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Pol. J. Environ. Stud. Vol. 31, No. 4 (2022), 3789-3796

Original Research

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DOI: *10.15244/pjoes/143115* ONLINE PUBLICATION DATE: 2022-05-30

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Received: 24 July 2021
Accepted: 15 October 2021

Abstract

Researchers are continuously focusing and exploring new aspirants in the field of natural dyeing to reduce the application of synthetic dyes and their harmful effect on living organisms via natural dyes. This aimed the evaluation on dyeing ability of a novel natural dye extracted from root bark of *C. polygonoides*, their pharmacological significance and phytotoxicity. Biological analyses of methanolic

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dye extract were conducted in laboratory according to standard protocols. Phytochemicals analysis of the dye solution indicated that it was rich in natural phenolics. Antibacterial activities were carried out *in-vitro* against various strains of bacteria such as *Klebsiella pneumoniea, Staphylococcus aureus, Acinetobacter baumannii* and *Escherichia Coli*. Maximum zone of inhibition against *E. Coli* (13 mm), *K. pneumoniea* (20 mm), *A. baumannii* (17 mm) and *S. aureus* (15 mm) was recorded when dye was used at 3mg/ml in dimethyl sulfoxide (DMSO). For antifungal activity, the dye at 3 mg/ml in DMSO was highly effective against *A. niger* showing 17 % inhibition in fungal growth and *A. flavous* showing 4% inhibition in linear growth. We used DPPH radical scavenging assay to document the antioxidant potential of the dye extract. The maximum scavenging potential of dye extract was found at 400 µg/ml (48.3%±0.3). Phytotoxic effect of *C. polygonoides* dye was conducted by the germination of maize in various concentrations (250-1000 ppm). The dye did not show any phytotoxic effect on the development and growth maize seedlings. We also checked the dye for its dying potential on cotton fibers. It was observed that the dye stained the cotton fabric as bright red using acetic acid as a mordant. Moreover, the dye was resistant to continuous washing with tap water and after that dye was considered as not washable. We concluded from these studies that dye extracted from root bark of *C. polygonoides* is antibacterial, shows antioxidant properties, non-toxic and efficient in dyeing of cotton fibers.

Keywords: dyeing, cotton fibers, biological investigation, phytotoxicity, *C. polygonoides*

Introduction

Synthetic dyes are widely used in textile industries for the coloration of cellulosic and cotton fibers. Compared with natural dyes, synthetic dyes can produce fairly good color fastness, a variety of bright shades and dyeing reproducibility [1]. But, some synthetic dyes contain heavy metals which are harmful to living organisms and their environment [2]. Natural dyes are defined as the dyes derived from plants, minerals or invertebrates. The most common sources of natural dyes are parts of some plants, like bark, leaves, roots, berries and wood and some organic sources such as lichens and fungi [3]. Antiquity was a time when the practise of natural dye was a very industrialised skill, and the pieces of coloured fabrics found in the tombs of early Romans and Egyptians are proof of that. The use of plants such as buckthorn (*Frangula*), safflower (*Carthamus*) weld (*Reseda*), saffron (*Crocus*), and use of mineral pigments (like hematite, limonite, copper sulphate, ochre) are confirmed by the examination of these fabrics. Application of mordants like tartar and alum, is also known since ancient time. Natural dyes are mostly nontoxic and provide protection from ultraviolet light because fabrics dyed absorb harmful rays coming from the sun [4]. The remaining left after extraction of dyes are biodegradable and used as fertilizer [5]. Cotton fabrics treated with extracts of tannin-rich pomegranate coating provide UV protection [6]. Cellulosic (cotton) are frequently treated with tannins and probably display UV protection [7].

In the food industry, anthocyanins (E163) play a very important role. Anthocyanins are utilized in food colouring (dairy products, beverages, confectionery) for strong dyeing ability and relative health safety. Quinone dyes are derived from benzoquinone, naphthoquinone and anthraquinone. Most of the quinoid dyes that are used for dyeing textiles, in cosmetics and food industry have a vegetable origin, except cochineal carmine. Red carthamin is present in safflower (*Carthamus tinctorius*), it is also used in various food as colorant (Natural Red 26). Besides textile dyeing it is used as a food colorant (Natural Red 4, E120), but because cochineal carmine often causes allergic reactions, it has been replaced by synthetic one [8].

The chemical lawsone is present in *Lawsonia inermis* therefore, it is used as antifungal. The same chemical provides its coloring properties. Because of its dyeing properties, it is still widely used in cosmetology [9]. Some plants have been provided an environment friendly coloring substance for both food and cloth. *Curcuma longa* has naturally occurring yellow dyes, which is the brightest and used as antiseptic. Researchers have investigated plant as source of natural dye; red dye of *Bixa orellana* L. seeds [10], yellow dye of *Curcuma domestica* rhizome [11], Brown dye of *Solanum nigrum* L. Fruit [12]. Black dye of *Lithocarpus pachyphylla* Kurz. [13], Brown dye of *Strobilanthes flaccidifolius* Nees. Leave [14]. Brown dye of *Parkia timoriana* (A.DC.) Merr. Fruit peel [15]. Black dye of *Terminalia chebula* Retz. Fruit [16]. Synthetic dyes are used in food, agriculture, medicines and textile industry due to their easy use and effectiveness. However, recently it is reported that many of the synthetic dyes are toxic to human beings and ecosystem. Therefore, to current research has been made on the use of natural dyes due to their non-toxic nature, sustainability and medicinal value. Information are not available on extraction of natural dye from bark of *C. polygonoides* and its use in textile and pharmaceutics industry.

C. polygonoides is a common plant of sand dunes, found in certain area of Pakistan including district Bannu, in Southern Baluchistan and Trans-Indus plains. *C. polygonoides* is commonly known as phog

in Punjab and Balanza in district Bannu. The ash of *C. polygonoides* along with tobacco used in the formation of snuff having sedative effect. Wood of *C. polygonoides* is used as fuel. During the driest months, it can tolerate extreme drought conditions by the loss of leaves and branches [16].

The *C. polygonoides* is a small shrub belonging to family Polygonaceae; usually 5 feet to 7 feet high but sometimes may reach even 10 feet in height. The young branches are green and fleshy and appear during March to April. The plant is fed to cattle and flowers are believed to contain high amount of proteins [17]. The *C. polygonoides* is becoming gradually rare due to its indiscriminate use for its roots, which are used to make charcoal. Its charcoal is used to melt iron. Many medicinal properties have also been reported in the plant. Decoction is used for the treatment of sore gums. Flower buds of *C. polygonoides* are effective in sun stroke. Flowers are also used for the cure of cough and cold [18, 19] have described the ethnic uses and potentials, as well as the need for germplasm conservation of phog (*C. polygonoides*) for a diverse range of habitats in Rajasthan, India. *C. polygonoides* is used as fuel wood; fodder and for analysis of degraded lands [16]. Formerly, plants were only used for shelter, food and for medicine but now they are investigated for other purposes.

So far, no work has been reported on the dyeing of cotton fiber or other cellulosic fibers using C. *polygonoides*. The aim and objectives of this research is to explore the root bark of this plant for the extraction of dye, to document phytotoxicity, to evaluate the antimicrobial activity and dyeing of cotton fabric.

Materials and Methods

Plant Collection and Identification

C. polygonoides plant specimen was collected from Thal Wazir, Bermi khel, district Bannu, Khyber Pakhtunkhwa, Pakistan, and was identified by Dr. Sultan Mehmood, Department of Botany, UST Bannu, Pakistan. After identification the root bark was collected from 50 Plants growing in wild. The bark was dried in shade for 20-25 days and converted to powder (2-3 mm particle size).

Preparation of the Crude Extract

50 g root bark fine powder was put in 450 ml (70%) methanol and shaken for 40 hours. The solution was filtered after 48 hours using a whiatman No.1 filter paper. The extract obtained was dried by evaporating methanol in a rotary evaporator. The thick gummy extract having dye of C. *polygonoides* root bark was stored at 4ºC for future use.

Phenolic Content of Dye

Methanolic crude extract of *C. polygonoides* L. with 4 ml of 3% solution of iron chloride was mixed. Presence of black or blue-green coloration indicated the presence of phenols [20].

Antibacterial Bioassay

The methanolic dye extract of *C. polygonoides* root bark was conducted against bacterial strains by using the procedure of Usman et al., [21]. The media with petri plates, cotton swab and a borer were autoclaved for 20 minutes at 121ºC and 17 psi pressure. Stock solution of methanolic dye extract of *C. polygonoides* root bark was prepared in dimethyl sulfoxide (DMSO).

Microorganisms

Staphylococcus aureus, Klebsiella pneumoniea, Acinetobacter baumannii and *Escherichia Coli* were used as bacterial strains. The selected bacterial strains were preserved at 4ºC on the medium.

Antifungal Activity

The antifungal activity of methanolic dye extract of *C. polygonoides* was carried out through the agar tube dilution method by using the protocol by Duraipandiyan and Ignacimuthu [22].

Microorganisms Used A. niger and A. flavous

Media for Antifungal Assay

SDA (MERCK) was used to grow fungus for inoculums preparation. Sabouraud dextrose agar was composed of 10 g/l peptone complex, 40 g/l and 15 g/l agar.

Preparation of Samples

The sample of antifungal activity were prepared from initial stock of 12 mg/ml to get final concentration of 200 µg/ml. 12 mg/ml solutions of terbinafine in DMSO was prepared for positive control. Pure DMSO was used as negative control.

Antioxidant Activity

The DPPH assay was done by using the protocol of [19]) with some modification. Stock solution was prepared by dissolving 2.6 mg DPPH with 100 ml methanol and stored at 21ºC. The solution was obtained by diluting DPPH solution with methanol to obtain an absorbance of about 0.980 at 517 nm using the spectrophotometer. 3 ml aliquot of this solution was mixed with 100 µl of the fraction at varying concentrations. The solution in the test tubes were

shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was taken at 517 nm.

Scavenging effect (%) = [(Sample absorbance - Control absorbance) / (Control absorbance)] × 100.

Phytotoxic Assay

The dye extract was taken and dissolved in 1.5 and 10 mg/ml methanol. The autoclaved petri plate having filter paper were moistened with 0.5 ml of the dye solutions separately. A positive control Petri plates were moistened only with 0.5 ml pure methanol and negative control with distilled water. The moistened filter papers were dried in an oven by evaporating methanol. The Petri plates were taken to a fume hood and seeds of maize were arranged into filter papers (5 seeds per Petri plate) separately. All the Petri plates were autoclaved in distilled water and kept in control.

The following parameters were studied.

Seed Germination (%)

Seed germination was noted for 10 days. After 10 days seed germination for all treatments was determined.

$$Germination\ (\%) = \frac{\text{No of seeds found germinated}}{\text{All the number of seeds grown}} \times 100$$

Root and Shoot Fresh and Dry Weight

Root and shoot were separated and their weight was determined directly by using a balance (digital), for dry weight analysis. The samples of root and shoot were first dried at 72ºC for 75 hours in an oven and then their weight was calculated.

Photosynthetic Pigments

A fresh leaf piece weight 0.5 gram was taken and crushed in 10 ml 80% acetone. After keeping for one night at 4ºC, the supernatant was used for determination of chlorophyll *a*, *b*, and carotenoids measuring optical densities at 645,663 and 480 nm [20].

Staining of Cloth Fibers

25 g of *C. polygonoides* L. root bark powder was boiled in 500 ml (70%) methanol and the volume was decreased to half. Then a cotton cloth $(1\times1\ \text{foot})^2$ was treated with 20% solution of acetic acid (boiling) for half an hour. Then the cloth piece was directly shifted to 70% methanolic boiling solution and treated for 1hour. After an hour, the cloth piece was directly washed with cold water (thrice) and then with commercially available detergent (twice). The shade of color was noted.

Statistical Analysis

Analysis of the data was done with the help of standard statistical software and analysis of variance and means were compared by least significant difference test.

Results

The present studies were conducted to extract a natural dye from root bark of *C. polygonoides*; investigate phytochemicals profile of the dye extract, study utilization of *C. polygonoides* dye in dyeing of cotton fibers. This study also evaluated the antimicrobial activity of *C. polygonoides* dye, evaluated the antioxidant activity of *C. polygonoides* dye and also determined the phytotoxic activity of the *C. polygonoides* dye.

Phenolic Content of Dye

We determined phenolic concentration in dye extract of *C. polygonoides*. We found that phenolic content was highest in dye solution at concentration of 1000 ppm followed by 500 ppm and 250 ppm dye solutions respectively.

Antibacterial Studies

The natural dye was tested for antibacterial potential against pathogenic bacterial strains viz. *A. baumannii, E. coli*, *K. pneumoniea* and *S. aureus* in different concentrations. Maximum zone of inhibition against *E. Coli* (13 mm), *K. pneumoniea* (20 mm), *A. baumannii* (17 mm) and *S. aureus* (15 mm) was recorded when dye was used at 3mg/ml in DMSO. It is worthy to mention here that our extracted dye was more effective in growth inhibition of *K. pneumoniea* than other bacterial strains. The susceptibility of bacterial strain *A. baumannii* to dye was higher than *S. aureus* at 3 mg/ml, 1.5 mg/ml,0.75 mg/ml and 0.375 mg/ml in terms of growth inhibition. The *K. neumoniea* and *A. baumannii* showed similar response to dye at 0.375 mg/ml (Table 1).

Antifungal Activity

The toxicity of dye against fungal strains such as *A. niger* and *A. flavous* was conducted in different concentration (3 mg/ml, 1.5 mg/ml, 0.75 mg/ml, 0.375 mg/ml). The dye at 3 mg/ml in DMSO was highly effective against *A. niger* 17 %. While the concentration used at 0.375 mg/ml against *A. flavous* was less effective causing 4% in linear growth of fungi (Table 1).

Table 1. Bacterial growth inhibition (mm) potential and Fungal inhibition (mm) of methanolic dye extract of *C. polygonoides*.

Concentration of extract	% inhibition in linear growth (mm)				%Fungal inhibition (mm)	
	A. baumannii	*S. aureus*	*K. pneumoniea*	*E. coli*	*A. niger*	*A. flavous*
3 gm/ml	17±0.41	15±0.53	20±0.47	13±0.51	17±0.51	14±0.53
1.5 mg/ml	12±0.51	13±0.56	14±0.71	11±0.41	13±0.41	10±0.56
0.75 mg/ml	10±0.46	12±0.49	13±0.51	9±0.31	10±0.31	7±0.49
0.375 mg/ml	14±0.51	13±0.46	14±0.71	9±0.37	7±0.37	4±0.46
Cm 100 µg/ml	44±0.58	38±0.61	35±0.56	44±0.51	44±0.51	38±0.61

Cm stands for Clarithromycin and ± indicates standard error value

Table 2. Antioxidant activity of red dye extracted from root bark of *C. polygonoides*.

Concentration of extract	Dye (DPPH % inhibition of free radicals)	Ascorbic acid (DPPH % inhibition of free radicals)
150 µg/l	22.5±0.4	72.3±0.1
250 µg/l	31.2±0.1	76.1±0.0
350 µg/l	38.4±0.1	81.1±0.3
400 µg/l	48.3±0.3	89.1±0.1

± indicates standard error value

Antioxidant Activity

Antioxidants are the substances which nullify the negative effects on cellular components. Free radical damage cell membrane as well as contributing in many fatal diseases. (DPPH) radical scavenging evaluate was used to document the anti-oxidant activity. The (DPPH) free radical scavenging potential of the red dye extract of *C. polygonoides* along with the standard ascorbic acid was observed. The maximum scavenging potential of dye extract was found at 400 µg/ml was (48.3%±0.3). While the lowest scavenging potential was 22.5%±0.4 at 150 µg/m dye concentration (Table 2).

Phytotoxicity

The phytotoxic potential of dye was investigated on the following growth parameters of maize under in vitro conditions (Table 3).

Seed Germination (%)

We observed non-significant effect of the dye in all concentration (250-1000 ppm) on seed germination (%) of maize (Table 3).

Root and Shoot Fresh Weight and Dry Weight

The dye extracted from root bark of *C. polygonoides* did not affect fresh weight and dry weight of both root and shoot in comparison to that of respective control (Table 3).

Leaf Chlorophyll and Carotenoids Content

All the treatment means for chlorophyll a and b as well as carotenoid content showed that they were not significantly different from each other and control. This indicated that dye did not affect photosynthetic pigments content of maize seedlings (Table 3).

Total Soluble Phenolics Content of Leaf

Like antioxidant content, the leaf Phenolics content was also not significantly affected due to treatment of maize plant with dye extract of *C. polygonoides* (Table 3).

Dying Potential of C. polygonoides Dye on Cotton Fibers

We evaluated dying potential of the *C. polygonoides* dye on cotton cloth. The mordant used was 80% acetic acid. The *C. polygonoides* stained the cotton fabric as red. (Plate 2). Moreover, the dye was resistant to continuous washing with tap water (5 times) and after that dye was considered as not washable (Table 3).

Discussion

Plants, animals and minerals are resources of natural dyes. It has been used for dyeing of textile, hair, body, leather, in cosmetology and craft food colourings.

Table 3. Effect of CPMDE on seed germination (%), root and shoot fresh weight and dry weight, photosynthetic pigments, Phenolics (mg Gallic acid eq/g f.w) content of maize.

Treatments	Germination (%)	Root fresh weight (g), Root dry weight (g)	Shoot fresh Weight (g), Shoot dry weight (g)	Chlorophyll a (mg/gf.w)	Chlorophyll b (mg/gf.w)	Carotenoid (mg/gf.w)	Total soluble Phenolics of leaf (mg gallic acid eq/g.fw)
Control	60.0 A± 0	0.16A±0.05 0.01A±0.01	0.28A±0.02 0.06A±0.02	1.20A±0	2.24A±0.79	20.43AB±7.48	64.76A±12.86
CPMDE (1000 ppm)	66.67A± 6.66	0.17A±0.07 0.02A±0.02	0.27A±0.06 0.08A±0.04	0.98A±0.52	1.37A±1.15	14.13AB±6.61	50.20A±2.97
CPMDE (500 ppm)	46.67A±6.66	0.21A±0.11 0.01A±0.01	0.46A±0.14 0.08A±0.01	2.06A±1.067	3.70A±1.64	32.33A± 9.28	57.29A±11.27
CPMDE (250 ppm)	60.0 ± 11.55	0.15A±0.05 0.03A±0.02	0.23A±0.05 0.04A±0.01	0.39A±0.18	0.29A±0.09	5.13B±0.69	39.92A±19.26

Means with same English letters are statistically similar±indicates values of standard errors.
CPMDE = *C. polygonoides* methanolic dye extract.

The dye yielding plants have many economic values and medicinal values. It has also been reported that the natural dyes are eco-friendly and not harmful in nature. Dye yielding remedial plants are used by the people for not only dyeing but also in treatment of numerous human diseases like strangury, chronic ulcers, diabetes, diarrhoea, piles, itches, skin disease and many more common as well as fatal diseases [23].

A large number of plant species have been explored for extraction and sources of natural dyes. In our studies we have evaluated pharmacological significance, phytotoxicity and dyeing ability of a natural red dye extracted from bark of *C. polygonoides*.

In our studies we found that red dye extracted in methanol from bark of *C. polygonoides* was rich in Phenolics. Similarly, Phenolics were abundant in natural dye extract of *Curcuma domestica* [24].

We tested *C. polygonoides* dye against bacterial and fungal pathogens. Our result revealed that *C. polygonoides* dye extracted inhibited growth of bacterial species like *S. aureus, K. pneumoniea, A. baumannii, E. Coli* and fungal pathogen. The results of our study showed that methanolic dye extract had inhibitory effect on both the bacteria and fungi pathogens. In medicinal plants phenolic compounds are present. Therefore, they show strong antimicrobial activity [25, 26]. In our studies *C. polygonoides* dye extract was rich in natural phenolics which might have contributed in its antimicrobial properties. The oxidants are those substances which show high reactivity towards the other substances and having short life span. These reactive oxygen species cause damages to cellular materials and destroy proteins and DNA. The compounds which prevent action of reactive oxygen species are called anti-oxidants [27].

The red dye extracted from root bark of *C. polygonoides* exhibited strong antioxidant properties. The scavenging capability of methanolic dye extract of this plant could be due to the presence of phenolic constituent. The maximum scavenging potential of our studied dye extract found at 400 µg/ml was (48.3%±0.3). While the lowest scavenging potential was 22.5%±0.4 at 150 µg/m dye concentration. Our result showed similarities with the result obtained [28] as they have reported that the medicinal plants have high potential for scavenging oxidants. The scavenging capability of methanolic red dye of *C. polygonoides* could be due to the phenolic constituent which have strong antioxidant properties.

Application of natural colorants in textile industry has gained interest recently due to their biodegradable and non-toxic nature. In our studies *C. polygonoides* root bark dye stained cotton fibers as bright red in the presence of mordant acetic acid. Usually metallic salts are used as mordants for natural dyes. However, many of the such salts are reported as highly toxic to living organisms [29]. In our study acetic acid in diluted form was used as a mordant. The dye was not washable after successive washings with detergent. This will

further improve environment friendly nature of the dye extracted from root bark of *C. polygonoides*.

Phytotoxicity is defined as plant poisoning. The poisons effect can result from products such as plant protection products, herbicides and pesticides. This negative effects may be observed on the crop at emergence or during growth or may be expressed at harvest. The effects can include abnormal growth, discoloration of plants or death of plants Khan et al., [16]. Majority of the compounds having hazardous effects on plants are phenolics in nature. Moreover, Phytotoxicity assay can used to study environmental hazards of a chemical substance. In our studies the red dye extracted from root bark of *C. polygonoides* did not show any phytotoxicity on maize seedlings. This indicated that our dye is environmentally safe and its release into the environment is not toxic for plants. Further studies on the toxicity evaluation of this dye in animal models will further assist in the commercial production and industrial use of this dye.

Conclusion

The dye extracted in methanol from root bark of *C. polygonoides* was rich in phenolics, exhibited antimicrobial and antioxidant properties. The dye was bright red in color, effective in dyeing of cotton fibers and acetic acid was used as a mordant. Moreover, the red dye did not show any phytotoxicity on seedling growth of maize. We concluded that dye extracted from root bark of *C. polygonoides* is highly medicinal, effective in dyeing of cotton fibers and has no toxicity on growth of maize seedling. Some recommendation of dye extracted from root bark of *C. polygonoides* for dyeing of cotton fibers on industrial scale. Due to its non-toxic nature the dye can be used in dyeing and making of food products after checking in animal models. Furthermore, to explore complete phytochemicals profile of the dye, Isolation, purification and identification of the major red pigments found in *C. polygonoides* root bark dye solution is needed using advanced chromatographic techniques like HPLC, LCMS and GCMS.

Acknowledgments

The authors gratefully acknowledge the support received from the Department of Botany AWKUM Mardan, Pakistan. This research work received no specific grant from any donor agency in the public, commercial, or nonprofit sectors, and these organizations have had no involvement in the analysis and interpretation of data, in the writing of the draft, or in the decision to submit the article for publication.

Conflict of Interest

The authors declare no conflict of interest.

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