

Zingiber officinale (ginger) extract as a histological dye for muscle fibers and cytoplasm

Ayodeji Blessing Ajileye, Afoke Kingsley Iteire, Queen Bisi Arigi

Department of Basic Medical Sciences, Achievers University, Owo, Ondo State, Nigeria.

Correspondence to: Ayodeji Blessing Ajileye, E-mail: ayobless05@gmail.com

Received February 21, 2015. Accepted June 22, 2015

Abstract

Background: The rhizome of *Zingiber officinale* belongs to the family Zingiberaceae, which has a deep yellow color. It is generally called ginger and consumed whole as a medicine or used as a spice in cooking, manufacturing drinks, and tea. Previous studies have shown the importance of this plant in traditional medicine for the treatment of several ailments and even in the dye industry. The later use could be owing to its deep yellowish coloration, which could benefit the histologist and histoscientist when used in an improvised manner. Therefore, this study was carried out to determine the staining potential of crude ethanolic extract of *Z. officinale* on tissue sections taken from the heart, lungs, spleen, and liver of Wistar rats.

Objective: To study *Z. officinale* (ginger) extract as a histological dye for muscle fibers and cytoplasm.

Materials and Methods: Tissue samples were obtained from the heart, spleen, lungs, and liver of adult Wistar rats. The tissues were histologically processed and sectioned with a rotary microtome. *Zingiber officinale* was obtained from a local store in Owo, Ondo state, Nigeria, and extracted with 90% ethanol, thereby giving rise to a crude extract, which was then used to counterstain the sections earlier stained with alum hematoxylin. The control tissue sections were correspondingly stained with hematoxylin and counterstained with eosin.

Result: The ethanolic extract of *Z. officinale*, when used as a counterstain for alum hematoxylin, stained the muscle fibers and the cytoplasm yellowish, and the nuclei stained deeply green within 4 min. The staining reaction was suspected to be similar to alum hematoxylin and eosin, except for its greenish yellow color.

Conclusion: *Zingiber officinale* is a promising histological natural dye that is not only cheap but also readily available.

KEYWORDS: *Zingiber-officinale*, ethanolic, liver, heart, spleen

Introduction

The rhizome of *Zingiber officinale* belongs to the family Zingiberaceae, which has a deep yellow color when compared with *Curcuma longa*, which is a similar species. The characteristic odor and flavor of ginger root is caused by a mixture of zingerone, shoals and gingerols, and volatile oils

that is composed of about 1%–3% of the weight of fresh ginger. *Zingiber officinale* contains up to 3% of a fragrant essential oil, with sesquiterpenoids with zingiberene as the main components.^[1] Small amounts of other sesquiterpenoids (β -sesquiphellandrene, bisabolene, and farnesene) and a small fraction of monoterpenoid (β -phelladrene, cineol, and citral) have also been identified.^[1] *Zingiber officinale* contains flavonoids, which is a polyphenolic compound, several coloring compounds, and phenols are acidic owing to their ability to release the hydrogen from their hydroxyl group. Furthermore, Evans^[2] stated that the characterization of the constituents of a polysaccharide fraction of *Z. officinale* demonstrated new acid glycans designated as ukanons A,B,C, and D, in addition to small amount of peptide moieties. Dyes are compounds that impact color on another substance.^[3] The advent of stain had made cellular differentiation of tissues more

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2015.21022015296	

International Journal of Medical Science and Public Health Online 2015. © 2015 Ayodeji Blessing Ajileye. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), allowing third parties to copy and redistribute the material in any medium or format and to remix, transform, and build upon the material for any purpose, even commercially, provided the original work is properly cited and states its license.

accurate, because stains increase the optical differentiation of cellular elements either by an alteration of contrast or impartation of color.^[4] Dyes are classified into natural and synthetic dyes.^[4] Hematoxylin, which is obtained from the Mexican tree *Haematoxylon campechianum*, is an example of a natural dye that is widely used in histology and histochemistry, while eosin is a synthetic dye also used in histology.^[5] Synthetic dyes are more efficient but have been reported to be hazardous to human health, thereby resulting to the cutback in their usage.^[6] With the global concern over the use of ecofriendly and biodegradable materials, the use of natural dyes has again gained importance.^[7] Moreover, as many developing countries can no longer afford the ever increasing cost of synthetic dyes, the use of cheaper, naturally occurring dyes from plants are being viewed as an alternative to synthetic dyes.^[8] There are several researchers who have examined the potential of natural dyes for use in histopathology; Avwioro et al.^[9] were able to ascertain the staining ability of *Curcuma longa* (ginger) extract as a histological dye for collagen fibers and red blood cells.^[9]

Materials and Methods

Extraction of *Zingiber officinale* Staining Solution

Rhizomes of the Allepey cultivar of *Z. officinale* were bought fresh from Oja Oba market in Owo, Ondo state, Nigeria. The rhizomes of *Z. officinale* were washed in water to remove sand and other dirt, after which they were peeled and cut into smaller pieces. The pieces were mixed with 90% alcohol (ethanol). After 24h, it was filtered with the aid of a funnel and some cotton wool and latter with Whatman filter paper.

Study Area

This research work was carried out at the Department of Basic Medical Sciences, Achievers University, Owo, Ondo State, Nigeria.

Experimental Animals

Ten adult Wistar rats of both sexes were obtained from the animal house of the Department of Basic Medical Sciences, Achievers University, Owo, Ondo State, Nigeria. The animals were in healthy conditions and weighed 150–250g. On transfer to the experimental location, the rats were allowed to acclimatize for 2 weeks under favorable climatic conditions and were properly fed.

Ethical Clearance

This study was approved by the research ethics committee of the Department of Basic Medical Sciences, Achievers University, Owo, Ondo state, Nigeria, and the experiment was carried out in strict accordance with the guidelines for the care and use of animals for research.

Samples Collection and Preparations for Histology

At the end of the acclimatization period, the rats were killed, and the hearts, spleens, lungs, and livers were harvested. The tissues were fixed immediately in 10% formal

saline for 24h, and standard manual tissue-processing techniques were used. Sections of about 3–5µm were cut by a SLEE medical rotary microtome. Some of the tissue sections from the heart, lungs, liver, and spleen were stained with alum hematoxylin (Harris hematoxylin) and counterstained with 90% ethanolic extract of *Z. officinale*, while the remainder were stained with alum hematoxylin (Harris hematoxylin) and counterstained with eosin, which served as the control. The stained slides of both the groups were viewed and analyzed with the aid of a light microscope for histological similarities and differences,^[2] thereafter, captured with a Brunel light microscope, 20 mega pixels (Brunel SP35 Digital Trinocular).

Staining Procedure (using Harris Hematoxylin and *Zingiber officinale*)

- Tissue sections of lungs, heart, liver, and spleen were dewaxed in xylene for 15 min.
- Sections were hydrated through descending grades of absolute (95%, 80%, and 70%) alcohol.
- Sections were rinsed in water briefly.
- Sections were stained in Harris hematoxylin for 4 min.
- Sections were rinsed in Scott's tap water to remove excess stain.
- Sections were differentiated in 1% acid alcohol for 3 s.
- Sections were blued in running tap water for 15 min.^[4]
- Sections were now counterstained with 90% ethanolic extract of *Z. officinale* stain for 4 min.
- Sections were finally rinsed in water, dehydrated in ascending grades of absolute alcohol (70%, 80%, and 90%), cleared in xylene, and mounted in a synthetic mountant.
- Prepared slides were allowed to air dry and examined under a light microscope, and photomicrographs were taken.

Staining Procedure for Control Section

Control sections for general tissue structure were stained by hematoxylin and eosin techniques.

- Tissue sections of lungs, heart, liver, and spleen were dewaxed in xylene for 15 min.
- Sections were hydrated through descending grades of absolute (95%, 80%, and 70%) alcohol.
- Sections were rinsed in water briefly.
- Sections were stained in Harris hematoxylin for 4 min.
- Sections were rinsed in Scott's tap water to remove excess stain.
- Sections were differentiated in 1% acid alcohol for 3 s.
- Sections were blued in running tap water for 15 min.
- Sections were now counterstained with eosin.
- Sections were finally rinsed in water, dehydrated in ascending grades of absolute alcohol (70%, 80%, and 90%), cleared in xylene, and mounted in a synthetic mountant.
- Prepared slides were allowed to air dry and examined under a light microscope, and photomicrographs were taken.^[4]

Reagents Used

Harris hematoxylin, 90% alcohol, *Z. officinale* extract stain, eosin, and 1% acid-alcohol.

Result

Zingiber officinale extract solution stained the muscle fibers and cytoplasm yellow and the nuclei deep green within 4 min when it was used as a counterstain for hematoxylin. The staining reaction was observed to be similar to eosin, except for its greenish yellow color [Figures 1–8].

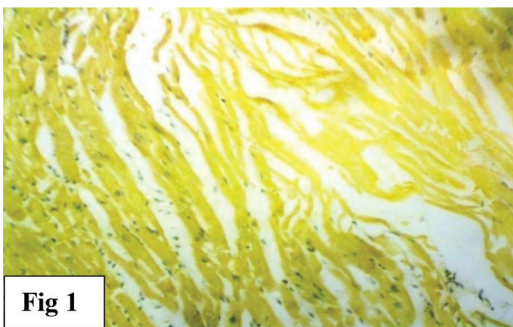


Fig 1

Figure 1: A heart tissue section counterstained with 90% ethanolic extract of *Z. officinale*; the muscle fibers were stained yellow and the nuclei deep green

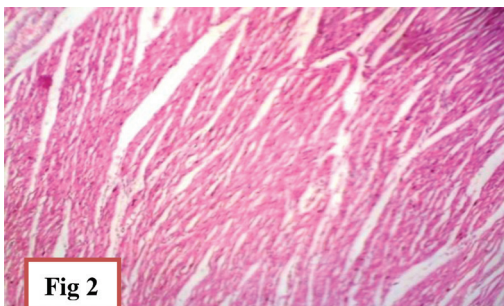


Fig 2

Figure 2: A section of the rat heart stained with hematoxylin and eosin consisting of cardiac muscle, intercalated disc, and cytoplasmic striations.

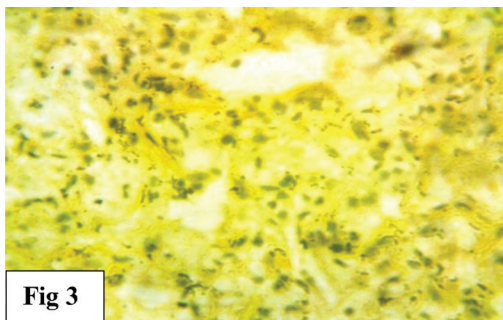


Fig 3

Figure 3: A lung tissue section stained with 90% ethanolic extract of *Zingiber officinale*; the lungs showed the respiratory bronchioles, which contained a small number of alveoli, and the alveolar sacs were stained yellow.

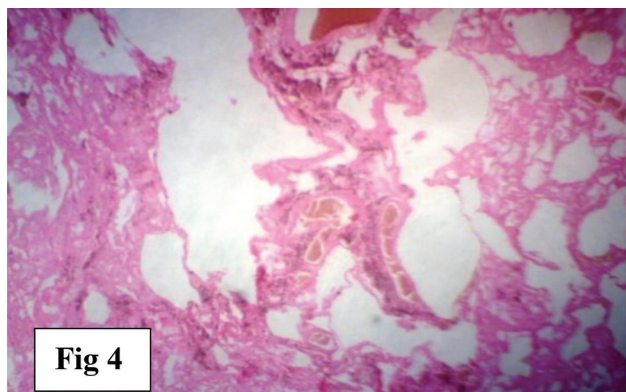


Fig 4

Figure 4: A rat lung tissue section stained with hematoxylin and eosin. The section showed the terminal bronchiole, respiratory bronchioles, and the alveolar sac, which also stained pinkish

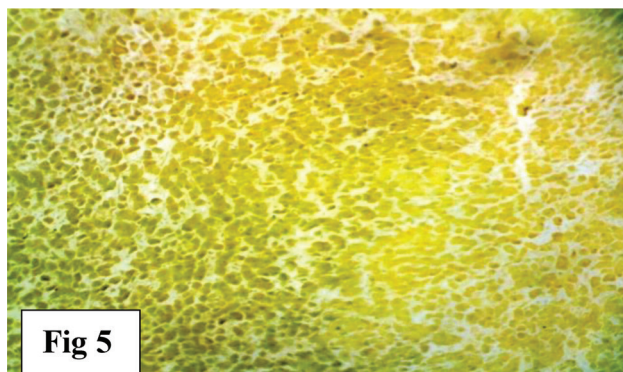


Fig 5

Figure 5: A liver tissue section counterstained with 90% ethanolic extract of *Z. officinale*. The section showed the portal tract, and the collagenous tissue were stained greenish yellow, while the nuclei were stained deep green

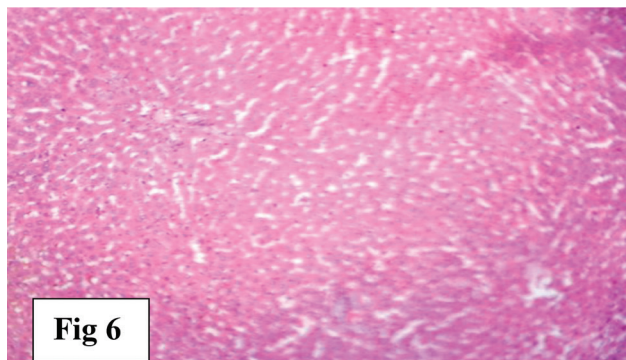


Fig 6

Figure 6: A section of the rat liver stained with hematoxylin and eosin consisting of hepatic vein and portal tract.

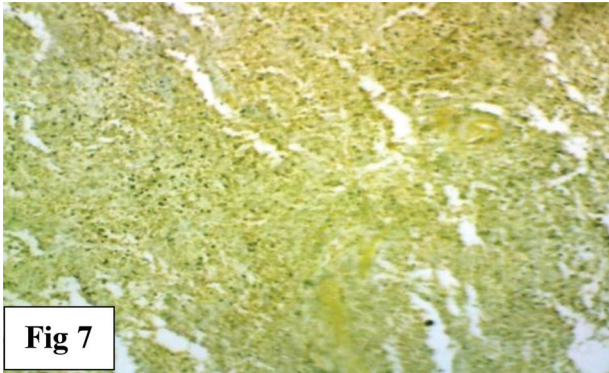


Fig 7

Figure 7: A spleen tissue section counterstained with 90% ethanolic extract of *Z. officinale*. The section showed the central artery, which was stained yellow. The white pulp and red pulp were not properly stained and seen.

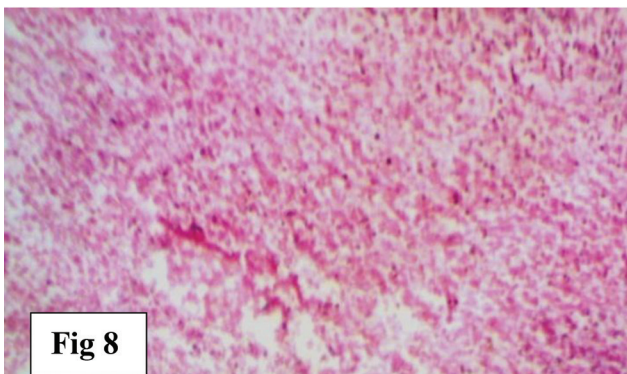


Fig 8

Figure 8: A section of the rat spleen stained with hematoxylin and eosin.

Discussion

Zingiber officinale contains several coloring compounds. The ability of a dye to stain specific tissue structure is determined by certain factors, one of which is the acidity of the stain.^[6] Acidic structures would be stained by basic dyes, while basic structures would be stained by acidic dyes.^[4,5,8] Owing to the strong affinity of *Z. officinale* for the cytoplasm, it can be inferred that the plant is acidic in nature, because it stained muscle fibers yellow, cytoplasm yellow, and the nuclei deeply green. *Zingiber officinale* contains flavonoids, which are typically polyphenolic compounds. Phenols are acidic because of their ability to release hydrogen from their hydroxyl group. Hence, this gives the extract solution of *Z. officinale* the ability

to stain the basic part of the cell. When *Z. officinale* extract was used as a counterstain for hematoxylin, the nuclei took deep green coloration, which enabled a clear contrast to be made between the different structures of the cells.

This shows that the reaction of the *Z. officinale* stain is similar to the reaction of eosin in the hematoxylin and eosin technique, except for its deep yellow coloration.

Conclusion

In conclusion, the ethanolic extract of *Z. officinale* is a promising natural histological dye that will stain both the muscle fibers and the cytoplasm of a tissue section. An 90% ethanolic extract of *Z. officinale* is cheap to prepare and readily available, and it could serve as a useful alternative to eosin in developing countries.

References

1. Bhuyan R, Saikia CN. Isolation of colour components from natedye bearing plants in north eastern India. *Bioresour Technol* 2005;96(3):363–75.
2. Evans A. *Pharmacopoeal and Related Drugs of Biological Origin Pharmacognosy*. London, UK: W.B Saunders, 1998.
3. Avwioro OG. *Histochemistry and Tissue Pathology*, 1st edn. Ibadan: Clavarianum Centre, 2002. pp. 134–40.
4. Carleton H. *Histological Techniques*, 4th edn. Oxford, UK: Oxford University Press, 1967. pp. 437–50.
5. Baker FJ, Silverton RE, Pallister CJ. *Introduction to Medical Laboratory Technology*. London, UK: Bounty Press limited, 1998. pp. 220–42.
6. Avwioro OG, Aloamaka PC, Ojiana MV, Oduola T, Ekpo EO. Pterocarpous osun stains at neutral red pH. *African J Biotech* 2005;460–2.
7. Eom S, Shin D, Yoon K. Improving the dye ability of natural colourants on cotton by cationization. *Ind J FibreText Res* 2001;26:425–31.
8. Singer M. Staining of tissue sections with acid and basic dyes. *Int Rev Cytol* 1952;1:211–55.
9. Avwioro OG, Onwuka SK, Moody JO, Agbadahunsi JM, Oduola T, Ekpo OE, et al. *Curcuma longa* extract as a histological dye for collagen fibres and red blood cells. *J. Anat* 2007;210(5):600–3.

How to cite this article: Ajileye AB, Iteire AK, Arigi QB. *Zingiber officinale* (ginger) extract as a histological dye for muscle fibers and cytoplasm. *Int J Med Sci Public Health* 2015;4:1445-1448

Source of Support: Nil, **Conflict of Interest:** None declared.