC with respect to remaining fractions. The alteration in the phenolic contents may be owing to the occurrence of essential oils exist in the plant. Phenolic compounds plentiful in medicinal plants contribute considerably to the antioxidant properties⁴².

The combined effect of phenolic compounds, flavonoids and other reducing agents in the plant extracts are well represented by TAA⁴³. The phosphomolybdenum method is mainly based on the reduction of molybdenum, Mo (VI) to Mo (V) by the action of antioxidant compounds and the formation of a green phosphate, Mo (V) complex with a highest absorption at 695 nm⁴⁴. Here CME of *S. podophyllum* L. leaves reported highest total antioxidant activity. In the study of antioxidant activity of *Torilis leptophylla* L. Saeed *et al.*, reported promising outcome⁴⁵.

In case of reducing power assay, reducing power of the test specimen is depicted by the changes of the color of the test solution from yellow to green⁴⁶. The existence of the reducing agents in the solution causes the reduction of the ferric form (Fe³⁺) to the ferrous form (Fe²⁺). Hence, the ferrous form can be observed by absorbance measurement at 700 nm⁴⁷. In this study CME of *S. podophyllum* L. leaves reported highest iron reducing capacity. The higher absorbance of CME extracts indicates its possible strong reducing power potential. The reducing power of the extracts is probably due to the biologically active compounds in the extract which contain potent electron donating abilities. An earlier study in the study of antioxidant profile of *Uvaria littoralis* Blume. also reported auspicious action⁴⁸.

DPPH radical purple-colored solution bleaching is often used to measure the electron donation capacity of natural products⁴⁹. The method is mainly based on the principle of scavenging of DPPH through the introduction of radical species or antioxidant that decolorizes the DPPH solution. In this method, the concentration and potency of

the antioxidants is proportional to the extent of color change. Moreover, substantial free radical scavenging activity of the compound under test is indicated by a great decrease in the absorbance of the reaction mixture⁵⁰. The results of this study recommended the highest DPPH radical scavenging activity in CME of *S. podophyllum* L. leaves. In the study on antioxidant activity of *Streblus asper* leaves, Ibrahim *et al.*, reported almost comparable findings⁵¹.

The replacement of synthetic with natural antioxidants may be advantageous. This study divulge a prominent *in vitro* antioxidant actions of *S. podophyllum* L. leaves, which may be a forceful natural source of healing agents for the controlling of oxidative stress related disorders.

CONCLUSION

The denouements obtained in the present study, assessed notable antioxidant activities of the *S. podophyllum* L. leaves. The CME of *S. podophyllum* L. leaves possessed highest antioxidant activity compared to remaining fractions. In this *in vitro* study a high association is reported between the TPC and the antioxidant activities. However, this is an initial study and further study needs to be carried out for the isolation of promising compound(s) and elucidate mechanism for the respective effects.

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