

Original Article : Open Access

Evaluation of nuclear staining potential of the medicinal *Hibiscus sabdariffa* L. flower extract in animal and poultry tissues

Riton Patra[♦], Santwana Palai^{*}, Srinivas Sathapathy, Nripendra Singh, Manish Gautom and Swagat Mohapatra^{**}

Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar-751 003, Odisha, India

^{*}Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar-751 003, Odisha, India^{**}Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar-751 003, Odisha, India.

Article Info

Article history

Received 12 April 2025

Revised 30 May 2025

Accepted 31 May 2025

Published Online 30 June 2025

Keywords

Hibiscus sabdariffa L.

Histology

Natural dye

Phytochemicals

Anthocyanins

Abstract

Staining histological slides with a dye is essential for their proper interpretation. Dyes of both natural and synthetic origin have long been employed in histological and cytological staining. However, the allergic and harmful side effects of synthetic dyes used in histology are prompting researchers to explore natural alternatives. Natural dyes are eco-friendly, affordable, non-toxic and visually appealing. The goal of this study was to evaluate the staining potential of antihypertensive and antihyperlipidemic *Hibiscus sabdariffa* L. flower extract, as a natural alternative to nuclear stain for animal and poultry tissues, and to identify the phytochemicals contributing to its staining properties. Phytochemical analysis using FTIR and UV-Vis characterization revealed absorption peak values for various functional groups, such as phenols, carboxylic acids, anthocyanins, and flavonoids in the extracts. Both methanolic and aqueous-methanolic extracts showed better nuclear staining activity (with different shades of blue) in all the tissue compared to aqueous extract. The methanolic extract of *H. sabdariffa* flower, mordanted with iron alum and applied for 30 min, produced a very satisfactory result, comparable to that of standard haematoxylin. The nuclei were clearly defined with the natural stain in both animal and poultry tissues. Thus, *H. sabdariffa* (Roselle) flower, being one of the richest natural anthocyanin resource, can contribute to the scaling-up of natural colorant production apart from its medicinal properties, which is important not only for histological labs but also for the food, pharmaceutical, and cosmetic industries.

1. Introduction

Natural dyes had been successfully replaced by commercially available artificial dyes, which led to a reduction in the use of natural dyes and the number of claims made about them (Henry, 2006). The poisonous and allergic effects of synthetic dyes employed in histology staining and fixation are damaging and represent a threat not only to researchers but also to ecosystems. Staining tissue sections is a subfield of histology. It is possible to make dyes that are non-allergic, non-toxic, and favourable to the environment in order to prevent the use of synthetic colours that could be detrimental. Natural dyes do not pose any health hazards, are easy to extract and purify, do not cause any waste, have a high level of sustainability, and employ gentle dyeing conditions (Samanta and Agarwal, 2009). Additionally, natural dyes are derived from renewable sources. They do not produce any harmful by-products, are safe for the environment, are pleasing to the eye, and exude a singular scent. The use of a mordant during the dyeing process might help minimize the downsides of lower colour reproducibility, changing composition, and moderate

washing fastness (Jabar *et al.*, 2020). Mordanted dyes have better colour fastness than non-mordanted dyes.

Hibiscus sabdariffa L., often known as roselle, is a member of the Malvaceae family and can be found growing wild in almost all warm countries, including India, Sudan, Thailand, Mexico, Philippines, Egypt, and China. In addition to its use as food and medicine, the seeds, leaves, fruits, and roots of the plant can all be consumed. It is used to treat conditions such as sore throats and coughs, as well as heart and liver conditions, neurological and cardiovascular disorders, metabolic syndrome, hyperlipidemia, hypertension, and cancer (Da-Costa-Rocha *et al.*, 2014). According to research, hibiscus calyx extracts possess powerful antioxidant, antihypertensive, antihypercholesterolaemic, antinociceptive, antibacterial, emollient, antipyretic, diuretic, antihelmintic, and sedative properties (Ali *et al.*, 2011). As a result, the use of this plant in the production of more advanced functional, useful, and therapeutic products is promising.

Because of their high content of polyphenols and anthocyanins, the calyces of the *H. sabdariffa* flower are commonly used as nutraceutical supplements. This is because they can improve the quality of fatty acids, preventing the oxidation of milk and meat, which would otherwise alter the products' colour, aroma, and flavour. Roselle acquires its antioxidant effect due to the presence of polyphenols (Jamrozik *et al.*, 2022). There are several different types of flavonoids exhibiting various medicinal properties, including flavonols, isoflavones, flavon-3,4-diols, flavones, and anthocyanidins (Palai *et al.*, 2020). Coumarins are another type of flavonoid.

Corresponding author: Dr. Riton Patra

Assistant Professor, Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India

E-mail: ritunpatra@ouat.ac.in

Tel.: +91-7978989299

Copyright © 2025 Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com

The majority of the phytochemical studies conducted on the components of *H. sabdariffa* have concentrated on the characterization of pigments, more notably anthocyanins (Jabeur *et al.*, 2017). Hibiscin, delphinidin, gossypicyanin, and cyanidin can all be found in extracts of Hibiscus flowers (Beye *et al.*, 2017). Malvidin, delphinidin, cyanidin, pelargonidin, peonidin, and malonyl acids, as well as malon, are examples of anthocyanins that may be discovered in plants and fruits (Mattioli *et al.*, 2020). In general, calyces of *H. sabdariffa* are used as natural food colorants (Pinela *et al.*, 2019). The characteristic red colour of these calyces is due to the presence of anthocyanins.

Better sources of extraction, identification, characterization, and reasonable recovery techniques are necessary in order to turn bio colorants into actual economic alternatives for their widespread use as synthetic equivalents. This will allow bio colorants to compete on an equal economic footing with synthetic histological staining dyes. Using FTIR and UV-Vis analysis, the goal of this study is to enhance the extraction and identification of anthocyanins found in the calyces of *H. sabdariffa* flowers, as well as the use of these anthocyanin as natural colorants in histology staining. In order to extract these colouring compounds in the most effective manner, we used the three varieties of solvents, *i.e.*, methanol, aqueous and aqueous-methanol (1:1) that were most pertinent to the process.

2. Materials and Methods

2.1 Collection and authentication of the *H. sabdariffa* flower

The hibiscus calyces were collected from mature *H. sabdariffa* plants in and around the areas of Bhubaneswar (latitude: 23.4855, longitude: 51.7927). The plant was authenticated by Dr. Debasis Dash, Assistant Professor, Department of Botany, College of Basic Science and Humanities, OUAT, and the herbarium was deposited in the same Department for future reference. The Voucher Specimen Number is 766/13.04.2024.

2.2 Extraction of the *H. sabdariffa* flower

Fresh flowers weighing 25 g each were used for extraction by maceration with various solvents (50 ml each), such as methanol, water, and water-methanol (1:1), at room temperature for 7 days. The crude extract was filtered through Whatman's filter paper (50 µm; Sigma, Bangalore, India), and the resultant extracts with different solvents were used in further investigation. Iron alum was then added at 1% to each type of extract. In total, 6 varieties (3 mordanted and 3 non-mordanted) of extracts were prepared to be used as staining agents. The reagents were of analytical grade.

2.3 FTIR characterization of the *H. sabdariffa* flower extracts

The Fourier transform infrared spectrometer (Model UTAR TWO, Perkin Elmer) was used to determine the functional groups of possible

phenolic compounds in various fresh hibiscus calyx extracts. The spectrum of the ATR sample base plate diamond instrument was an average of 8 scans, with a spectral resolution of 4 cm⁻¹. The spectral measurement range was 4000 to 450 cm⁻¹.

2.4 UV-Visible Spectrophotometry of the *H. sabdariffa* flower extracts

The filtered extracts were scanned using a UV-Visible Spectrophotometer (Make: Perkin Elmer, Model: Lambda 365), where the sample was illuminated with electromagnetic rays of 700-190 nm wavelengths to determine their λ-max using UV Express Version 4.1.1 software.

2.5 Collection of tissue sample, preparation of tissue sections and their staining with *H. sabdariffa* flower extract

The tissue samples, *i.e.*, skin, brain, liver, intestine, trachea, and adrenal gland of goat and poultry, were collected in 10% buffered neutral formalin from the local slaughter house as soon as the animals were sacrificed. They were processed routinely to obtain 6 µm thick paraffin sections. The tissue sections were stained and mounted according to the procedure for routine hematoxylin and eosin staining, except that instead of hematoxylin, the six types of *H. sabdariffa* flower extracts were used. For each extract, three durations were tested: 10 min, 20 min, and 30 min. After staining with each extract, the slides were dipped briefly in distilled water to remove any residue.

3. Results

3.1 *H. sabdariffa* flower extraction and characterisation

Natural colours are available in very small amounts in natural products. To extract dye from its original source, a specialized process with specific solvents must be used. In the present experiment, the materials were macerated using specific solvents, namely methanol and water, in order to extract natural colours from their parent sources. For the successful commercial use of natural dyes, it is necessary to characterize the dyes.

3.2 Physical characteristics and percentage yield of *H. sabdariffa* flower extracts

The extraction yield of *H. sabdariffa* in different solvent systems ranges from 13.71% to 22.78%. The extraction yields were 17.81%, 13.71%, and 22.78% for methanol, aqueous, and methanolic-aqueous (1:1) solvent systems, respectively. The colours of the extracts were light maroon, reddish maroon, and bluish maroon for the methanol, aqueous, and methanol-aqueous solvent systems, respectively. The texture of all the extracts was sticky and slimy (Table 1).

Table 1: Physical characteristics and % yield of *H. sabdariffa* calyx extracts

S. No.	Solvent	Colour of extract	Texture of extract	Percentage yield
1.	Methanol	Light maroon	Sticky and slimy	17.81
2.	Water	Reddish maroon	Sticky and slimy	13.71
3.	Methanol:water:1:1	Bluish-maroon	Sticky and slimy	22.78

3.3 FTIR analysis of *H. sabdariffa* flower extracts

The FTIR spectra showed 20 peaks in the methanolic extract, 10 peaks in the aqueous extract, and 11 peaks in the aqueous-methanolic extract. Peaks ranging from 3800 to 2500 cm^{-1} were typically associated with the O-H elongation vibration group, as evidenced by the peaks at 3307.2, 2953.03, 2842.08, and 2511.61 in the methanolic extract; 3326.18 in the aqueous extract; and 3309.61, 2884.3, and 2171.87 in the methanolic-aqueous extract. The presence of a ketone (C=O) group is observed in the peaks at 1643.98 cm^{-1} in the

methanolic extract, 1635.01 cm^{-1} in the aqueous extract, and 1639.24 cm^{-1} in the methanolic-aqueous extract (Figure 1).

The existence of (O-H bend and C-O stretch) is indicated by the peaks at 1450.45, 1408.95, and 1113.01 cm^{-1} in the methanolic extract and at 1450.9, 1409.05, and 1112.58 cm^{-1} in the aqueous-methanolic extract, which were absent in the aqueous extract. Further, more peaks were found in the methanolic extract and the methanolic-aqueous extract than in the aqueous extract in the range of 1650-400 cm^{-1} , indicating the presence of saccharides (Table 2).

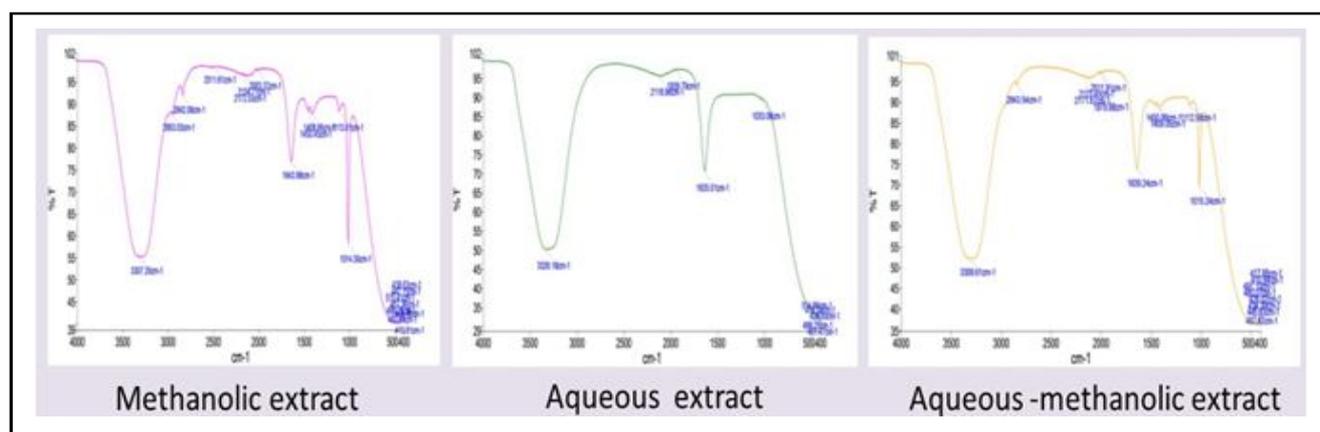


Figure 1: FTIR analysis (graphical form) of different extracts prepared from *H. sabdariffa* calyx.

Table 2: FTIR analysis of *H. sabdariffa* calyx extracts

Peak No.	Absorption (cm^{-1})		
	Methanolic extract	Aqueous extract	Methanolic-aqueous extract
Total number of peaks	20	10	11
1	3307.2	3326.18	3309.61
2	2953.03		
3	2842.08		2843.54
4	2511.61		
5	2172.53		2171.87
6	2134.71	2118.96	2123.45, 2011.91
7	2003.37	1939.79	1979.98
8	1643.98	1635.01	1639.24
9	1450.45		1450.9
10	1408.95		1409.05
11	1113.01		1112.58
12	1014.3	1033.06	1015.24
13	515.41	514.86	
14	499.25	499.25	491.1
15	482.59	475.74	480.07
16	457.36	451.47	457.62
17	447.15		445.63
18	438.63	426.5	434.39, 426.02
19	413.81		417.88
20	406.46		410.84

3.4 UV-Vis analysis of *H. sabdariffa* flower extracts

There were more peaks in the methanolic extract. Within the range of 230-285 nm, the methanolic extract exhibited peaks at 214.50, 220.05, 230.55, 242.90, 262.60, and 269.35 nm, while the aqueous extract

showed peaks at 215.75 and 260.60 nm. An absorption peak was recorded at 620.05 nm in the methanolic extract, 618.80 nm in the methanolic-aqueous extract, and 618.80 nm in the aqueous extract, all within the spectral range of 400 to 780 nm (Figure 2) (Table 3).

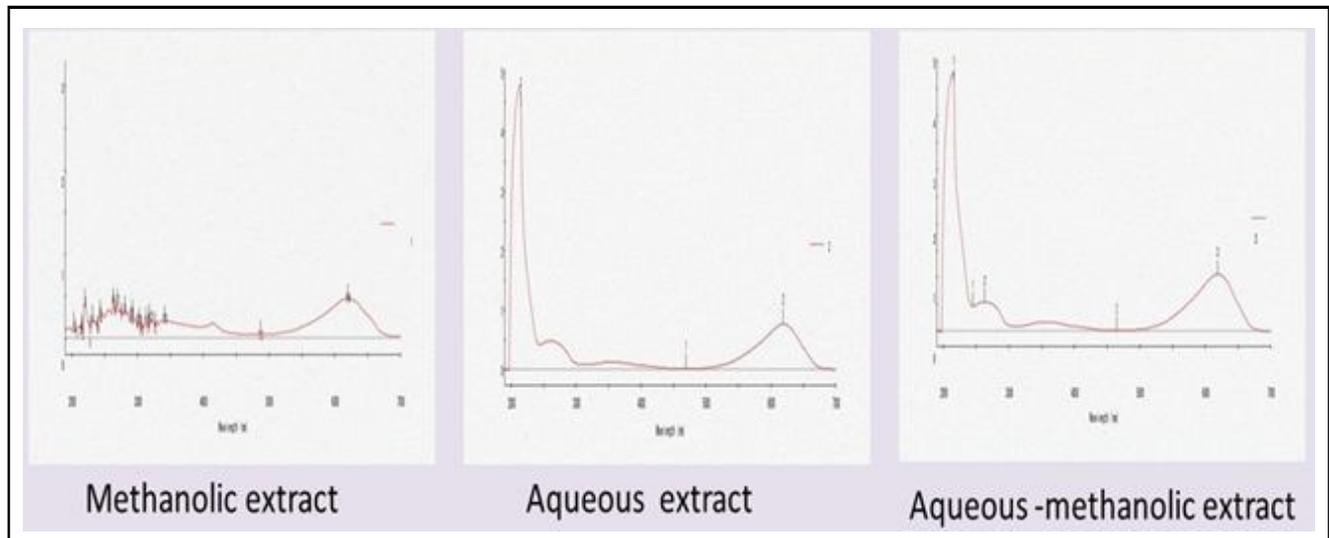


Figure 2: UV-Vis peaks (graphical form) of different extracts prepared from *H. sabdariffa* calyx.

Table 3: UV-Vis peaks of *H. sabdariffa* calyx extracts

Peak No.	UV-Vis peak positions (nm)		
	Methanolic extract	Aqueous extract	Methanolic-aqueous extract
1	203.40		
2	214.50	214.50	215.75
3	220.05		
4	230.55		
5	242.90		
6	262.60		262.60
7	269.35		
8	281.15		
9	292.35		
10	303.45		
11	313.30		
12	318.25		
13	340.45		
14	620.05	618.80	618.80

3.5 Histological staining evaluation of *H. sabdariffa* flower extracts

After staining the slides of different tissues from goat and poultry with various extracts of *H. sabdariffa* flower extracts, the slides were observed under the microscope and compared with those of hematoxylin and eosin-stained slides (taken as control slides). The extracts from *H. sabdariffa* flower imparted a bluish colour of different shades to the nucleus of tissue sections of both goat and

chicken (Figures 3a, b, c, d). The potential of the nuclear staining ability of wild hibiscus extracts was graded into four categories: poor, good, very good, and excellent. This grading was based on the observation of the stained slides under the microscope, taking into account criteria such as the appearance of the nuclear membrane, the density of the bluish tint in the nucleus, the staining contrast of the nucleus compared to the counterstain (*i.e.*, eosin), and the resolution of the image. Staining with each extract showed a positive result.

The methanolic extract of *H. sabdariffa* flower, moderated with iron alum, gave the best nuclear staining when applied for half an hour. All the tissue sections (*i.e.*, from skin, brain, liver, intestine, trachea, and adrenal gland) of both goat and chicken showed clear nuclear

staining with a combination of time periods of 20 and 30 min (Figures 4a, b, c, d). The three extracts without mordant (iron alum) gave poor and very faint nuclear staining when applied for only 10 min (Table 4).

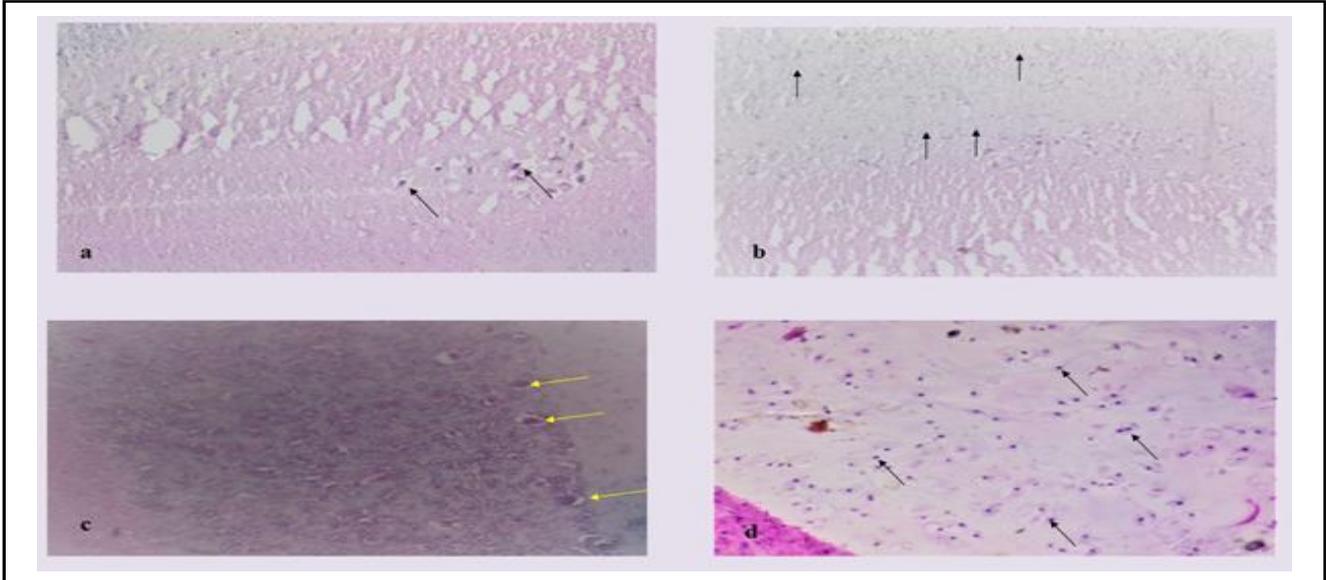


Figure 3: a. Photomicrograph of cerebrum of chicken showing prominent nucleus (arrow) of nerve cells stained deep blue with methanolic extract of rosella mordanted with iron alum, stained for 30 min. 100X, b Photomicrograph of cerebrum of chicken showing the nucleus (arrow) of nerve cells visible as light blue colour stained with methanol- aqueous (1:1) extract of rosella for 30 min. 100X, c Photomicrograph of cerebellum of goat showing the nucleus (arrow) of purkinje cells visible prominently as bluish-violet with methanolic extract of rosella mordanted with iron alum, stained for 30 min. 400X, d Photomicrograph of trachea of goat showing the nucleus (arrow) of chondrocytes visible prominently as deep blue colour with methanolic extract of rosella mordanted with iron alum for 30 min 100X.

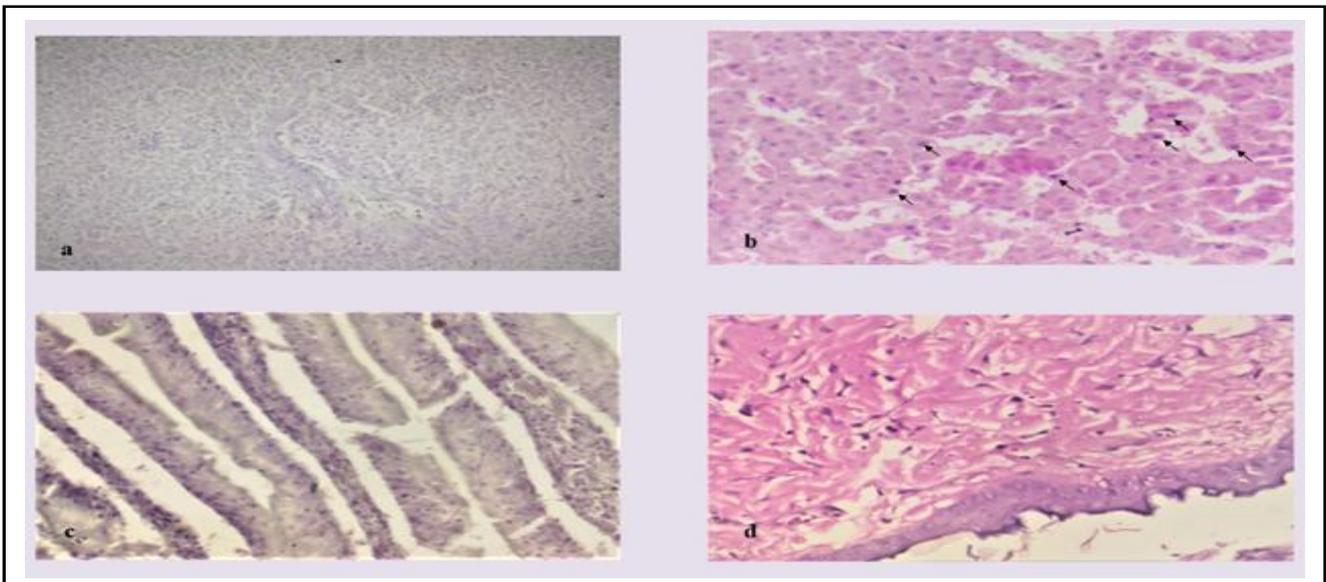
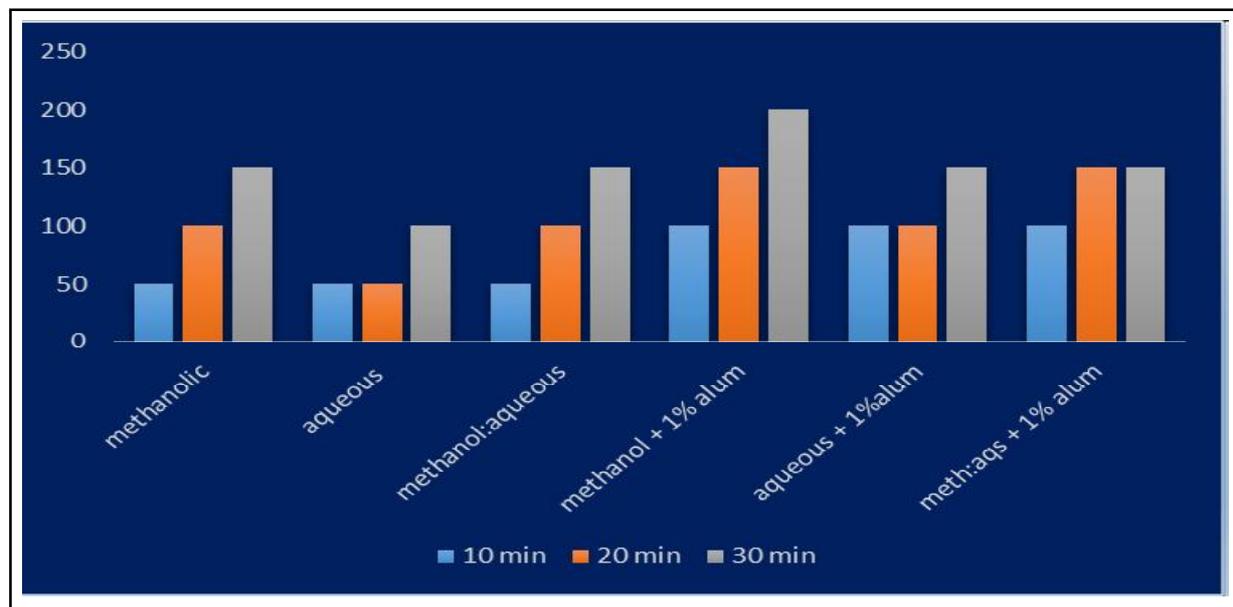


Figure 4: a. Photomicrograph of liver of goat showing the nucleus of hepatocytes visible as light blue colour with aqueous extract of rosella mordanted with iron alum stained for 20 min. 100X, b Photomicrograph of adrenal gland of goat showing the nucleus of cells of adrenal cortex (arrow) visible as light blue colour with methanolic- aqueous (1:1) extract of rosella mordanted with iron alum stained for 30 min. 400X, c Photomicrograph of duodenum of chicken showing the nucleus of enterocytes and the cells in the lamina propria visible as bluish violet colour with methanolic extract of rosella mordanted with iron alum, stained for 20 min. 100X, d Photomicrograph of skin of goat showing the nucleus of epidermal cells as faint blue colour and nucleus of connective tissue cells of dermis as deep blue colour with methanolic extract of rosella mordanted with iron alum, stained for 30 min 400X.

Table 4: Gradation of staining potential of *H. sabdariffa* flower extract on nucleus of goat and poultry tissue

Type of extract from <i>H. sabdariffa</i>	Duration of application as nuclear stain	Grading of staining efficacy	Scoring of the staining quality as per eye estimation
Methanolic extract	10 min	Poor	+
	20 min	Good	++
	30 min	Very good	+++
Aqueous extract	10 min	Poor	+
	20 min	Poor	+
	30 min	Good	++
Methanol:Aqueous (1:1)	10 min	Poor	+
	20 min	Good	++
	30 min	Very good	+++
Methanol extract with 1% iron alum	10 min	Good	++
	20 min	Very good	+++
	30 min	Excellent	++++
Aqueous extract with 1% iron alum	10 min	Good	++
	20 min	Good	++
	30 min	Very good	+++
Methanol:Aqueous (1:1) with 1% iron alum	10 min	Good	++
	20 min	Very good	+++
	30 min	Very good	+++

**Figure 5:** Bar diagram presenting gradation of staining efficacy of different *H. sabdariffa* flower extracts as nuclear stain. 50 = + (poor), 100 = ++ (good), 150 = +++ (very good), 200 = ++++ (excellent).

4. Discussion

The extract yields and colours were significantly different depending on the type of extraction solvent used. Characterization of the fresh *H. sabdariffa* flower extract was accomplished using FTIR spectroscopy to determine the presence of potentially bioactive components and assess the extract's potential for use in technological

applications such as histological staining. The peaks at 1014.3 cm^{-1} (methanolic extract), 1033.06 cm^{-1} (aqueous extract), and 1015.24 cm^{-1} (methanolic-aqueous extract) were located between 1100 and 1071 cm^{-1} . These peaks suggest the presence of anthocyanins (cyanidin-3-O-sambubioside and delphinidin-3-O-sambubioside) (Paraiso *et al.*, 2020). Additionally, in the range of 1650-400 cm^{-1} ,

more peaks were observed in the methanolic extract and methanolic-aqueous extract compared to the aqueous extract. These results indicate that the primary distinctive peaks of anthocyanin standards are present, supporting the combination of multiple-coloured compounds (Choong *et al.*, 2019).

In the spectrum range of 3800-2500 cm^{-1} , the methanolic extract showed higher peaks than the aqueous extract. This region corresponds to the phenol group (O-H elongation stretch), which is characteristic of natural compounds such as flavonoids responsible for antioxidant, antihypertensive properties (Choong *et al.*, 2019, Pradhan *et al.*, 2023). The IR spectra of solvent extracts from fresh hibiscus calyces revealed the presence of phenol, carboxylic acid, alkyl halides, alkanes, and ketones. FTIR and GCMS analyses have indicated that these constituents are responsible for the staining properties of the calyces (Alara and Abdurahman, 2019). A variety of solvents and aqueous crude extracts of bioactive compounds can form complexes by interacting with proteins and nucleic acids. These complexes can bridge dye molecules to tissue proteins, facilitating successful tissue-dye interactions (Veuthey *et al.*, 2014). This process is aided by bridging chemicals called mordants, which anchor the dye molecules to the tissue.

UV characterization of the *H. sabdariffa* flower was conducted to determine the capacity of dye molecules to absorb UV wavelengths and to assess the fading characteristics of dyes when exposed to various solvents. In *H. sabdariffa*, the peaks at 214.50 nm (methanolic extract), 214.50 nm (methanolic-aqueous extract), and 215.75 nm (aqueous extract) correspond to the presence of various natural moieties in the extract (Alshehri *et al.*, 2017). The findings revealed that the flavonoid spectral range of 230-285 nm showed a greater number of peaks in the methanolic extract (Kalaichelvi and Dhivya, 2017). The absorption peak observed at 620.05 nm (methanolic extract), 618.80 nm (methanolic-aqueous extract), and 618.80 nm (aqueous extract) within the spectrum range of 400 to 780 nm corresponds to absorption spectra at approximately 516 nm in the hibiscus dye extract. The different colours of the extracts and the types of functional groups present in the anthocyanins contributed to the variances in absorption properties (Mansa *et al.*, 2014).

The effectiveness of any histological staining depends largely on the clarity of the cell membranes, nuclear membranes, the clarity of the cytoplasm, and the contrast between the nucleus, cytoplasm, and extracellular matrix (Mohamed *et al.*, 2012). If, all of these criteria are clearly visible and no noticeable artefacts are present, the staining potential is rated as excellent. The grade of the staining is classified as very good, good, or poor, depending on whether there is a lack of clarity in one, two, or three of the cell components listed above. Based on this concept, which was applied in this experiment to evaluate the staining ability of various *H. sabdariffa* flower extracts, the methanolic extract of hibiscus calyx demonstrated outstanding nuclear staining capability when stained for 30 min with 1% iron alum as the mordant. This suggests that the mordant plays a role as an agent that bridges the gap between the nucleus and the dye component in the extract. Therefore, this extract could be used as a powerful alternative to synthetic stains in nuclear staining processes. The natural dye derived from *H. sabdariffa* flower is readily available, easy to extract, inexpensive, and environment friendly. It also has a wide range of applications in nutrition and therapy (Alshamer and Dapson, 2020). It has also been reported that methanolic extracts

from *H. sabdariffa* flower can be used as a staining agent for some fungi (Asenge *et al.*, 2012), helping to reduce the complications associated with over-dependence on toxic, expensive, and rarely available synthetic stains. These findings corroborate the results of the present study, confirming their accuracy. In a study of rabbit testicular tissue, a water extract of *H. sabdariffa* was used as a nuclear stain, albeit with slight adjustments. Mordanting the water extract with iron alum produced successful results (Egbujo *et al.*, 2008). When applied to formaldehyde fixed paraffin tissue sections (4 micrometers thick), an extract made by combining *H. sabdariffa* flower extract with ferric chloride, sodium chloride, and glacial acetic acid exhibited nuclear staining comparable to routine hematoxylin. Due to its excellent nuclear staining properties, it has the potential to serve as a progressive alternative to traditional nuclear stains (Benard *et al.*, 2015). This result supports the conclusions drawn in the current study.

This work demonstrates that the colours derived from *H. sabdariffa* flowers belong to the anthocyanin group, which is responsible for the efficient staining of nuclear material. When using methanol, aqueous, and a mixture of methanol and aqueous solvents to dilute the colours, the methanol and aqueous solvents provided the highest efficiencies for anthocyanin extraction from *H. sabdariffa* (Al-Alwani *et al.*, 2017; Rosli *et al.*, 2021). Phytochemical analysis of *H. sabdariffa* extracts revealed the presence of secondary metabolites such as flavonoids responsible for antioxidant, antimicrobial, and antihyperlipidaemic properties (Abdel-Shafi *et al.*, 2019; Salem *et al.*, 2021).

5. Conclusion

Given the need for alternative synthetic pigments in histological staining, it was assumed that natural plant extracts would be of interest. According to this study, all extracts obtained from *H. sabdariffa* (Roselle) flowers contain several phytochemicals such as phenols, flavonoids, anthocyanins, and others. The higher the polarity of the solvent, the more phytochemicals was detected in the methanolic extract. Natural pigments are generally less stable than synthetic colorants; however, this can be remedied by using mordants. So in the present research work, the methanolic extract mordanted with 1% iron alum gave the excellent nuclear staining with nuclear detail in all the tissues taken. FTIR and UV-Vis fingerprinting confirmed the presence of flavonoids and anthocyanins in various extracts of *H. sabdariffa* flower, which may contribute to the stability of the plant pigments. Thus, our findings suggest that *H. sabdariffa* calyces can be used as a viable alternative to chemical dyes in the production of bio-based colorants. Due to its high concentration of anthocyanins, it may contribute to the growth of natural colorant manufacturing, which is of interest in veterinary diagnostics (histopathology) and for industrial suppliers in the food, pharmaceutical, and cosmetic industries.

The *H. sabdariffa* flower extract can be further exploited to assess its efficacy as a histochemical stain, specifically whether it has the ability to stain a particular cell or component of a tissue. Exploring this as natural stain both in form of a nuclear stain and histochemical stain would help the researchers to avail a better contrast for better differentiation in histopathological, histological and toxicological studies or diagnostic studies at less cost without any harmful residual effect.

Acknowledgments

The authors express heartfelt thanks to the Central Instrumentation Facility (CIF), OUAT, Bhubaneswar-751003, Odisha for providing instrumental facilities.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

- Abdel-Shafi, S.; Al-Mohammadi, A.R.; Sitohy, M.; Mosa, B.; Ismaiel, A.; Enan, G. and Osman, A. (2019). Antimicrobial activity and chemical constitution of the crude, phenolic-rich extracts of *Hibiscus sabdariffa*, *Brassica oleracea* and *Beta vulgaris*. *Molecules*, **24**(23):4280.
- Alara, O.R. and Abdurahman, N.H. (2019). GC-MS and FTIR analyses of oils from *Hibiscus sabdariffa*, *Stigma maydis* and *Chromolaena odorata* leaf obtained from Malaysia: Potential sources of fatty acids. *Chemical Data Collections* **20**:100200, <https://doi.org/10.1016/j.cdc.2019.100200>.
- Ali, M.K.; Ashraf, A.; Biswas, N.N.; Karmakar, U.K. and Afroz, S. (2011). Antinociceptive, anti-inflammatory and antidiarrheal activities of ethanolic calyx extract of *Hibiscus sabdariffa* Linn. (Malvaceae) in mice. *Journal of Complementary and Integrative Medicine*, **9**(6):626-631. doi: 10.3736/jcim20110608.
- Al-Alwani, M.A.M.; Mohamad, A.B.; Kadhum, A.A.H.; Ludin, N.A.; Safie, N.E.; Razali, M.Z.; Ismail, M. and Sopian, K. (2017). Natural dye extracted from *Pandanus amaryllifolius* leaves as sensitizer in fabrication of dye-sensitized solar cells. *International Journal of Electrochemical Science*, **12**(1):747-761, <https://doi.org/10.20964/2017.01.56>.
- Alshamer, H.A. and Dapson, R.W. (2020). Use of roselle extracted from *Hibiscus sabdariffa* for histological staining: A critical review and rational stain formulation. *Biotechnic and Histochemistry*, **96**(2):94-101. DOI:10.1080/10520295.2020.1769864.
- Alshehri, A.; Malik, M.A.; Khan, Z.; Al-Thabaiti, S.A. and Hasan, N. (2017). Biofabrication of Fe nanoparticles in aqueous extract of *Hibiscus sabdariffa* with enhanced photocatalytic activities. *RSC Advances*, **7**:25149–25159. <https://doi.org/10.1039/c7ra01251a>.
- Asenge, I.G.H.; Abioye, J. and Dick, S. (2012). Application of methanolic extracts from *Hibiscus sabdariffa* L. a biological staining agent for some fungal species. *The International Journal of Plant, Animal and Environmental Sciences*, **2012**(2012): <https://api.semanticscholar.org/CorpusID:90292626>.
- Benard, S.; Abdurrahman, O.L.A.M.; Fowotade, A.A. and Afolabi, O.O. (2015). *Hibiscus sabdariffa* extract as haematoxylin substitute in the histological demonstration of brain tissues. *African Journal of Cellular Pathology*, **5**:32-35.
- Beye, C.; Hiligsmann, S.; Tounkara, L.S. and Thonart, P. (2017). Anthocyanin content of two *Hibiscus sabdariffa* cultivars grown in Senegal. *Agronomie Africaine Sp.*, **29**(1):63-68.
- Choong, Y.K.; Mohd Yousof, N.S.A.; Jamal, J.A. and Wasiman, M.I. (2019). Determination of anthocyanin content in two varieties of *Hibiscus sabdariffa* from Selangor, Malaysia using a combination of chromatography and spectroscopy. *Journal of Plant Science and Phytopathology*, **3**:67-75. <https://doi.org/10.29328/journal.jpssp.1001034>.
- Da-Costa-Rocha, I.; Bonnlaender, B.; Sievers, H.; Pischel, I. and Heinrich, M. (2014). *Hibiscus sabdariffa* L. : A phytochemical and pharmacological review. *Food Chemistry*, **165**(2):424-443, <https://doi.org/10.1016/j.foodchem.2014.05.002>.
- Egbujo, E.C.; Adisa, O.J. and Yahaya, A.B. (2008). A study of the staining effect of roselle (*Hibiscus sabdariffa*) on the histologic section of the Testis. *International Journal of Morphology*, **26**(4):927-930. <https://doi.org/10.4067/S0717-95022008000400022>.
- Henry, S.W.H. (2006). A review of his life, work and legacy. *Coloration Technology*, **122**:235-251. <https://doi.org/10.1111/j.1478-4408.2006.00041.x>.
- Jabar, J.M.; Ogunmokun, A.I. and Taleat, T.A.A. (2020). Color and fastness properties of mordanted *Bridelia ferruginea* B dyed cellulosic fabric. *International Journal of Interdisciplinary Research*, **7**(1):1-13. <https://doi.org/10.1186/s40691-019-0195-z>.
- Jabeur, I.; Pereira, E.; Barros, L.; Calheta, R.C.; Sokovic, M.; Oliveira, M.B.P.P. and Ferreira, I.C.F.R. (2017). *Hibiscus sabdariffa* L. as a source of nutrients, bioactive compounds and colouring agents. *Food Research International*, **100**(1):717-723. <https://doi.org/10.1016/j.foodres.2017.07.073>.
- Jamrozik, D.; Borymska, W. and Kaczmarczyk-ebrowska, I. (2022). *Hibiscus sabdariffa* in diabetes prevention and treatment does it work? An evidence-based review. *Foods*, **11**(14):2134. <https://doi.org/10.3390/foods11142134>.
- Kalaichelvi, K. and Dhivya, S.M. (2017). Screening of phytoconstituents, UV-VIS spectrum and FTIR analysis of *Micrococca mercurialis* (L.) Benth. *International Journal of Herbal Medicine*, **5**(6):40-44.
- Mansa, R.F.; Govindasamy, G.; Farm, Y.Y.; Bakar, H.A.; Dayou, J. and Sipaut, C.S. (2014). Hibiscus flower extract as a natural dye sensitizer for a dye-sensitized solar cell. *Journal of Physical Science*, **25**(2):85-96.
- Mattioli, R.; Francioso, A.; Mosca, L. and Silva, P. (2020). Anthocyanins: A Comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. *Molecules*, **25**(17):3809. doi: 10.3390/molecules25173809.
- Mohamed, B.B.; Sulaiman, A.A. and Dahab, A.A. (2012). Roselle (*Hibiscus sabdariffa* L) in Sudan, cultivation and their uses. *Bulletin of Environment, Pharmacology and Life Sciences*, **1**(6):48-54.
- Palai, S.; Dehuri, M. and Patra, R. (2020). Spices boosting immunity in COVID-19. *Ann. Phytomed.*, **9**(2):80-96.
- Paraiso, C.M.; dos Santos, S.S.; Ogawa, C.Y.L.; Sato, F.; dos Santos, O.A.A. and Madrona, G.S. (2020). *Hibiscus sabdariffa* L. extract: Characterization (FTIR-ATR), storage stability and food application. *Emirates Journal of Food and Agriculture*, **32**(1):55-61. <https://doi.org/10.9755/EJFA.2020.V32.I1.2059>.
- Pinela, J.; Prieto, M.A.; Pereira, E.; Barreiro, M.F.; Barros, L. and Ferreira, I.C.F.R. (2019). Optimization of heat- and ultrasound-assisted extraction of anthocyanins from *Hibiscus sabdariffa* calyces for natural food colorants. *Food Chemistry*, **275**:309-321. <https://doi.org/10.1016/j.foodchem.2018.09.118>.
- Pradhan, S.; Palai, S.; Dash, J.R.; Patra, R.; Sahoo, P.R.; Behera, P.C. and Parija, S.C. (2023). Characterisation of selenium nanoparticles and *Phyllanthus niruri* Hook. selenium nanoparticles with histopathological investigation of their effects on cadmium-induced gastric toxicity in wistar rats. *Ann. Phytomed.*, **12**(1):367-75.

Rosli, N.; Sabani, N.; Shahimin, M.M.; Juhari, N.; Shaari, S.; Ahmad, M.F. and Zakaria, N. (2021). Dyes extracted from *Hibiscus sabdariffa* flower and *Pandanus amaryllifolius* leaf as natural dye sensitizer by using an alcohol based solvent. Journal of Physics: Conference Series, 1755:012025. <https://doi.org/10.1088/1742-6596/1755/1/012025>.

Salem, M.A.; Zayed, A.; Beshay, M.E.; AbdelMesih, M.M.; Ben Khayal, R.F.; George, F.A. and Ezzat, S.M. (2021). *Hibiscus sabdariffa* L.: phytoconstituents,

nutritive, and pharmacological applications. Advances in Traditional Medicine, 2021:1-1.

Samanta, A.K. and Agarwal, P. (2009). Application of natural dyes on textiles. Indian Journal of Fibre and Textile Research, 34(4):384-399.

Veuthey, T.; Herrera, G. and Dodero, V.I. (2014). Dyes and stains: from molecular structure to histological application. Front Biosci (Landmark Ed), 19(1):91-112. doi: 10.2741/419, PMID: 24389174.

Citation

Ritun Patra, Santwana Palai, Srinivas Sathapathy, Nripendra Singh, Manish Gautam and Swagat Mohapatra (2025). Evaluation of nuclear staining potential of the medicinal *Hibiscus sabdariffa* L. flower extract in animal and poultry tissues. Ann. Phytomed., 14(1):1131-1139. <http://dx.doi.org/10.54085/ap.2025.14.1.113>.