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Value of *Hibiscus Rosa-Sinensis* Petal Extract as Biological Stain in Plant Systematic Research

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Abstract: The significance of *Hibiscus rosa-sinensis* petal extract as stains in plant systematic research was investigated. This was borne out of the desire to obtain cheap, available stains from effective and safe bio-sources to replace hazardous synthetic chemicals employed as stains in plant systematic research. 100g of petals obtained from the plant was homogenized using a mortar and pestle. Extraction was done using methanol and ethyl acetate in a sonicator for 1 hour. The extract was filtered using whatman filter paper and concentrated at 40^{o}C using a thermostatic water bath. The efficacy of this dye was tested on eighty (80) foliar epidermal peel slides prepared from two plant specimens (*Ixora parviflora* and *Allium cepa*). Eighty (80) epidermal peel slides were also prepared and stained with safranin. All slides were viewed under the compound light microscope. Images were captured using digital camera under x40 objective lens. Results from the two sources of stain were compared. *Hibiscus* stain revealed better contrasting features of stomata, guard cells and epidermal cells than safranin. Epidermal cell walls and different shapes of the epidermal cells were more vividly elucidated on the abaxial and adaxial surfaces of *I. parviflora*. The different types of stomata present in all preparations under *Hibiscus* stain were more conspicuously seen without muddle. All images emanating from this stain were excellent and appealing with consistent brownish effect. *I. parviflora* specimens stained with safranin on the other hand yielded less contrasting anatomical features. Images were less appealing and generally inconsistent with reddish effect. Independent staining effect on *Allium cepa* also showcased the superiority of *Hibiscus* stain over safranin by producing sharper images with more enhanced contrasts. The ability of *Hibiscus* stain to vividly reveal cell wall demarcations, guard cells, stomata and general outline of the epidermal cells has

suggested its significance in plant anatomical research. The stain is effective, user friendly, non-hazardous, easily prepared, cheaply available, generally accessible and environmentally friendly. This cheaper biological source of stain may reduce importation of stain into the country and help save time and money.

Keywords: *Hibiscus rosa-sinensis,* Extract, Safranin, Biological stain, Plant anatomy, Systematics.

INTRODUCTION

H. rosa-sinensis of the family Malvaceae is a bushy, evergreen shrub or small tree growing 2.5-5m (8-16ft) tall and 1.5-3m (5-10ft) wide, with glossy leaves and solitary, brilliant red flowers[1, 2]. The flower is usually dark red in colour and non- fragrant. Inflorescence is solitary as in cymes, but sometimes they are in panicle raceme, regular polypetalous, bisexual, hypogenous, conspicuously mucilaginous with a whorl of bracteoles known as epicalyx. It is a perennial plant in the tropical and sun-tropical region. Its members may be herbs shrubs, or trees with mucilage. It possesses properties desirable of breeding practices.

These include: vigour, attractive foliage, and strong root system, ease of maintenance, adaptability, excellent flowering and attractive ornamentation. Copious medicinal and industrial uses of this plant have been reported in different parts of the world [3-5, 21-22]. The flower is used in epilepsy, leprosy, bronchial catarrh and diabetes [2]. The flower also possesses anti spermatogenic, anti tumour and anticonvulsant activities [6]. Flower extract has been reported to be anti-diabetic and antiglycemic [7]. There are numerous reports on the internet concerning the use anthocyanin pigment of *Hibiscus* species as natural dye employed in textile industries (www.onlinelibrary.wiley.com)[21-22.] *Hibiscus* species and henna (*Lawsonia*) dyes are also used in coloring hair for beautification [8].

Staining is an auxiliary technique used in cytology, cytogenetics, histology, systematics and anatomy to enhance contrast of the microscopic image [9-11]. The use of dyes as stain is employed in this regard. By causing certain cells or structures to take on contrasting colour (s), their form (morphology) or position within a cell or tissue can be easily observed and studied [8, 11]. Therefore, main purpose of staining is to reveal cytological details that might otherwise not be apparent. Stains may be applied singly or as combined dyes on specimens (counterstaining). Most stains used in biological research are purely synthetic as products of chemical combinations. Some synthetic stains are toxic to living organisms, hence are not used as vital stains.

Many stains are expensive, rigorous to prepare, genotoxic, mutagenic and unfriendly to users [12]. Inimical effects of chemicals generally on the environment are core parts of research in environmental studies today, most especially the implication on loss of biodiversity [13, 14]. Laboratories in Nigeria are often in short supply of stains due to high dependence on importation of laboratory materials including reagents. According to Aguoru *et al* [12], a good biological stain must be effective, cheap, readily available, user friendly, environmentally acceptable and easily prepared from accessible sources. Also, the dye must not be toxic to the user or to the laboratory specimen. It must also be locally produced in large quantity for quick accessibility to students and researchers. Therefore, it becomes necessary to divert attention from chemically imported sources of stain to cheaper biological sources, yet efficacious.

The idea of potential uses of pure plant extract as stains in cytological work was first reported by Aguoru *et al* [12] in Nigeria. In their findings, ethanolic leaf extract of *Lawsonia inermis* was more efficacious in staining onion epidermal cells than the chemically made safranin stain. In the same vein, the idea of potential uses of the *H. rosa-sinensis* floral extract as stain in microscopy was borne out of curious observation of the brilliant red flower of the plant. This is because they have been reported to be used as dyes in colouring fabrics. This innovative and maiden research was aimed at confirming the staining efficacy of *H. rosa-sinensis* extract (biological source) on anatomical specimens prepared from two different plants (*Ixora parviflora* and *Allium cepa*). The results would be compared with staining effect of safranin as chemical source.

MATERIALS AND METHODS

Fresh red flowers of *Hibiscus rosa-sinensis* were collected from the Horticulture garden close to Commissioner's Village in Makurdi, Benue State, Nigeria. The material was gently placed in sterile polythene bag and transported to the Biological Laboratory of the Federal University of Agriculture, Makurdi, Nigeria. The petals were detached and rinsed with distilled water in readiness for extraction. 100g of the sample was homogenized using a mortar and pestle. Extraction was done using methanol and ethyl acetate in a sonicator for 1 hour. The extract was filtered using whatman filter paper and concentrated at 40^{o}C using a thermostatic water bath. Extract was transferred to a sterile bottle and stored at room temperature. Forty (40) foliar anatomical slides were prepared from *Ixora parviflora* on the abaxial and adaxial surfaces (20 each) following the methods of Aguoru *et al* [12, 15, 16]. Another forty (40) epidermal peels were also prepared from *Allium cepa*.

In total, eighty (80) slides were stained with *H.rosa-sinensis* stain. The above steps were repeated where another set of eighty (80) slides of *Ixora parviflora* and *Allium cepa* were stained with safranin. All slides were viewed on the compound light microscope using low and high power objective lenses. Images were captured using digital camera (with microchip) under x40 objective lens. All photomicrographs were transferred to a computer system for critical analysis. Results from the two sources of stain were compared. Significance of the *H. rosa-sinensis* dye as biological stain was ascertained for its efficacy.

RESULTS AND DISCUSSION

Photomicrographs of *Hibiscus*-stained specimens are shown in **Plates 1a-5a**. Safranin stained specimens are shown in **Plates 1b-5b**. Foliar anatomical preparations from *Ixora parviflora* where the two dye types were independently employed are given in **Plates 1-3** irrespective of the alphabetical notation. Results revealed that *Hibiscus* stain gave better contrasting features of stomata, guard cells and epidermal cells (**Plates 1a, 2a and 3a**). Epidermal cell walls and different shapes of the epidermal cells were more vividly elucidated on the abaxial and adaxial surfaces of the leaf. The different types of stomata present in all preparations under *Hibiscus* stain were conspicuously seen without muddle. All images emanating from *Hibiscus* stain were generally appealing, consistent and excellent. Consistency was maintained with uniformity in brownish staining effect. *I. parviflora* specimens stained with safranin on the other hand yielded less contrasting anatomical features (**Plates 1b, 2b and 3b**). Images of this dye were less appealing and generally inconsistent in revealing all features. Inconsistent staining of safranin yielded reddish effects that were darker at some points than other points, but not due to contrasting effects.

Onion epidermal peels independently stained with *Hibiscus* dye and safranin are shown in **Plates 4 and 5**. Here again, *Hibiscus* stain produced sharper images with more enhanced contrasts than safranin stain (**Plates 4a and 4b**). Cellular components such cell wall, middle lamella and cytoplasmic granules were vividly revealed within the resolution limit of light microscopy as a whole. The intercellular connectivity forming the overall tissues was better elucidated using *Hibiscus* stain than safranin. Moreover, the nucleus could be seen pushed to one side of each adjacent cell wall close to the centrally placed vacuole (Plate 4a).

From the results, it is glaring that *Hibiscus* stain produced quality images with consistent staining effects that revealed appealing and contrasting features of the anatomical preparations from the two plants studied. It thus appears that consistency in staining may be due to the easy flow and diffusibility of the stain across the specimen in a uniform manner. It is also possible that the stain contains both hydrophilic and hydrophobic regions interacting with all cellular components to have yielded consistent staining. This property alone makes it an excellent stain. Secondly, the stain is non-toxic neither to the user nor to the specimen. Hence it could be applied as vital stain to study living specimens [11] and this makes it applicable in bacteriology, phycology and mycology. The ability to vividly reveal cell wall components, guard cells, stomata and general outline of the epidermal cells has suggested its potential significance in plant anatomy. This finds application in microtaxonomic work where anatomic evidences from leaf, root and stem play pivotal roles in taxa description, circumscription, delimitation and determination of phylogenetic relationships among organisms [17, 18].

Aguoru *et al* [12] enumerated basic properties of an excellent stain in the report on staining efficacy of *Lawsonia inermis* dyes on onion epidermal structure. These qualities include: effectiveness, non-toxicity, user-friendliness, ease of preparation, availability, accessibility, cost effectiveness, environmental friendliness and mass production. All these characteristics are embedded in the dyes extracted from *Hibiscus rosa-sinensis* in the present study. The stain originates from plant source (*Hibiscus*) that can be cultivated and produced in large quantity. This makes the stain relatively cheap, available and accessible.

The dye is easily extracted from the petal. Hence, the rigorous protocols of combining and mixing chemicals to synthesize hazardous dyes are eliminated [19]. It would also reduce importation of stain into the country as well as the associated costs. Most synthetic stains such as safranin and methylene blue contain hazardous methylated groups that can induce mutation and alter the reading frame of the genetic code [19, 20]. Safranin, as a potential mutagen, is an azonium group of 2, 8 dimethyl-3, 7 diamino phenazine [19, 20]. The use of biological sources as stain may reduce exposure to these chemicals.

From this report, the relevance of *Hibiscus rosa-sinensis* as a potential source of biological stain in plant anatomy and general plant biology is solidly confirmed. Therefore, it is ideal for routine light microscopic research under safe laboratory practices in schools and colleges. University students and researchers may take advantage of this cheap source of staining plant materials. More research may be undertaken to further confirm the efficacy of this stain on chromosome, nucleic acid and animal tissue. This would boost research work in cytogenetics, molecular biology and histology respectively if confirmed potent in these fields.

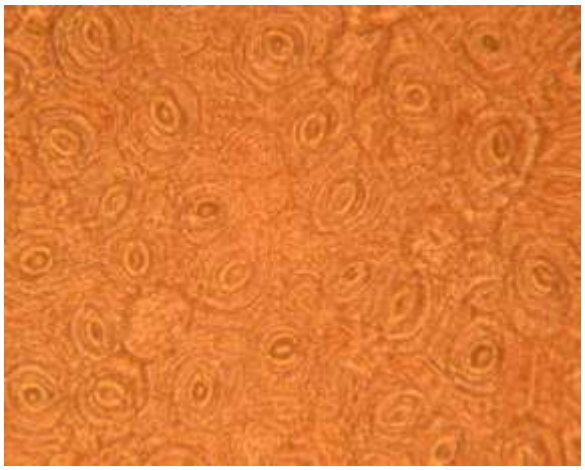

Plate 1a: *I.paviflora* (abaxial) stained with *Hibiscus*

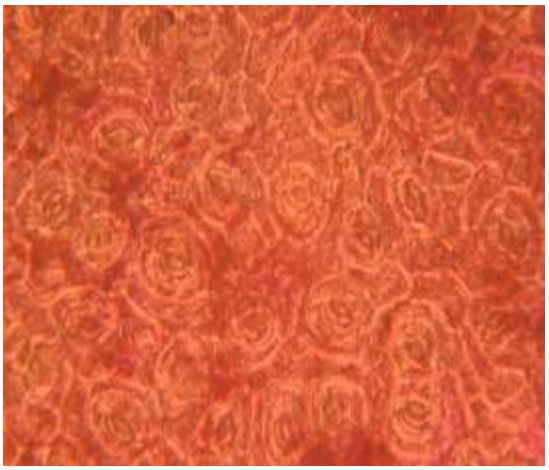

Plate 1b: *I.paviflora* (abaxial) stained with safranin

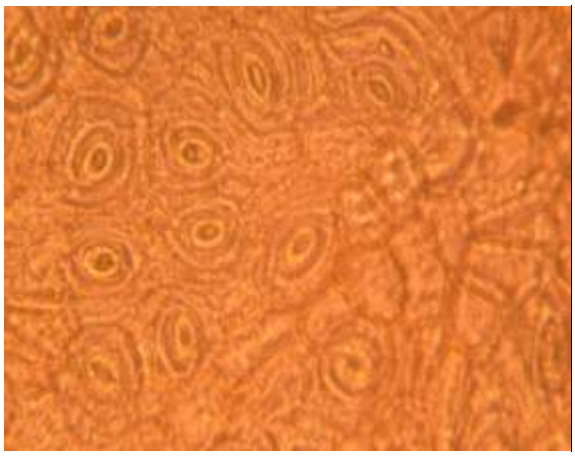

Plate 2a:*I.paviflora* (adaxial) stained with *Hibiscus*

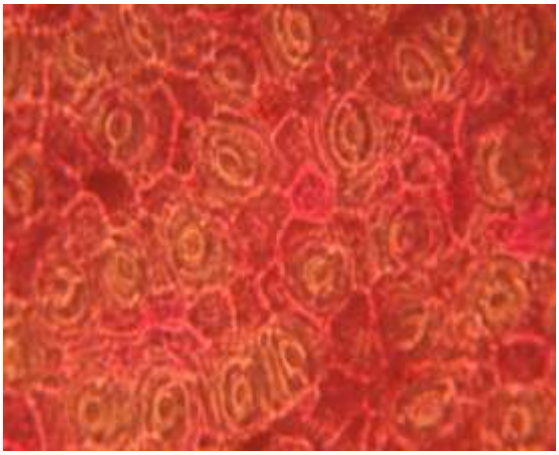

Plate 2b: *I.paviflora* (adaxial) stained with safaranin

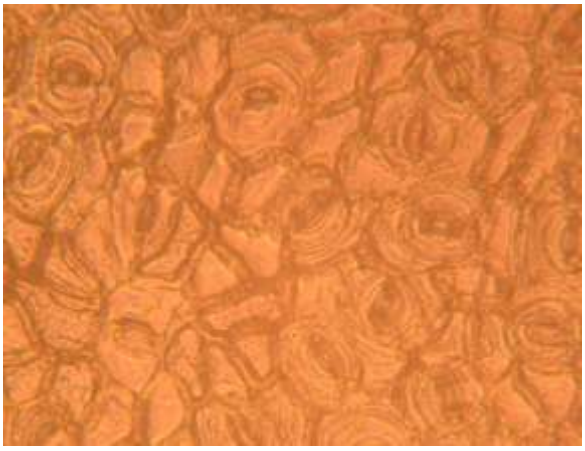

Plate 3a: *I.paviflora* (adaxial) stained with *Hibiscus*

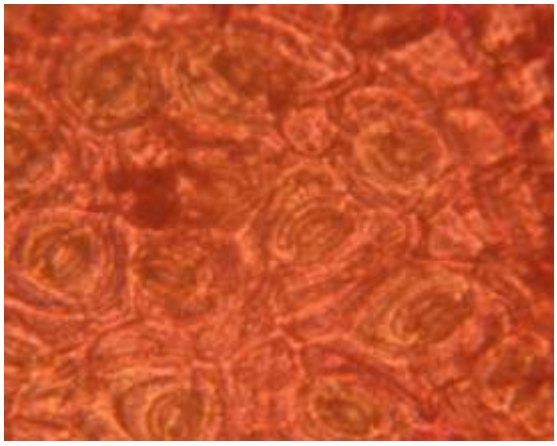

Plate 3b: *I.paviflora* (adaxial) stained with safranin

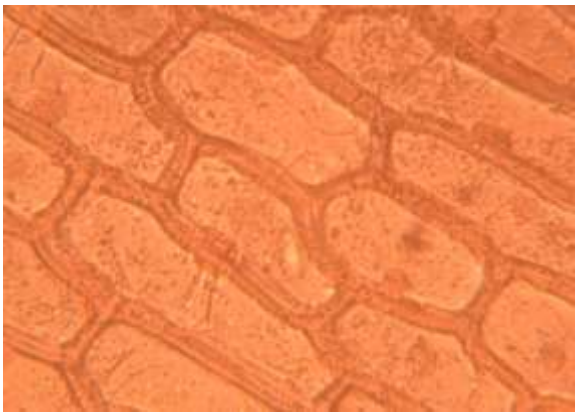

Plate 4a: *A.cepa* epidermis stained with *Hibiscus*

Plate 4b: *A.cepa* epidermis stained with safranin

Plate 5a: *A. cepa* epidermis stained with *Hibiscus*

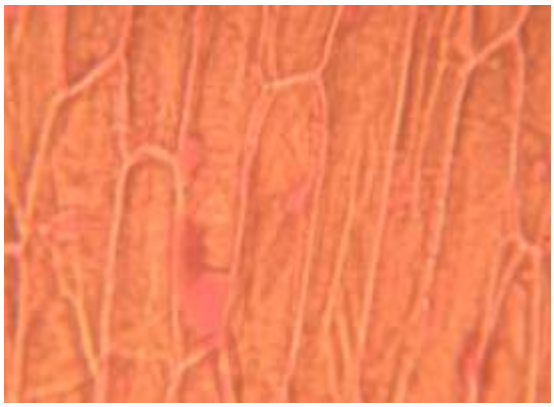

Plate 5b: *A. cepa* epidermis stained with safranin

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