



Original Research Article

Comparative Staining Efficacy of Safranin and *Lawsonia inermis* L. Aqueous Ethanolic Leaf Extract on Epidermal Cells of *Allium cepa* L.

C.U. Aguoru*, F.O. Okpe and J.O. Olasan

Department of Biological Sciences, University of Agriculture, Makurdi, Nigeria

*Corresponding author.

Abstract	Keywords
<p><i>Lawsonia inermis</i> L. plant, popularly known for its dye properties was investigated as a source of dye for cytological studies using onion bulb epidermal cells. This study evaluated the efficacy of the ethanolic leaf extract of <i>L. inermis</i> stain for elucidation of cellular features using onion epidermal cells in comparison with safranin, an imported stain for cellular studies in Nigeria. Maceration method was used in the extraction of the dye. 100 fresh leaves of <i>L. inermis</i> were harvested from the plants from various parts of Benue State, Nigeria and prepared using ethanolic extraction. Twenty different slides of stained epidermal cells of onion prepared with <i>L. inermis</i> stain as well as slides of same using safranin and 20 slides without any form of stain as control were prepared. These were observed under the microscope and photomicrographs of cellular features revealed by each stain were made. The epidermal cellular features were more elucidated under the microscope using <i>L. inermis</i> extract when compared to the features revealed by staining with safranin. This study reveals and suggests that <i>L. inermis</i> ethanolic leaf extract could be a local source of cheap stain for cellular studies and anatomical studies and eliminate importation of other stains applied in such studies. It could also be a source of earning by engagement in its farming and industrial production of stain from <i>L. inermis</i>. It shall also eliminate over dependence on safranin and other stains applied in such studies.</p>	<p><i>Lawsonia inermis</i> extract Onion epidermal cells Safranin Staining dyes</p>

Introduction

Lawsonia inermis L. is a perennial shrub native to tropical and sub-tropical regions of Africa, Southern Asia, Northern Australia and semi-arid zones. The plant has been introduced to other parts of the world as exotic plants with high invasiveness. The common

names are 'henna', 'mignonette' and 'Egyptian privet' in English. In Nigeria, it is called 'Laali' in Yoruba and 'Leeli' in Hausa. It is an angiosperm and belongs to the family Lythraceae. It is a tall shrub of 2-6m that grows at a temperature higher than 11⁰C and it grows

better in arid regions producing leaves, flowers, fruits and seeds (Bardi and Burkinshaw, 1993). *L. inermis* is a much-branched glabrous shrub with tipped branchlets in most cases. Young branches are quadrangular green but turn red with age. The leaves are opposite in arrangement, entire in margin, elliptic to broad in shape with net venation on the depressed dorsal surface. The leaf contains the active colouring agent called lawsone. The morphology of the flower shows small size, white colour, four sepals and four petals. The fruits are small (4-8mm in diameter) and brown in colour with 32-49 seeds per fruit. The plant is dioecious and anemophilous (Kumari et al., 2011).

It is propagated by seed or stem cuttings. The plant grows on any type of soil, from loam to clay loam. It tolerates a slight alkalinity in the soil. Propagation by seed is done by soaking the seeds in water for 8-10 days to allow for imbibitions and proper germination when planted (Orwa et al., 2009). *L. inermis* grows mainly along waterways and in semi-arid regions and it is physiologically adapted to a wide range of environmental conditions such as low humidity and drought conditions and this is because it requires high temperature to grow maximally (Orwa et al., 2009). Several studies had already been conducted on the plant to elucidate its medicinal values such as in the treatment of ulcer, anaemia, diabetes and jaundices (Reddy, 1988). However, studies on the staining efficacy of the plant are limited.

In Nigeria, most especially in the Northern parts, *L. inermis* is commonly used for aesthetic purposes to beautify the human body especially among the women. The dye obtained from the plant may be fixed on finger nails, faces, palms, hands, legs, stomach and any other part of the body. In extreme cases, it is part and parcel of the culture of the people to apply the dye on body parts as symbols of identity (Chengaiyah and Rao, 2006). Microscopic preparations of biological specimens involve: fixation, dehydration, clearing, embedding, sectioning, staining and mounting (Taylor et al., 2007). Most biological structures are transparent, hence there is need to employ means of obtaining contrast between cellular structures. Therefore, staining of specimen using dye(s) is an important stage in cytological and histological studies as a method of revealing and differentiating cellular components as they respond differentially to certain stains. For instance, eosin dye stains the cytoplasm pink red while Feulgen's stain gives red colour to the

chromosome. Leishman's stain gives red-pink colour to blood cells while safranin stains nuclei red (Taylor et al., 2007). Dyes used on plant cells include but not limited to aniline blue (for fungal hyphae), fast green (for cellulose) and phloroglucinol (for lignin). Animal stains include borax carmine, haematoxylin and Leishman's stain among other synthetic chemical dyes. Some dyes such as fast green and Feulgen's stain can be applied on both plant and animal cells to view the cytoplasm and chromosome respectively (Taylor et al., 2007).

Most synthetic stains are expensive as they are synthesized from mixtures of various chemicals, there is need to explore other cheaper and natural sources of obtaining dyes used as biological stains. For this purpose, plant sources such as *L. inermis* may be explored since they contain dye called lawsone used among women to enhance their beauty (Chengaiyah and Rao, 2006). On this note, a detailed cytological study was carried out on onion epidermal cells as model plant cells in cell biology and cytogenetics using the dye extracted from *L. inermis* plant. The use of onion (*Allium cepa*) epidermal cells is normal in biological research. Aguoru and Okoli (2004) attempted the use of ethanolic extract of leaves of *L. inermis* as a chromosome stain.

This study therefore aimed at the use of *L. inermis* aqueous extract for the staining of plant epidermal cells to investigate its ability to elucidate the epidermal cellular components vis-à-vis the well-established safranin stains which are always imported.

Materials and methods

Plant collection

One hundred (100) fresh leaves of *L. inermis* were collected from thirty plants from various parts of Benue State, Nigeria. Plant identification was done by experts in the department of Biological Sciences, University of Agriculture Makurdi, Nigeria.

Plant material extraction

The leaves were sun dried for one week. The dried leaves were broken into pieces by pounding with pestle in a mortar. 50g of the dried leaves was weighed out using a digital scale. Extraction of stain was by maceration method. Cold extraction using ethanol was

carried out by soaking the 50g leaf material in 500ml of ethanol for 48 hours in 500ml beaker with stopper. After 48 hours, filtration was carried out to obtain the filtrate. The filtrate was exposed to allow the ethanol to evaporate to obtain dry mass of *L. inermis* stain. The dye extract was obtained following the methods of Sastyajit et al. (2005) and Samuelsson (2004).

Staining the onion epidermal cells

The following materials were used in staining onion epidermal cell Glycerine, Safranin solution (red colour), *L. inermis* dye extract (brown), Forceps, Needle and brush, Cover slip, Blotting paper, Onion, Glass slide and Watch glass.

Onion epidermal peels were transferred into safranin solutions using forceps and allowed to stand for 5 minutes. A drop of glycerine was poured at the centre of a dry glass slide. The epidermal peels were transferred onto glass slides containing glycerine and gently covered with the cover slips. Twenty different slides of stained epidermal cells of onion prepared with *L. inermis* stain as well as slides of same using safranin and 20 slides without any form of stain as control were prepared. These were observed under the microscope and photomicrographs of cellular features revealed by each stain were made. The images were compared and recorded. Magnification was achieved by multiplying the ocular lens ($\times 10$) with the objective lenses used ($\times 4$, $\times 10$ and $\times 40$).

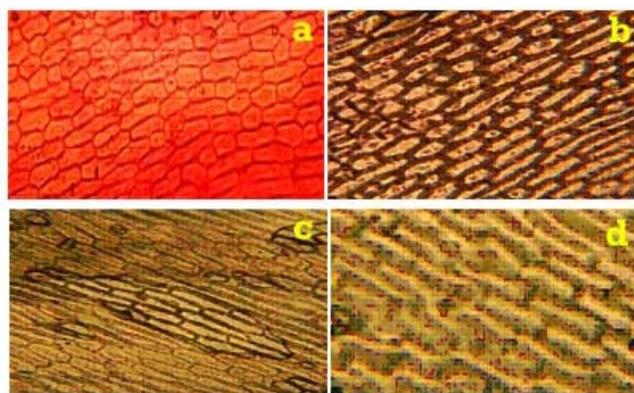
Results and discussion

Safranin stain produced red images while those obtained from *L. inermis* dye were brown in color. Selected micrographs obtained from the slides are presented in Figs. 1(a) to 1(d). Fig. 1(a) shows the photomicrograph of the onion epidermal cells using safranin stain while those of onion epidermal cells using *L. inermis* stain are displayed in Figs. 1(b), 1(c) and 1(d).

The use of stain to improve contrast in cellular structures has been substantiated in this work. Figs. 1(b) to 1(d) clearly confirm the efficacy of *L. inermis* dye in staining onion cells and even gave better contrasts when compared with safranin staining technique. The use of onion as a typical representative of plant cell is a common practice in biological experiments especially in cytological, cytogenetic and

toxicological studies (Muhammed and Muhammed, 2005). Therefore, effective staining technique, as tested and confirmed using *L. inermis* dye in this report, is required for detailed revelation of plant cellular components (Taylor et al., 2007). For example, Fig. 1(b) has revealed the cellular arrangements and interconnectivity of the onion epidermal cells joined together by the cell walls to form a tissue of connected cells. This is similar to the pattern of cell arrangements in other plant tissues such the parenchyma and collenchyma tissues (Taylor et al., 2007). This result thus suggests the efficacy of *L. inermis* dye in staining and elucidating the basic structural outline of the cell.

Fig. 1: Staining of onion epidermal cells using (a) safranin stain as control ($\times 400$) and *L. inermis* extracts at (b) $\times 400$, (c) $\times 100$ and (d) $\times 40$.



Khargbude and Agarwal (2000) stated that a good biological stain must be effective, cheap, and less toxic and the source must be available. Many chemists have also argued that natural dyes are more environmentally friendly, less toxic and cheaper than synthetically produced chemical dyes (Khargbude and Agarwal, 2000). All the above criteria are fully met by *L. inermis* stain being cheap, natural, environmentally friendly and generally available as the plant source is common thereby suggesting the use of this plant as a potential source of biological stain.

Most stains are specific in action which could either be used on plant cells or on animal cells (Taylor et al., 2007). It should be noted that stains that can be applied on both plant and animal cells are more commonly used in routine biological experimental work being more versatile than plant or animal specific stain (Taylor et al., 2007). All micrographs

obtained in this work have clearly proven the potential use of *L. inermis* leaf as a source of obtaining cheap biological stains that may offer huge contribution to our knowledge of cytology, cytogenetics, histology and anatomy [Figs. 1(b), 1(c) and 1(d)]. This suggests the efficacy of the *L. inermis* dye in staining plant cell type and it also confirms the use of onion bulb as a model plant specimen in biological research (Aguoru and Okoli, 2004; Muhammed and Muhammed, 2005). This study reveals and suggests that *L. inermis* ethanolic leaf extract could be a local source of cheap stain for cellular studies and anatomical studies and eliminate importation of other stains applied in such studies. It could also be a source of earning by engagement in its farming and industrial production of stain from *L. inermis*. It shall also eliminate over dependence on safranin and other stains applied in such studies.

In conclusion, *L. inermis* stain is natural, environmentally friendly and effective with ease of extraction from cheap plant source. This may be commercially produced in large quantity to be used as routine biological stain to replace the costly and imported synthetic dyes in the nearest future. However, it is strongly recommended more elaborate studies should be carried out to fully establish the efficacy of the dye on diverse types of organelles, cells and tissues in plant and animal.

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