DOI: 10.7860/JCDR/2026/84858.23923

Original Article

Dentistry Section

A Comparative Evaluation of *Beta Vulgaris* and *Zingiber officinale* Staining with Conventional Haematoxylin and Eosin: An Experimental Study

SANGEETHA RAMU[1], INCHARA[2]

ABSTRACT

Introduction: Exfoliative cytological staining is a fundamental technique in pathology, providing contrast to cellular components for microscopic examination. Haematoxylin and Eosin (H&E) stain is the gold standard in cytopathology; however, concerns about the health and environmental hazards of synthetic stains necessitate the exploration of natural alternatives. Beetroot (*Beta vulgaris*)and ginger (*Zingiber officinale*) possess natural staining properties, potentially serving as substitutes for haematoxylin and eosin, respectively.

Aim: To evaluate the staining efficacy of beetroot (*Beta vulgaris*) and ginger (*Zingiber officinale*) extracts as alternatives to conventional H&E staining in exfoliative cytology smears from oral white lesions.

Materials and Methods: The present experimental study was conducted on exfoliative buccal smears obtained from patients with oral white lesions at the Department of Oral Medicine and Oral Pathology, Dayananda Sagar College of Dental Sciences, Bengaluru, Karnataka, India.The study duration was from February 2023 to October 2023, with a total sample size of 80 buccal smears from patients aged 18-60 years with clinically diagnosed oral white lesions (n=20 per group). Group 1 (Control) received conventional H&E staining, Group 2 received beetroot extract and eosin, Group 3 received ginger extract and haematoxylin, and Group 4 received beetroot and ginger extracts with vinegar mordant. Staining quality was scored (0-2 scale) by a calibrated pathologist for presence/absence of staining, nuclear staining intensity, cytoplasmic staining intensity, and clarity/crispness. Data were analysed using Statistical Package for the Social Sciences (SPSS) Software version 26.0. Chi-square test was used for Intergroup comparisons ($p<0.05$ considered significant).

Results: Presence of staining was observed in 20/20 (100%) of smears (Group 1), 17/20 (85%) (Group 2), 17/20 (85%) (Group 3), and 15/20 (75%) (Group 4) ($p=0.136$). Strong nuclear staining was observed in 18/20 (90%) (Group 1), 14/20 (70%) (Group 2), 17/20 (85%) (Group 3), and 13/20 (65%) (Group 4) ($p=0.413$). Strong cytoplasmic staining occurred in 16/20 (80%) each for Groups 1-3 and 15/20 (75%) (Group 4) ($p=0.990$). Clarity and crispness were present in 18/20 (90%) (Group 1), 17/20 (85%) (Group 2), 16/20 (80%) (Group 3), and 16/20 (80%) (Group 4) ($p=0.799$). All Intergroup differences were statistically non significant ($p>0.05$).

Conclusion: Beetroot and ginger extracts provide staining efficacy comparable to conventional H&E across all parameters, offering biosafe, cost-effective, and ecofriendly alternatives for exfoliative cytology applications.

Keywords: Beet root extract, Exfoliative cytology, Ginger extract, White lesions

INTRODUCTION

The use of ecofriendly and biodegradable materials has been a key agenda for global advocacy. Most of the biomedical reagents used in hospitals, laboratories for histological, histopathological, biochemical and imaging techniques are hazardous to human health and environment upon exposure [1]. Among the enormous number of dyes used in histopathology, H&E dyes remain to be the gold standard for routine staining. Haematoxylin is a natural dye obtained from the barks of *Haematoxylin campechianum* [2], while eosin which is a synthetic xanthene dye, is highly effective stain but has harmful effects on both animal and human health [3].

In addition, the economic burden is high due to the expensive nature of routine laboratory reagents. These have stirred the search for natural materials that are ecofriendly, biodegradable, inexpensive and safe for routine commercial use in laboratories. Hence, the use of natural material is experiencing resurgence in biomedical research in recent times [4].

Natural dyes, generally supposed to be a cheap, non toxic, renewable and sustainable resource with minimal environmental impact, have attracted the attention of the scientist community to use them in a variety of traditional and newly discovered application disciplines 2. It is known that natural dyes have been used for colouring textile materials, however, there are also studies with natural dyes on the staining of tissues, which are a different discipline [5].

Beet root is cherished for its nutritional and medicinal value. Beetroot has a long history as dye for fabrics and woods; it impacts purple to red colour [4]. Beetroot has also been widely used industrially as food colourant due to its red colour from the pigment betalain mainly from betanin, betanidin, and betaxanthin [6].

The rhizome of *Zingiber officinale*, a member of the Zingiberaceae family, has a deep yellow colour. *Zingiber officinale*, also known as ginger, is commonly used as a whole medicinal plant, as a spice in cooking, in beverage manufacturing, and for making tea. Flavonoids, a polyphenolic compound, contribute to its acidic nature, and colouring compounds that impart colour are found in *Zingiber officinale*. The extracts of *Zingiber officinale* (ginger) were also used as a histological dye to stain various tissues as an alternative to routinely used eosin[7].

Although beetroot and ginger possess favourable properties, few studies have explored their staining potential in histology. This study aimed to evaluate the staining efficacy of beetroot (*Beta vulgaris*) and ginger (*Zingiber officinale*) extracts as alternatives to

conventional H&E staining in exfoliative cytology smears from oral white lesions. The objectives were to observe the staining capacity of beetroot and ginger on exfoliative smear and to compare the staining capacity of beetroot and ginger with conventional H&E for intensity of nuclear staining, intensity of cytoplasmic staining, and clarity and crispness of staining. The research question addressed whether there is any difference in staining efficacy between beetroot/ginger extracts with conventional H&E in exfoliative cytology smears from oral white lesions. The null hypothesis (H_0) stated there is no significant difference in presence/absence of staining, nuclear staining intensity, cytoplasmic staining intensity, and clarity/crispness between beetroot/ginger extracts and conventional H&E, while the alternative hypothesis (H_1) stated there is significant difference in presence/absence of staining, nuclear staining intensity, cytoplasmic staining intensity, and clarity/crispness between beetroot/ginger extracts and conventional H&E.

MATERIALS AND METHODS

The experimental study was conducted on exfoliative buccal smears obtained from patients with oral white lesions at the Department of Oral Medicine and Oral Pathology, Dayananda Sagar College of Dental Sciences, Bengaluru, Karnataka, India from February 2023 to October 2023. Ethical approval was obtained from the Institutional Review Board (IRB), Dayananda Sagar College of Dental Sciences (IRB No: 181-IRB-2023) written informed consent was obtained from all participants prior to sample collection.

Sample size calculation: The sample size was estimated using G*Power software version 3.1.9.4 (Franz Faul, Universität Kiel, Germany). A medium effect size (f=0.40) was assumed based on Cohen's classification of effect sizes [8], calculated using the formula $f=\sqrt{(\eta^2 / (1 - \eta^2))}$ where η^2 represents the eta-squared value derived from expected staining quality differences.This was combined with a power of 80% (1-β=0.80) and an alpha error probability of 0.05, yielding a minimum required sample size of 76, which was rounded up to 80 for equal allocation across the four study groups.

Inclusion and Exclusion criteria: The study population included patients aged 18-60 years who presented with clinically diagnosed oral white lesions. Participants were selected based on predefined inclusion and exclusion criteria. Individuals with systemic conditions known to affect the oral mucosa, a history of chemotherapy or radiotherapy, traumatic oral lesions, and those with clinically normal oral mucosa were excluded from the study. Oral white lesions were specifically chosen for evaluation as exfoliative cytology is a non-invasive, first-line investigative procedure used to assess nuclear and cytoplasmic alterations. Assessing staining efficacy in these lesions provides clinically relevant information compared to normal mucosa, while avoiding additional risk or discomfort to patients.

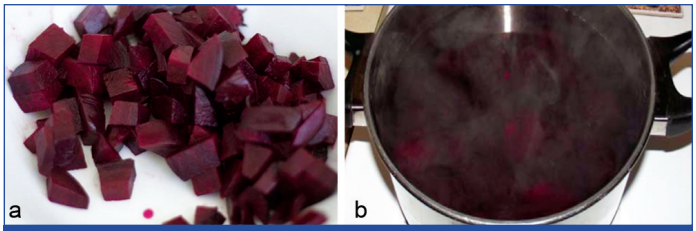
A total of 80 buccal smears were collected using disposable wooden ice-cream sticks and fixed by air-drying. The samples were randomly divided into four groups (n=20 per group):

- **Group 1** (Control): Conventional H&E
- **Group 2:** Beetroot extract and eosin
- **Group 3:** Ginger extract and haematoxylin
- **Group 4:** Ginger and beetroot extract with vinegar as mordant

Study Procedure

Preparation of natural stains was done in department of oral and maxillofacial pathology.

Beetroot stain preparation: Fresh beetroot was washed, cubed, and blanched at 80°C for 3-5 minutes (50 g/100 mL water). The extract was filtered using Whatman No.1 filter paper and oven-dried to obtain a concentrated dye and stored in amber- coloured bottle [Table/Fig-1a,b] [4,6].



[Table/Fig-1]: a) Collection of fresh beetroot; b) Blanching of beetroot.

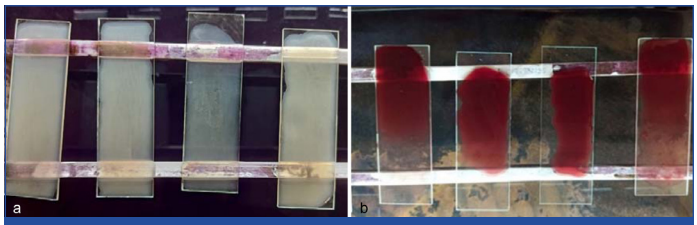
Ginger stain preparation: Fresh ginger rhizomes were cleaned, dried, powdered, and 20 g of powder was extracted in 100 mL of 70% alcohol for 24 hours. The supernatant was filtered and stored in amber-coloured bottle [Table/Fig-2a,b] [3,7].

[Table/Fig-2]: a) Collection of ginger; b) Preparation ginger dye.

Both stains were stored at 4°C and had a shelf life of 3-4 days.

Staining procedures

- **Group 1 (H&E):** Smears were stained with haematoxylin for five minutes, blued under running tap water for 15 minutes, counterstained with eosin for four minutes, mounted with DPX.
- **Group 2 (Beetroot extract and eosin):** Smears were stained with beetroot extract for 15 minutes, rinsed, counterstained with eosin for four minutes, mounted [6].
- **Group 3 (Ginger extract and haematoxylin):** Smears were stained with haematoxylin for five minutes, blued for 15 minutes, counterstained with ginger extract for eight minutes, mounted [7].
- **Group 4 (Ginger and beetroot extract with vinegar):** Smears were briefly rinsed in vinegar, stained with beetroot extract for 15 minutes, counterstained with ginger extract for eight minutes, mounted [Table/Fig-3a,b] [6,7].



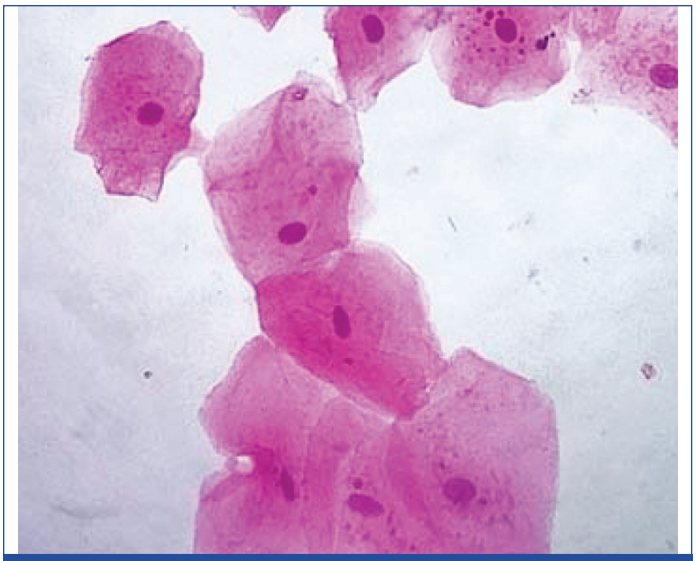
[Table/Fig-3]: a) Buccal smear stained with ginger extract; b) Buccal smear stained with beetroot extract.

Imaging: All slides were examined under a light microscope at 40× magnification, and photomicrographs were captured. Representative images for each group were documented and are shown in [Table/Fig-4-7].

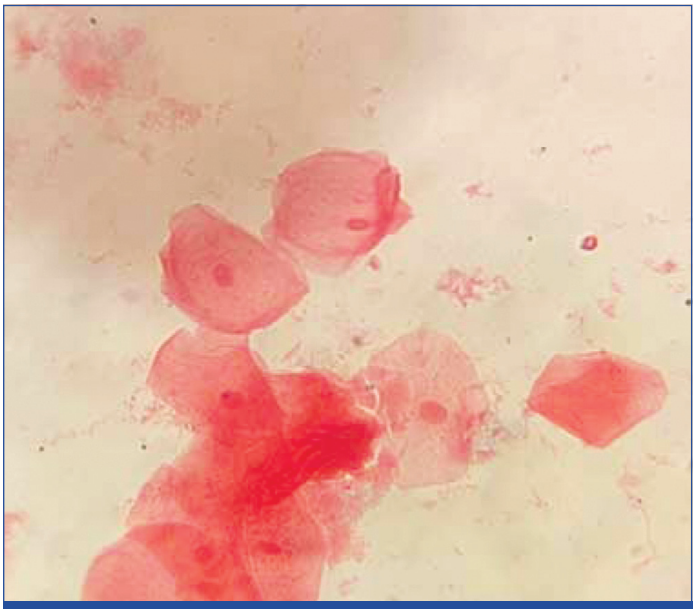
Slide evaluation and scoring: All slides were evaluated by a single trained and calibrated oral pathologist. Calibration was performed by re-evaluating 10 randomly selected slides after one week, achieving a Cohen's kappa value>0.8. The scoring system used for the evaluation of staining efficacy was partly adapted from previously published cytological staining assessment studies and partly self-developed, with minor modifications to suit exfoliative cytology smears[3,4,6,7,9,10].

Clarity and crispness of staining was defined as the distinct and sharp visualisation of cellular and nuclear details without background

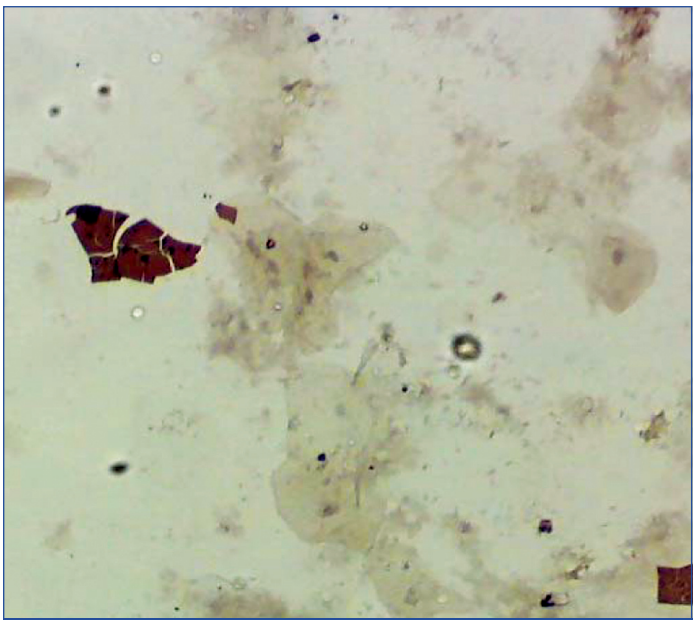
staining or dye diffusion. A score of "present" was assigned when nuclei and cytoplasm showed clear delineation, uniform staining intensity, and well-defined cellular borders. A score of "absent" was assigned when staining appeared diffuse, uneven, poorly contrasted, or when cellular and nuclear details were indistinct or obscured by background artefacts [2].



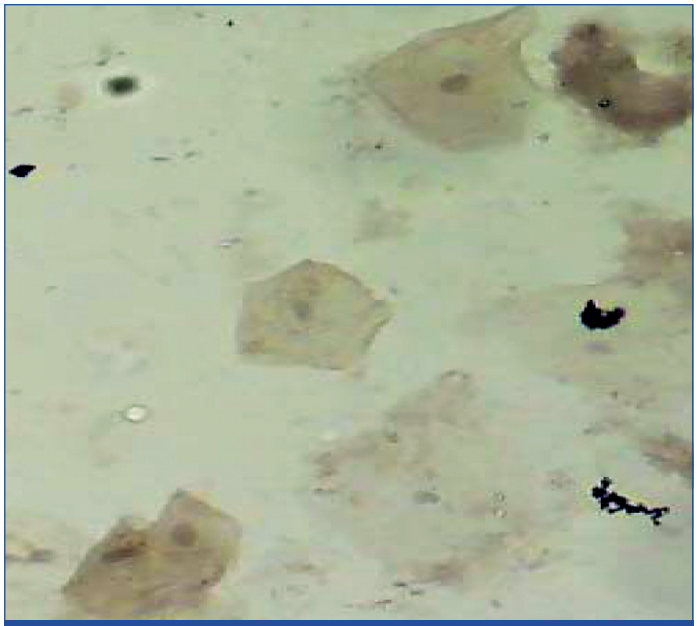
[Table/Fig-4]: Photomicrograph of buccal smear stained with haematoxylin and eosin (40×).



[Table/Fig-5]: Photomicrograph of buccal smear stained with beetroot extract and eosin (40×).



[Table/Fig-6]: Photomicrograph of buccal smear stained with ginger extract and haematoxylin (40×).



[Table/Fig-7]: Photomicrograph of buccal smear stained with beetroot and ginger extract (40×).

Assessment parameters

A Presence or absence of staining:

- 0=Absent
- 1=Partially present
- 2=Present

B Intensity of nuclear and cytoplasmic staining:

- 0=Weak
- 1=Moderate
- 2=Strong

C Clarity and crispness of staining:

- 0=Absent
- 1=Present

Outcome Measures

- Presence or absence of staining
- Intensity of nuclear staining
- Intensity of cytoplasmic staining
- Clarity and crispness of staining

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Data were expressed as frequencies and percentages. Intergroup comparisons were carried out using the Chi-square test. A p-value <0.05 was considered statistically significant and corresponding test values and p-values are reported in the tables.

RESULTS

A total of 80 exfoliative buccal smears were evaluated, with 20 smears in each group. The staining outcomes were assessed based on presence or absence of staining, intensity of nuclear and cytoplasmic staining, and clarity and crispness of staining.

Presence or Absence of Staining

All smears in Group 1 demonstrated staining, with 100% showing complete presence of stain.

In Groups 2 and 3, staining was present in 85% of smears, while partial staining was observed in 15% of cases in each group. In Group 4, staining was present in 75% of smears, partial staining was seen in 15%, and absence of staining was noted in 10% of samples. The difference in staining presence among the four groups was not statistically significant (p=0.136) [Table/Fig-8].

Group	Absent	Partially present	Present	χ^2 value	p-value
Group 1	0	0	20 (100)	5.62	0.136
Group 2	0	3 (15)	17 (85)		
Group 3	0	3 (15)	17 (85)		
Group 4	2 (10)	3 (15)	15 (75)		

[Table/Fig-8]: Comparison of presence or absence of staining among groups 1, 2, 3, and 4 in exfoliative buccal smears.
Data are expressed as number (percentage); statistical analysis was performed using the Chi-square test (χ^2=5.62); a p-value <0.05 was considered statistically significant. Abbreviations: χ^2- Chi-square value; p- probability value.

Intensity of Nuclear Staining

Intensity of nuclear staining was evaluated only in smears that demonstrated complete or partial staining, as staining intensity cannot be assessed in smears with complete absence of stain. Strong nuclear staining predominated across all groups. Group 1 showed strong nuclear staining in 90% of smears, with weak and moderate staining observed in 5% each. In Group 2, strong nuclear staining was observed in 70% of smears, moderate staining in 20%, and weak staining in 10%. Group 3 demonstrated strong nuclear staining in 85% of smears, with moderate and weak staining in 10% and 5%, respectively. Strong nuclear staining was observed in the majority of smears across all groups. Group 1 showed strong nuclear staining in 90% of samples, with weak and moderate staining observed in 5% each. In Group 2, strong nuclear staining was seen in 70% of smears, moderate staining in 20%, and weak staining in 10%. Group 3 demonstrated strong nuclear staining in 85% of samples, while moderate and weak staining were observed in 10% and 5% of smears, respectively. Group 4 showed strong nuclear staining in 65% of cases, with moderate staining in 15% and weak staining in 10%. The intergroup comparison for nuclear staining intensity was not statistically significant (p=0.413) [Table/Fig-9].

Group	Weak	Moderate	Strong	χ^2 value	p value
Group 1	1 (5)	1 (5)	18 (90)	3.93	0.413
Group 2	2 (10)	4(20)	14 (70)		
Group 3	1 (5)	2 (10)	17 (85)		
Group 4	4 (10)	3 (15)	13 (65)		

[Table/Fig-9]: Comparison of conventional haematoxylin and eosin stain with beetroot and ginger extract stains for intensity of nuclear staining in exfoliative buccal smears.
Data are expressed as number (percentage); statistical analysis was performed using the Chi-square test (χ^2=3.93); a p-value <0.05 was considered statistically significant. Abbreviations: H&E: Haematoxylin and Eosin; χ^2- Chi-square value; p- probability value.

Intensity of Cytoplasmic Staining

Strong cytoplasmic staining was observed in 80% of smears in Groups 1, 2, and 3, while 75% of smears in Group 4 demonstrated strong staining. Weak and moderate cytoplasmic staining was observed in smaller proportions across all groups. There was no statistically significant difference in cytoplasmic staining intensity among the four groups (p=0.990) [Table/Fig-10].

Clarity and Crispness of Staining

Clarity and crispness of staining were present in 90% of smears in Group 1, 85% in Group 2, and 80% each in Groups 3 and 4. Absence of clarity and crispness was observed in 10% of samples in Group 1, 15% in Group 2, and 20% in Groups 3 and 4. The difference among the groups was not statistically significant (p=0.799) [Table/Fig-11].

Group	Weak	Moderate	Strong	χ^2 value	p-value
Group 1	1 (5)	3 (15)	16 (80)	1.27	0.990
Group 2	2 (10)	2 (10)	16 (80)		
Group 3	2 (10)	2 (10)	16 (80)		
Group 4	2 (10)	3 (15)	15 (75)		

[Table/Fig-10]: Comparison of conventional haematoxylin and eosin stain with beetroot and ginger extract stains for intensity of cytoplasmic staining in exfoliative buccal smears.
Data are expressed as number (percentage); statistical analysis was performed using the Chi-square test (χ^2=1.27); a p-value <0.05 was considered statistically significant. Abbreviations: H&E: Haematoxylin and Eosin; χ^2- Chi-square value; p- probability value.

Group	Absent	Present	χ^2 value	p-value
Group 1	2 (10)	18 (90)	1.02	0.799
Group 2	3 (15)	17 (85)		
Group 3	4 (20)	16 (80)		
Group 4	4 (20)	16 (80)		

[Table/Fig-11]: Comparison of conventional haematoxylin and eosin stain with beetroot and ginger extract stains for clarity and crispness of staining in exfoliative buccal smears.
Data are expressed as number (percentage); statistical analysis was performed using the Chi-square test (χ^2=1.02); a p-value <0.05 was considered statistically significant. Abbreviations: H&E: Haematoxylin and Eosin; χ^2- Chi-square value; p- probability value.

DISCUSSION

The present study evaluated the staining efficacy of beetroot (*Beta vulgaris*) and ginger (*Zingiber officinale*) extracts as natural alternatives to conventional H&E in exfoliative buccal smears. Overall, the findings demonstrated that both plant-derived extracts provided staining characteristics comparable to routine H&E, aligning with prior studies assessing the utility of natural dyes in histological and cytological applications [3,4].

Beetroot extract showed nuclear staining comparable to haematoxylin, consistent with observations by Obeta U et al., who reported satisfactory nuclear affinity of *Beta vulgaris* extracts [9].This nuclear staining property has been attributed to betanin, the principal betalain pigment present in beetroot, which possesses strong chromophoric properties and affinity for cellular components, as described in biochemical and nutritional studies of *Beta vulgaris* pigments [10]. Obeta U et al., also demonstrated effective nuclear staining using beetroot-based dyes as substitutes for haematoxylin in histological sections [9]. Similarly, in cytological preparations, Singnarpi S et al., reported that vegetable-derived stains provided nuclear detail comparable to conventional H&E, supporting the present findings [11].

Despite these positive outcomes, variability in staining intensity and occasional partial or mixed staining has been reported in earlier studies. Udonkang MI et al., and Singh A et al., highlighted that such inconsistencies are influenced by factors including extraction method, dye concentration, pH, and staining duration [6,12]. Hartika G et al., similarly emphasised the lack of standardisation as a key limitation for routine application of natural dyes [13]. The minor variations observed in the present study are therefore in agreement with the literature and do not detract from the overall staining efficacy of beetroot extract.

Regarding cytoplasmic staining, ginger extract demonstrated eosin-like properties, consistent with the findings of Sudhakaran A et al., who observed cytoplasmic staining intensity of *Zingiber officinale* comparable to eosin in tissue sections [7]. Prajapati MR et al., also reported acceptable cytoplasmic contrast with ginger relative to other vegetable dyes [3], while Singnarpi S et al., confirmed satisfactory cytoplasmic staining using plant-based dyes in exfoliative cytology [11]. The cytoplasmic staining behaviour of ginger has been attributed to its content of polyphenols and flavonoids, weakly acidic compounds capable of binding basic cytoplasmic proteins, as described in earlier studies [7,14].

A distinct aspect of the present study is the use of human exfoliative buccal smears, providing a clinically relevant cytology model

compared to previous studies that primarily used animal tissues or general histological sections. Additionally, this study evaluated both nuclear staining using beetroot extract and cytoplasmic staining using ginger extract within the same samples, thereby mimicking the conventional H&E staining workflow. This combined approach allows for a comprehensive assessment of natural dyes as an H&E alternative, whereas earlier studies often focused on individual dyes in isolation. The present work also provides direct side-by-side comparison with conventional H&E, highlighting the practical applicability of these natural dyes for screening and teaching purposes in oral cytology, a context less explored in previous research. Observations regarding minor variations in staining reinforce real-world considerations, such as dye preparation, stability, and ease of use, offering practical insights for potential implementation in routine laboratory settings.

Comparative assessment of staining clarity and crispness revealed that, while conventional H&E provides superior sharpness, natural dyes, including beetroot and ginger extracts, offer sufficient morphological detail for screening and educational purposes [3-5,7]. Hartika G et al., also emphasised that plant-derived dyes are better suited for preliminary diagnostic applications and teaching rather than routine clinical use [13].

Overall, the present study supports earlier reports that beetroot and ginger extracts can serve as viable natural alternatives to H&E, particularly for screening-based cytological applications [3,7,9,15]. All Intergroup comparisons yielded non significant p-values ($p > 0.05$), supporting acceptance of the null hypothesis (H_0) that there is no significant difference in staining efficacy between the natural extracts and conventional H&E.

Natural dyes such as beetroot and ginger are inexpensive, easily available, biodegradable, and less hazardous compared to synthetic stains. Their successful application in cytological staining suggests potential use in screening procedures, teaching laboratories, and resource-limited settings. Future studies should focus on standardising extraction protocols, evaluating long-term stability, assessing staining performance on histopathological sections, and comparing diagnostic accuracy with conventional stains using larger sample sizes and multiple observers.

Limitation(s)

While the present study provides useful insights, certain limitations should be acknowledged. The investigation was limited to exfoliative cytology smears; hence, extrapolation of the findings to other cytological preparations or histological tissues should be attempted with caution. Quality of staining was evaluated by a sole observer to ensure internal consistency; however, the lack of inter-observer assessment could restrict the evaluation of variability and is acknowledged as a possible confounding factor. Furthermore, the research centered mainly on immediate staining effectiveness and did not assess the long-term stability or durability of stains, which could affect their use in standard laboratory procedures. Similar constraints, including the lack of standardised formulations and limited shelf life of natural stains, have been reported in earlier studies. Furthermore, like most existing studies in this area, the present work primarily evaluated staining efficacy, and more detailed assessment of subtle nuclear and cytoplasmic morphological alterations was beyond its scope, warranting further systematic investigations.

CONCLUSION(S)

Within the limitations of the study, beetroot and ginger extracts demonstrated staining properties comparable to haematoxylin and eosin, respectively, in exfoliative cytology smears. These findings suggest that natural dyes can serve as safe, eco-friendly, and cost-effective alternatives to conventional synthetic stains, particularly for preliminary screening and educational purposes. Further research is warranted to validate their routine diagnostic applicability and long-term performance.

REFERENCES

[1] Pereira L, Alves M. Dyes-environmental impact and remediation. In: Malik A, Grohmann E, editors. Environmental protection strategies for sustainable development. Dordrecht: Springer Science + Business Media B.V.; 2012. p. 111-54.
[2] Culling CF, Allison RT, Barr WT. Cellular pathology technique. 4th ed. Wellington: Butterworth & Co Ltd; 1985. p. 148-49.
[3] Prajapati MR, Agarwal P, Jadav B. Exploring the staining potential of Zingiber officinale extracts (ginger) as a natural alternative to eosin in haematoxylin and eosin staining: A cross-sectional study. J Clin Diagn Res. 2023;17(10):ZC49-ZC52.
[4] Finbarrs-Bello E, Ojo OP, Agu CK. A bioprospective study on histological staining potential of (beetroot)extract. J Exp Res. 2019;7(4):01-05.
[5] Ashis KS, Adwaita K. Dyeing of textiles with natural dyes. In: Natural dyes. Europe: Intech Open; 2011. p. 29-52.
[6] Udonkang MI, Inyang IJ, Ukorebi AN, Effiong F, Akpan U, Bassey IE. Spectrophotometry, physicochemical properties, and histological staining potential of aqueous and ethanol extracts of beetroot on various tissues of albino rat. Biomed Hub. 2018;3(3):1-10.
[7] Sudhakaran A, Hallikeri K, Babu B. Natural stains Zingiber officinale Roscoe (ginger) and Curcuma longa L. (turmeric)—a substitute to eosin. Ayu. 2018;39(4):220-25.
[8] Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale: Lawrence Erlbaum Associates; 1988.
[9] Obeta U, Dajok G, Ejinaka O, Deko B, Yusuf BN, Ukpabi M, et al. Beetroot and turmeric as alternative dyes for haematoxylin and eosin in histological staining. SRJ Clin Med Sci. 2022;2(1):54-58.
[10] Tom C, Glyn H, Daniel JW, Emma JS. The potential benefits of red beetroot supplementation in health and disease. Nutrients. 2015;7(4):2801-22.
[11] Singnarpi S, Ramani P, Natesan A, Sherlin HJ, Gheena S, et al. Vegetable stain as an alternative to H&E in exfoliative cytology. J Cytol Histol. 2017;8:469.
[12] Singh A, Ganesapillai M, Gnanasundaram N. Optimization of extraction of betalain pigments from Beta vulgaris peels by microwave pretreatment. IOP Conf Ser Mater Sci Eng. 2017;263:032004.
[13] Hartika G, Zulharmita Z, Asra R. Utilization of natural dye substances for histological staining: A review. Asian J Pharm Res Dev. 2021;9(1):149-58.
[14] Sutradhar P, Bhattacharya C. Use of the natural pigments of red beetroot pomace (Beta vulgaris L.) to develop a mycological stain: An eco-friendly alternative substitute. J Sci Res. 2021;65:73-77.
[15] 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;10:1445-48.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Oral and Maxillofacial Pathology, Dayananda Sagar College of Dental Sciences, Bengaluru, Karnataka, India.
2. Intern, Department of Oral and Maxillofacial Pathology, Dayananda Sagar Dental College and Hospital, Bengaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Sangeetha Ramu,
Professor, Department of Oral and Maxillofacial Pathology, Dayananda Sagar College of Dental Sciences and Hospital, Shavige Malleshwara Hills, Kumaraswamy Layout, Bengaluru-560078, Karnataka, India.
E-mail: sangeethar@dscds.edu.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Oct 31, 2025
- Manual Googling: Mar 07, 2026
- iThenticate Software: Mar 09, 2026 (4%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Oct 13, 2025**
Date of Peer Review: **Jan 01, 2026**
Date of Acceptance: **Mar 11, 2026**
Date of Publishing: **Jul 01, 2026**