

STAINING EFFECT OF DYE EXTRACTED FROM DRY LEAVES OF *LAWSONIA INERMIS* LINN (HENNA) ON ANGIOSPERMIC STEM TISSUE

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Abstract

A natural dye lawsone (2-hydroxy-1, 4-naphthoquinone) was isolated from dry leaves of *Lawsonia inermis* L., by extraction with clove oil, ethyl alcohol, water and its effectiveness as staining agent for angiospermic stem tissue was studied. *Lawsonia* revealed meta-chromatic property of staining. A 10%w/v crude extract of *Lawsonia inermis* L., leaves in ethyl alcohol and water were used for staining stem cross section of angiospermic plants like *Helianthus annuus* L., and *Zea mays* L. Dye extracted from *Lawsonia inermis* leaves in ethyl alcohol profusely stained vascular bundle (sclerenchyma) of the angiospermic stem cross section of *Helianthus annuus* L., however, ground parenchyma was stained very lightly Dye extracted from *Lawsonia inermis* leaves in 10%w/v water though stained the sclerenchyma of *Zea mays* stem cross section more effectively than parenchyma in *Helianthus annuus*, but not as profusely as result of *Lawsonia inermis* in ethyl alcohol. Within each vascular bundle xylem cells were stained very effectively but the cortex and medulla were stained less effectively. However staining power of the extracted dye of *Lawsonia inermis* L., with respect of different stem tissue was apparently variable according to the solvent used, but the extracted dye of *Lawsonia inermis* L., proved to be weak staining agent for parenchyma of angiospermic stem tissue in both the species of *Helianthus annuus* L., and *Zea mays* L., as members of dicot and monocot respectively. This open a new avenue of research and further probes to discover better method of extraction of the dyes in concentrated form in more different solvents and better method of preparing stains from them, will finally prove their validity and will be useful addition in botanical staining materials.

Introduction

Small shrub of henna (*Lawsonia inermis* Linn.) belongs to the family Lythraceae is widely cultivated in Middle Eastern and northern African countries. The plant has been introduced widely throughout the tropics and sub-tropics as to enhance beauty, as a dyestuff and elsewhere as a commercial crop. The plant grows at temperatures higher than 11°C and needs around 5 years to mature as well as achieves a height of 8 to 10 feet. The major pigment in henna leaf is lawsone (2-hydroxy-1, 4-naphthaquinone), having a fast-dyeing property. The plant bears great medicinal importance. Al-Arnaout *et al.*, (1987) reported in the book of "Prophetic Medicine" that henna usage is very obvious at the beginning of Islamic traditions, where the medicinal usages of *Lawsonia*, have been noted by the supporters and others that were close to Prophet Mohammed (PBUH) in his family were recorded. According to Pinto *et al.*, (1977) *Lawsonia* plant possessing naphthoquinones have been used in treating a disease like cancer by American and Indians. Pink *et al.*, (2000) also declared that several research reports have also confirmed cancer treatment activity of *Lawsonia*. De Almeida *et al.*, (1990) described

naphthoquinones being anti-inflammatory, fungicidal, (Gafner *et al.*, 1996), virucidal (Heinrich *et al.*, 2004), bactericidal (Binutu *et al.*, 1996), trypanocidal, and anti-malaria (De Moura *et al.*, 2001). Several reports declared that lawsone is the major constituent of *Lawsonia inermis* and can treat tuberculosis (Sharma, 1990). From Pakistan such information has been compiled and reported (Shinwari, 2010; Gilani *et al.*, 2010). Prasirst *et al.*, (2004) also demonstrated that lawsone is useful against oral *Candida albicans* separated from patients with HIV/AIDS among human. Growth and replication of retroviruses (HIV) were found to be inhibited through naphthoquinones (Koyama, 2006). Endrini *et al.*, (2002) demonstrated anticancer trait of Henna's using an extract of *Lawsonia inermis* in chloroform by the culture tetrazolium salt (MTT) test on the breast of human, liver and colon normal cell lines and carcinogenic liver cell lines. Nawaf *et al.*, (2003) demonstrated that the greater usage of henna for body ornamentation and hair dyeing, can cause allergic reactions to some contaminants present commercially in natural henna powders. *Lawsonia* leaves are used in many countries for hair dyeing, fingernails and eyebrows during religion festivals and marriages. The use of henna for dyeing the palms and fingernails is a gifted custom (Probu & Senthilkumar, 1998). Since time immemorial natural dyes have become a part of human life. From very beginning the alchemy of colors has started its use (Vankar, 2000). Kumar & Bharti, (1998) pointed out that natural dyes with the environment show better biodegradability and usually have a better compatibility. They show lower toxicity and allergic reactions than synthetic dyes. Histological staining of stem cross section of the plant tissue provides a satisfactory method for quick and cheap microscopic observation of their internal organization. Drury & Wallington, (1976) reported that natural as well as synthetic both dyes are used for cross section staining. Fessenden & Fessenden, (1998) reported that the presence of quinones in henna, gives its dyeing properties. Al-Abri *et al.*, (unpublished) have demonstrated that henna extract could be used in histological preparations as a naturally occurring stain. Habbal *et al.*, (2007) reported that henna extract contains a naturally occurring pigment naphthoquinone. Dye uptake increases with increase in pH (Badri & Burkinshaw, 1993) and it stains tissue preparations in histological paraffin sections of different organs (Veereshkumar, 2005). Aim of the present investigation was to scrutinize the efficiency of dye extracted from the dry leaves of *Lawsonia inermis* L., for staining the vascular stem tissue of *Zea mays* L., and *Helianthus annuus* L., as representative of angiospermic plants.



Material and Method

a. Plant material: Fresh plants of *Lawsonia inermis* L. were collected from the fields of Shamshi khel, in district Bannu, Khyber Pakhtunkhwa Pakistan. Plant was identified and a specimen was submitted vide Voucher No. H-105 to the herbarium in the Department of Botany, Govt. Post Graduate College Bannu, Khyber Pakhtunkhwa Pakistan for future reference.

b. Dye extraction: The plant of *Lawsonia inermis* was dried in shade for several days at room temperature. The leaves are grinded and powdered mechanically for effective extraction. Dye was extracted with 1%, 5% and 10% solution using solvents, that is water, ethanol and clove oil.

c. Tissue staining: Analyzing the staining property of *Lawsonia inermis* Linn, stem tissue of *Helianthus annuus* L., and *Zea mays* L, representatives of angiosperm were selected. Microtome cut stem section of *Zea mays* and *Helianthus annuus* were prepared in water, ethanol, clove oil, acetic acid and formalin. Stem tissues of *Zea mays* and *Helianthus annuus* were stained with extract of *Lawsonia inermis* leaves using water, ethanol and clove oil (Rozin, 1999).

d. Microscopy: Stained slides of *Zea mays* and *Helianthus annuus* were studied under simple light microscope (Olympus BX51) and their staining intensity were identified (Lux *et al.*, 2005).

Results and Discussion

Solution of dye prepared from crude extract of *Lawsonia inermis* Linn., leaves in clove oil, water and ethanol were found to stain the vascular tissue of stem in *Zea mays*. Water extract showed significant effect as compared to ethanol and clove oil extracts (Table 3). The color of the dye extracted from *Lawsonia* leaves with water was dark brown while that extracted with ethanol and clove oil were light brown. The dye extracted in water imparted grayish green color to the sclerenchyma and parenchyma stem tissue of cross section (Fig 1). A 10% (w/v) extract of dye from *Lawsonia* in water was found to be effective in staining dicotyledonous stem tissues. With in each vascular bundle, xylem cells were stained very effectively, while the cortex and medulla were stained less effectively. But the 10% (w/v) extract of dye in water was found to be more effective in staining sclerenchyma, but not as profusely as results of *Lawsonia inermis* in ethanol, while less effective in staining parenchyma of dicotyledonous (*Helianthus annuus* L.). The same extract of dye also showed slight effect on monocotyledonous stem tissues (Tables 1 and 2). The dye extract of *Lawsonia inermis* leaves in clove oil does not show their effect to stain the stem vascular tissues of angiosperm. The solubility of the dye in water, ethyl alcohol and clove oil was quite evident, however in clove oil solubility was not equally profuse.

A 10% (w/v) dye extract of *Lawsonia* leaves with ethyl alcohol imparted greenish color to the sclerenchyma and parenchyma of monocotyledons stem cross section (*Zea mays* L.) and produced a very interesting but connecting xylery rays among the stem vascular tissue and would be useful addition in new research studies (Fig. 2). Although, the staining effects were more prominent on the former tissue (sclerenchyma). A 10% (w/v) extract of *Lawsonia* dye in ethyl alcohol was found to be more effective in staining

sclerenchyma but less effective in parenchyma of monocotyledons (*Zea mays* L). However dye extract of *Lawsonia* in clove oil was not found to be effective for staining monocot stem tissue. In this investigation *Lawsonia* revealed meta-chromatic property of staining. A stain that dyes certain tissue and imparts different color other than the dye solution is a metachromatic stain.

Table 1. Staining effect of *Lawsonia inermis* L., leaves dye on stem tissues of *Zea mays* L.

<i>Lawsonia inermis</i> leaves extract	Tissue stained	Intensity of staining
I. In water		
1%	—	—
5%	Parenchyma	+
10%	Sclerenchyma	++
II. In ethanol		
1%	—	—
5%	Parenchyma	+
10%	Sclerenchyma	+++
III. In clove oil		
1%	—	—
5%	Parenchyma	Trace
10%	Sclerenchyma	Trace

Table 2. Staining effect of *Lawsonia inermis* L., leaves dye on stem tissues of *Helianthus annuus* L.

<i>Lawsonia innermis</i> leaves extract	Tissue stained	Intensity of staining
I. In water		
1%	—	—
5%	Parenchyma	+++
10%	Sclerenchyma	++++
II. In ethanol		
1%	—	—
5%	Parenchyma	+
10%	Sclerenchyma	++
III. In clove oil		
1%	—	—
5%	Parenchyma	Trace
10%	Sclerenchyma	Trace

Table 3. Variation in staining intensity of *Lawsonia inermis* L., leaves dye on *Zea mays* L., and *Helianthus annuus* L.

Dyes (stain)	Staining intensity (<i>Zea mays</i> , L.)		Staining intensity (<i>Helianthus annuus</i> , L.)	
	Parenchyma	Sclerenchyma	Parenchyma	Sclerenchyma
<i>Lawsonia</i> in water	+	++	+++	++++
<i>Lawsonia</i> in ethanol	+	+++	+	++
<i>Lawsonia</i> in clove oil	Trace	Trace	Trace	Trace

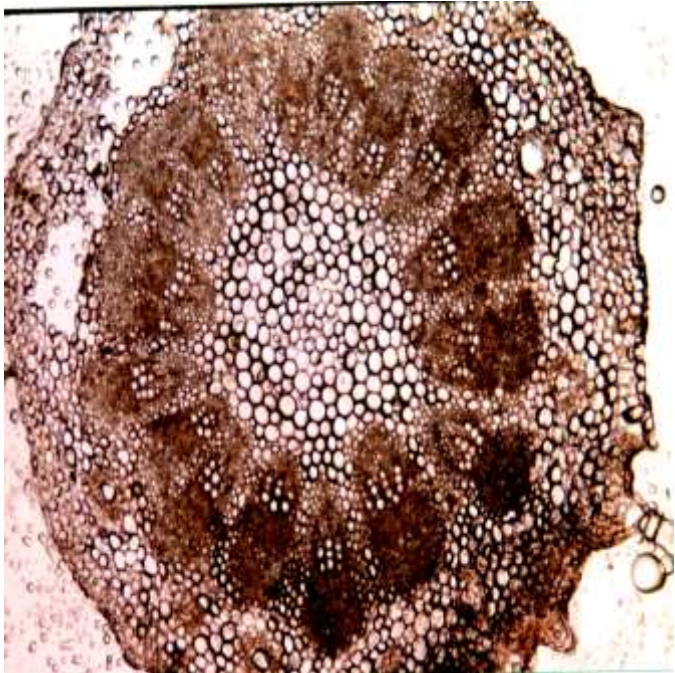


Fig. 1. Stem cross section of *Helianthus annuus* L., stain with extract of *Lawsonia inermis* L., in water.

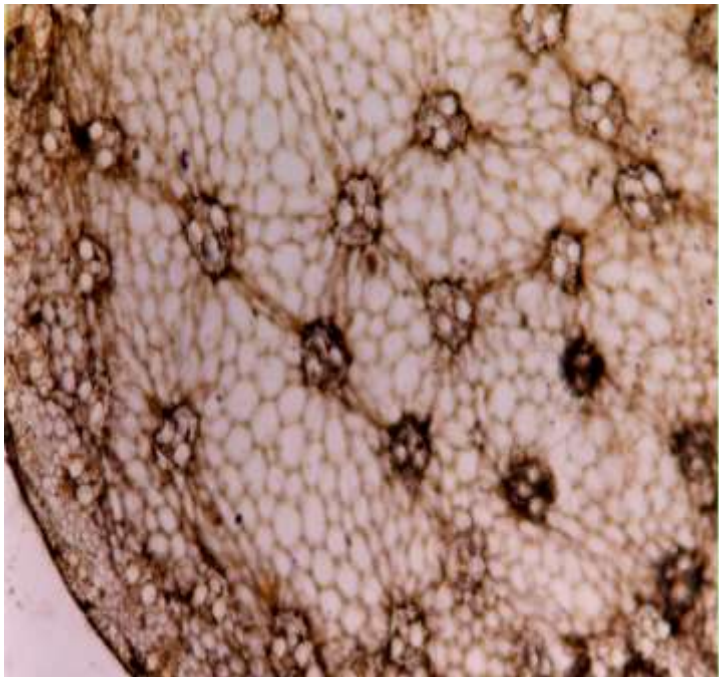


Fig. 2. Stem cross section of *Zea mays* L., stain with extract of *Lawsonia inermis* L., in ethyl alcohol.

The result analysis declared that the extract of dye from *Lawsonia* in ethyl alcohol and water could be used effectively to stain lignified plant tissues when employed in single staining. Avwioro *et al.*, (2005) has provided similar results for dye extracted from wood of *Pterocarpus osun* and Jan, (2004) for dye extracted from wood of *Berberis*, that can be used as an effective histological stain without addition of oxidants, mordants and accelerators, used for increasing the intensity of staining. Lux *et al.*, (2005) reported that berberin show effective staining for root tissue when dissolved in lactic acid. Cytoplasm of the cell is usually stained with Acidic stains, while the basic stains usually stain the nucleus of the cell (Baker & Silverton, 1976). From this observation it can be estimated that the dye extracted from leaves of *Lawsonia* is acidic in nature. Using advanced chromatographic techniques, further research are required to recognize the accurate nature of the brown dye extracted in water and ethyl alcohol from *Lawsonia* leaves, as majority of the natural dyes contains several impurities (Banerjee & Mukherjee, 1981). The recognition of dynamic ingredients of dye will open a new way of research in the field of dyeing. The dye extracted in water and ethyl alcohol could be tested for bacteria and fungi as histological stain. In addition, investigation of diverse solvents for dye extraction and their use as staining agent is also desired.

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