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INFLUENCE OF HERBAL DYE EXTRACTED FROM DRY WOOD OF INDIGENOUS *BERBERIS PACHYACANTHA* KOCHNE IN PLANT HISTOLOGICAL STAINING

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Abstract

The staining capacity of *Berberis pachyacantha* Kochne extract on histological sections of angiosperms stem was determined. A yellow pigment extracted from the powder of dried stem of *B. Pachyacantha* with different solvents. Staining dye was chemically extracted from the dry wood of *Berberis pachyacantha* Kochne in solvents like clove oil, ethanol, water, and its influence as histological staining agent for angiospermic plants was studied. The dye in 10% w/v clove oil was found more effective, showing high intensity to stain stem tissue of monocots (*Zea mays* L.) section, while the dye extracted from wood of *Berberis pachyacantha* Kochne in 10% w/v ethanol was found highly effective and showing high intensity to stain stem tissue of dicot using *Helianthus annuus* L. stem cross section. Staining power of the extracted dye of *Berberis pachyacantha* Kochne with respect of different stem tissue was actually variable according to the solvent used, but they proved to be weak staining agent for parenchyma of both monocot and dicot stem tissue. The recognition of dynamic ingredients of dye will open a new way of research in the field of dyeing. Current research work would also be helpful to explore better solvents for the means of dye extraction. In the dye extracts further research on the analysis of the active chemical substances, improvement of colour imparting, the extracts shelf life, attribute through the preamble of mordant into the dye extract is convenient. Present breakthrough will go a long way in minimizing over-dependence on toxic, as well as synthetic, expensive and non available exotic stains in the future.

Introduction

Many techniques and procedures are utilized to stain various plant tissues (Johansen, 1940 and Jensen, 1962). The genus *Berberis* belong to the family Berberidaceae and its various species are medicinally important plant, and most commonly used as purgative and also for the treatment of jaundice, chronic diarrhea, cholera and dysentery etc. Such information has been compiled and reported from Pakistan (Shinwari, 2010; Gilani *et al*., 2010). The bark of *Berberis* is bitter, astringent, and has been used with an advantage as a tonic. Moreover the bark of *Berberis* has been found of service as a wash in aphthous sore mouth, and in chronic ophthalmia (Harvey *et al.*, 1998). *Berberis* is a more commonly growing shrub, native to tropics of Asia, Indonesia sub continent, Nepal, Himachal Pardesh, Uttar Pradesh, and mountainous region of Pakistan. An important antibacterial alkaloid Berberine and Berbamine ($C_{18}H_{19}NO_3$) is present in the wood of *Berberis* (Kong *et al.,* 2004). Dissolving Berberine in a clearing agent lactic acid can be used as a staining material (Lux *et al*., 2005). Plant kingdom provides an unlimited number of natural dyes which can be extracted from various parts of the plant such as flowers, seeds, fruits, barks, roots and leaves. Plenty of plant species possessing dye-yielding properties are found in the sub-Himalayan region of northeastern India. Some people of the rural area traditionally, extract dyes from various plant species parts such as barks, roots, flowers or leaves by the process of scrapping, powdering, boiling and mixing with other materials to get the desired color. Onal *et al.,* (1999) have reported results on the extraction of color components from various dye consisting plant species.

There are two types of dyes. The dyes which can be obtained from natural sources are natural dye while those that can be produced synthetically through chemical reactions are synthetic dyes (Carleton *et al*., 1976). Some of the dyes necessitate the primary addition of, oxidants, accelerators mordants and pH adjustment. While majority of the dyes do not need these substances to stain tissues. For example, simple aqueous or ethanol solutions of the dyes can be used as staining agent and is known as simple stains (Avwioro, 2002). Most of the synthetic dyes are carcinogenic and produce allergy-like symptoms and are prohibited. Recently first choice for naturally derived colorants is due to their healthfulness and superb performance. Currently natural dyes due to no side effects, UV protection and anti-aging properties are frequently used in the cosmetic industry. (Chengaiah *et al*, 2010). Haematoxylin is an important commonly used natural dye which can be obtained from the wood of Mexican tree, *Haematoxylon campechianum* (Baker & Silverton, 1976). Several other sources of plant dyes rich in naphthoquinones such as lawsone from *Lawsonia inermis* L. (Henna), are reported to exhibit antibacterial and antifungal activity from Pakistan (Jan *et al*., 2011).

Though synthetic dyes are very proficient but their applications are restricted due to their harmful consequences to human health and other animals. This hazardous effect has resulted in the removal of some dyes as they have become known (Bhuyan & Saikia, 2004). World wide natural dyes have been used historically. But gradually the use of natural dyes has decreased considerably due to the introduction of synthetic dyes. Naturally occurring dyes obtained from plants are less expensive, is being used as a sustitute to synthetic dyes. (Sewekow,. 1988) find out that synthetic dyes, have carcinogenic effects on human health and other organism. According to (Padhy & Rathi, 1990) the worldwide use of eco-friendly and biodegradable materials, over the use of natural dyes has once again gained interest.

Aim of the present investigation was to assess the effectiveness of yellow dye extracted from the dry wood of *Berberis pachyacantha* Kochne for staining histological stem tissues of monocotyledonous and dicotyledonous plants. This work would be helpful to examine the efficiency of indigenous eco friendly and non toxic herbal stains for staining plant tissues with the aim of improvising for the expensive, toxic, and non available synthetic exotic stains used in plant histology. The present research work would also help and describes the detail information about the major pigments and their importance, found in naturally occurring dye yielding plants. Which would be helpful to further development of new way of research of preparing stains from the natural dyes.

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Material and Method

Plant material: Fresh *Berberis pachyacantha* Kochne Plants were collected from hills of district Mansehra Khyber Pakhtunkhwa Pakistan. Plant was identified with the help of Flora of Pakistan, (Jafri, 1975) and a specimen was properly mounted, preserved and tagged on standard size herbarium sheet after standard method of (Judd *et al*., 2002). Identified specimen was submitted vide voucher No. H-12O to the herbarium in Department of Botany, Govt. Post Graduate College Bannu, Khyber Pakhtunkhwa Pakistan for future reference.

Dye extraction: The plant of *Berberis pachyacantha* Kochne was dried in shade for two months at room temperature. The plant was grinded and powdered mechanically. Dye was extracted with 1%, 5% and 10% solution in three different solvents, ethanol (Ethyl alcohol), clove oil, and water.

Staining procedure and slide preparation: The extracted *Berberis pachyacantha* Kochne wood dyes in clove oil, ethanol, and water were tested on Microtome cut sections of *Zea mays* and *Helianthus annuus* (Rozin, 1999). The plant sections were stained in the extracted dyes for 3 to 5 minutes. The cut sections were washed in water for simple staining before handling in series of ethyl alcohol to remove water molecules and to remove excess stain. The dehydrated and differentiated sections were transferred into absolute xylole in two series to take away last traces of water, to clear the cut section for making it more transparent and to remove last traces of ethyle alcohol and since xylole is the solvent of the mountant (DPX) used. It removes cloudiness of the slide and makes slide to dry fast since xylole is a volatile solvent. Finally the sections were mounted on glass slide in DPX mountant.

Microscopy and photography: Observation of all the prepared slides were made and recorded microscopically. Stem cross section already stained slides of Zea mays and *Helianthus annuus* were studied and observed under simple light microscope (Olympus BX51) and their staining intensity were identified (Lux *et al*., 2005). Photomicrographs were taken from the stained slides through photomicroscope. Best results were obtained when microphotographs were taken with in a few hours of staining.

pH Determination: Digital BMF/ pH meter were used for determination of pH value of each dye.

Results and Discussion

Certain factors are responsible to determine the ability of a dye to stain specific tissue structures. Usually pH of the stain is one of the major factors among them. According to Avwioro, (2002), basic tissue structures would be stained by using acidic dyes while acidic structures would be stained by using basic dyes. With the selective use of various natural and synthetic stains cellular structures differentiation and contrast are enhanced. In some instances to display the presence of some tissue structures or cellular inclusions there may be need to apply a combination of these stains.

Solution of the dye prepared in solvents like clove oil, water and ethanol were found to stain the stem tissues like sclerenchyma and parenchyma of angiospermic representative *Zea mays* L. plant (Table 1). Although in clove oil, extract of *Berberis pachyacantha* Kochne showed significant effect for monocotyledonous stem tissue as compare to extracts prepared with Ethanol (Ethyl alcohol) as well as water (Table 2). The colour of the dye extracted with water was light yellow in colour while dye extracted with ethanol and clove oil were dark yellow in colour. The clove oil extract of dye imparted light yellow colour to the sclerenchyma and parenchyma stem tissue of cross section. But the 10% (w/v) extract of dye in clove oil was found to be more effective in staining sclerenchyma, while less effective in staining parenchyma of monocotyledon (*Zea mays* L.) Fig. 2. The same extract of dye does not show any effect on dicotyledonous stem tissues.

Table 1. Staining effect of *Berberis pachyacantha* Kochne wood dye on stem tissues of *Zea mays* L.

***Berberis* wood extract**	**Tissue stained**	**Intensity of staining**
I. In clove oil		
1%	-	-
5%	Parenchyma	+ +
10%	Sclerenchyma	+ + + +
II. In ethanol		
1%	-	-
5%	Parenchyma	+
10%	Sclerenchyma	+
III. In water		
1%	-	-
5%	Parenchyma	+
10%	Sclerenchyma	+ + +

Table 2. Staining effect of *Berberis pachyacantha* Kochne wood dye on stem tissues of *Helianthus annuus* L.

***Berberis* wood extract**	**Tissue stained**	**Intensity of staining**
I. In clove oil		
1%	-	-
5%	Parenchyma	Trace
10%	Sclerenchyma	Trace
II. In ethanol		
1%	-	-
5%	Parenchyma	+ + +
10%	Sclerenchyma	+ + + +
III. In water		
1%	-	-
5%	Parenchyma	+
10%	Sclerenchyma	+

The 10% (w/v) ethanol extract of dye imparted dark yellow colour to the sclerenchyma and parenchyma of dicotyledonous stem cross section (*Helianthus annuus* L.) Fig. 1. Although, the staining effects were more prominent on the prier tissue. The 10% (w/v) extract of dye in ethanol was found to be more effective in staining sclerenchyma but less effective in parenchyma of dicotyledon (*Helianthus annuus* L.).

Dye extract from *Berberis pachyacantha* Kochne in ethyl alcohol proved highly selective because of its excellent inherent affinity for fibre and other lignified cells. However, the same extract of dye in clove oil and water was not effective for staining dicot stem cross section. (Table 3). In respect of histochemical behaviour of *Berberis pachyacantha* Kochne dye extract in ethyle alcohol and clove oil did not show similar effects, because

both the dyes prepared in the said solvents imparted their colours indiscriminately on all cells but fibre, vessel members, other lignified cells and thick walled cells taken stain more genuinely. Curcumin and other related curcuminoids are reported to be responsible for yellow colour of the dye (Jan *et al*., 2011).

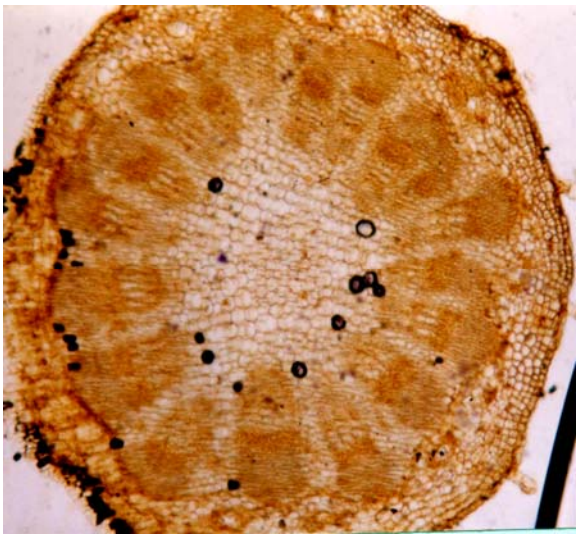

Fig. 1. Stem cross section of *Helianthus annuus* L., stain with 10% w/v Extract of *Berberis pachyacantha* Kochne.in ethanol.

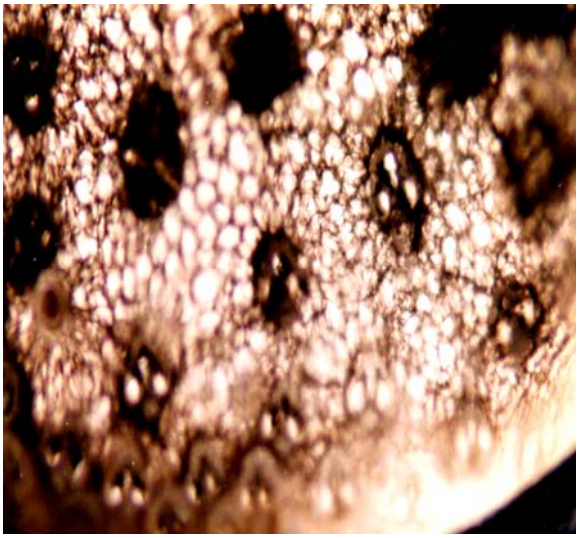

Fig. 2. Stem cross section of *Zea mays* L., stain with 10% w/v Extract of *Berberis pachyacantha* Kochne.in clove oil.

Table 3. Variation in staining intensity of *Berberis pachyacantha* Kochne wood dye on *Zea mays* L. and *Helianthus annuus* L.

Dyes (Stain)	Monocotyledonous tissue (*Zea mays* L.)		Dicotyledonous tissue *(Helianthus annuus* L.)	
	Parenchyma	Sclerenchyma	Parenchyma	Sclerenchyma
Berberis in clove oil	+ +	+ + + +	Trace	Trace
Berberis in ethanol	+	+	+ + +	+ + + +
Berberis in water	+	+ + +	+	+

The declared that the dye extract of *Berberis pachyacantha* Kochne in ethanol could be used effectively to stain lignified plant tissues when used in single staining. (Avwioro *et al.*, 2005) has provided similar results for dye extracted from wood of *Pterocarpus osun* 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) compiled and reported that alkaloid berberin found in *Berberis* plant material declare effective staining for root tissue when dissolved in lactic acid.

Acidic stains usually stain cytoplasm of the cell, while the basic stains usually stain the nucleus (Baker & Silverton, 1985). In observation of this, it can be estimated that the dye extracted from wood of *Berberis* is acidic in nature. Using advanced chromatographic techniques, further research are required to recognize the accurate nature of the yellow dye extracted in clove oil and ethanol from *Berberis* wood as majority of the natural dyes contains several impurities (Banerjee & Mukherjee, 1981).

(Singh *et al.*, 2005) studied the antimicrobial activity of some natural dyes. The recognition of dynamic ingredients of dye will open a new way of research in the field of dyeing. Even though the dyes extracted in clove oil and ethanol 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. Berberis contains an antimicrobial alkaloid berberin and is effective in staining plant tissues as staining agent (Kong *et al.*, 2004). Present research work has recognized the fact that herbal stain from *Berberis pachyacantha* Kochne could be successfully utilized for plant histological evaluation. Further more the results also revealed that a high performance of *Berberis* dye extracts is obtainable with the utilization of ethyle alcohol as a solvent for their extraction. In detecting presence or absence of cell inclusions there is also a need to explore the potential of the dye extracts.

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