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Spectrophotometry and Histological Staining Potential of Ethanol Extract of *brassica Oleracea* (Red Cabbage) on Selected Organs of *Sprague Dawley* rat

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A B S T R A C T

Objective: Plants with coloring and dyeing effects have widely been used in histological staining. Natural dyes are less expensive, nontoxic, renewable and sustainable resources, with minimal environmental impact compared to synthetic stains. Red cabbage is one of such natural dyes. The red color of the red cabbage derived from anthocyanin pigments confers a great advantage to red cabbage. In this study, the physiochemical and spectrophotometric characteristics of red cabbage as well as its histological staining potentials on various tissues were carried out to determine its tissue specificity.

Methods: The ethanol extract of red cabbage was prepared using 100% ethanol, then pH, spectrophotometry, and concentration of the red cabbage ethanol extract were measured before the solution was divided into two equal volumes, and one part was added with 2ml glacial acetic acid while the other part was added with 10g of aluminum sulfate. The two solutions were used to stain 10% neutral-buffered formalin-fixed, paraffin-wax-embedded tissue sections separately. Stained sections were viewed with a photomicroscope.

Results: The ethanol extract was acidic at a pH of 4.89 and the spectrophotometry graph showed 3 peak absorbances at the wavelengths of 395nm, 545nm and 670nm. The maximum absorbance was 0.795 at the wavelength of 395nm and this is indicative of the pigment cyanidin which is the anthocyanin present in red cabbage. The red cabbage ethanol extract with both glacial acetic acid and aluminum sulfate showed good histological details in tissues of kidney, stomach, lungs, liver, spleen, small intestine and skin. There was no difference between the red cabbage ethanol extract and the conventional hematoxylin and eosin stain ($P>0.05$). Our study showed that red cabbage ethanol extract with acetic acid and aluminum sulfate is stable at 4°C, with color amber to light gold with a pH of 4.89 and a concentration of 1000 which gave good histological details. In general, our result indicates that red cabbage ethanol extract is an efficient stain for histological tissue structures and can be used as an eco-friendly alternative to eosin.

Conclusion: Red cabbage ethanol extract stained basic structures of the cell such as the cytoplasm, muscle fibers and mucins. Therefore, red cabbage ethanol extract can be used in histological staining when appropriate modifications are done.

Keywords: Red Cabbage, Anthocyanin, Spectrophotometry, Histology, Staining, Ethanol

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INTRODUCTION

Red cabbage (*Brassica oleracea*) belongs to the family Crucuferae. The plant is popular as a vegetable that is eaten either raw in salads or cooked in many dishes because of its high fiber content and low calories. Consumption of red cabbage has numerous health benefits against cancer, inflammation, diabetes, and constipation. Red cabbage anthocyanins are distinct from others because they provide varying colors to the leaves at different soil pH. Natural dyes have been very effective in the staining of cells in histological studies, and they are non-toxic, less expensive and eco-friendly[1]. Dyes used in histological studies can be categorized into four groups, including synthetic dyes, dyes made similar to natural sources, inorganic dyes and natural dyes. Synthetic dyes that are mostly used in the staining of tissues including safranin-O and methylene blue are relatively easy to use in

microscopy studies. However, the use of these dyes harms the human body, and safranin-O is a cationic dye and its waste can cause dangerous contaminations [2].Moreover, safranin-O dyes can cause allergic reactions to the skin. Methylene blue causes dizziness, fever, headache, and cardiovascular problems. Methylene blue is a carcinogenic compound and very difficult to decompose. To address the aforementioned problems, it is important to provide an alternative solution. One of such solutions is the use of natural dye extracts of *Brassica oleracea* (red cabbage) which has been reported to be more eco-friendly, non-carcinogenic, non-toxic and biodegradable [3]. In general, plants such as red cabbage have great potential as a source of natural dyes, especially in the roots, bark, leaves, fruit, wood, seeds, and flowers. Natural dyes offer several advantages over their synthetic counterparts. They are typically biodegradable, renewable, and environmentally friendly, aligning with the increasing emphasis on sustainability and safety in scientific research [4]. *Brassica oleracea*, encompassing vegetables like cabbage, kale, broccoli, and cauliflower, presents an intriguing natural source of pigments suitable for histologic staining. The choice of *Brassica oleracea* ethanolic extract for this study is particularly intriguing due to its ready availability, affordability, and well-documented safety for human consumption.

The aim of this study was to determine the staining potential of red cabbage ethanol extract on the histology of various tissues of *Sprague dawley* rats. The ethanol extract of red cabbage appeared amber to light gold in color and maintained staining ability when stored in the refrigerator at 4^{o}C. This is similar to other reports of plant extracts being used for staining. The extracts showed an acidic pH of 4.89 when examined with a pH meter. This indicates that the extract will have affinity for the basic structures of the cells. It is a known fact that the juice of red cabbage can be used as a home-made pH indicator. and at an ideal pH, certain tissue components will show a relative acidophilia whereas others display relative basophilia.

Materials and Methods

Red cabbage (*Brassica oleracea*) was obtained from Spar Mall, Calabar, in Nigeria. It was identified and authenticated with the number- Bot/Herb/UCC/307.

Procedure for Ethanol Preparation

1.0 gram of red cabbage was weighed with a digital weighing balance and grinded in a mortar by adding 25ml of ethanol until complete crushing and extraction of the pigment was achieved. The remaining 5ml of ethanol was used to rinse the remaining plant material in the mortar. The solution was filtered using Wattman No. 1 filter paper into a beaker and labeled 100% ethanol red cabbage extract.

Preparation of Dilution Series (50, 25, and 12.5%)

Three test tubes each for the 50, 25 and 12.5% dilutions were set up for the ethanol red cabbage extract. For the 50% solution, 4ml of the 100% solution was put in a test tube and 4ml of ethanol was added. For the 25% solution, 4ml of the 50% solution was transferred into the test tube and 4ml of ethanol was added. While for the 12.5% solution, 4ml of the 25% solution was transferred into the test tube and 4ml of ethanol was added.

Spectrophotometric Analysis

The wavelength of the SP-2000 spectrophotometer (Power 85-264 VAC, 50/60Hz) was set to 320nm. Ethanol in a cuvette was used to blank the spectrophotometer, before absorbance of the solution was read at intervals of 25nm wavelength between 320 and 795nm for ethanol extracts.

Concentration of Red Cabbage Dye

The concentration of the fresh red cabbage in the ethanol was measured with SP 2000 spectrophotometer.

pH Measurement

The pH of the solution was determined using the OHAUS starter 2100 pH meter.

Preparation of Red Cabbage Stain

The staining solution was prepared using 459g red cabbage (blended to powder) dissolved in 400ml of ethanol. The red cabbage was washed carefully with running tap water to remove dust and dirt. The cabbage was then shredded and blended using a blender. Ethanol was added to enable extraction of the pigment from the red cabbage. The solution was then filtered and refrigerated at 4^{o}C. The staining solution which is now 400ml of the red cabbage ethanol extract was then divided into two halves. To enhance the staining capability of the solution, 2ml of Glacial Acetic Acid (GAA) was added to 200ml of the staining solution in a beaker and labeled 100% red cabbage glacial acetic acid extract and 10g of alum sulfate was added to the other remaining 200ml of the prepared staining solution in a beaker and labeled 100% red cabbage alum ethanol extract respectively.

Animal material and Tissue Processing

A *Sprague dawley* rat was bought from the Animal house of the College of Medical Sciences, University of Calabar, Nigeria. The rat was sacrificed by using chloroform inhalation method. For the microscopic study, 7 tissues were removed after the sacrifice. Tissues include; Kidney, stomach, lungs, liver, spleen, small intestine and skin. The tissues of the rats were washed with normal saline to remove blood and then fixed in 10% neutral buffered formalin for a duration of 48 hours to enable complete fixation of the tissues. All tissues were processed using the routine paraffin wax tissue processing procedure in the histopathology laboratory. The process included tissue processing, embedding, microtomy - where 3 sections of the same organ were cut before proceeding to staining).

Staining Techniques

Tissue sections were divided into three (3) experimental Groups A, B, C.

Group A was stained with red cabbage ethanol extract containing acetic acid. Group B was stained with red cabbage ethanol extract containing alum sulphate. Group C was stained with the conventional Cole's Hematoxylin and Eosin staining solution.

For group A and B procedure, The sections were dewaxed and hydrated in descending grades of alcohol before rinsing in water and stained with red cabbage ethanol extract containing acetic acid for Group A or stained with red cabbage containing alum sulphate for Group B for 30 minutes respectively. The tissue sections were rinsed in tap water, allowed to air dry, then cleared in xylene before mounting in Dibutyl phthalate polystyrene xylene (DPX) and a cover slip was used. For group C, tissue sections were dewaxed and hydrated, and stained with alum hematoxylin for 30 minutes;this was followed with differentiation with 1% acid alcohol for 2 seconds and blued in tap water for 10 minutes, counterstained with eosin for 2 minutes before rinsing in water. The slides were dehydrated, cleared in xylene and mounted with Dibutyl phthalate polystyrene xylene (DPX) and a cover slip was used to cover the slides.

Statistical analysis

The red cabbage ethanol extract with glacial acetic acid alongside the extract used with aluminum sulfate were compared with hematoxylin and eosin stain using chisquare test and probability value (p-value) test based on the demonstration of general tissue morphology. The statistical package for social sciences (SPSS) version 21.0, IBM incorporated, USA, was used for all analysis. Significant difference was established at P value <0.05.

Result

The concentration of the extract solution was 1000; pH at 4.89, and the extract was soluble in ethanol giving a color of amber to light gold.

The red cabbage ethanol extract gave three (3) peak readings at 395nm, 545nm and 670nm wavelength respectively.

Stained tissue sections acquired varying degrees of staining with red cabbage ethanol extracts as compared to Hematoxylin and Eosin control sections. The color extract was absorbed by tissues, but intensity differed based on internal structures.

The result for the grading of the staining potentials for red cabbage ethanol extract with glacial acetic acid as a mordant (Stain 1) compared to the grade of the hematoxylin and eosin stain (stain 2) is presented in Table 2. In this table, the staining potential of both stains were compared and the Chi-square =3.116 andprobability value (p=0.876)indicating that there is no significant difference (P>0.05) between the red cabbage ethanol extract and the conventional hematoxylin and eosin stain.

The result of the grading of the staining potentials for red cabbage ethanol extract with Aluminum sulphate as a mordant (stain 1) compared to the grading of hematoxylin and eosin stain (stain 2) is presented in Table 3. As shown in the results, the staining potential of both stains showed a chisquare = 2.433 and p=0.876. Also indicating there is no significant difference (P>0.05) between the red cabbage ethanol extract and the conventional hematoxylin and eosin stain.

Table 1: shows the concentration of the extract solution was 1000, pH at 4.89, and the extract was greatly soluble in ethanol giving a color of amber to light gold.

Parameters	Result
Concentration	1000g/dl
pH	4.89
Solubility	Soluble in Ethanol
Color	Amber to Light Gold

Table 2.0 Grading of the staining results for Ethanol Red Cabbage Extract with Glacial Acetic Acid and Hematoxylin and Eosin.

Staining Potential **N=7**	**Nuclear Staining**	**Cytoplasm staining**	**Histological details**	**Clarity of staining**	**Integrity of Staining**	**Total score**	**Statistics**
Stain 1	15	15	16	11	13	70	X^2=3.116
Stain 2	19	19	21	14	16	89	P=0.876

Stain 1: Red Cabbage extract with glacial acetic acid
Stain 2: Hematoxylin and Eosin Stain
Organs: 1-Kidney, 2-Stomach, 3-Lungs, 4-Liver, 5-Spleen, 6-Small Intestine, 7-Skin.
Chi Square: X^2 = 3.116; df (6); p = 0.794
Key:
Scoring used was
1 – Poor staining
2 – Moderate
3 – Good

Table 3.0 Grading of the staining results for Ethanol Red Cabbage Extract with Aluminum Sulphate and Hematoxylin and Eosin

Staining Potential N=7	Nuclear Sta	Cytoplasmic staining	Histological details	Clarity of sta	Integrity of St	Total sc	Statistics
Stain 1	15	15	18	12	14	74	X^2=2.433
Stain 2	19	19	20	14	15	87	P=0.876

Stain 1: Red Cabbage extract with Aluminum Sulphate

Stain 2: Hematoxylin and Eosin Stain

Organs: 1-Kidney, 2-Stomach, 3-Lungs, 4-Liver, 5-Spleen, 6-Small Intestine, 7-Skin.

Chi Square: X^2 = 2.433; df (6); p = 0.876

Key:

Scoring used was

1 – Poor staining
2 – Moderate
3 – Good

Figure 1: Red cabbage plant (*Brassica oleracea*).

Figure 2: Red cabbage bulb cut in half.

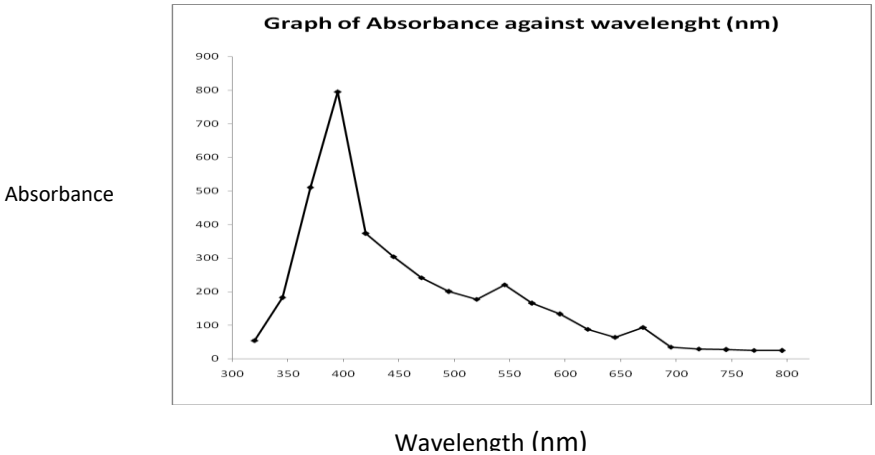


Figure 3: Spectrophotometric curve of ethanol extract of red cabbage.

Plate 1a shows histology of the kidney stained with hematoxylin and eosin with a clear nucleus stained purple and cytoplasm stained pink. Plate 1b shows the photomicrograph of a kidney section stained with red cabbage extract with alum. Good nuclear staining, cytoplasmic staining (where the cells of the glomerulus showed good staining contrast.) and clarity of the stain were observed, while the histological details and stain intensity were relatively poor. Plate 1c shows the photomicrograph of a kidney section stained with red cabbage extract combined with acetic acid. Good nuclear staining, cytoplasmic staining, and histological details were achieved, while the clarity and intensity of the stain were poor.

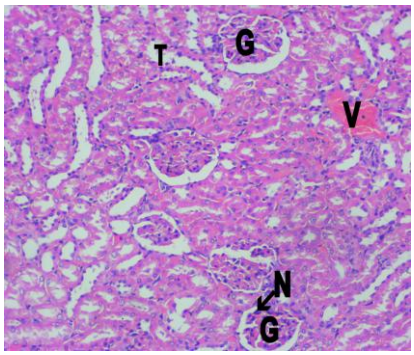


Plate 1a:

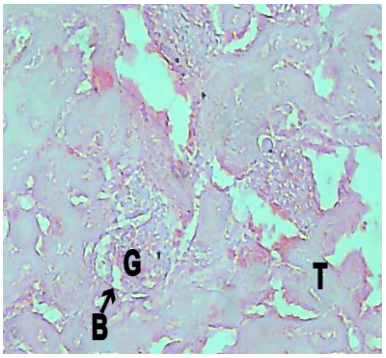


Plate 1b:

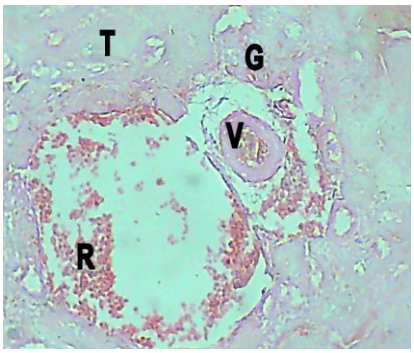


Plate 1c:

Plate 1a: Kidney of rat stained with Hematoxylin and Eosin. There is purple staining of the nucleus (N) of the tubule (T) and glomerulus (G) and orange staining of red blood cell (R) in blood vessel (V). Hematoxylin and eosin x100 magnification.

Plate 1b: Kidney of rat stained with ethanol extract of Red cabbage and ethanol alum. There is light pink staining of the connective tissue of the basement membrane (B), tubules (T) and glomerulus (G). Red cabbage and ethanol alum, x100 magnification.

Plate 1c: Kidney of rat stained with ethanol extract of Red cabbage/ethanol acetic acid. There is light pink staining of the connective tissue of the blood vessel (V), basement membrane (B), tubules (T) and glomerulus (G) and orange staining of red blood cells (R). Red cabbage and ethanol acetic acid, x100 magnification.

Plate 2a shows the histology of the stomach of the rat stained with hematoxylin and eosin. The purple staining of nucleus was distinct and pink staining of connective tissue of submucosa and muscularis external. Plate 2b and Plate 2c show the photomicrograph of a stomach sectioned stained with red cabbage ethanol acetic acid extract and red cabbage ethanol alum extract respectively, the both showed a distinctive light pink staining of cytoplasm of the mucosa epithelium and connective tissue of the submucosa and muscularis external, also, the clarity of staining and the staining intensity was good.

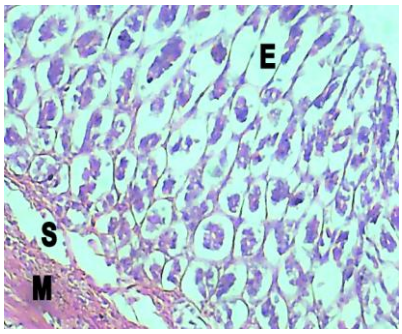


Plate 2a:

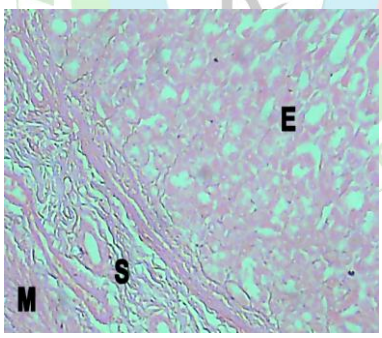


Plate 2b:

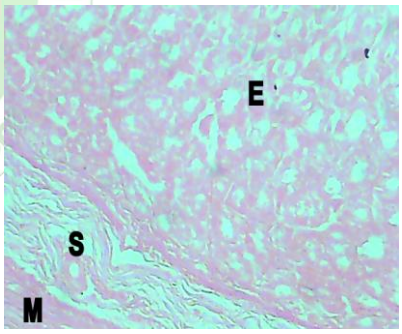


Plate 2c:

Plate 2a: Stomach of rat stained with Hematoxylin and Eosin. There is purple staining of nucleus (N) of the mucosa epithelium (E) and pink staining of connective tissue of submucosa (S) and muscularis externa (M). Hematoxylin and eosin x100 magnification.

Plate 2b: Stomach of rat stained with ethanol extract of Red cabbage and ethanol acetic acid. There is light pink staining of cytoplasm of the mucosa epithelium (E) and connective tissue of the submucosa (S) and muscularis externa (M). Red cabbage and ethanol acetic acid,x100 magnification.

Plate 2c: Stomach of rat stained with ethanol extract of Red cabbage and ethanol alum. There is light pink staining of cytoplasm of the mucosa epithelium (E) and connective tissue of the submucosa (S) and muscularis externa (M). Red cabbage and ethanol alum, x100 magnification.

Plate 3a shows a section of a lung of a rat stained with hematoxylin and eosin where the light pink staining of cytoplasm of the mucosa epithelium and connective tissue of the basement membrane, alveolar wall, and blood vessel was stained showing of their details. Plate 3b shows a section of a lung of a rat stained with red cabbage ethanol acetic acid extract where the nuclear, cytoplasmic, intensity of the staining, clarity of staining and histological details where prominent and distinctive. Plate 3c show a section of a lung of a rat stained with red cabbage ethanol alum extract, where just the histological details were quite distinct (the aveoli spaces, pulmonary vessels, bronchus were observed) but other features such as the nuclear staining, staining intensity and clarity was relatively poor.

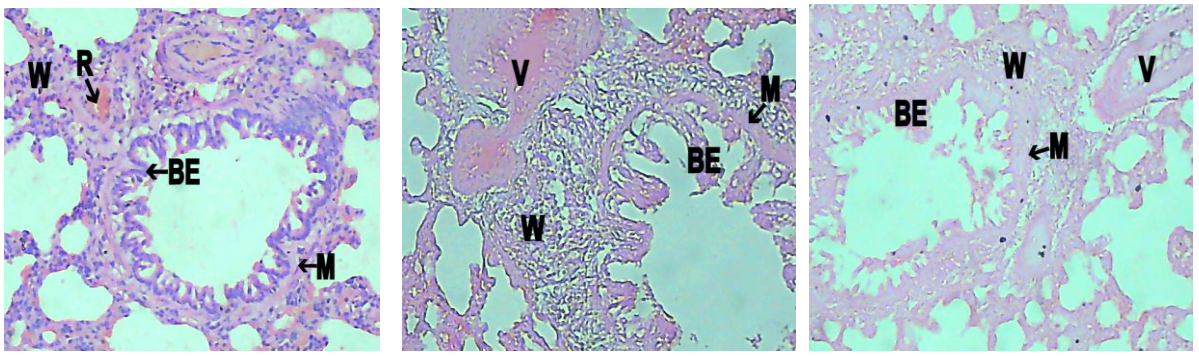


Plate 3a: **Plate 3b:** **Plate 3c:**

Plate 3a: Lung of rat stained with Hematoxylin and Eosin. There is light pink staining of cytoplasm of the mucosa epithelium (BE) and connective tissue of the basement membrane (M). Alveolar wall (W), and blood vessel (V). Red cabbage and ethanol acetic acid,x100 magnification.

Plate 3b: Lung of rat stained with ethanol extract of Red cabbage and ethanol acetic acid. There is light pink staining of cytoplasm of the mucosa epithelium (BE) and connective tissue of the basement membrane (M). Alveolar wall (W), and blood vessel (V). Red cabbage and ethanol acetic acid, x 100 magnification.

Plate 3c: Lung of rat stained with ethanol extract of Red cabbage and ethanol alum. There is light pink staining of cytoplasm of the mucosa epithelium (BE) and connective tissue of the basement membrane (M). Alveolar wall (W), and blood vessel (V). Red cabbage and ethanol alum, x100 magnification.

Plate 4a shows a section of a liver of a rat stained with hematoxylin and eosin. The deep blue staining of the nucleus and pink staining of cytoplasm of the hepatocyte (H) and connective tissue around the portal triad (T). Plate 4b shows a section of a liver of a rat stained with red cabbage ethanol acetic acid extract where all the features, nuclear, cytoplasmic, histological details, clarity of staining and staining intensity of the section was observed distinctively. Compared to Plate 4c of the same section of the liver stained with red cabbage ethanol alum extract which also showed good features but not as outstanding as 4b.

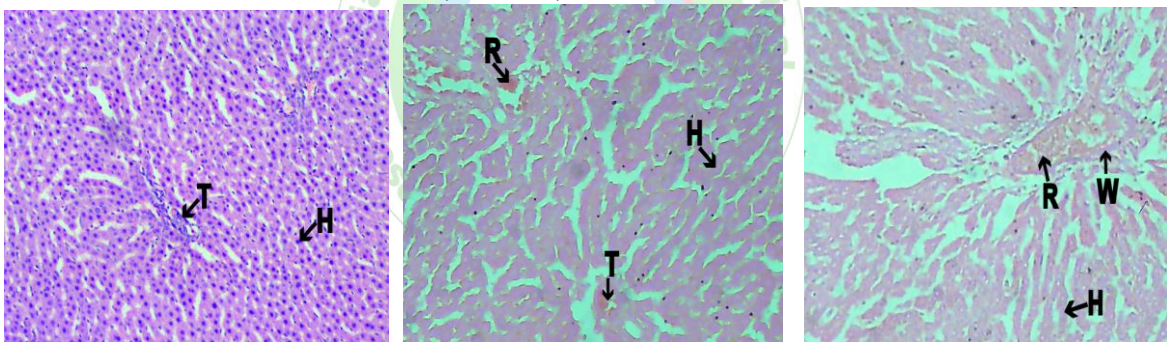


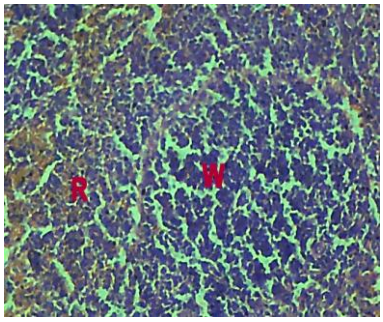
Plate 4a: **Plate 4b:** **Plate 4c:**

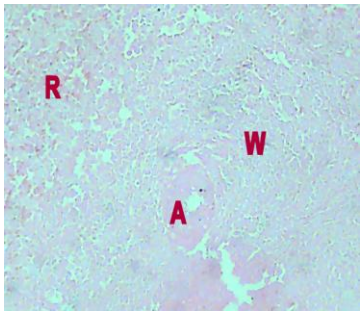
Plate 4a: Liver of rat stained with Hematoxylin and Eosin. There is deep blue staining of the nucleus and pink staining of cytoplasm of the hepatocyte (H) and connective tissue around the portal triad (T) and central vein with red blood cell (R). Hematoxylin and eosin x100 magnification.

Plate 4b: Liver of rat stained with ethanol extract of Red cabbage and ethanol acetic acid. There is faint blue staining of the nucleus and pink staining of cytoplasm of the hepatocyte (H) and connective tissue around the developing portal triad (T) and central vein with red blood cell (R) staining orange. Red cabbage and ethanol acetic acid, x100 magnification.

Plate 4c: Liver of rat stained with ethanol extract of Red cabbage and ethanol alum. There is faint blue staining of the nucleus and pink staining of cytoplasm of the hepatocyte (H) and connective tissue around the wall of the central vein (W) and light orange staining of red blood cell (R). Red cabbage and ethanol alum, x100 magnification.

Plate 5a shows a section of a spleen of a rat stained with hematoxylin and eosin where the stain distinctively outlined the features of the spleen such as the central arteries, the white pulp and red pulp. Plate 5b shows a section of the same spleen but stained with red cabbage ethanol alum extract which showed just a good staining feature as well, where the red pulp, the white pulp and central arteries were observed; then plate 5c which was the same spleen section but was stained with red cabbage ethanol acetic acid extract.





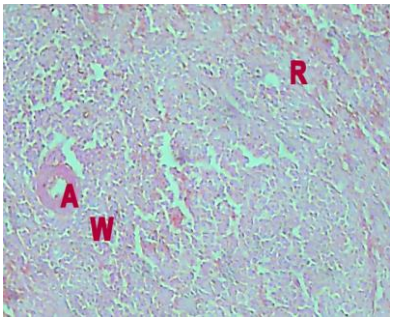


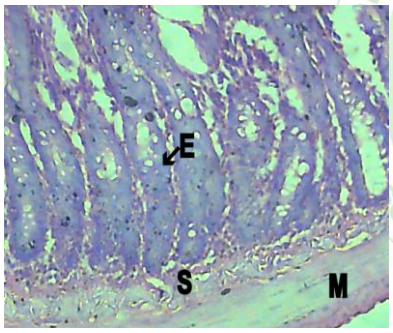
Plate 5a: **Plate 5b:** **Plate 5c:**

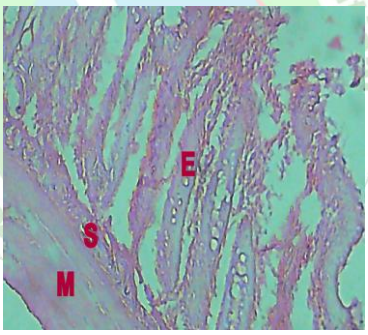
Plate 5a: Spleen of rat stained with Hematoxylin and Eosin. There is deep purple staining of the lymphocytes in the white pulp (W) and orange staining of red blood cells in the red pulp (R). Hematoxylin and eosin x100 magnification.

Plate 5b: Spleen of rat stained with ethanol extract of Red cabbage and ethanol acetic acid. There is deep purple staining of the lymphocytes in the white pulp (W) and orange staining of red blood cells in the red pulp (R). Red cabbage and ethanol alum, x100 magnification.

Plate 5c: Spleen of rat stained with ethanol extract of Red cabbage and ethanol acetic acid. There is deep purple staining of the lymphocytes in the white pulp (W) and orange staining of red blood cells in the red pulp (R). Red cabbage and ethanol acetic acid, x100 magnification.

Plate 6a shows a section of the small intestine of rat stained with hematoxylin and eosin. There is purple staining of the nucleus of the mucosa epithelium and pink staining of connective tissue of submucosa and muscularis external which were all distinctive. Both plate 6b and 6c shows the section of a small intestine of a rat stained with red cabbage ethanol acetic acid extract and red cabbage ethanol alum extract respectively, both showed very good nuclear staining, cytoplasmic staining, histological details, clarity of the staining and intensity of staining.





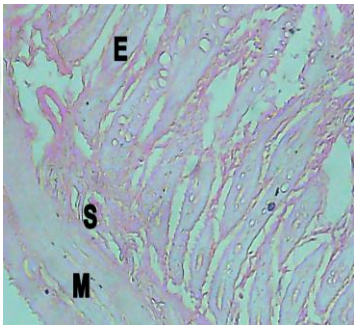


Plate 6a: **Plate 6b:** **Plate 6c:**

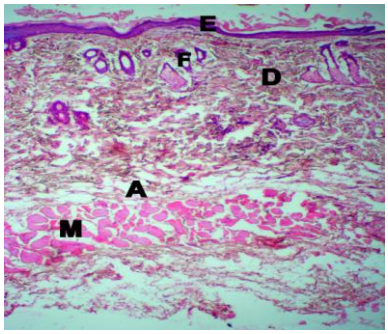
Plate 6a: Small intestine of rat stained with Hematoxylin and Eosin. There is purple staining of nucleus (N) of the mucosa epithelium (E) and pink staining of connective tissue of submucosa (S) and muscularis externa (M). Hematoxylin and eosin x100 magnification.

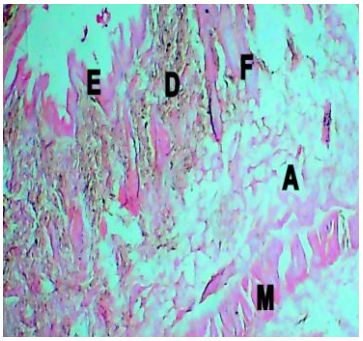
Plate 6b: Small intestine of rat stained with ethanol extract of Red cabbage and ethanol acetic acid. There is light pink staining of cytoplasm of the mucosa epithelium (E) and connective tissue of the submucosa (S) and muscularis externa (M). Red cabbage and ethanol acetic acid, x100 magnification.

Plate 6c: Small intestine of rat stained with ethanol extract of Red cabbage and ethanol alum. There is light pink staining of cytoplasm of the mucosa epithelium (E) and connective tissue of the submucosa (S) and muscularis externa (M). Red cabbage and ethanol alum, x100 magnification.

Plate 7a shows a section of the skin of a rat that was stained with hematoxylin and eosin stain, which showed every detail feature of the section excellently. Plate 7b and plate 7c show the same section of the skin of the rat which was stained with red cabbage ethanol alum extract and red cabbage ethanol acetic acid extract respectively. The two stained sections showed peculiar staining features except the clarity of the stain which was very poor in the two section

s.





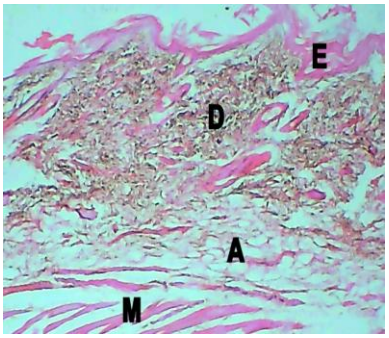


Plate 7a: **Plate 7b:** **Plate 7c:**

Plate 7a: Skin of rat stained with Hematoxylin and Eosin. There is purple staining of the epidermis (E) and hair follicle (F) and muscle (M) stains pink. The adipose subcutaneous tissue is (A). The collagen fibres of the dermis stain light yellow to brown. Hematoxylin and eosin x100 magnification

Plate 7b: Skin of rat stained with ethanol extract of Red cabbage and ethanol alum. There is pink staining of the epidermis (E), hair follicle (F) and muscle (M). The adipose subcutaneous tissue is (A). The collagen fibres of the dermis stain light yellow to brown. Red cabbage and ethanol alum, x100 magnification.

Plate 7c: Skin of rat stained with ethanol extract of Red cabbage and ethanol acetic acid. There is pink staining of the epidermis (E), hair follicle (F) and muscle (M). The adipose subcutaneous tissue is (A). The collagen fibres of the dermis stain light yellow to brown. Red cabbage and ethanol acetic acid, x100 magnification.

Discussion

Histological staining techniques play a pivotal role in the fields of pathology and biology, enabling the visualization of intricate tissue structures and cellular components. While traditional histological dyes, such as hematoxylin and eosin, have been effective in histological staining due to their established efficacy and reliability, growing concerns about the environmental impact and potential health risks associated with synthetic dyes have sparked interest in natural alternatives.It is known that plants with coloring and dyeing effects have been used in histological staining [5]. In this context, the potential utilization of *Brassica oleracea* ethanolic extract as a histologic dye warrants exploration and evaluation when compared to conventional dyes. This was investigated in this study.

In our result, the red cabbage extracts had an acidic pH of 4.89 which enables the staining of basic structures like cytoplasm, muscle fibers, and mucins. Thus the pH of the extract could be the reason explaining why some structures in the tissue pick up the color and stains more clearly than others.

The spectrophotometric curves of the red cabbage ethanol extract showed 3 peaks at 395, 545, and 670nm.[6] stated that the presence of more than 1 peak in an absorption curve is an indication of a mixture of more than one (1) dye and also for a dye to be established as a stain, it must have an absorption maximum, thus our result is in agreement with the suggestion by[7]. The maximum absorbance was 0.795 at the wavelength of 395nm and this is anindicative of the pigment cyanicilin which is the most common anthocyanin present in red cabbage. The other two pigments of anthocyanin are delphinidin and malvidin which showed absorbance at 0.220 at the wavelength of 545nm and 0.093 at the wavelength of 670nm wavelength respectively.

The histological staining of different tissue structures by anthocyanins of red cabbage extracts may be attributed to their physiochemical compositions and spectral properties. The addition of glacial acetic acid and aluminum sulfate acts as a mordant which tends to bind the dye with the tissue and also increase the color intensity of the stain[8]. Also, the majority of nuclei stained loosely indicating pH selectively as nuclei are mostly stained at a pH less than 4.0 as stated by[9]. This suggests that nuclear staining may have better results with adequate acidification.

Preliminary results from this study indicate that *Brassica oleracea* (Red Cabbage) ethanolic extract exhibits promising histologic staining properties. The pigments extracted from this source provide sufficient contrast and clarity of cellular structures, almost mirroring the performance of traditional dyes like the Hematoxylin and Eosin stain. This finding indicates the potential of *Brassica oleracea* ethanolic extract as a natural histologic dye, while also raising questions about the optimization of staining protocols and compatibility with various tissue types and staining techniques.

Future studies should focus on a wider range of histological applications, including specialized staining techniques and the staining of specific cellular components. Long-term studies are essential to investigate the stability and longevity of staining using *Brassica oleracea* ethanolic extract. Ultimately, the exploration of natural dyes like *Brassica oleracea* ethanolic extract opens new possibilities for environmentally responsible and safe histological practices.

The evaluation of *Brassica oleracea* ethanolic extract as a histologic dye in this study presents a promising natural alternative to traditional dyes, potentially contributing to ecofriendly and safer histological practices. While further research is essential to optimize protocols and assess practicality, the adoption of natural dyes in histology aligns with current sustainability trends in scientific research.

CONCLUSION

In conclusion, the study showed that red cabbage ethanol extract with acetic acid and aluminum sulphate is stable at 4^{o}C, with a colour or amber to light gold with a pH of 4.89 and a concentration of 1000 which gave good histological details to the extract stain basic structures of the cell such as the cytoplasm, muscle fibers and mucins.

Therefore, red cabbage ethanol extract can stain histological tissue structures and can be used as an ecofriendly alternative to eosin.

Declarations

Plant identification and ethical approval and consent to participate: The red cabbage was identified and authenticated in the Department of Plant and Ecological Studies, Faculty of Biological Sciences, University of Calabar, Calabar and given the identification number Bot/Herb/UCC/307. The Faculty Animal Research Ethics Committee of the Institution gave an outright approval for this research study to be carried out. The letter for this approval is listed as Figure 7.

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Conflict of interest: None

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