ORIGINAL ARTICLE

Evaluation of black plum fruit dye as a cost-effective substitute for hematoxylin in nuclear staining in resource-limited laboratories

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Abstract

Background: Hematoxylin remains an essential nuclear stain in histopathology, yet its cost and limited availability often challenge laboratories in resource-constrained regions. Natural plant-based dyes offer sustainable, economical alternatives. This study evaluates the staining performance and cost-effectiveness of *Syzygium cumini* (black plum) fruit dye as a substitute for hematoxylin. *Aim and Objectives:* To assess and compare the staining quality of black plum dye with conventional Hematoxylin and Eosin (H&E) stains in histopathological, cytological, and exfoliative cytology specimens. *Materials and Methods:* Ninety samples (30 each of histopathology, cytology, and exfoliative cytology) were included. Ripened black plum fruits were washed, crushed, and extracted using distilled water and 45% glacial acetic acid. The filtrate was stored at 4°C until use. Standard H&E staining served as the control. Staining characteristics like nuclear clarity, chromatin pattern, cytoplasmic transparency, and background uniformity were independently evaluated by two pathologists. Statistical analysis was performed using the Chi-square test and Quality Index (QI) scores. *Results:* The black plum dye required 20–30 minutes for extraction and approximately 37 minutes for staining. Compared with conventional H&E, cytology and exfoliative cytology smears demonstrated significant correlation for nuclear and chromatin details ($p < 0.001$). Histopathological sections showed comparable nuclear staining, though slightly reduced chromatin detail. *Conclusion:* Black plum fruit extract offers an economical, biodegradable, and effective natural alternative to hematoxylin. With standardization of extraction stability and concentration, it may serve as a sustainable nuclear stain in diagnostic laboratories, particularly in low-resource settings.

Keywords: *Syzygium cumini*, black plum, natural dye, hematoxylin substitute, nuclear stain, eco-friendly histology

Introduction

Hematoxylin is one of the most widely used nuclear stains in cytology, histology, histopathology, and histochemistry. It plays a crucial role in identifying cellular details and diagnosing malignant changes in tissue lesions [1]. Hematoxylin can be obtained from both synthetic and natural sources. After appropriate chemical processing, it is commercially utilized as a routine nuclear stain.

The natural form of hematoxylin is derived from *Haematoxylum campechianum* (the logwood tree), which is native to Central America and the Caribbean regions [1, 2]. Large-scale extraction of natural hematoxylin from logwood is commonly performed using two methods: the French process, which involves boiling wood chips, and the American process, which utilizes steam and pressure. Although hematoxylin can be synthesized chemically—similar to other plant-derived compounds such as quinine and morphine, its artificial synthesis remains more expensive than extraction from natural sources [1].

A major limitation of hematoxylin obtained from the heartwood of the logwood tree is its restricted geographical availability. Consequently, the supply of this dye may fluctuate due to climatic, political, or economic factors [2].

Although synthetic dyes provide excellent staining properties, they are often associated with toxic, carcinogenic, and environmental hazards. Hence, there is an increasing demand for eco-friendly, biodegradable, and sustainable natural dyes for histological applications. Plants are valuable sources of natural pigments, and numerous trees, herbs, and fruits have been explored for their dyeing potential [1–3]. Among them, *Syzygium cumini* (black plum), an evergreen tropical tree of the Myrtaceae family, has shown promise. It is commonly cultivated across India, Pakistan, Bangladesh, Nepal, Thailand, and Indonesia. The deep violet, oblong berries of the black plum are edible and contain a pinkish pulp rich in anthocyanins and tannins, compounds known for their coloring and medicinal properties [4].

Dyes used in histological staining are broadly classified into two categories: Synthetic dyes, produced through complex chemical reactions, and natural dyes, derived from plant or biological sources.

However, limited studies have investigated the extraction and staining potential of natural dye derived from black plum fruit. Moreover, the optimal extraction conditions and staining parameters have not been thoroughly standardized. Therefore, the present study was undertaken to extract and characterize the natural dye from *Syzygium cumini* (black plum), using different solvents, and to evaluate its staining efficacy on histopathological sections and cytological smears under varying staining durations.

Material and Methods

Study design and sample selection

This was an observational, comparative study conducted in the Department of Pathology at a tertiary care medical institution. Ninety samples were included: 30 histopathology sections, 30 cytology smears, and 30 exfoliative cytology specimens collected over a period of three months. Sample size was determined for a paired-comparison design. For a paired t-test with two-sided alpha=0.05 and power=80%, detection of a medium standardized effect size (Cohen's dz = 0.5) requires approximately 32 paired specimens. Given the study aim to compare staining across three specimen types (histopathology, cytology, exfoliative cytology), we enrolled 30 cases per specimen category (total n = 90) as a pragmatic sample for a pilot comparative evaluation.

Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections with adequate preservation, routine cytology smears with sufficient cellularity, and exfoliative cytology smears from buccal mucosa and cervical samples were included. Poorly fixed or autolyzed tissue samples and smears with inadequate cellular material or thick background were excluded.

Preparation of black plum dye

Fresh, ripened fruits of *Syzygium cumini* were thoroughly washed to remove surface impurities. The pulp was separated, crushed, and macerated with two types of solvents: (1) distilled water and (2) 45% glacial acetic acid in a 1:2 (w/v) ratio. The mixture was filtered through muslin cloth and Whatman No. 1 filter paper, and the extract were divided into two portions: one half was used immediately for staining examinations, while the other half was stored in a dark area at 4 degrees

Celsius for two weeks before being utilized for staining. The dye remained stable for approximately 14 days. The procedure as shown in figure 1.

Standardizing staining solution

Staining procedure

Standard hematoxylin and eosin staining served as the control. Sections and smears were deparaffinized, rehydrated, and stained using the prepared black plum dye. Staining time was optimized at 37 minutes to achieve optimal nuclear coloration. Sections were subsequently counterstained with eosin for cytoplasmic contrast and mounted with DPX. Two experienced pathologists independently evaluated the stained slides under a light microscope (×40 objective). The following parameters were assessed for nuclear clarity for distinctness of nuclear boundaries: chromatin pattern for visibility and texture of chromatin; cytoplasmic transparency for clarity of background and cytoplasmic features; staining uniformity for evenness of color distribution; and presence of artifacts for precipitates or uneven staining. Each parameter was graded on a 4-point scale (0–3). The overall Quality Index (QI) was calculated as the mean of all parameter scores.

Statistical analysis

Data were analyzed using IBM Statistical Package for the Social Sciences, version 22. Descriptive statistics were applied to compare staining characteristics between the black plum dye and standard hematoxylin. The Chi-square test was used to determine associations between categorical parameters, and a p-value < 0.05 was considered statistically significant.

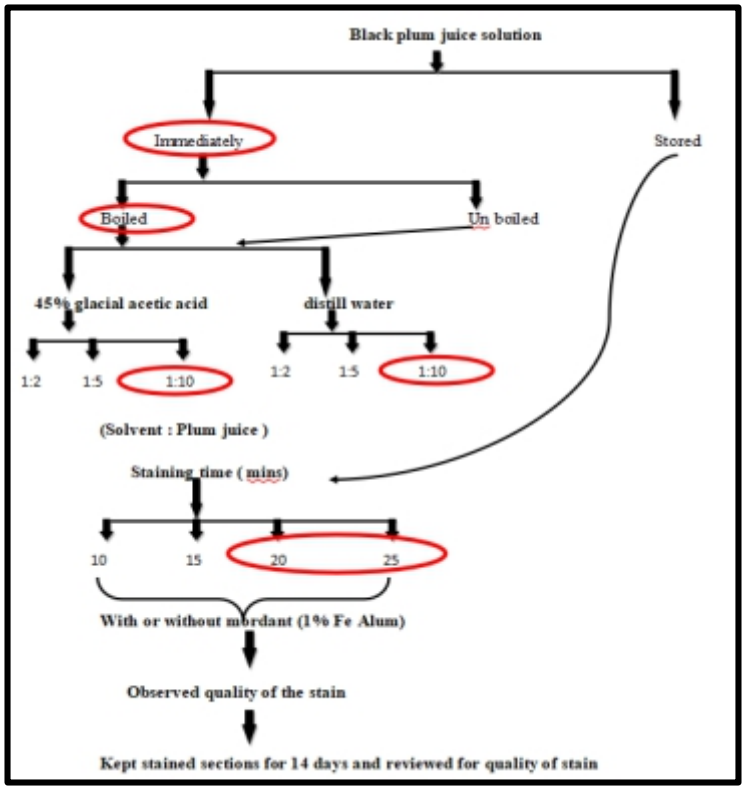

Figure 1: Preparation of black plum dye

Results

A total of 90 patient samples were collected, with two smears stained using routine Hematoxylin and Eosin (H&E) and black plum stains for interpretation. The minimum age of the patients was 27 years, and the maximum was 65 years, resulting in a mean age of 37 years. The majority of patients were in the age group of 31-40 years.

The distribution of cases included 30 cytology cases, 30 exfoliative cytology cases, and 30 histopathology cases. Among the cytology cases, there were 16 benign and 14 malignant lesions. In the exfoliative cytology cases, there were 21 cases of Negative for Intraepithelial Lesion or Malignancy (NILM), 2 cases of Low-grade Squamous Intraepithelial Lesions (LSIL), 1 case of High-grade Squamous Intraepithelial Lesion (HSIL), and the remaining cases were squamous cell carcinoma. For the histopathology cases, the breakdown included 5 liver lesions (autopsy cases), 4 cases of reactive lymphadenitis, 5 cases of buccal mucosa squamous cell carcinoma, 4 cases of fibroadenoma, 5 cases of placentas, 4 cases of chronic cervicitis, and 3 cases related to the endometrium. Both staining techniques and smears were compared and analyzed (Table 1).

The mean QI for the stains revealed that routine H&E staining had a value of 0.45, while black plum juice staining had a value of 0.40. Chi-square analysis indicated statistically significant differences between the two staining techniques, routine H&E and the new nuclear staining showing values of 76.25 for nuclear staining and 47.14 for chromatin density ($p < 0.001$). In cytology cases, the Chi-square values were 60 for nuclear staining and 42.22 for chromatin density ($p < 0.001$), also statistically significant. Although malignant lesions could be identified, typing them was not possible (Figure 1).

In histopathology cases, Chi-square values showed a significant difference between the stains for nuclear staining (40.57, $p < 0.001$) while chromatin density showed no statistically significant difference (3.42, p-value = 0.180). There was difficulty in diagnosing malignant lesions due to the chromatin not being sufficiently crisp for accurate assessment (Figure 2).

In exfoliative cytology cases, Chi-square analysis revealed a statistical difference for nuclear staining (8.606, $p = 0.013$) and chromatin density (9.042, $p = 0.010$). Again, there was difficulty in diagnosing malignant lesions due to chromatin clarity issues (Figure 3).

Table 1: Association between H&E and black plum juice stain in histopathology, cytology cases, exfoliative cytology and overall staining quality

Quality parameters	Scores		Histopathology		Cytology		Exfoliative Cytology		Overall	
			H&E stain	Black plum Stain	H&E stain	Black plum Stain	H&E stain	Black plum Stain		
Cell morphology										
Not Preserved	0	10	—	—	—	—	—	—	0	11
Moderately Preserved	51	57	—	—	—	—	—	—	57	63
Well preserved	39	23	—	—	—	—	—	—	43	27
Chi square *p*			—		—		—		**14.46 <0.001**	
Nuclear characteristics										
Dull	0	27	0	53	0	37	3	0	0	30
Moderately crisp	15	48	13	40	0	63	27	63	17	53
Crisp	75	15	80	7	100	0	70	37	83	17
Chi square *p*			**40.57 <0.001**		**60 <0.001**		**8.606 0.013**		**84.29 <0.001**	
Overall staining										
Bad	3	32	10	47	0	47	3	10	3	36
Moderately good	66	45	47	40	100	33	74	87	73	50
Good	21	13	43	13	0	20	23	3	23	14
Chi square *p*			**12.04 0.002**		**30 <0.001**		**5.88 0.054**		**29.88 <0.001**	
Chromatin Density										
Dull	4	42	7	33	0	20	7	27	5	47
Moderately crisp	47	36	60	54	33	57	60	66	52	40
Crisp	39	12	33	13	67	23	23	3	43	13
Chi square *p*			**3.425 0.180**		**42.22 <0.001**		**9.04 0.010**		**47.14 <0.001**	

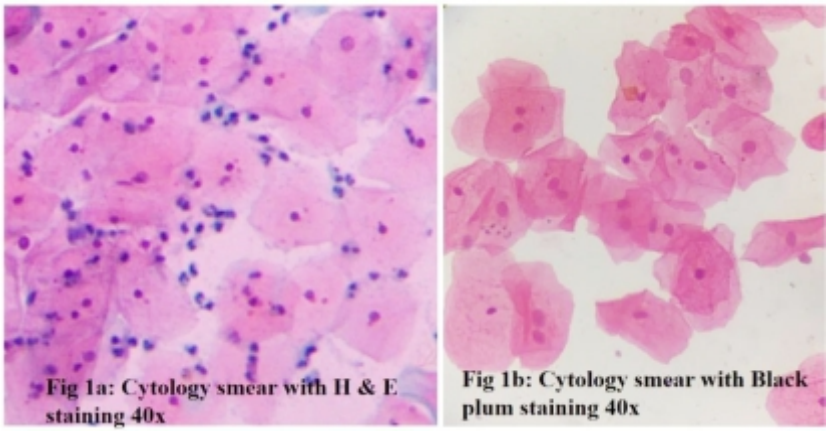

Figure 1a:Cytology smear, H & E staining 10×
Figure 1b:Cytology smear Black Plum stain and Eosin, 10× magnification

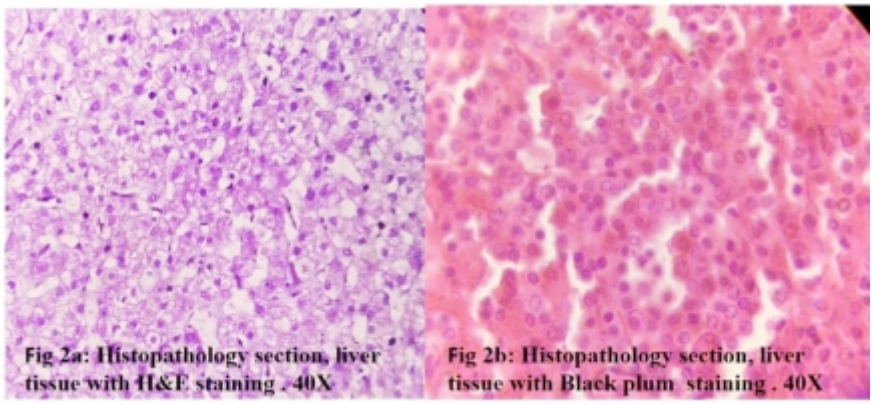

Figure 2a:Histopathology section (Liver) H&E staining 10×
Figure 2b:Histopathology section (Liver) Black Plum stain and Eosin, 40× magnification

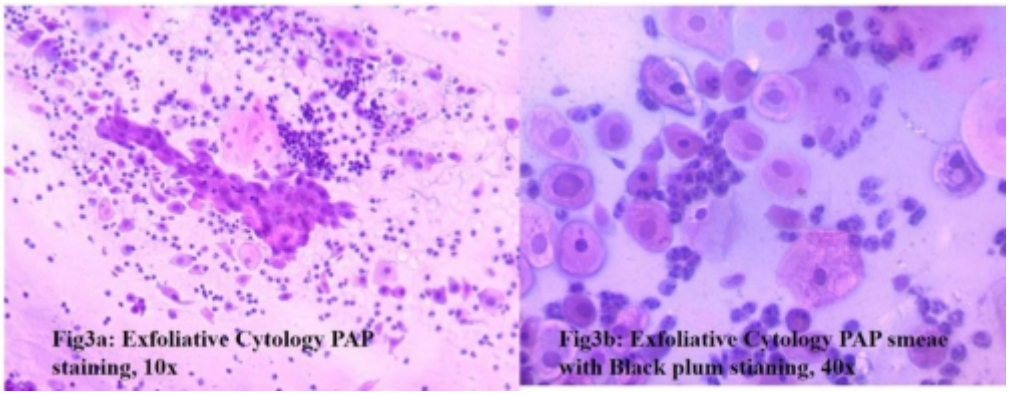

Figure 2a:Exfoliative Cytology smear, Pap staining using H &E dye, 10×.
Exfoliative Cytology smear. Black Plum stain and Eosin, 40×

Discussion

Syzygium cumini (black plum), a tropical evergreen fruit tree native to South and Southeast Asia, has long been recognized for its rich anthocyanin pigments, responsible for its characteristic deep purple coloration. These natural pigments have shown promise as eco-friendly substitutes for synthetic and commercially prepared dyes. However, systematic documentation on the extraction and histological application of *S. cumini* fruit dye remains limited in scientific literature [4].

Hematoxylin, derived from the heartwood of *Haematoxylum campechianum* (logwood), has remained the cornerstone of nuclear staining for more than a century [1, 2]. The oxidation of hematoxylin to hematein, either by natural aging or chemical oxidants produces a compound capable of forming strong coordination complexes with metal mordants such as aluminum or iron. These complexes selectively bind to basophilic nuclear chromatin, producing the classical blue-violet nuclear coloration characteristic of H&E staining [2, 8, 9]. Despite its reliability, recent global shortages of logwood and rising costs have drawn attention toward developing sustainable, locally sourced alternatives [1, 2]. The current study explored the staining potential of *S. cumini* fruit extract as a nuclear dye and demonstrated that both acidic and neutral extraction media yielded effective pigment recovery. The dye exhibited satisfactory nuclear affinity and retained staining

Table 2: Comparison of quality of stains with other studies

Authors	Stains	Nuclear details	Chromatin details
Suabjakyong *et al.* (2011)	**Routine H&E stain**	Optimal	Optimal
	Black Plum dye	Optimal	Optimal
Present study N=(90)	**Routine H&E stain**	Optimal	Optimal
	Black Plum dye	**Optimal for cytology smears and histopathology sections**	**Optimal for cytology smears. Suboptimal for histopathology sections**

Table 3: Comparison of costs and time with other studies

Authors	Stains	Time (minutes)	Costs per slide (Rs)
Suabjakyong *et al.* (2011)	**Routine H&E stain**	12+/-5	50
	Black Plum dye	15+/-5	10
Present study	**Routine H&E stain**	12+/-5	60
	Black Plum dye	20+/-5	12

intensity during counterstaining, suggesting its potential comparability to conventional hematoxylin. Similar to findings by Suabjakyong *et al*. [4], variations in extraction pH showed minimal influence on staining properties, implying that anthocyanin pigments within black plum maintain stability across a moderate pH range. (Tables 2 & 3)

Although mordants often enhance dye–tissue interactions, the inclusion of 1% ferric alum in this study did not significantly alter staining quality. This finding contrasts with traditional hematoxylin chemistry, where mordant–dye complexes are essential for nuclear selectivity [2, 8-10]. The difference could be attributed to the intrinsic chelating ability of anthocyanins, which may independently bind to nuclear components without requiring extensive metal coordination [11-14].

The stability profile of the black plum extract emerged as a key limitation. The dye exhibited reduced intensity after two weeks of cold storage at 4 °C, although slides stained and stored under dark conditions retained acceptable color for a similar duration [4, 15]. These findings echo concerns in earlier natural-dye studies that emphasized short shelf life as a practical constraint [4]. Preservative additives, controlled pH storage, or lyophilization of the extract could improve its longevity. From an operational standpoint, black plum dye presents a viable, low-cost alternative in regions where hematoxylin procurement is challenging. Studies published have successfully evaluated crystal violet and other plant-based stains as nuclear alternatives, supporting the feasibility of substituting conventional dyes in diagnostic histopathology [16-21]. Incorporating *S. cumini* extracts into this growing body of work aligns with the ongoing emphasis on sustainable and indigenous innovations in laboratory science.

Future research should focus on optimizing solvent systems, mordant concentrations, and fixation compatibility to enhance reproducibility. Broader validation using varied tissue types and digital colorimetric analysis could further substantiate diagnostic reliability. Additionally, pilot cost– benefit analyses comparing locally prepared natural stains to imported hematoxylin could quantify economic advantages for resource-limited laboratories.

Conclusion

This study demonstrates that black plum (*Syzygium cumini*) fruit dye is a feasible, economical, and biodegradable substitute for hematoxylin in nuclear staining. The prepared dye produced satisfactory nuclear and chromatin detail across histopathological, cytological, and exfoliative cytology specimens. Although minor variations in chromatin intensity were observed, diagnostic interpretation remained uncompromised. Its local availability, low cost, and non-toxic nature make it an appropriate alternative for laboratories in resource-limited settings. Further studies focusing on dye stability, pH optimization, and mordant enhancement are recommended to standardize large-scale use.

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