EXTRACTION AND EVALUATION OF A NATURAL DYE FROM BISTORTA AMPLEXICAULE

A. Fatima, G. K. Raja, M. A. Shah*, I. Gul, S. Tabassum, S. Batool, W. Ahmed, M. S. Ahmad

Department of Biochemistry
*Department of Pathobiology, Faculty of V&AS, MAS-Arid Agriculture University Rawalpindi, Pakistan
Corresponding author's email: dr.sheeraz@uaar.edu.pk

ABSTRACT: Natural pigments extracted from plants are raised as a vital substitute to artificial dyes. The present study was conducted to extract pigment from *Bistorta amplexicaule* and evaluate its dyeing property, antimicrobial activity and toxicity. The extraction was done by maceration and sonication methods with 7.5% and 5% yield, respectively. The extracted pigment appeared bright-amber colored which was used for dyeing fabrics and food using traditional methods. The suitable dying was achieved using 4g dye powder in 100ml water for fabrics and 250mg in 26.2g of rice. The extract did not indicate antibacterial activity against *Staphylococcus aureus, Klebsiella sp.*, *Escherichia coli* and *Serratia marcescens* by disc-diffusion method. Dye extract using albino mice assay did not show any mortality throughout the study period (15 days). The natural plant pigment extracted from *B. amplexicaule* had a potential as natural dye for food and fabric.

Keywords: Natural dye, *Bistorta amplexicaule*, Fabric, Antibacterial.

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INTRODUCTION

Polygonaceae is the family of flowering plants known as knotweed or smartweed. There are 50 genera and 1200 species in this family (Batool *et al.,* 2015 and Mabberley, 2008). Flowering plants of this family are distributed worldwide, but most of the species are found in north temperate zone *(*Takhtajan, 2009).

Bistorta consists of 300 species and most of the species have medicinal value like *B. amplexicaule* and *B. multiflorum*. *Polygonum* produces high contents of antioxidants like phenolics, terpenoids, flavonoids, cardiac glycosides, etc. (Isobe *et al*., 1981). Polygonaceae plants are used as laxative, nettle antidote, astringent and stimulant in the management of abnormalities like sores, hurts, cancer and tumors, etc.(Nahar *et al.,* 2010).

Species of *Bistorta* are reported in the treatment of various conditions such as snake-bites, inflammation, cancer, microbial infections, diabetes, hypertension, hyperlipedemia, jaundice, hemorrhage, cough, fever, ulcers, anemia, diarrhea and various urologic disorders (Muhamed, 2014).

One of the important species from this genus is *amplexicaule*, herbaceous perennial plant that grows up to 1.2m (4ft) in height and produces rose-red or white flower in summer. Its leaves are wide, pointed and heart shaped. *B. amplexicaule* is also known as *Persicaria amplexicaule.* It is is widely distributed in the northern Pakistan and has a common name 'maslon'. It is reported to be used as medicine to treat inflammation, dysentery, promoting blood circulation, fractures, diuretic, pain and hemorrhage (Mudasir *et al*., 2012). *B. amplexicaule* contains high concentration of antioxidants (Ahmad *et al.,* 2013; Qurashi *et al.,* 2007 and Uniyal *et al*., 2002). This plant is used as a herbal tea for the treatment of dysentery, leucoeehoea, ulcers, fever and various heart problems (Matin *et al*., 2001). It is documented that its extracted root sap can be applied on fresh wounds in the eye (Uniyal *et al*., 2006).

In the last sixty years, a number of research findings on plants have been reported by (Ahmed *et al*., 2016; Amjid *et al*., 2016; Niaz *et al*., 2016 and Raza *et al*., 2016). Natural dyes that were shoved into the contextual by synthetic pigments are currently making a comeback again due to customer interest. Mostly these pigments contain azo-group and are reported as carcinogens by (Prabhu, 2012).

The plant under study has already been proven to have chemical compounds of antioxidant potential and medicinal importance. It is observed that rhizome of the plant contains an amber colored pigment. This natural dye is so intense that even a thin layer gives strong color. Keeping in view the dyeing potential of the pigment, current study was designed to extract and determine the applications and toxicity of the natural dye from *B. amplexicaule.*

MATERIALS AND METHODS

Preparation of dye extract: The whole plant of *B. amplexicaule* was collected from Murree hills. The rhizome of the plant was separated, washed, dried and powdered to an average size of 20µm, mixed with pure methanol and was subjected for dye extraction by cold

maceration and ultra-sonication method (Simon *et al*., 1998).

Textile dyeing: Cotton fabric purchased from local market of Rawalpindi was used for dyeing. The dye potential of the pigment was analyzed by dyeing pure cotton fabric under normal conditions. The fabric was washed using a solution of 0.5g/L sodium carbonate and 2g/L non-ionic cleanser solution at 50°C for 15min, washed under tap water and dried at room temperature. This dried fabric was re-soaked in clean water and was treated with copper sulphate and alum for pre-mordanting. The 2% mordant was liquefied to make a liquor ratio of 1:40 and afterwards soaked sample cloth was added into the mordant solution and then heated at 80°C for 30 minutes (Saravanan *et al.*, 2011).

Dye powder (4g) was added in 100ml water (4%) for the preparation of dye extract liquor. Dyeing was done by the method as described above. The mordant fabric was instantly added in staining solution at 80°C for 25min. After dyeing, cold water washing was done and dyed cloth was dried at room temperature. For better fixing of dye, the cloth was dipped in brine. Color intensity was measured with the help of Chromameter i.e. CR-400, Konica Minolta.

Food dyeing: Food dyeing potential of the dye was analyzed using rice purchased from local market of Rawalpindi. For this purpose, 26.2g rice were boiled in three beakers (each containing 26.2g rice) and dye was added during boiling of the rice. In one beaker 250mg of dye was added, in second beaker 500mg and in the third beaker 750mg of dye was added to get desirable color intensity. The amount of dye which gave maximum color was selected for further studies. The color intensity was measured before and after rinsing with tap water with Chromameter CR-400, Konica Minolta (Ayala-silva *et al*., 2005).

Antibacterial activity: Antibacterial activity was analyzed against *Staphylococcus aureus*, *Klebsiella sp.*, *Escherichia coli* and *Serratiam arcescens* by disc diffusion method (Ahmad *et al*., 2013).

Whatman's membrane filter paper (0.45 µm pore size) was punched into 5mm discs and sterilized. Then, each sterilized disc was incorporated with 20-60µl (10mg/ml of stock) of dye extract using micropipette. To prevent the flow of solvent extract from discs, small quantities were applied and were allowed to air dry. After drying, another dose of extracts was applied on discs. These discs were then stored at 4°C till used.

Disc diffusion method: Freshly cultured bacterial cultures were streaked on Muller Hinton agar (MHA). The discs were coated with different concentrations of extract (20, 30, 40, 50 and 60µl) and were incubated for 24 hours at 37°C. The diameter of inhibitory zone made around each disc was measured in mm (Siva *et al*., 2011).

Toxicity assay (Albino mice assay): Female albino mice were used to evaluate *in vivo* acute oral toxicity of the dye extract of *B. amplexicaule*. The mice were divided into five groups i.e. one control and four treated groups each group consisted of five mice.

The extract dose of 200mg, 100mg, 20mg and 10mg/Kg of body weight was administered to four treatment groups by oral route along with one control group (without extract).

Mice were closely observed for any toxic symptom for 8 to 10 hours daily. Animal's survival was observed daily for any change in their physical appearance, injury, behavioural pattern, mortality or any other sign.

Experimental design

Group 1: Control (negative)
Group 2: 200mg/Kg body weight
Group 3: 100mg/Kg body weight
Group 4: 20mg/Kg body weight
Group 5: 10mg/ Kg body weight

Biochemical parameters: Blood serum was used for biochemical analysis. Total bilirubin, alanine transferase (ALT), alanine phosphatase (ALP), urea, creatinine, creatinine kinase (CK) and creatinine kinase-myoglobin (CK-MB) levels were estimated.

RESULTS AND DISCUSSION

The dried dye extract of *B. amplexicaule* was weighed and its calculated percentage yield was 7.5%. As methanol is a good solvent of many polar and some non-polar compounds, so extraction of pigment from *B. amplexicaule* with pure methanol gave very intense color. Maceration a traditional technique used for many years in the extraction of many natural products and did not require any special equipment.

The percentage yield of the *B. amplexicaule* pigment obtained after ultra-sonication was 5%. The pigment gave amber color in pure methanol (Figure-1). Ultrasound assisted extraction technique was considered as an efficient, less time-consuming and a good alternative than other conventional ones. Its apparatus and operation was easier and inexpensive than other methods. For instance, microwave and super critical fluid extractions (Vikhu *et al*., 2008).

Extraction with maceration was higher (7.5%) than the ultra-sonication (5%). In both the methods, the solvent used was 100% methanol, it was relatively inexpensive and lot of polar and non-polar compounds dissolved in it and easily evaporated. When maceration method was assisted with regular shaking, extraction efficiency also increased (Handa *et al*., 2008)). In the present experiment maceration method resulted in higher dye yield as the maceration was supported with regular shaking.

Dyes are the substances that impart color by some process that change the crystal structure of the colored substances which may be temporary or permanent. The dyes attach to the compatible surfaces in solution by forming bonds, covalent or ionic or by mechanical retention or physical adsorption. The dyeing process is a key factor in successful trading of many products such as textile, food and pharmaceuticals (Gurses *et al*., 2016 and Chequer *et al*., 2013)

The rhizome of the plant showed intense amber color and was easily extracted by maceration method. For the fixation of color, some chemical and natural substances were used as mordant to impart color. Dyeing without mordant would lead to fade color with washing or by exposure to light as it aids the chemical reaction between dye and the fiber.

The washed cotton fabric was treated with chemical mordant *i.e*. copper sulphate and alum at 50°C for 25 minutes, prior to dyeing. Afterwards, the fabric was dipped in sample extract solution in 1:40 ratio for another 30 minutes. Uptake of the dye was suitable. As previously documented that *B. amplexicaule* pigment showed good dyeing result when silk fabric was used (Srivastava *et al.,* 2008).

After completion of the procedure, the material was air dried and then the intensity of the absorbed colors was measured by chromameter (CR-400, Konica Minolta). The values obtained were L54.58, A7.48 and B14.70 where, L represents the brightness, A represented green to red color and B represented blue to yellow color.

According to the results, the extracted pigment was bright which reflected red and yellow colors (figure-2). The substance that gives color to any material must display high affinity, resistance to fading, uniform color and economically viable (Siva, 2007).

Food dyeing: In the present study rice were used for analysis of food dyeing potential of the pigment. Colorant was added during boiling of the rice. The concentration of the colorant used was 250mg, 500mg and 750mg for 26.2g of rice in three beakers. After completion of boiling process, rice were rinsed and change in color was observed before and after the rinse.

The Color obtained was appealing and got very intense with increasing the amount of the pigment. There was substantial difference in colors when the dyed boiled rice was rinsed with water (Fig-3). The values of dyed rice before and after rinsing with tap water were measured by chromameter. Before rinsing of 0.25mg, 0.5mg and 0.75mg of boiled rice, the level of brightness was 69.30±1.19, 72.62±1.26 and 81.08±1.10 respectively whereas, the level of brightness after rinsing of aforementioned quantities of rice the level of brightness was 56.01±6.8, 64.60±1.92 and 73.81±0.52 respectively (Table-1).

The use of this color in food items not only makes them more presentable but also provides natural antioxidant and antibiotic quality in food. The natural colorants used in different food items impart antibiotic quality to the food as well. In food industry, many of the natural and synthetic dyes can be used to impart color in many processed food items as confectionaries (Gregory *et al*., 2017 and Sivakumar *et al*., 2011).

Antibacterial activity: Antibacterial activity of the colored extract of *B. amplexicaule* was investigated by using disc diffusion method against *Staphylococcus aureus, Serratia marcescens, Bacillus subtilis* and *Klebseilla pneumonia*. Pigment did not inhibit the growth of the tested pathogens. Pigments used as food colorants showed toxicity to microorganism (Dufosse, 2009) but certain bacteria and fungi are source of pigments as well. These microbes use pigments as secondary metabolites (Chidambaram *et al*., 2013), so it is not mandatory for all pigments or colorants to show toxicity to microbes. Why our dye is non-toxic to above mentioned bacterial species is still not known, further characterization of the dye is needed.

Toxicity analysis: In recent years, there is an increasing trend towards natural products due to their fewer side effects, low pollution and less toxicity. Above all, they are environment friendly and recyclable. The toxicity of the extracted pigment was investigated using albino mice assay (*in vivo*). The albino mice assay was efficient for evaluating toxicities of many natural and chemical products (Rajalakshm *et al*., 2014).

The study revealed that the dye extract of *B. amplexicaule* did not produce any mortality throughout the study period of 15 days, even when the dose was up to 200mg. No significant behavioral and physical changes were observed between the control and pigment treated animals. Although, there was a slight increase in the weight of the treated groups compared with control one. On completion of the study, one mouse from each group was selected randomly and the blood was drawn by cardiac puncture to measure biochemical parameters (Table-2).

The results revealed that the tested dye extract showed some variations in ALT, ALP and cardiac enzymes (CK and CK-MB). There were no significant changes in the values of total bilirubin and creatinine (Table-2). Based on the acute toxicity study, it was concluded that the tested doses 200mg, 100mg, 20mg and 10mg of the pigment *B. amplexicaule* were non-toxic. There was not any behavioral or physical change observed during the entire study period.

Pigments obtained from the rhizomes of *B. amplexicaule* can be used to recover the old art of dyeing using natural dyes. Plant rhizomes dried out under shade and crushed to powder provided suitable material for extraction of dye. For dyeing silk material, washing with

detergent followed by air drying and used to get rid of the unwanted impurities. However, the fabric dyeing required treatment with different natural and metallic mordants before dying. Color shades obtained from natural mordants like Katha and Amla were reported for positive and intense colors (Shanker, 2007).

A **B**

Figure 1. Colored pigment obtained from methanolic extract of *B. amplexicaule,* a) air dried at room temperature b) dried pigment placed on paper.

Figure 2. Dying of cotton fabric using 4% dye powder for the preparation of dye extract liquor and dyeing was by traditional method.

A **B**

Figure 3. Coloring of boiled rice using 250mg of extract in 26.2 g of Rice a) before rinsing with water b) after rinsing with water

Table 1. Chromameter values of dyed boiled rice before and after rinsing with tap water.

Sr. #	Parameters	dyed rice before rinsing			dyed rice after rinsing		
		0.25mg	0.5mg	0.75mg	0.25mg	0.5mg	0.75mg
1	L	69.30±1.19	72.62±1.26	81.08±1.10	56.01±6.8	64.60±1.92	73.81±0.52
2	A	11.80±0.79	12.46±0.34	18.05±0.58	9.14±0.17	9.84±0.57	12.05±1.96
3	B	18.64±0.84	21.05±2.75	25.05±0.93	12.36±3.06	15.64±2.21	21.11±1.06

L= level of brightness, A= green to red color, B= blue to yellow color

Table 2. Biochemical parameters of Albino mice blood serum.

Sr. #	Parameters	Control	Dye extract doses given per kg of mouse weight			
			Dose 1 (200mg)	Dose 2 (100mg)	Dose 3 (20mg)	Dose 4 (10mg)
1	**Total bilirubin** (U/L)	0.7±0.02	0.8±0.02	0.7±0.015	0.8±0.02	0.7±0.02
2	**ALT** (U/L)	45±0.35	56±0.81	22±0.53	68±1.18	44±0.76
3	**ALP** (U/L)	206±1.53	108±1.53	175±1	211±3.06	178±1.53
4	**Urea** (mg/dL)	13±0.46	13±0.42	15±1.04	15±1.4	19±0.96
5	**Creatinine** (mg/dL)	0.2±0.01	0.4±0.02	0.4±0.02	0.3±0.03	0.4±0.02
6	**CK** (U/L)	187±1.53	-	168±1.53	155±0.72	278±2.52
7	**CK-MB** (U/L)	172±2.08	-	121±1.06	114±1.53	274±2.52
8	**Mortality**	0.0	0.0	0.0	0.0	0.0

ALT= Alanine transferase, ALP= Alanine phosphataseCK= Creatinine kinase, CK-MB= Creatinine kinase-Myoglobin, - = not determined

Conclusion: The extracted dye has intense bright red and yellow colors. The dye can be used in textile industry due to its appealing nature. The dye is safer, non-toxic and has no antibacterial activity therefore can be used for food and pharmaceutical products. This research may pave the way to know the complete information about the extracted dye that we are working in next project.

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