

SHORT COMMUNICATION

BIOCHEMICAL STUDIES ON PROTEIN, PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITIES OF *SIDA CORDIFOLIA* EXTRACTS

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ABSTRACT. The study aimed to characterize the antioxidant properties regarding the *Sida cordifolia* with special reference to its detailed biochemical analysis. The study revealed that chlorophyll A (0.9 ± 0.3 mg/g), total chlorophyll content (3.0 ± 0.7 mg/g), total carotenoid content (0.3 ± 0.1 mg/g), total soluble proteins (7.5 ± 0.1 mg/g), and total phenolic contents (5.6 ± 1.3 mg/g) were found highest in flower tissue of *S. cordifolia*. However, peroxidase (POD) contents (118 ± 31 units/g), superoxide dismutase (SOD) activity (64 ± 1.5 units/g) were maximum in the leaf tissues, while catalase (CAT) contents (133 ± 25 units/g), ascorbate peroxidase (APX) contents (145 ± 44 units/g) were also found more in the flowers of *S. cordifolia* rather than other parts. Our results conclude that leaves, stem, flower of *S. cordifolia* could be exploited in pharmacology due to presence of different antioxidants reflected in flower and leaf extract make them potent and profound therapeutic agents.

KEY WORDS: Alkaloids, Flavonoids, Phenolics, Antioxidant, *Sida cordifolia*

INTRODUCTION

Plants grow with significant vigor and great ability to spread in areas, which controlled by humans because in most cases they are widespread species highly modified to the environment. They can create competition with developed pastures in terms of living space, nutrients, water, sunlight and CO₂ [1]. India has widely rich heritage in the use of medicinal plants as clinical practices since earliest times. In other countries like China, Africa and Brazil, the medicinal plants are usually used in the treatment of diseases for centuries. Physicians prescribe medicinal plants for different purposes in traditional system uses by the 80% of world population and modern medicine and 30% of herbal medicines. WHO estimates that most populations use products of medicinal plants for primary health care in developing countries. Homeopathic, Siddha, Naturopathy, Unani as alternative medicine systems also contain different products attained from plants. There are thousands of various plant species, which may be used in clinical practices. Several plant parts like roots, stem, bark, leaves, flowers, fruits, seeds and whole plants are used in different remedies. They may be used separately or in combined form with other plant parts. People living in rural areas very well know plants and their parts for clinical treatment. These studies may use mainly development of new and cheaper types of treatment for different sicknesses. By using modern tools phytochemical, biochemical, biotechnological,

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bioinformatics in which traditionally essential molecules may be evaluated for their activities [2].

From the 19th century, many bioactive chemical constituents have been reported from the plants that used as potent of present medicine [3]. Phytochemicals classified as primary and secondary metabolites. Protein, carbohydrates, chlorophyll are classified as primary metabolites and bioactive secondary metabolites are flavonoids, steroids, alkaloids, terpenes, coumarins and phenolic compounds for the medicinal purposes [4]. There are some natural antioxidants present in plants like alkaloids, flavonoids and phenols plays vital role in health care system. Phenolic acids, flavonoids, biflavonoids, anthocyanins and isoflavonoids are subclasses of phenols have many properties of antioxidants and works against allergies, ulcers, tumors, platelet aggregation, and cardiovascular diseases and can reduced the cancer risk [5-7]. Currently, research on plants has been increased worldwide and important in traditional system.

Sida cordifolia (Family: Malvaceae) is annual subshrub plant contains 88 genera and 2,300 species. It is delineated by 19 genera and 94 species in Pakistan. It is widely distributed in the tropical and temperate regions. The taste of this herb is highly bitter. The different plant parts such as seeds, leaves, and root are commonly used as herbal medicine in the Indian subcontinent [8]. *Sida cordifolia* is known as Brazilian malva Blanca (white mallow) or malva Blanca sedosa (silky white mallow) and is used as Brazilian folk medicine [9, 10]. It is mostly traditionally used for treatment of asthma, hypoglycemic cardiovascular effects [11, 12], arrhythmia, hemiplegia, sciatica, neuritis, neuralgia, epilepsy, rheumatism, anorexia, fatigue, impotence, gonorrhoea, cystitis, leucorrhoea, urinary frequency, diabetes, diarrhea, dysentery, hemorrhoids, and chronic fever [13]. It has been reported that *S. cordifolia* contains many compounds with pharmacological activities including hypoglycemic, anti-asthmatic, anti-rheumatic, anti-pyretic, laxative, diuretic, anti-inflammatory and analgesic [14, 15], anti-viral, anti-microbial, anti-oxidant [16, 17] and anti-fungal activities. It also contains ephedrine that affects the heart and central nervous system (CNS) [18]. Therefore, it is necessary to study the biochemical analysis and antioxidant properties of *Sida cordifolia*. The objectives of this study were to (i) investigate the antioxidant activities of the aqueous extracts of dry leaves, stems and flower *S. cordifolia*, and (ii) the biochemical activities of the aqueous extracts of the above plant towards protein and estimation of total phenolic contents were also investigated.

EXPERIMENTAL

Plant material. The whole plant of *Sida cordifolia* was collected from Lake View Park, Islamabad, Pakistan and identified by Dr. Iqbal Hussain, Associate Professor, Department of Botany, Government College University, Faisalabad where a voucher specimen number has been deposited.

Sample extraction. Fresh plant samples (0.2 g) were carefully washed each alone under tap water and cut into tiny pieces and extracted in 10 mL of KH₂PO₄ (potassium dihydrogen phosphate) buffer (50 mM; pH 7.2). Fresh plant parts at 4 °C were centrifuged for 10 min at 14000×g (MIKRO-200R; Hettich GmbH & Co. KG). For different biological analysis, supernatant was isolated. Three concordant readings were taken for all data by using UV-VIS (Hitachi U-2910) spectrophotometer.

Photosynthetic pigments. Chlorophyll (Chl.) A, B, total Chl. and total carotenoids contents of fresh plant material was measured by using the method of Yoshida [18]. 10 mL of 80% acetone used for the homogenized fresh plant material (0.1 g) and by using UV-VIS (Hitachi U-2910) spectrophotometer supernatant absorbance measured at 480, 645 and 663 nm wavelength. Carotenoids were determined with the method as reported by Davies and Taylor [19].

$$\text{Chl. A (mg/g FW)} = [12.7 (\text{OD}_{663}) - 2.69 (\text{OD}_{645}) \times V/1000 \times W]$$

$$\begin{aligned} \text{Chl. B (mg/g FW)} &= [12.9 (\text{OD}_{645} - 4.68 (\text{OD}_{663}) \times V/1000 \times W] \\ \text{Total chl. (mg/g FW)} &= [20.2 (\text{OD}_{645} - 8.02 (\text{OD}_{663}) \times V/1000 \times W] \end{aligned}$$

$$\text{Carotenoids (mg/g FW)} = [\text{OD}_{480} + (0.114 \times \text{OD}_{663}) - (0.638 \times \text{OD}_{645})]$$

Total soluble proteins (TSP) contents. Total protein contents in *Sida cordifolia* fresh materials (0.1 g) were determined by using the 2 mL of phosphate buffer saline (pH 7.2) as described by Bradford [20] and then homogenized material was centrifuged for 10 minutes at 16128×g (MIKRO-200R; Hettich GmbH & Co. KG). The sample was incubated at room temperature for 30 min. The optical density (OD) of the mixture was estimated at 595 nm using UV-VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan) and BSA was used as standard.

Total phenolic contents (TPC). A sample of fresh material (0.5 g) of *S. cordifolia* was homogenized with 1 mL acetone (80%) solution. The homogenate was centrifuged at 16128×g (MIKRO-200R; Hettich GmbH & Co. KG) for 15 min, then; 100 µL of the supernatant, 0.5 mL of Folin-Ciocalteu's phenol reagent, and 2.5 mL Na₂CO₃ (20%) was added to test tube and shaken, then added distilled water to make 5 mL final volume and vortexed. After 20 min, the absorbance was determined at 750 nm. The quantity of phenolics was determined as explained by Tulkunen-Tiitto [21].

Antioxidant enzymes assay. For the antioxidant enzyme extraction fresh material (shoot, stem and flower) of the *Sida cordifolia* were grinded in cooled mortar and pestle in the presence of cooled phosphate buffer (50 mM; pH-7.0) and dithiothreitol (1 mM). This solution was centrifuged at 25200 xg for about 20 min at 4 °C and the supernatant was used for measuring the activities of antioxidant enzymes [22].

Superoxidase dismutase (SOD) contents. The determination of SOD activity in *Sida cordifolia* fresh material was done with minor modifications in line with the method of Gong *et al.* [23]. The glass vials containing the reaction mixture were exposed under 15 watts fluorescent lamps for 15 min at 78 µmol m⁻² s⁻¹. The absorbance was assessed at 560 nm.

Catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) contents. The CAT and POD activities were done with minor modifications in line with the method of Cakmark *et al.* [24]. The absorbance of the reaction mixture was estimated at 240 nm every 20 s. The decline in absorbance of reaction solution of CAT and POD at 240 nm and 470, respectively, followed the decomposition of H₂O₂. The enzyme activity was indicated in units mg⁻¹ protein (U = 1 mM of H₂O₂ reduction min⁻¹ mg⁻¹ protein). Bradford [20] method was used to determine the protein contents from the sample. Dixit method used to measure the ascorbate peroxidase activity [22]. Assay buffer was composed by mixing 0.5 M EDTA (ethylenediamine tetraacetic acid), 200 mM buffer with pH 7 and 10 mM ascorbic acid. APX activity was estimated by assay solution composed of 0.5 M EDTA, 10 mM ascorbic acid (AsA), 1 mL of H₂O₂ (hydrogen peroxide), 200 mM potassium phosphate buffer and 50 µL of supernatant. The absorbance after every 30 s was measured spectrophotometrically (Beckman 640 D, USA) at 290 nm [25] and EU mg⁻¹ protein expresses the APX activity. One unit of APX is the quantity 175 of protein used to break down 1.0 µmol of substrate per min at 25 °C.

Statistical analysis. For analysis and organization of resulting data, descriptive statistics was applied. Whole data was reported as mean ± SD. To analyze the data two-way ANOVA (analysis of variance) with replications was used. The difference among means was determined

with Tukey (HSD) Test at 5% probability level ($p \leq 0.05$) by using STATISTIX software (version 8.1).

RESULTS AND DISCUSSION

The different studied parameters of tested plants mean values are presented in Table 1. The total soluble proteins (TSPs) level was the highest (7.5 ± 0.1 mg/g FW) in the flowers of *S. cordifolia* rather than other parts of plant as shown in Table 1. Earlier study reported the pollens of *S. cordifolia* possess (1.4 ± 0.8) protein [26], whereas, in the present study TSP was found higher in the fresh flower as compared to the pollens of earlier reported study. The enzymatic antioxidants such as POD (118 ± 31 units/mg), SOD (64 ± 1.5 units/mg) were the highest in leaves, while CAT (133 ± 25 units/mg) contents were found more in the flowers of *S. cordifolia* as shown in Table 1. POD used as an ordinary skin caring constituent in cosmetic products among antioxidants to remove the H_2O_2 (hydrogen peroxide) from the tissues. SOD contents are also known as the antioxidant defense in the body. Because they reduced, the oxidative stress which cause diseases like atherosclerosis, heart attack, stroke, various age-related disorders and acute as well as chronic inflammatory conditions [27, 28].

Table 1. Compression of different parameters in several parts of *S. cordifolia*.

Sr #	Parameters	Leaves (Mean±SE)	Stem (Mean±SE)	Flower (Mean±SE)
1	TSP (mg/g FW)	6.5 ± 0.15	6 ± 0.02	7.5 ± 0.1
2	TPC (mg/g FW)	2.8 ± 0.4	2.6 ± 1.0	5.6 ± 1.3
3	SOD (units/mg)	64 ± 1.5	61 ± 2.9	58 ± 3
4	CAT (units/mg)	106 ± 15	118 ± 24	133 ± 25
5	POD (units/mg)	118 ± 31	114 ± 3	89 ± 12
6	APX (units/mg)	108 ± 5.6	82 ± 5.7	145 ± 44
7	Chlorophyll A ($\mu\text{g/g}$ FW)	0.6 ± 0.1	0.8 ± 0.7	0.8 ± 0.3
8	Chlorophyll B ($\mu\text{g/g}$ FW)	1.3 ± 0.2	1.8 ± 0.2	2.2 ± 0.4
9	Total chlorophyll ($\mu\text{g/g}$ FW)	1.9 ± 0.3	2.6 ± 0.28	3.0 ± 0.7
10	Carotenoids (mg/g FW)	0.16 ± 0.02	0.2 ± 0.03	0.3 ± 0.1

There are different toxins in the body that can also be oxidized by catalase such as formic acid, phenols, alcohols and formaldehyde [29-30]. The level of the valuable enzymes could not be improved easily in the ancient times while now by taking nutritional supplements of useful enzymes can be raised up the weak antioxidants easily [27]. Biological properties of phenolic components are improvement of endothelial function, anti-atherosclerosis, anti-inflammation, anti-apoptosis, antiaging, cardiovascular protection, and anti-carcinogen and inhibition of angiogenesis properties [31-32]. In the treatment of different diseases, which involve high oxidative stress by observed high antioxidant potential of *S. cordifolia* parts can be oppressed for parallel applications. Crude extract of flower of this plant can also be explored for remedial purpose to the people may prefer use natural bio constituents [33]. Serine protease also called tissue plasminogen activator that can be used for the treatment of ischemic stroke and can help in debridement process which required to cure the wound healing by removing the damage cells. Mostly processes of the body are protease dependent [34, 35]. The α -protease shows the molecular function of carboxylesterase activity and its deficiency accountable for the non-Hodgkin lymphoma and B-cell lymphocytic leukemia [36]. Monocytes tumor killing activity may reduce if protease shown by organophosphates [37, 38].

The above-mentioned applications can be observed by the enzyme activity of ascorbate peroxidase (APX) in *S. cordifolia* parts. Ascorbate peroxidase contents measured by the method of ABTS as ascorbic acid was observed to be maximum (145 ± 44 units/mg FW) in the flowers of *S. cordifolia* as shown in Table 1. The *S. cordifolia* plant leaves have been reported in previous study of radical scavenging activity of crude methanol extract [39]. The ABTS assay is

used to determine the antioxidant activity of hydrogen donating and chain-breaking antioxidants. All fractions of *S. cordifolia* showed a strong scavenging activity against ABTS radicals [40].

More total phenolic contents (5.6 ± 1.3 mg/g) were found in the flowers of *S. cordifolia* as shown in Table 1. There are many reports, which indicate the phenolic play a very important role in protecting plant against different stresses in biological system. Sakihama *et al.* [41] found that the phenolic compounds can act as antioxidants by donating electrons to POD having the property to scavenge ROS in plants. Phenolic compounds synthesize with as exposure through stress after signaling [42, 43]. The chlorophyll A (0.9 ± 0.3 mg/g), chlorophyll B (2 ± 0.4 mg/g), total chlorophyll (3 ± 0.7 mg/g), and carotenoids (0.3 ± 0.1 mg/g) were higher in the flowers of *S. cordifolia* (Table 1). Liquid supplements of chlorophyll show vital and useful role in enhancing energy, liver detoxification and act as body odor elimination as bad breath, cleaning the gastrointestinal region, helping to reduce the risk of liver cancer [44]. Carotenoids plays an important role as antioxidant activity and have many important properties also known as their intense coloring abilities, as well as use as precursors of vitamin A. Carotenoids can take as food supplements and also used for nutraceutical purposes as safe chemical [45]. For the plants and animals' carotenoids are very important for their normal growth and development. These enzymes not synthesize within the body of animals. That is why animal's body needs the daily ingestion of carotenoids, which they take it from plant-based products or plants [46]. There are such main carotenoids that can be prepared synthetically in nutraceutical industries on large scale such as zeaxanthin, b-carotene, canthaxanthin, astaxanthin and lycopene. These carotenoids are widely used in cosmetics, food products and health products supplements that are related to vitamins as well as food additives for shellfishes, cattle, poultry and fish [47, 48]. In future study, detected pigments can further be purified, characterized from flowers of *S. cordifolia* for its applications.

CONCLUSION

Generally, higher enzymatic antioxidant potential was shown in flowers and leaves of *S. Cordifolia* rather than other parts of this plant. Especially flowers can be used for the development of pharmaceutical herbal drugs. The results showed that *S. Cordifolia* had substantial antioxidant activity and free radical-scavenging activity. Our study concludes that leaves, stem, and flower of *S. cordifolia* could be used in pharmacology due to presence of total phenolic, TSP and different antioxidants reflected in flower and leaf extract make them more potent and profound therapeutic agents. Nevertheless, the evaluation of their antioxidant activities and bioactive compounds in plants is essential.

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