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Short Communication

Staining Effect of Yellow Dye Extracted from Wood of *Berberis vulgaris* L. on Angiospermic Stem Tissues

Faizanullah[a], Asghari Bano[a]* and Yunus Dogan[b]
[a]Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan
[b]Faculty of Education, Department of Biology, Dokuz Eylul University, Buca / Izmir, Turkey

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Abstract. Yellow dye was chemically extracted from wood of *Berberis vulgaris* L. using water and ethanol and its effectiveness as staining agent for angiosperm stem tissues was studied. The dye stained the lignified tissues of both monocotyledonous as well as dicotyledonous stem cross sections. However, the dye extracted in ethanol (10% w/v) was found more effective to stain the lignified tissues of plants.

Keywords: yellow dye, *Berberis vulgaris* L., angiospermic stem tissues

Berberis vulgaris L., belonging to family Berberidaceae, is a shrub commonly growing in the southern Europe, northwest Africa and western Asia. It bears great medicinal importance. It was formerly used as a source of yellow dye and contains an antibacterial compound berberine (Kong *et al*., 2004). Berberine can be used as staining agent when dissolved in lactic acid (Lux *et al*., 2005).

Staining anatomical sections of plant tissues provides an adequate method for rapid and inexpensive microscopic observation of their internal structure. For section staining both natural and synthetic dyes are used (Drury and Wallington, 1976). The commonly used natural dye is haematoxylin, obtained from wood of Mexican tree, *Haematoxylon campechianum* (Baker and Silverton, 1985). Although synthetic dyes are very effective but their utilization is limited due to their harmful effects on human and animals. Some synthetic dyes have been in disuse due to their recognized adverse effects (Bhuyan and Saikia, 2004). Owing to the global demand for the use of environmental friendly and biodegradable materials, the use of natural dyes has once again gained interest (Garg *et al*., 1991). The aim of the present investigation was to explore the effectiveness of yellow dye extracted from the wood of *B. vulgaris* L. for staining stem tissues of angiospermic plants.

Air dried wood of *B. vulgaris* L. was extracted with petroleum ether and after evaporating the petroleum ether, solutions were made using crystals in water and ethanol and used for staining. Free hand stem sections of *Pennesetum typhoides* (Burm.f.) Stapf. and C.E.Habb. syn: *P. glaucum* (L) R.Br. and *Chenopodium murale*, were prepared in ethanol, water, acetic acid and formalin, and stained with Berberis wood extracts (water and ethanol) (Ruzin, 1999). Then they were observed under light microscope (Olympus BX51) and the intensity of staining was determined (Lux *et al*., 2005).

The solutions of dye made in water and ethanol were found to stain the lignified tissues of both *P. typhoides* and *C. murale* stem cross sections (Table 1 and 2). However, ethanolic extract was more effective than aqueous extract. The colour of the dye extracted with water was light yellow while that extracted with ethanol was dark yellow. The ethanolic extract of dye imparted dark yellow colour to vascular tissues of the stem cross sections. The dye extract 10% (w/v) in ethanol was found to be more effective in staining vascular bundles of monocotyledonous as well as dicotyledonous stem cross sections; however, the staining effects were more pronounced on the latter. The results further revealed that the dye extract in ethanol could be used successfully to stain lignified plant tissue. Similar results were obtained by Avwioro *et al*. (2005) for dye extracted from wood of *Pterocarpus osun* and Faizanullah (2004) for dye extracted from leaves of Henna

Table 1. Staining effect of *Berberis vulgaris* L. wood dye on stem tissues of *Pennesetum typhoides*

Berberis wood extract	Tissue stained	Intensity of staining
I. In ethanol		
1%	Vascular	+
5%	Vascular	+ +
10	Vascular	+ + + +
II. In water		
1%	Vascular	Traces
5%	Vascular	Traces
10	Vascular	Traces

*Author for correspondence; E-mail: asgharibano@yahoo.com

Table 2. Staining effect of *Berberis vulgaris* L. wood dye on stem tissues of *Chenopodium murale*

Berberis wood extract	Tissue stained	Intensity of staining
I. In ethanol		
1%	Vascular	+
5%	Vascular	+ +
10	Vascular	+ + + + +
II. In water		
1%	Vascular	Traces
5%	Vascular	Traces
10	Vascular	Traces

(*Lawsonia alba* Lam.) which can be used as an effective histological stain even in absence of mordants. The search for suitable mordants would further improve the feasibility of the extracted dye in ethanol for usage in plant micro techniques. The basic stains generally stain the nucleus while cytoplasm is stained by acidic stains (Baker and Silverton, 1985). In view of this, it can be expected that the dye extracted from wood of Berberis is acidic in nature. More experiments are needed to identify the exact nature of the yellow dye extracted in ethanol from Berberis wood by utilizing advanced chromatographic techniques as majority of the natural dyes contain several impurities and other dye fractions (Banerjee and Mukherjee, 1981), the identification of active ingredients of dye will open a new avenue of research in the field of dyeing. Although the dye extracted in ethanol was not tested against microorganisms, it could be tested as histological stain for bacteria and fungi. Moreover, search for different solvents for dye extraction and their implication as staining agents is also needed. Lux *et al*. (2005) reported that solving berberine in lactic acid was highly effective in staining the root tissues in plants.

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