Hindawi
Journal of Chemistry
Volume 2020, Article ID 3865139, 8 pages
https://doi.org/10.1155/2020/3865139

Research Article

The Phytochemical Screening and Antioxidants Potential of *Schoenoplectus triqueter* L. Palla

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Received 5 March 2020; Revised 9 June 2020; Accepted 23 June 2020; Published 30 September 2020

Guest Editor: Yunus Alparslan

Over the centuries, humans use different types of therapeutic plants to treat several diseases. Cyperaceae family has a significant number of monocotyledon plants, and Schoenoplectus is one of the genera that belong to this family; about forty-nine compounds are isolated. Our current study was evaluated on *Schoenoplectus triqueter* L. Palla to show the potential of its antioxidants and confirm the phytochemical constituents in this plant species. Fully powdered plant taken for successive extraction process in hot continuous process for Soxhlet was 20 g plant in porous bag manually prepared; the constant temperature provided was 40–50°C. In the maceration extraction method, 30 g plant was taken in a closed jar and the solvent placed for extraction was 300 mL of ethanol; the extract gets filtered and fractioned to different solvents such as water, dichloromethane, ethyl acetate, and *n*-hexane fraction. Important types of phytochemicals found in this species are alkaloids, proteins, amino acids, flavonoids, phenols, terpenoids, tannins, saponins, and carbohydrates. All the entire extracted fractions which are water, dichloromethane, ethyl acetate, and n-hexane possess noticeable activity at various concentrations of 31.25, 62.5, 125, 250, and 500 μg/mL by the dilution method. The ethyl acetate extract holds greater median inhibitory concentration ($IC_{50} = 3.52 \pm 0.01$), and water showed $IC_{50} = 3.61 \pm 0.01$ percent potential as compared to the standard ascorbic acid which possesses $IC_{50} = 2.27 \pm 0.01$. Their potential may be enhanced or lowered with the purification of extracts which might be useful in biological activities.

1. Introduction

Over the centuries, humans use different types of therapeutic plants to treat or cure diseases. Such therapeutic plants are very important and play a key role in a better and happy life, for health at every stage [1]. Cyperaceae family has a significant number of monocotyledon plants but the taxonomist did not give proper attention to this family [2]. The family Cyperaceae mostly contain halophytic plants and most of them are fibrous and found in Pakistan [3]. *Schoenoplectus* is one of the genera that belong to this family; about forty-nine compounds are isolated from the plant *Schoenoplectus lacustris* in alcohol fraction and water fraction; all the compounds are applied one by one to green algae, i.e., *Selenastrum capricornutum* (a unicellular organism that is normally used in tests of toxicity as a bioindicator of eutrophic spots) in such a way to test the phytotoxic effect of all forty-nine compounds which are isolated from the *Schoenoplectus lacustris*. Out of all the most active compounds is (negative) catechin displaying similar inhibition to the algaecide copper sulphate [4]. There is no such a presence of any antifungal compound in *S. triqueter* because it does not inhibit many fungi such as (a) *Pestalotia palmarum* Cooke., (b) *hylotialateripes* (Ellis & Ev) Guba, and (c) *Polyschema olivacea* (Ellis & Everh.) while it acts as a host for the abovementioned fungi [5]. Batool and Hameed reported three different species from the Cyperaceae family collected from different areas of Punjab. All the species were introduced to salt stress; under very crucial and high saline conditions, the *S. triqueter* showed high advanced root adaptation [6]. Fy et al. examine the basic species of Schoenoplectus from the midway river with the help of APLG fingerprint. Among the species, the *S. lacustris* is dominant in all hybrids which are studied from all rivers and

the additivity was proved among the fingerprints of *S. triqueter* [7]. Jiménez-Mejías et al. reported that in Pakistan, India, and Africa, *Schoenoplectus corymbosus* (Cyperaceae) is widely distributed. Its presence in the wetland of Morocco and Spain is proved recently. We know the availability of this plant in the extreme of the Mediterranean. Since 1999, it has not been collected due to its medium-large size [8]. Roalson studied the vast variation in the chromosome number of family Cyperaceae among the genera. Recently, studies indicate that there are 4231 chromosomes in this family. About 16% of species recently have been known/recognized [9]. Antioxidants are those substances that prevent most of the oxidation reactions which are initiated with the production of free radicals. Antioxidants captured free radicals thereby delaying or preventing damage to the cells and tissues of the living organisms. Antioxidants are also called reducing agents [10–12]. Antioxidants have also a lot of applications on an industrial level such as preservation of food, cosmetics, and prevention of gasoline and rubber degradation. Various rich sources of antioxidant compounds such as carotenoids, flavonoids, lutein, and polyphenols are vegetables and fruits [13, 14]; plant extraction or isolation produces some potent compounds or substances that are accountable for biological activity [15]. Modern medicine, food supplements, and folk and traditional medicine are mainly obtained from plants [16]. In medicinal plants, important constituents such as vitamins A, C, and E, flavonoids, lignins, and tannins which are phenolic compounds are found and represent antioxidant potentials [17]. Our current study evaluated the extraction and fractionation of *Schoenoplectus triqueter* L. Palla plant, performed *in vitro* antioxidants 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging potential, and confirmed the phytochemical constituents.

2. Materials and Methods

The present study was conducted to determine the *in vitro* antioxidant potential and phytochemicals screening of the species *Schoenoplectus triqueter* (L.) Palla from the genera *Schoenoplectus*. The plant *S. triqueter* was identified by a botanical export Professor Muhammad Israr of Botany Department, Government Post Graduate College, Mardan 23200, KP, Pakistan, and was also confirmed through various literature studies and comparison data obtained from different floras.

2.1. Plant Collection and Drying. The given plant was collected during the month of January-February winter season at Katlang from river areas: geographical location: Mardan, KP, Pakistan, Asia, and geographical coordinates: 34° 21′ 38″ North, 72° 4′ 49″ East. Only a healthy stem part of the plant was collected and washed with sterilized water to remove any surface dust and contaminants. The collected plants were stored under shades and protected from light exposure and contaminants in the class store for three weeks to completely dry under room temperature and humidity. After drying, they were then ground with a normal grinder, and a fully powdered sample was obtained with a greater surface area for a better extraction process.

2.2. Soxhlet Extraction. The fully powdered plant taken for the successive extraction process in hot continuous process for Soxhlet [18] was 20 g plant in porous bag manually prepared from the sterilized filter paper. In the round bottom flask of Soxhlet was taken 250 mL of ethanol, and a manually prepared cellulose bag was placed in the thimble chamber of Soxhlet. The upper part was fitted with a condenser where cool water inflow and outflow were provided for liquid condensation throughout the process of successive extraction. All the apparatus was fitted over the Mantoux heater at a constant temperature of 40–50°C. The process continued until the liquid droplet from the siphon arm does not leave any residues during the cycling. The extract was obtained and then filtered and fractioned after a controllable water bath to water, dichloromethane, ethyl acetate, and *n*-hexane fraction. The dried fraction was then evaluated for further analysis.

2.3. Maceration Extraction. In the maceration extraction [18] method, the 30 g plant was taken in a closed jar made from Pyrex glass. The solvent placed for extraction was ethanol 300 mL. The jar with a closed cap was placed under the shade at room temperature for 30 days only. At least twice and trice a day, the plant was shaken with continuous stirring to dissolve the plant sample and release the soluble metabolites to the solvent. After that, the extract was then filtered and dried under a controllable water bath and then fractioned to different solvents such as water, dichloromethane, ethyl acetate, and *n*-hexane fraction. The dried fraction was then evaluated for further analysis.

2.4. Phytochemical Screening. The medicinal plants contain some important types of biologically active compounds called phytochemicals which are accountable and show potency towards the biological activity. Their confirmations, identifications, and characterization are very important, and to detect these phytochemicals, a standard procedure previously reported in [19–22] was followed as given hereinafter.

2.4.1. Carbohydrates and Tannins. Aqueous extract (2.5 mL) in a test tube was treated with a Molisch reagent (0.5 mL) followed by 3 drops of concentrated H_2SO_4; a violet ring showed the presence of carbohydrates. While treating with ferric chloride (0.5 mL) solution, green color in a condensed form showed tannins.

2.4.2. Alkaloids and Saponins. Fresh dilute acidic extracts (HCl) (2.5 mL) added were treated with reagents (Wagner, Hager, and Mayer's) (0.5 mL); color precipitation in solution showed positive results for alkaloids. Aqueous extract (4 mL) vigorously in the test tube was shaken for 2 minutes; a froth of about 5 cm formed in about 10 minutes showed saponins.

2.4.3. Flavonoids. Acidic extracts (HCl) (2.5 mL) were added to two test tubes; sodium hydroxide (NaOH) (1.5 mL) was added to one tube and 1.5 mL of distilled water was added to the other one; yellow color confirmed flavonoids. While treating with lead acetate (1.5 mL), yellowish precipitate also showed flavonoids.

2.4.4. Sterols and Triterpenes. Salkowski. While 2.5 mL of ethanolic extract was directly treated in a test tube with the addition of 3 drops of concentrated sulphuric acid (H_2SO_4), reddish-brown color with an absence of green layer showed positive results. *Lieberman.* 2.5 mL of fresh ethanolic extract in a test tube was treated with 0.5 mL of acetic anhydride and also the addition of 3 drops of concentrated sulphuric acid (H_2SO_4), and the appearance of brown-reddish color at the upper layer showed a positive result.

2.4.5. Anthraquinone Glycosides and Resin. Fresh ethanolic extract (2.5 mL) was mixed with an ammonia solution (0.5 mL) in a test tube, and a combination of light rose color with more green color indicates a positive result of anthraquinone glycosides. While 2.5 mL of water extract was treated with 1 : 1 (water and acetone), no turbidity indicates the absence of resin.

2.4.6. Proteins and Amino Acids. Xanthoproteic. 2.5 mL of the extract was directly treated with 3 drops of concentrated nitric acid (HNO_3) in a test tube, and yellow color indicates the protein-positive result. *Biuret.* 4 mL of the extract in a test tube followed by sodium hydroxide NaOH (10%) and 1 mL was heated and treated with copper sulphate ($CuSO_4$) (10%), and 0.5 mL solution indicates protein-positive results.

2.4.7. Diterpene Fats and Fixed Oils. 4 mL of the extract in a test tube was treated with 3 drops of copper acetate solution, and bright green color appearance indicates diterpenes positive result. While pressing 3 mL of water extract within filter paper, no stain of oil was formed or seen which showed a positive result and absence of fixed oils and fats.

2.4.8. Phenol Content Determination. The total phenol content in aqueous extraction and in other different fractions of *S. triqueter* was mainly obtained on the basis of the last-mentioned described procedure [23]. After this, 2.5 mL of 10% Folin–Ciocalteau's reagent was mixed with about 2 mL of 2% sodium carbonate solution (Na_2CO_3), along with the addition of 0.5 mL of aqueous extract and some amount of *S. triqueter* (1 mg/mL); the above mixture is placed in an incubator machine for at least 15 minutes at 45°C; at 765 nm, the absorbance of the mixture takes place. The identification of quantity was done with respect to the standard of gallic acid at various concentrations (1, 0.5, 0.25, 0.125, 0.063, and 0.031 mg/mL). The content of the total phenolic compounds was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

2.4.9. Flavonoid Content Determination. By using aluminum chloride with the help of the colorimetric method, the total flavonoid content was obtained [24]. In such a method, about 1 mL of crude extract fractions of *S. triqueter* was added to 3 mL solution of methanol, followed by the addition of 0.2 mL of 10% aluminum chloride ($AlCl_3$), about 0.2 mL of potassium acetate (1 M), and 5.6 mL of distilled water, and then left at room temperature for at least 35 min. Absorbance takes place at 420 nm. Here, the quantification was done with respect to the standard of gallic acid at various concentrations (1, 0.5, 0.25, 0.125, 0.063, and 0.031 mg/mL); again, the total phenolic content was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample.

2.5. Antioxidant Activity. The antioxidant activity was performed according to the previously reported one in [25–28] using a stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging power assay for all the entire fraction extracts such as *n*-hexane, water, dichloromethane, and ethyl acetate. All the extracts were first dissolved in suitable solvent dimethylsulfoxide (DMSO) at various concentrations of 31.25, 62.5, 125, 250, and 500 μg/mL by dilution method. Before at least 2 hrs, 0.0238 grams of solid DPPH was taken by weight in 100 mL of methanol solvent, and the standard solution was covered with aluminum foil in the dark at room temperature to avoid light decomposition. First, all the entire fraction concentration was mixed with DPPH solution sample extracts (10 μL) and standard solution (90 μL) stored for incubation for 30 minutes at 37°C in the dark. Then, at 517 nm of wavelength, absorbance was measured at least three times. The positive control media were treated the same as sample extracts with DPPH; in negative cases, DMSO was placed. The spectrophotometer was used to measure the absorbance, and the percentage of DPPH free radical scavenging activity was calculated according to the equation of [29, 30], where Y' is the absorbance of sample extracts and X' is actually the standard sample absorbance:

$$\text{DPPH \% scavenging activity} = \frac{X' - Y'}{X'} \times 100. \quad (1)$$

2.6. Statistical Analysis. All the data were calculated in triplicate as a standard ± mean using test statistics and standard deviation. The latest *Graph pad prism* software was used for the evaluations of the median inhibitory concentration values in spite of the statistical regression and correlation analysis.

3. Results and Discussion

3.1. Phytochemical Screening. All the important phytochemicals were confirmed and predicated in this species according to the previous methods in [19–22]. The most important types of phytochemicals found in this species are alkaloids, proteins, amino acids, flavonoids, phenols,

terpenoids, tannins, saponins, carbohydrates, etc. positively detected during phytochemical confirmation as shown in Table 1. Each phytochemical showed potency towards some biological action; for example, flavonoids play a role in antioxidant potential [31]; alkaloids are important in antimicrobial, analgesic, and other antispasmodic actions [31–33] and inflammatory potency was found with steroids [32]. According to [34–38], plants contain phytochemicals which were purified and mainly used to treat some types of health-related diseases and also utilized in making dietary supplement and nutrients. Each phytochemical shows novel biological behavior which may increase the chances of the discovery of new compounds like antibiotics against pathogens [18, 39–41] and only the flavonoids which are polyphenols and play a role in antibiotics activity [42, 43] because flavonoids make complexes with bacterial proteins, cell wall, and other ingredients which are accountable for biological action [44]. Also, many other compounds such as terpenoids, tannins, steroids, and saponins also show the plants' potential towards antimicrobial (both bacterial and fungus) activity [45]. Steroids make the lipid-bilayer membrane rupture and release liposome [46] while saponins from the cell of microbes release enzymatic protein [47] and terpenoids are involved in the weakening of cell wall and tissue of the microorganisms [48].

The flavonoids and phenolic content were determined by the Folin Ciocalteu method and the phenol content was quantitatively estimated and ranged from 5.33 ± 0.05 to 16.45 ± 0.02 (mg 55GAE/g) as shown in Table 2. The entire fraction contained an appreciable amount of phenolic content; the water fraction possesses noticeable greater content as compared to ethyl acetate fraction while other fractions such as *n*-hexane and DCM possess lower content of phenol. In the determination of flavonoid content, water fraction was found dominant among all other fractions and ranged from 3.73 ± 0.02 to 14.05 ± 0.5 (mg GAE/g); the *n*-hexane contained the least amount of flavonoid content as shown in Table 2.

Plant tissues are a rich source of phenolic compounds and are used as antioxidants. This antioxidant activity is good and effective for some diseases [49], because of the presence of hydroxyl groups, which play an important role in their scavenging ability. Thus, they have the ability to react with active oxygen radicals such as hydroxyl radicals [50]. Flavonoids are polyphenolic compounds, contain many phenolic groups, and are responsible for some of the health benefits of vegetables and fruits [51]. They are known to play an active role in the quenching of free radicals, in other words, to trap the free radicals to avoid any harm, because of their redox properties [52]. Tannins, however, are high molecular weight polyphenolic compounds that have also the best property and used as antioxidants [53].

3.2. Antioxidant Activity. The *S. triqueter* L. Palla plant antioxidant potential was performed and calculated via a standard protocol of [25–27] to check whether the fractions of this plant possess free radicals to destroy the effects and oxidative stress to keep the organisms safe [54].

Table 1: Phytochemical screening test results of *S. triqueter* L. Palla.

Serial no.	Phytochemical tested	Test results
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Protein and amino acids	+
5	Fats and oil	−
6	Tannins	+
7	Sterol	−
8	Terpenoids	+
9	Glycosides and anthraquinone	+
10	Resin test	−
11	Carbohydrates	+
12	Phenol	+

+ = *Positive* means present, − = *Negative* means absent.

All the entire extracted fractions such as water, dichloromethane, ethyl acetate, and *n*-hexane possess noticeable activity as shown in Table 3 at various concentrations of 31.25, 62.5, 125, 250, and 500 μg/mL by the dilution method. The ethyl acetate extract possesses greater median inhibitory concentration ($IC_{50} = 3.52 \pm 0.01$) and water showed $IC_{50} = 3.61 \pm 0.01$ percent potential as compared to the standard ascorbic acid which possesses $IC_{50} = 2.27 \pm 0.01$. The sample extracts are nearly closed to the standard in the activity of free radicals as shown in Figure 1. All the other relative fractions of *S. triqueter* showed wide ranges of antioxidant activity which were measured as mean and standard deviation taken in triplicate noting that DCM possesses $IC_{50} = 4.05 \pm 0.2$ while *n*-hexane showed $IC_{50} = 6.35 \pm 0.05$; this experimental study and [20, 55] have similar results. Elufioye et al. reported that ethyl acetate fraction had the highest DPPH radical scavenging activity, with a concentration providing 50% inhibition (IC_{50}) of 0.079 mg/mL [56]; among all fractions, ethyl acetate fraction exhibits the most potent scavenging activity at 0.5 mg/mL concentration. In comparison with other fractions, hexane has exhibited the second most potent in percent of inhibition at 0.5 mg/mL concentration [57]. In [19], the highest antioxidant activity was reported in ethyl acetate extract ($IC_{50} = 7.59\ \mu g\ mL^{-1}$) followed by ethanol extract ($IC_{50} = 74.80\ \mu g\ mL^{-1}$) and hexane extract ($IC_{50} = 189.89\ \mu g\ mL^{-1}$), and also Al-Muniri and Hossain [58] showed that the highest IC_{50} was found in the order of activity as chloroform >ethyl acetate >hexane >water >methanol >butanol extract. In [59], from the results of antioxidant assays *in vitro*, ethyl acetate was found to have the highest antioxidant activity. The 50% inhibition of DPPH radical scavenging effect (IC_{50}) was recorded as 8.67, 0.64, 0.31, and 0.14 mg/ml for the hexane fraction, ethyl acetate fraction, and ethanol fraction; in [22, 60], the water extract has the highest scavenging activity among all extracts. Khan et al. [30] revealed that the ethyl acetate fractions of *E. gerardiana* (root and stem) have significant free radical scavenging potential with values of 2.96 ± 0.39 and 2.73 ± 0.84. The lowest antioxidant activity was found in the *n*-hexane extract of the plant selected to be equivalent to DPPH [21, 61]. Free radicals contain single

Table 2: Determination of flavonoids and phenolic contents in *S. triqueter*.

Contents	Water	DCM	Ethyl acetate	*n*-hexane
Total flavonoids (mg GAE/g)	14.05 ± 0.5	8.16 ± 0.02	9.01 ± 0.005	3.73 ± 0.02
Total phenolic (mg GAE/g)	16.45 ± 0.02	10.01 ± 0.01	14.15 ± 0.01	5.33 ± 0.05

Table 3: DPPH activity results of different fractions of *S. triqueter* plant.

S. No	Samples extracts	IC_{50} mg/mL
1	Water fraction	3.61 ± 0.01
2	Ethyl acetate fraction	3.52 ± 0.01
3	*n*-Hexane fraction	6.35 ± 0.05
4	DCM fraction	4.05 ± 0.2
5	Control ascorbic acid	2.27 ± 0.01

The data were represented as mean via statistics as a standard deviation (SD) in triplicate under standard graph pad prism software (mean ± SD). The median inhibitory concentration plotted concentration with percent inhibition.

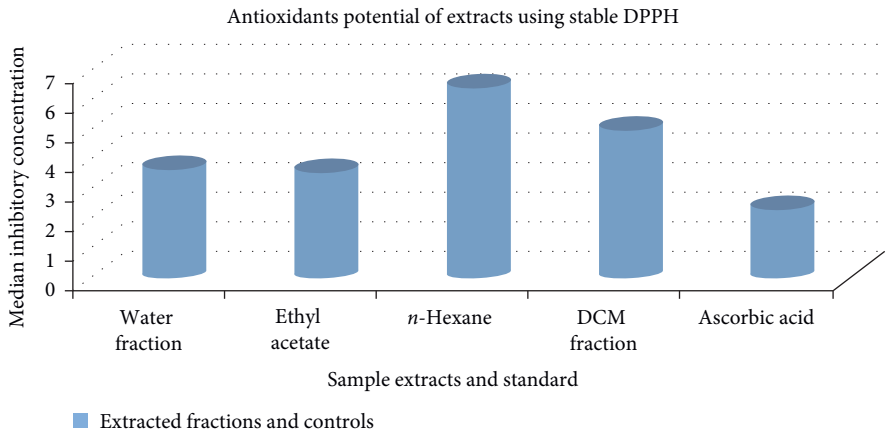

Figure 1: Comparisons of 50% inhibition of plant fractions and standard.

electron, which is produced by the broken down covalent bond between two entities because single electron free radical is too much unstable. The free radical containing oxygen is known as reactive oxygen species (ROS). Reactive oxygen species are also called active oxygen species because the oxygen containing species such as hydroxyl radicals ($OH^{\bullet}$) and superoxide ions ($O_2^{\bullet-}$) contains free radicals. Free radicals are formed generally during metabolic processes such as eating and breathing [62–64].

Antioxidants are mostly used for the protection of various diseases such as coronary heart disease, dementia, cancer, Alzheimer's disease, and arthritis [10–12]. Antioxidants have also a lot of applications on an industrial level such as preservation of food, cosmetics, and prevention of gasoline and rubber degradation. Various rich sources of antioxidant compounds such as carotenoids, flavonoids, lutein, and polyphenols are vegetables and fruits [13, 14]. It has been reported that synthetic antioxidants have high risks of side effects [65]; therefore, proper attention was given towards natural antioxidants. Interrogation on identifying natural antioxidants has become an important problem [66]. In the last few years, natural antioxidants are considered as a good preventive medicine [67]. Plants are the rich source of antioxidants, and every year, thousands of new antioxidant compounds have been invented. Plants are considered as a potential source of new compounds having antioxidant properties [63, 68, 69]. A stable free radical such as the compound of diphenyl picrylhydrazyl is demonstrated by the goodness of the delocalization of the free electrons over the whole molecule. A deep violet color appeared as a result of delocalization by the absorption band in the solution of methanol at about 517 nm. The substance that can donate a hydrogen atom is mixed with the DPPH solution; then, it gives the reduced form with a vanished violet color. In the end, pale color is indicated due to the presence of picryl residue [70–73].

Most of the bioactivities of these crude extracts especially high flavonoids and phenolic content are important. Flavonoids are good and highly effective scavengers for most of the oxidizing compounds such as singlet oxygen, and various types of other free radicals are implicated or involved in several diseases [74]. Flavonoids suppress reactive oxygen formation; in free radical production, chelate elements are specially used, and scavenge reactive species upregulate and protect antioxidant defenses [75]. Similarly, phenolic compounds confer oxidative stress tolerance in plants. Such types of crude extracts of fruits, herbs, vegetables, cereals, and other plant materials are fulfilled by phenolic contents which are highly used in the food industry and medicine industry for their antioxidative properties and for better

health. It is concluded that their potential towards a stable DPPH is because they contain flavonoids that are accountable for antioxidants potential.

4. Conclusion

It is concluded that *S. triqueter* plant contains almost all important types of phytochemical constituents and possesses antioxidant potential at various concentrations. The ethyl acetate and water fraction extracts had very high antioxidant activity, subsequently. Extracted fraction possesses the antioxidant potential and might be helpful in preventing or slowing the progress of various oxidative stresses. Further study and analysis are strongly recommended to isolate and purify the major phenolic compound as well as other bioactive compounds for further bioactivity tests which are mediated by free radicals; the isolation and identification of antioxidant component in the plant may lead to chemical entities with more potential for clinical use.

Data Availability

All the data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

All the authors thank Amir Hassan of the Chemistry Department, Government Post Graduate College, Mardan 23200, Khyber Pakhtunkhwa, Pakistan, for writing, designing, and publishing the manuscript. They also acknowledge Himayat Ullah for project assessment and evaluation.

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