



Original Research

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EVALUATION OF THE STAINING POTENTIAL OF AQUEOUS AND ETHANOLIC LEAF EXTRACT OF *Celosia argentea* ON LIVER, KIDNEY AND COLON TISSUES OF WISTAR RAT

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ABSTRACT

Background: Histological diagnosis relies heavily on synthetic dyes like Eosin, which are often expensive, hazardous to health, and environmentally non-degradable. There is a growing scientific interest in exploring eco-friendly, plant-based alternatives for routine tissue staining. *Celosia argentea* belongs to family Amaranthaceae, this plant is use for traditional purposes also it is known for its brilliant colour. It is an erect plant, mostly with much branches with annual growing up to 40-200 cm tall.

Aim: To evaluate the staining potential of aqueous and ethanolic leaf extract of *Celosia argentea* on liver, kidney and colon tissues of Wistar rat.

Materials and Methods: Physical and phytochemical analysis of the plant were determined. Fresh leaves were dried, pulverized, and extracted via maceration. Aqueous and ethanolic extracts were prepared, 1% and 2% were prepared from the extract. Liver, kidney and colon tissues of Wistar rats were harvested and processed. The tissues were stained using H and E as control, some were stained using 1%, 2% of the extracts with varying time of 5mins, 10mins for each tissue.

Results: The physical analysis of aqueous extract demonstrated a dried texture while ethanolic extraction demonstrated a gummy texture. Phytochemicals revealed the presence of carbohydrate, protein, alkaloid, flavonoids, cardiac glycoside, saponin, phenol, steroid and tannins in aqueous and ethanolic extract. Histological results revealed the aqueous extract demonstrated a good staining intensity compared to ethanolic extract, which exhibited poor staining. 2% aqueous extract stained for 10mins produced the good staining reaction compared to 5mins, effectively staining the cytoplasm of the three organs.

Conclusion: The results show that aqueous extract *Celosia argentea* at 2% concentration stained tissues at 10 minutes duration will give a good contrast of cytoplasmic staining of liver, kidney and colon tissues. Also, this study concluded that *Celosia argentea* might be a promising natural staining dye that can replace eosin as synthetic dye.

Keywords: *Celosia argentea*, Aqueous extract, Ethanolic extract, Liver, Kidney, Colon

INTRODUCTION

Staining is the key technique in pathology and histology that help to promote a good visualization and differentiating of cellular and tissue constituents under microscope. Staining plays a vital role in determining abnormalities at cellular level, as well it helps to distinguish microscopic structures and diagnosing different pathological conditions. Cells and tissues appeared transparent when viewed under light microscope because of their high-water content as well as uniform refractive indices, at the end differentiation is not possible, but with the advent of staining cells and tissues can easily be viewed and differentiated [1]. Histological staining is the application of chemicals or dyes that attaches exactly on to tissues or cellular structures, imparting colors and contrast. The

contrast helps in differentiating as well as identifying tissue and cellular morphology, classifying tissue and cellular types, identifying tissue and cellular abnormalities. Staining in histology is classified into general tissue staining, special staining and immunohistochemical staining. The most widely used general staining is Haematoxylin and Eosin (H&E) staining, which gives clear differentiation between cytoplasm and nucleus [2].

The staining of tissue is based on the principle of chemical reaction between the dye used and the tissue components. The anionic (Acidic dyes) attracts and stain the basic structures of the tissue such as cytoplasm of the cell, while cationic (Basic dyes) attaches and stain the acidic structures of the tissue such as nucleus of the cell [3]. In histological staining apart from general tissue staining, there is special staining that employed staining tissue in order to identify some specific tissue constituents such as carbohydrate, lipids, connective tissue, microorganisms and many more [4]. *Celosia argentea* is a plant generally known in Nigeria as “Soko yokoto” or “Silver Cockscomb”, this plant is herbaceous tropical plant mostly grown within Africa, Asia and some parts of South America. This plant belongs to the family of Amaranthaceae, extensively planted because of its nutritional, ornamental as well as its medicinal important [5]. Traditionally in Nigeria the leaves are consumed as vegetable, and also used as traditional medicine for the remedy of different disease conditions such as inflammatory conditions, diarrhea and wound healing [6]. The most popular and routine staining technique in histopathology is hematoxylin and eosin staining method. It plays a major role in identifying diseases in tissues and cells, hematoxylin is natural while eosin is synthetic. These dyes are imported to not produced locally in Nigeria. There is need to search for natural dye which is safe, less expensive to avoid biological hazards of using synthetic dyes. Because of the potential threat to hazardous and environmental pollutions from synthetic dyes that originated from chemicals, there is need to explore alternatives from the natural product such as plants to produce natural dyes that can replace the synthetic dyes.

MATERIAL AND METHOS

STUDY LOCATION

Extraction of *Celosia argentea* was conducted at the laboratory of Histopathology Department, School of Medical Laboratory Sciences, Usmanu Danfodiyo University Sokoto. Animal sacrifice and dissection was carried out at the Animal house of Pharmaceutical Sciences. Tissue processing and staining was conducted at the Service Laboratory Histopathology Department, Usmanu Danfodiyo University Teaching Hospital Sokoto.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethics and Research Committee, Usmanu Danfodiyo University, Sokoto. With ethical approval number Ref/ID No: HBREC/25/053.

PLANT PURCHASE AND IDENTIFICATION

Celosia argentea was purchased from a local market, kasuwa Dankure, Sokoto, Sokoto state. The plant was identified at the Herbarium of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences of Usmanu Danfodiyo University, Sokoto, Nigeria. A specimen was deposited at the herbarium, and a voucher number was obtained: PCG/UDUS/AMAR/0007.

PLANT PREPARATION

The collected leaves were washed thoroughly with distilled water to remove dust and debris, and then air-dried at room temperature for 2 weeks under shade to prevent pigment degradation. Once dried, the leaves were pounded into fine powder using a mortar and pestle, sieved and stored in a dry container.

PLANT EXTRACTION

Aqueous extraction

One hundred grams (100 g) of the pounded plant material was macerated in 1000 ml of distilled water at room temperature for 48 hours with intermittent agitation. The mixture was filtered through a muslin cloth to remove coarse debris, followed by filtration through Whatman No. 1 filter paper. The filtrate was concentrated to dryness using a hot air oven (Memmert 854 UK) at a controlled temperature to avoid thermal denaturation of the phytochemical constituents. The resulting dried extract was weighed to calculate percentage yield and stored in an airtight container at 4°C [7].

Ethanolic extraction

One hundred grams (100 g) of the grounded powder was macerated in 1000ml of 95% ethanol. It was agitated constantly for 72 h and filtered using a muslin cloth and Whatman's no. 1 filter paper. The filtrate was concentrated in a hot air oven to get the crude extract. The extract was stored in an airtight container at 4°C until needed [8].

PHYSICAL PROPERTIES AND PHYTOCHEMICAL ANALYSIS OF THE EXTRACTS

The physical properties and confirmatory qualitative phytochemical analysis of the aqueous and ethanolic leaf extracts of *Celosia argentea* was performed to identify some of the main compounds present in the extracts

1. Test for alkaloids (Wagner's test): about 2 ml of the methanolic leaves extract of *Jatropha tanjorensis* filtrate was mixed with 1ml of dilute hydrochloric acid, Wagner's reagent was then added in drops and shake well. Formation of reddish-brown precipitate was observed for, which indicates the presence of alkaloids [9].
2. Test for tannins: about 200 mg of the extract was boiled with 10 ml of distilled water, and 0.1% ferric chloride was added to the mixture, it was then observed for blue-black coloration which indicates presence of tannins [9].
3. Test for saponins: about 0.5 ml of the extract and 5 ml of distilled water was combined and agitated, the formation of foam was observed which indicates the presence of saponins [9].
4. Test for flavonoids: 200 mg of the extract was mixed with 10 ml of ethanol and filtered, 2 ml of the filtrate was then mixed with concentrated HCL and magnesium ribbon. The formation of red or pinkish color was then observed which indicates the presence of flavonoids [9].
5. Test for phenols: about 1 mg of the extract was combined with three drops of ferric chloride and 1 ml of potassium ferricyanide ($K_2Fe(CN_6)$). Formation of greenish-blue color was observed which indicates the presence of phenols [9].
6. Test for terpenoids: Five milliliters of the extract were combined with 2 ml of chloroform, after which 3 ml of concentrated sulfuric acid was carefully added to form a separate layer. The appearance of a reddish-brown color at the interface indicated a positive result for the presence of terpenoids [9].
7. Test for cardiac glycosides: Two milliliters of the extract were mixed with 1 ml of glacial acetic acid, one drop of 5% $FeCl_3$, and 1 ml of concentrated H_2SO_4 . The formation of a brown ring at the interface indicated the presence of cardiac glycosides [9].

ANIMAL SACRIFICE AND SAMPLE COLLECTION

One experimental Wistar rat was used for this research, which was purchased and kept in a well-ventilated cage at the animal house of Faculty of Pharmaceutical Sciences, UDUS. The animal was sacrificed by administering a chloroform vapor anesthesia. Using surgical blades, lateral

abdominal incision was done on the animal in order to harvest the liver, kidney and colon. The organs harvested were washed in normal saline, and fixed in 10% neutral buffered formalin.

STUDY DESIGN

One section each of liver, kidney and colon was stained with Haematoxylin and Eosin as the controls. The solutions of *Celosia argentea* extracts (aqueous and ethanolic) were made into 2 different concentrations (1% and 2%) and each was used to stain the liver, kidney and colon of Wistar rat at varying time (5 and 10 minutes).

HISTOLOGICAL ANALYSIS

Fixation of tissue samples

The liver, kidney and colon harvested from the Wistar rats were fixed immediately in 10% neutral buffered formalin for 48 hours [2].

Tissue processing

After fixing the samples for 48 hours, they were grossed and processed using the Automatic tissue processor (ATP) (Leica TP1020 Germany) based on the schedule below [2].

TABLE 1: Tissue processing schedule

BEAKER	STAGE	REAGENT USED	TIME
1	Fixation I	10% neutral buffered formalin	1 hour
2	Fixation II	10% neutral buffered formalin	1 hour
3	Dehydration I	70% alcohol	1 hour
4	Dehydration II	80% alcohol	1 hour
5	Dehydration III	90% alcohol	1 hour
6	Dehydration IV	Absolute alcohol I	1 hour
7	Dehydration V	Absolute alcohol II	1 hour
8	Clearing I	Xylene	30 min
9	Clearing II	Xylene	30 min
10	Infiltration I	Molten wax	30 min
11	Infiltration II	Molten wax	30 min
12	Infiltration III	Molten wax	30 min

Tissue embedding

The infiltrated tissue cassettes were retrieved from paraffin chamber of the ATP, the paraffin embedding station (Leica EG1160 USA) were preheated to 60°C to keep wax molten. The tissue was removed from the cassette and placed in a pre-warmed mold and oriented appropriately (cut edge facing downward). The mold filled with molten paraffin wax and the labeled cassette positioned on top. The filled mold was transferred onto a cold plate to rapidly solidify the wax. Once hardened the paraffin block was removed from the mold. The blocks were stored in a dry cool place until ready for sectioning [10].

Microtomy

Sectioning was carried out using a rotary microtome (Leica RT2125RT Germany) to obtain thin and uniform sections of the tissues for staining and microscopic analysis. The paraffin blocks were trimmed to expose the tissue surface. Each block was securely clamped into the specimen holder of the microtome, and the cutting thickness adjusted to 3 microns, a standard thickness for histological examination. Prior to sectioning, the paraffin blocks were cooled on an ice tray to improve ribbon formation and prevent crumbling. A sharp disposable microtome blade was used

to ensure clean, artifact-free cuts. As the microtome was being operated, a ribbon of serial tissue sections was obtained. These ribbons were carefully picked up using forceps and floated onto 20% alcohol and a float out water bath (Unicope SM801A UK) to flatten the sections and remove folds or wrinkles. Flattened sections were picked up on albumin-coated glass slides to ensure proper adhesion. The slides were placed on a hot plate for 30 minutes to ensure firm attachment of the tissue to the slide and melt residual wax. After drying, the slides were ready for staining [10].

STAINING TECHNIQUES

Haematoxylin and Eosin staining method (control)

1. The sections were deparaffinized in two changes of xylene for 5 minutes each
2. The sections were hydrated to distilled water by passing through descending grades of alcohol for 2 minutes each
3. The sections were stained with Haematoxylin for 10 minutes
4. The sections were washed in water for 2 minutes
5. The sections were briefly differentiated with 1% acid alcohol then blued in Scott's tap water
6. The sections were counterstained with Eosin for 2 minutes
7. The sections were dehydrated by passing through ascending grades of alcohol
8. The sections were cleared in xylene, mounted in DPX and cover slipped [10]

Haematoxylin and *Celosia argentea* staining method (experimental staining)

1. The sections were deparaffinized in two changes of xylene for 5 minutes each
2. The sections were hydrated to distilled water by passing through descending grades of alcohol for 2 minutes each
3. The sections were stained with Haematoxylin for 10 minutes
4. The sections were washed in water for 2 minutes
5. The sections were briefly differentiated with 1% acid alcohol then blued in Scott's tap water
6. The sections were counterstained with each of the extract solutions of *Celosia argentea* (aqueous and ethanolic) at varying concentrations (1% and 2%) and varying time (5 and 10 minutes)
7. The sections were dehydrated by passing through ascending grades of alcohol
8. The sections were cleared in xylene, mounted in DPX and cover slipped

MICROSCOPY

The stained slides were viewed under light microscope (Olympus H30 Germany) at different magnifications to choose the better sections. The slides were snapped using digital microscope camera (Autofocus SONY IMX290 UK)

RESULTS

PHYSICAL PROPERTIES OF *Celosia argentea* LEAF EXTRACTS

The aqueous and ethanolic leaf extracts of *Celosia argentea* showed brown color with a leafy odor. The aqueous extract had a dried texture and the percentage yielded was 21% (see table 2). The texture of the ethanolic extract was gummy with a percentage yield of 9.7% (see table 2).

Table 2: Physical Properties of *Celosia Argentea* Leaf Extract

Part of Plant	Type of Extract	Color	Odor	Texture	Percentage yield (%)
Leaf	Aqueous	Dark brown	Leafy odor	Dried	21
Leaf	Ethanolic	Dark brown	Leafy odor	Gummy	9.7

PHYTOCHEMICAL CONSTITUENTS OF *Celosia argentea* LEAF EXTRACT

The phytochemical screening of *Celosia argentea* leaf extracts was carried out using qualitative method of testing which showed the presence of the following bioactive compounds; Alkaloid, Flavonoid, Tannins, Phenol, Saponin, Steroid/Triterpenoid, Cardiac glycoside, carbohydrate and protein in the leaf extract (see table 3).

Table 3: Phytochemical Constituents of *Celosia Argentea* Leaf Extract

Constituents	Tests	Aqueous Extract	Ethanollic Extract
Alkaloid	Mayer's test	+	+
Flavonoid	Ferric chloride test	+	+
	Alkaline test	+	+
	Ferric chloride test	+	+
Tannin	Lead acetate test	+	+
	Ferric chloride test	+	+
Phenol	Froth's test	+	+
Saponin	Salkowski's test	+	+
Steroid/Triterpenoid	Keller-Kiliani test	+	+
Cardiac glycoside	Fehling's test	+	+
Carbohydrate	Xanthoproteic test	+	+
Protein			

HISTOLOGICAL RESULTS

Liver section stained with Hematoxylin & Eosin and Hematoxylin & aqueous extract

Section of liver stained with Hematoxylin and eosin as control (A). Section of liver stained with Hematoxylin and 1% aqueous *Celosia argentea* extract for 5 minutes (B), central vein, hepatocytes and sinusiodes are not well stained compared with control slide plate 1. Section of liver stained with Hematoxylin and 1% aqueous *Celosia argentea* extract for 10 minutes (C), central vein, hepatocytes and sinusoides are not well stained compared with control slide plate 1. Section of liver stained with Hematoxylin and 2% aqueous *Celosia argentea* extract for 5 minutes (D), central vein, hepatocytes and sinusiodes are well stained compared with control slide plate 1. Section of liver stained with Hematoxylin and 2% aqueous *Celosia argentea* extract for 10 minutes (E), central vein, hepatocytes and sinusiodes are well stained compared with control slide and all other slides plate 1.

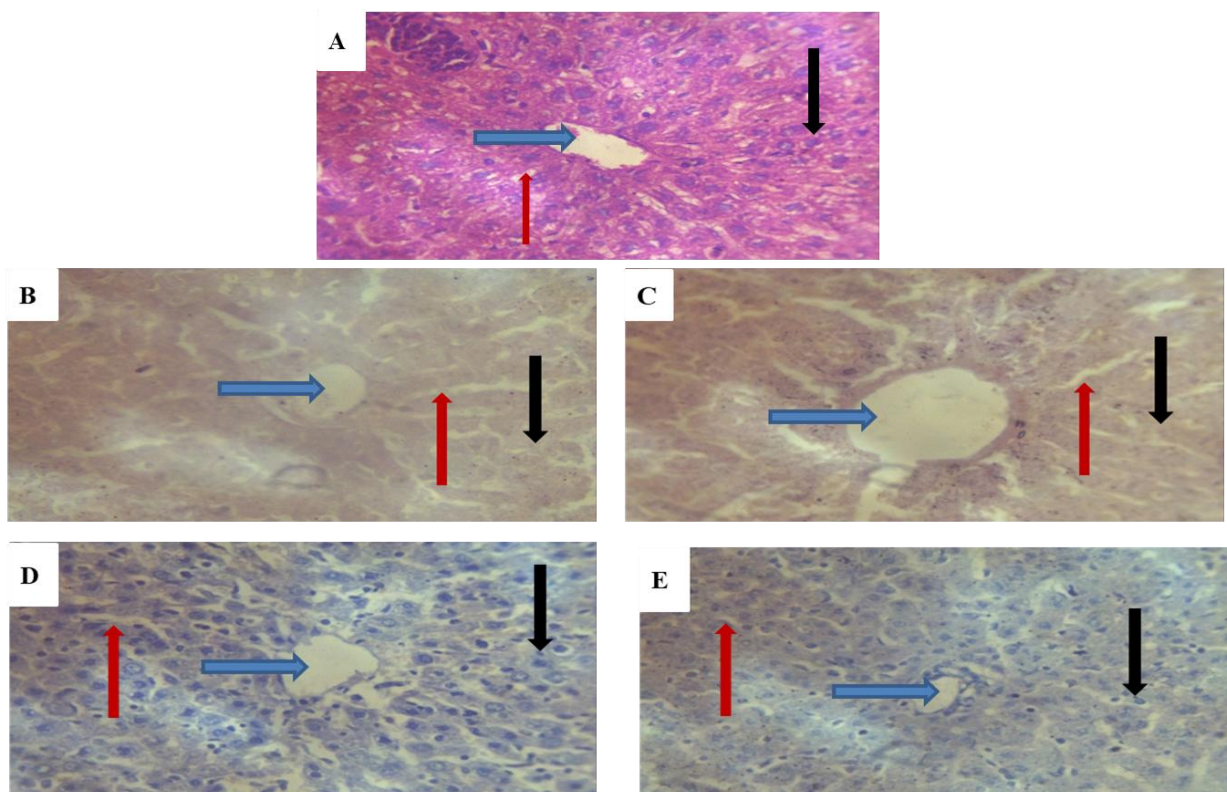


Plate 1: Liver sections X400. (A) H&E as control (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids). (B) 1% aqueous extract for 5 mins (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids). (C) 1% aqueous extract for 10 mins. (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids). (D) 2% aqueous extract for 5 min. (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids). (E) 2% aqueous extract for 10 mins (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids).

Liver section stained with Hematoxylin & Eosin and Hematoxylin & Ethanolic extract

Section of liver stained with Hematoxylin and eosin as control (A). Section of liver stained with Hematoxylin and 1% ethanolic *Celosia argentea* extract for 5 minutes (B) central vein, hepatocytes and sinusoids are not well stained compared with control slide plate 2. Section of liver stained with Hematoxylin and 1% ethanolic *Celosia argentea* extract for 10 minutes (C), central vein, hepatocytes and sinusoids are not well stained compared with control slide plate 2. Section of liver stained with Hematoxylin and 2% ethanolic *Celosia argentea* extract for 5 minutes (D), central vein, hepatocytes and sinusoids are well stained compared with control slide plate 2. Section of liver stained with Hematoxylin and 2% ethanolic *Celosia argentea* extract for 10 minutes (E), central vein, hepatocytes and sinusoids are well stained compared with control slide and all other slides plate 2.

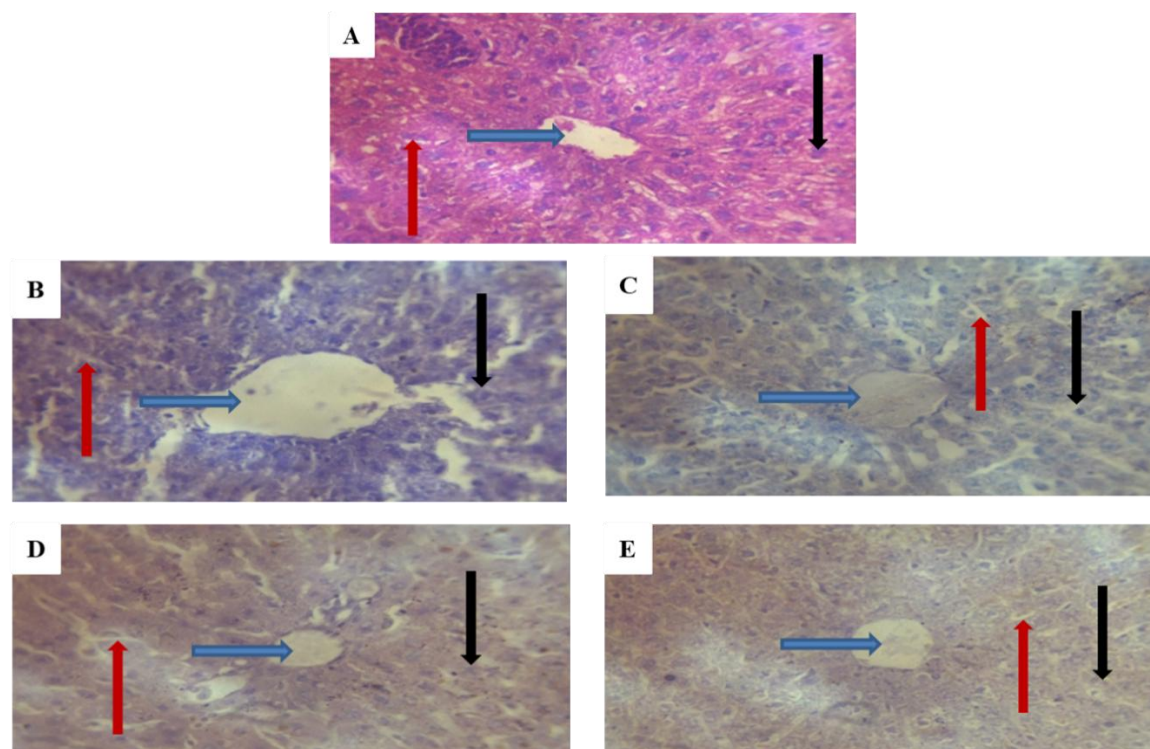


Plate 2: Liver sections X400. **(A)** H&E as control (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids). **(B)** 1% ethanolic extract for 5 mins (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids). **(C)** 1% ethanolic extract for 10 mins. (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids). **(D)** 2% ethanolic extract for 5 min. (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids). **(E)** 2% ethanolic extract for 10 mins (Blue arrow Central vein, Black arrow Hepatocytes, Red arrow Sinusoids).

Kidney section stained with Hematoxylin & Eosin and Hematoxylin & aqueous extract

Section of kidney stained with Hematoxylin and eosin as control **(A)**. Section of kidney stained with Hematoxylin and 1% aqueous *Celosia argentea* extract for 5 minutes **(B)**, glomerulus and glomerular space are not well stained compared with control slide plate 3. Section of kidney stained with Hematoxylin and 1% aqueous *Celosia argentea* extract for 10 minutes **(C)** glomerulus and glomerular space are not well stained compared with control slide plate 3. Section of kidney stained with Hematoxylin and 2% aqueous *Celosia argentea* extract for 5 minutes **(D)**, glomerulus and glomerular space are well stained compared with control slide plate 3. Section of kidney stained with Hematoxylin and 2% aqueous *Celosia argentea* extract for 10 minutes **(E)**, glomerulus and glomerular space are well stained compared with control slide and all other slides plate 3.

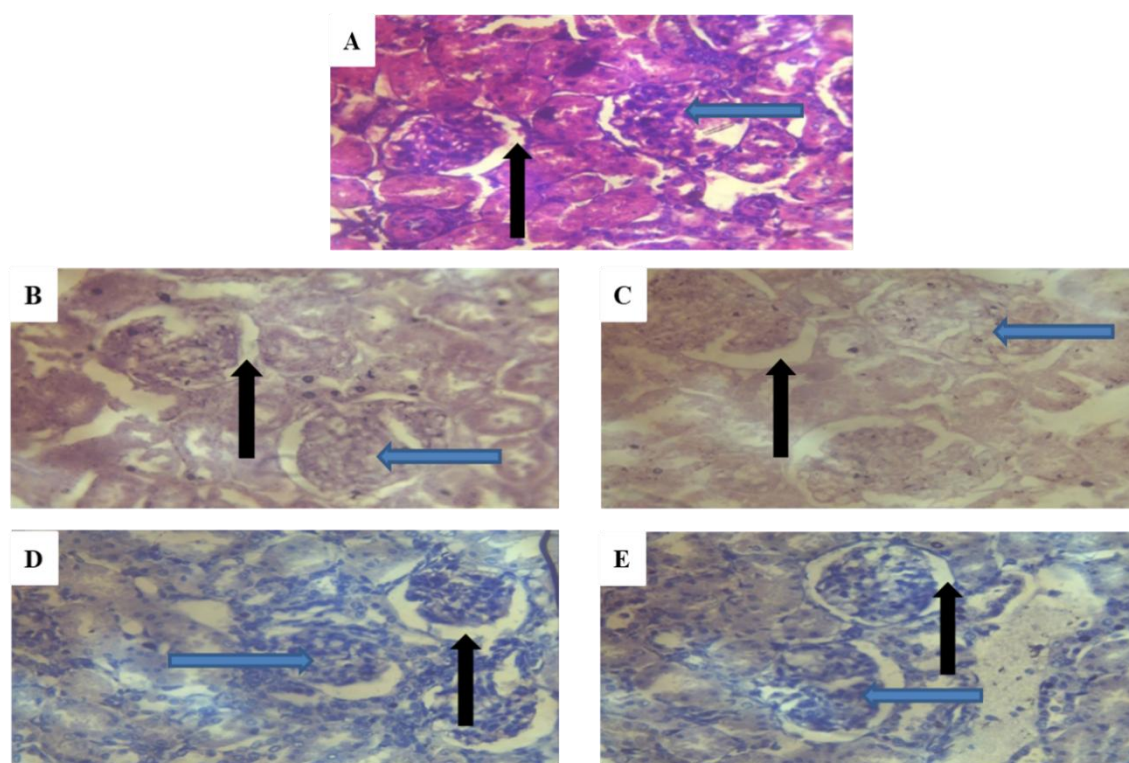


Plate 3: Kidney sections X400. **(A)** H&E as control (Blue arrow Glomerulus, Black arrow glomerular space). **(B)** 1% aqueous extract for 5 mins (Blue arrow Glomerulus, Black arrow glomerular space). **(C)** 1% aqueous extract for 10 mins (Blue arrow Glomerulus, Black arrow glomerular space). **(D)** 2% aqueous extract for 5 mins (Blue arrow Glomerulus, Black arrow glomerular space). **(E)** 2% aqueous extract for 10 mins (Blue arrow Glomerulus, Black arrow glomerular space).

Kidney section stained with Hematoxylin & Eosin and Hematoxylin & ethanolic extract

Section of kidney stained with Hematoxylin and eosin as control **(A)**. Section of kidney stained with Hematoxylin and 1% ethanolic *Celosia argentea* extract for 5 minutes **(B)**, glomerulus and glomerular space are not well stained compared with control slide plate 4. Section of kidney stained with Hematoxylin and 1% ethanolic *Celosia argentea* extract for 10 minutes **(C)**, glomerulus and glomerular space are not well stained compared with control slide plate 4. Section of kidney stained with Hematoxylin and 2% ethanolic *Celosia argentea* extract for 5 minutes **(D)**, glomerulus and glomerular space are well stained compared with control slide plate 4. Section of kidney stained with Hematoxylin and 2% ethanolic *Celosia argentea* extract for 10 minutes **(E)**, glomerulus and glomerular space are well stained compared with control slide and all other slides plate 4.

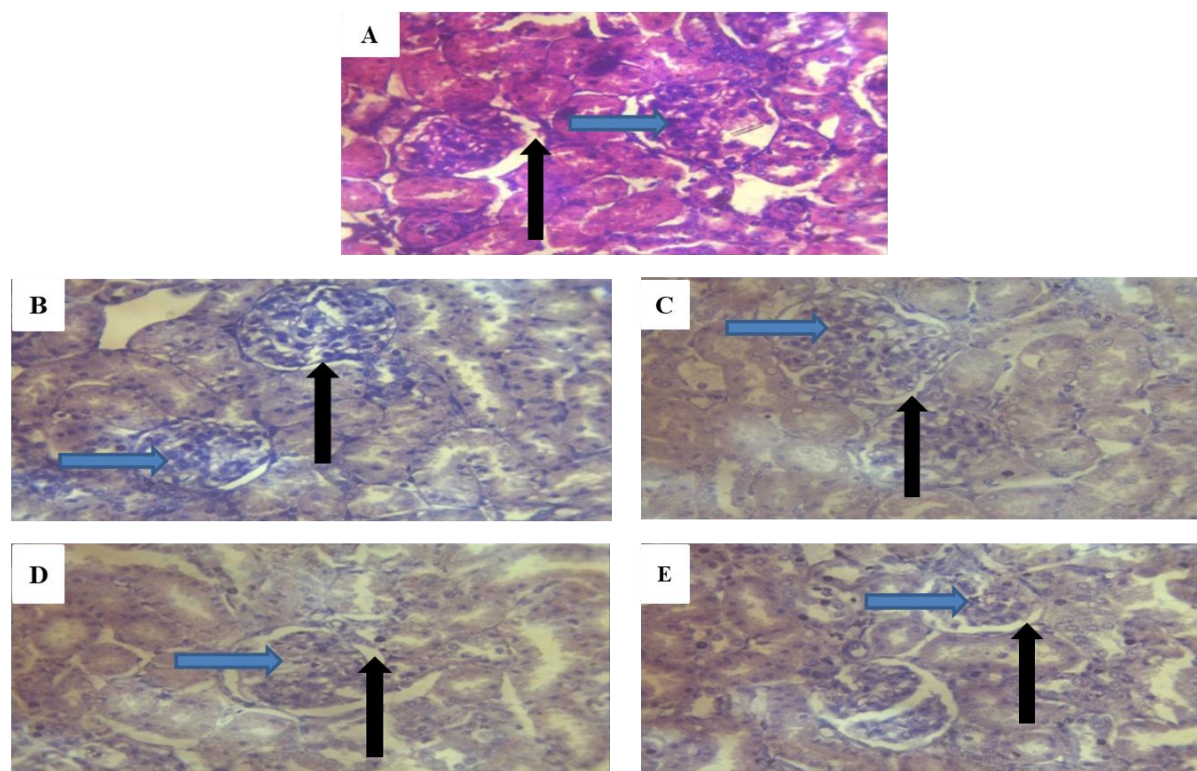


Plate 4: Kidney sections X400. (A) H&E as control (Blue arrow Glomerulus, Black arrow glomerular space). (B) 1% ethanolic extract for 5 mins (Blue arrow Glomerulus, Black arrow glomerular space). (C) 1% ethanolic extract for 10 mins (Blue arrow Glomerulus, Black arrow glomerular space). (D) 2% ethanolic extract for 5 mins (Blue arrow Glomerulus, Black arrow glomerular space). (E) 2% ethanolic extract for 10 mins (Blue arrow Glomerulus, Black arrow glomerular space).

Colon section stained with Hematoxylin & Eosin and Hematoxylin & aqueous extract

Section of colon stained with Hematoxylin and eosin as control (A). Section of colon stained with Hematoxylin and 1% aqueous *Celosia argentea* extract for 5 minutes (B), mucosa and goblet cells are not well stained compared with control slide plate 5. Section of colon stained with Hematoxylin and 1% aqueous *Celosia argentea* extract for 10 minutes (C), mucosa and goblet cells are not well stained compared with control slide plate 5. Section of colon stained with Hematoxylin and 2% aqueous *Celosia argentea* extract for 5 minutes (D), mucosa and goblet cells are well stained compared with control slide plate 5. Section of colon stained with Hematoxylin and 2% aqueous *Celosia argentea* extract for 10 minutes (E), mucosa and goblet cells are well stained compared with control slide plate 5.

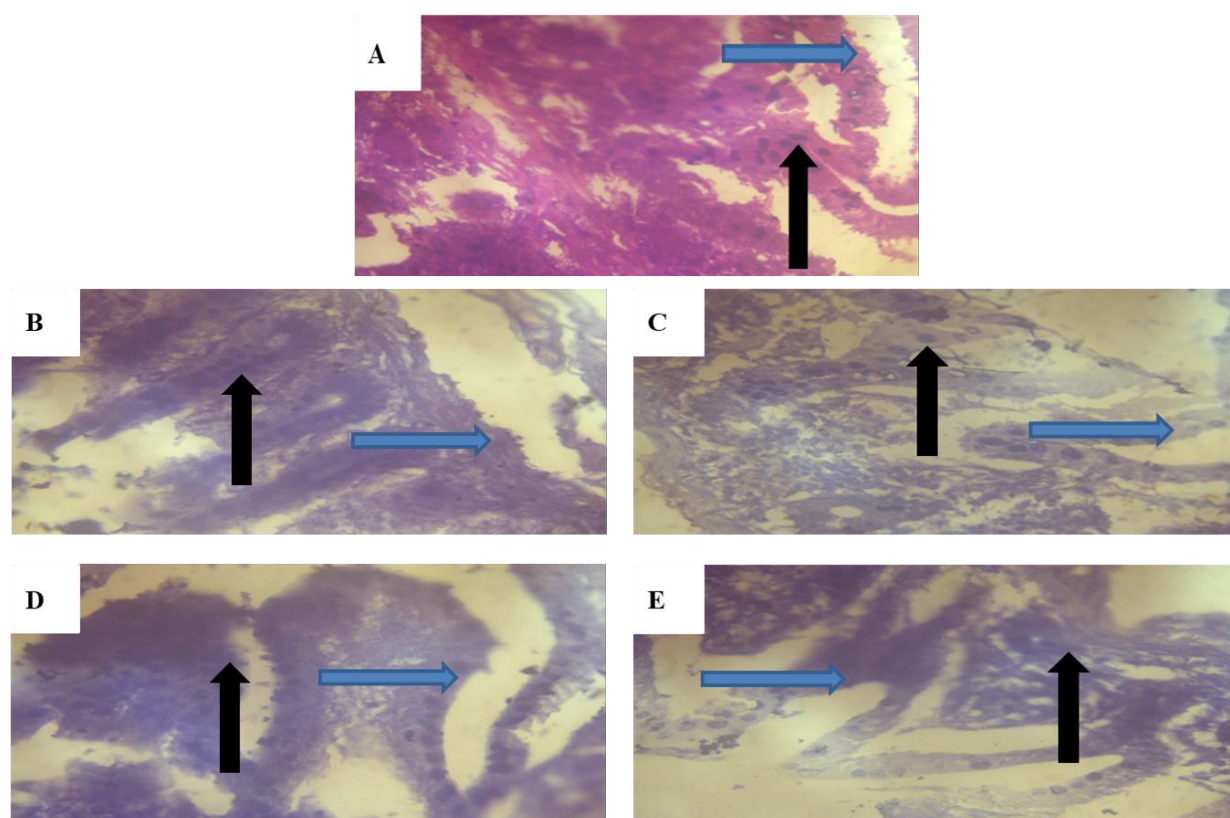


Plate 5: Colon sections X400. (A) H&E as control (Blue arrow mucosa, Black arrow Goblet cell). (B) 1% aqueous extract for 5 mins (Blue arrow mucosa, Black arrow Goblet cell). (C) 1% aqueous extract for 10 mins (Blue arrow mucosa, Black arrow Goblet cell). (D) 2% aqueous extract for 5mins (Blue arrow mucosa, Black arrow shows Goblet cell). (E) 2% aqueous extract for 10 mins (Blue arrow mucosa, Black arrow Goblet cell).

Colon section stained with Hematoxylin & Eosin and Hematoxylin & ethanolic extract

Section of colon stained with Hematoxylin and eosin as control (A). Section of colon stained with Hematoxylin and 1% ethanolic *Celosia argentea* extract for 5 minutes (B), mucosa and goblet cells are not well stained compared with control slide plate 6. Section of colon stained with Hematoxylin and 1% ethanolic *Celosia argentea* extract for 10 minutes (C), mucosa and goblet cells are not well stained compared with control slide plate 6. Section of colon stained with Hematoxylin and 2% ethanolic *Celosia argentea* extract for 5 minutes (D), mucosa and goblet cells are well stained compared with control slide plate 6. Section of colon stained with Hematoxylin and 2% ethanolic *Celosia argentea* extract for 10 minutes (E), mucosa and goblet cells are not well stained compared with control slide and all other slides plate 6.

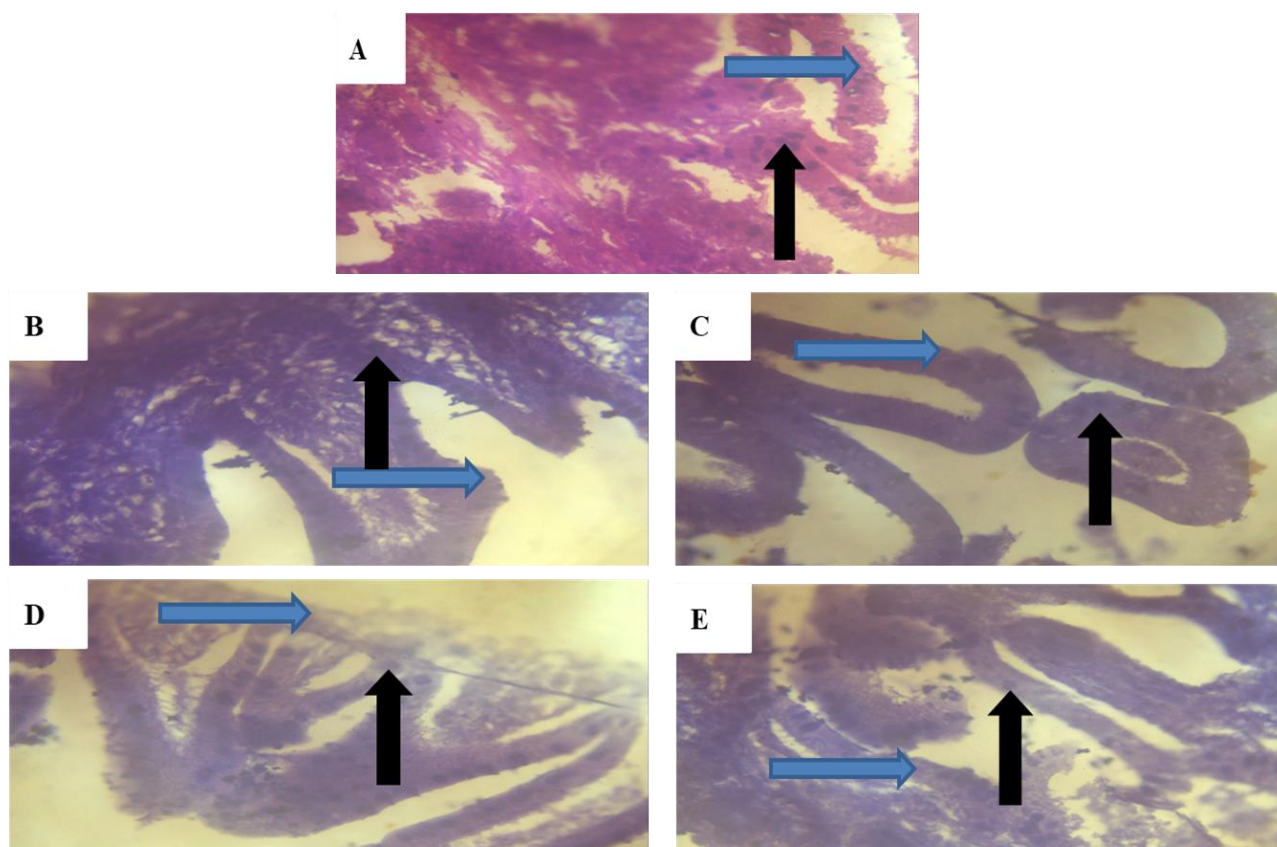


Plate 6: Colon sections X400. (A) H&E as control (Blue arrow mucosa, Black arrow Goblet cell). (B) 1% ethanolic extract for 5 mins (Blue arrow mucosa, Black arrow Goblet cell). (C) 1% ethanolic extract for 10 mins (Blue arrow mucosa, Black arrow Goblet cell). (D) 2% ethanolic extract for 5 mins (Blue arrow mucosa, Black arrow shows Goblet cell). (E) 2% ethanolic extract for 10 mins (Blue arrow mucosa, Black arrow Goblet cell).

DISCUSSION

Most of the dyes used in histopathology are synthetic dyes for the assessment of the tissue integrity, but there is problem regarding the waste management and disposal. The waste of toxic chemicals is harmful to aquatic ecosystem [11]. Waste from toxic chemical from the synthetic dyes could also be a problem to ecosystem, and as well may result to harmful effect to people handling it directly or indirectly [12]. But natural dyes from plants or animals are eco-friendly and are very important source of stains but most are not explored in histopathology [13]. In this study, *Celosia argentea* leaf extracts were employed specifically as counterstains, in place of conventional eosin, while a routine haematoxylin was used to provide nuclear staining. Thus, the primary expectation from the aqueous and ethanolic extracts was not to demonstrate strong nuclear affinity, but rather to act as an acidic dye capable of staining the cytoplasm and other basic tissue components, thereby providing contrast to the hematoxylin stained nuclei. Eosin an acidic dye is used as counterstain to hematoxylin that stain the nuclei of cell blue-purple. The combination of hematoxylin and eosin help to visualize cellular components and help as well in identification of various tissue structures. Eosin staining basically target structures in tissues that are basic in nature such as cytoplasm, cytoplasmic proteins as well as collagen fibers. The acidic property of eosin makes it a very good counterstain for basic components of the cells, producing a good contrast to the nuclear staining achieved with hematoxylin. This contrast help researchers and pathologists to assess cellular morphology, discern cell types as well as identifying pathological changes in the tissues [14].

The *Celosia argentea* leaf extracts used in this study showed distinct physical properties that are relevant to their behavior as histological stains. Both the aqueous and ethanolic extracts were

brown in color, suggesting the presence of conjugated plant pigments and polyphenolic compounds capable of absorbing visible light and imparting color to tissue sections. The aqueous extract dried to form a solid residue, whereas the ethanolic extract had a gummier consistency, indicating differences in the classes and solubility of phytochemicals extracted by the two solvents. In both cases, the extracts retained a leafy odor, consistent with the use of fresh plant material and minimal loss of volatile components during processing. The percentage yield of the aqueous extract (21%) was more than double that of the ethanolic extract (9.7%), indicating that water was a more efficient solvent for extracting the predominately polar constituents of *Celosia argentea* leaves under the conditions employed. This higher yield implies a greater abundance of water-soluble chromogenic compounds in the aqueous fraction, while the lower yield of the ethanolic extract suggests that a smaller proportion of ethanol soluble components were present. The phytochemical analysis of the *Celosia argentea* leaf extracts revealed the presence of carbohydrates, flavonoids, tannins, phenols, saponins, steroids, cardiac glycosides, proteins and alkaloids. The staining capability of *Celosia argentea* can be attributed to its high concentration of betalains, along with flavonoids and tannins. In comparison to other natural histological stains, such as *Hibiscus rosa-sinensis* which relies on anthocyanins [15], or *Lawsonia inermis* (Henna) which relies on lawsone (a naphthoquinone) [16]. Malomo *et al.* (2011) confirmed the presence of flavonoids and phenols in *Celosia argentea*, which often act as auxochromes, enhancing the binding of the primary pigment to tissue structures [7]. The richness in phenolic compounds such as flavonoids, tannins and general phenols is particularly significant for histological use, because these molecules often possess chromophoric structures and conjugated double bonds that can bind to tissue proteins and absorb visible light, thereby producing color [7]. Tannins and related polyphenols are also known to form stable complexes with proteins and metal ions, enhancing their affinity for tissue components and improving stain retention on slides. Saponins can influence the surface activity and permeability of the stain, potentially aiding tissue penetration. Proteins and carbohydrates may affect the viscosity and film-forming properties of the extract, while alkaloids and steroidal compounds may contribute to tissue binding through ionic and hydrophobic interactions [70]. This phytochemical profile distinguishes *Celosia* from non-betalain natural dyes, placing it in the same phytochemical category as *Beta vulgaris* extracts used in histology [17]. The phytochemical constituents identified in the current study is in conformity with study conducted by Divya *et al.*, (2019) in which phenols, tannins, flavonoids, alkaloid, cardiac glycosides, steroids, carbohydrates, protein were identified in *Celosia argentea* [18].

The pigments in *Celosia argentea* behave as anionic (acidic) dyes; the overall staining reaction is cytoplasmic and acidic. This is an acid-base reaction similar to that of Eosin. The stain highlights the cytoplasm in shades of brownish red to brownish yellow, while leaving the nuclei stained blue-black. Between the two extracts, the aqueous extract typically stains more intensely and effectively than the ethanolic extract for *Celosia argentea*, this might be as a result solubility. Betalains are highly water-soluble hydrophilic pigments. Water extracts these pigments more efficiently than ethanol, resulting in a higher concentration of the chromophore in the final staining solution. Udonkang *et al.* (2018) observed this exact phenomenon in *Beta vulgaris* (which shares the same pigments as *Celosia*), reporting that aqueous extracts produced distinct cytoplasmic staining, whereas ethanolic extracts resulted in faint or poor staining intensity [17], this is similar observed in the current study. Furthermore, ethanol can denature certain proteins or dehydrate the tissue differently during the staining process, which might inhibit dye penetration or binding affinity for these specific hydrophilic pigments. Therefore, the aqueous extract staining in this study shows a deeper, more defined contrast compared to the potentially washed-out appearance of the ethanolic extract, this is also seen from the previous study conducted by Nnaemeka *et al.*, (2021) for both aqueous and ethanolic extract of indigenous *Hibiscus sabdariffa* calyx, *Lawsonia inermis* leaves and *Vitex doniana* stem barks on liver and kidney tissue, the

aqueous extract give a good staining contrast compared to ethanolic extract [19]. The concentration of the extract played a significant role in the optical density and clarity of the stained sections. The results consistently demonstrated that the 2% concentration yielded superior staining intensity compared to the 1% concentration across both aqueous and ethanolic extracts. At the 1% concentration, the staining was generally faint, resulting in poor contrast between the cytoplasm and the background. This phenomenon can be attributed to the quantity of chromophores specifically flavonoids, tannins [17]. As indicated in the results, the aqueous extraction process yielded a significantly higher percentage of crude extract (21%) compared to the ethanolic extraction (9.7%). This suggests that the aqueous solvent was more efficient at retrieving the polar, water-soluble pigments necessary for staining. Consequently, when prepared at 2% concentration, the aqueous solution contained a critical mass of these hydrophilic chromophores, allowing for sufficient electrostatic bonding with the positively charged cytoplasmic proteins [17]. The concentration dependent behavior in the current study is in consistent with findings in similar studies on natural dye where Raheem *et al.* (2015) observed that crude plant extracts, such as those from *Hibiscus sabdariffa*, often require higher concentrations to achieve staining quality comparable to synthetic dyes like eosin. Unlike synthetic dyes, which are purified and highly concentrated, crude extracts contain non chromophoric impurities such as mucilage and sugars that effectively dilute the active staining agents [20]. Therefore, the 1% concentration likely felt below the saturation threshold required to fully coat the tissue binding sites, leading to the washed-out appearance observed in some of the lower-concentration. The 2% solution provided a higher density of pigment molecules, increasing the probability of dye-tissue collisions and resulting in the defined brownish-yellow cytoplasmic staining observed in the Liver, Kidney and Colon sections at this concentration.

The duration of staining was a determining factor in the quality of the histological images. The study compared a short staining interval (5 minutes) with a longer interval (10 minutes). The results indicated that the 5-minute duration was largely insufficient for optimal staining, particularly for the ethanolic extracts which exhibited a gummy texture. This texture likely impeded the rate of diffusion into the tissue matrix. In contrast, extending the time to 10 minutes significantly improved the definition of cellular structures, such as the hepatocytes in the liver and the tubular epithelium in the kidney. Bassey *et al.* (2012) reported similar results with *Curcuma longa* extract, noting that natural dyes require prolonged exposure to reach equilibrium within the tissue fibers [21]. While synthetic Eosin acts rapidly, often staining cytoplasm within 1 to 2 minutes [21], the *Celosia argentea* extract required the full 10 minutes to penetrate the dense protein structures of the liver and kidney. This extended time allowed the betalains and phenolic compounds to migrate through the tissue and form stable hydrogen and ionic bonds. The 10-minute timeframe appears to be the optimal window for this extract; it is long enough to ensure adequate saturation but short enough to prevent the non-specific background staining (overstaining) that can obscure nuclear details, a limitation frequently cited in the use of unrefined plant extracts [20]. The interaction between concentration and time was most evident in the specific organs stained. The liver, being a protein rich organ with dense cytoplasm, showed the best response to the combination of 2% concentration at 10 minutes. The high concentration ensured there was enough dye to bind to the abundant cytoplasmic enzymes, while the extended time allowed the dye to penetrate the hepatocytes fully. Conversely, the colon sections, which contain mucin in goblet cells, required the higher concentration to visualize the mucosal lining effectively against the pale background. The 1% ethanolic extract at 5 minutes represented the least effective combination, likely due to the low extraction yield of ethanol (9.7%) combined with insufficient time for the sparse pigments to penetrate the tissue.

CONCLUSION

The results of this study revealed that the aqueous extract of *Celosia argentea* showed significantly higher staining intensity than the ethanolic solution, which can be attributed to the higher percentage yield of water-soluble chromophores in the aqueous extract. Furthermore, the staining intensity was found to be time and concentration dependent. The study concludes that the 2% aqueous extract applied for 10 minutes provided the optimal staining reaction, adequately visualizing cytoplasmic structures. While the 1% concentration and 5 minutes duration yielded faint results, the optimized 2% aqueous solution of *Celosia argentea* to stain tissues at 10 minutes demonstrated sufficient contrast to serve as a potential natural substitute for Eosin.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

Authors have declared that no conflict of interest exist

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