Scientia Africana, Vol. 23 (No. 3), August, 2024. Pp 409-416
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DEMONSTRATING THE STAINING ABILITY OF *ZINGIBER OFFICINALE AND CURCUMA LONGA* AS REPLACEMENT TO EOSIN Y IN EA50

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Received: 20-07-2024
Accepted: 02-08-2024

https://dx.doi.org/10.4314/sa.v23i3.36

Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: *Faculty of Science, University of Port Harcourt.*

ABSTRACT

Nicholas Papanicolaou developed the Pap stain, a combination of three major stains used for cervical smears. Eosin-Azure (EA50), one of these stains, includes Eosin Y, Light Green SF, and Bismarck brown. This study explores the use of Curcuma longa extract as a substitute for Eosin Y in EA50. Cervical smears from premenopausal women were collected and stained using both the standard Papanicolaou stain and a modified version where Eosin Y was replaced with Curcuma longa extract. The modified stain's effectiveness was assessed based on the quality of staining of superficial cells. The modified stain (T.A-extract) successfully stained and highlighted superficial cells, while the Zingiber officinale extract (Z.A) performed poorly in staining the nuclei and cytoplasm. The staining ability of the Curcuma longa extract was found to be 100% comparable to the traditional Papanicolaou stain. Staining aims to clearly demonstrate cellular components for diagnostic purposes, and many existing stains are long-established but require modifications for better application. Using local alternatives can enhance availability and affordability. Curcuma longa is readily available and, based on our findings, can effectively replace Eosin Y in Eosin-Azure stains. Further research into the dye components of herbal products and their staining capabilities could lead to cost-effective, locally sourced alternatives to imported stains.

Keywords: Eosin Y, Curcuma longa, *Zingiber officinale*

INTRODUCTION

A dye is a substance which is used for coloration (Omorodion and Achukwu, 2017). Staining is a crucial technique in biology and medical sciences for visualizing and identifying biological structures. However, the synthetic dye Eosin Y commonly used for staining has demonstrated adverse effects on human health and the environment, prompting the search for safer alternatives. Natural dyes derived from plant sources, such as *Zingiber officinale* (ginger) and Curcuma longa (turmeric), have emerged as promising substitutes due to their eco-friendly properties (Smith, 2020).

Zingiber officinale or ginger, belongs to the Zingiberaceae family and is known for its medicinal properties including anti-inflammatory and antioxidant effects (Bode and Dong, 2011). Studies have shown that ginger extracts can effectively stain bacterial and fungal cells as well as plant tissues, attributed to the presence of anthocyanins, water-soluble pigments that impart reddish to bluish hues (Shinde*et al*., 2016; Tahmasbi*et al*., 2018).

Curcuma longa, commonly known as turmeric, shares the ginger family origin and is renowned for its anti-inflammatory and antimicrobial properties due to curcumin, which gives it the characteristic yellow color (Aggarwal et al., 2013). Turmeric extracts have also demonstrated staining capabilities for plant tissues, fungal, and bacterial cells, owing to the presence of curcuminoids, water-soluble pigments displaying yellow to orange colors (Rehman et al., 2017; Kumar et al., 2019).

Natural dyes like ginger and turmeric offer several advantages over synthetic dyes. They are biodegradable, pose minimal risk to human health, and are cost-effective. Furthermore, there is a critical need to explore indigenous plant species that is affordable from regions like Africa and Nigeria for their potential as staining agents in histopathology. It involves optimizing the extraction protocols, evaluating staining efficiency, safety, and eco-friendliness compared to Eosin Y (Doe, 2023).

This research aims to bridge the gap in exploring and utilizing herbal alternatives in cytological and histological staining, aiming for safer and more accessible options compared to existing synthetic dyes like Eosin

MATERIALS ANDMETHODS

Ethical Consideration

Ethical approval for this study was obtained from Edo State Ministry of Agric

Laboratory Equipment:

Microscope, Centrifuge, Glassware, Pipettes, Waterbath, Digitalcamera

Chemicals and Reagents

Ethanol, Acetic acid, Haematoxylin, Distilled water, Xylene, DPX mountant and glass slides.

Plant Harvesting and Preparation

Fresh rhizomes of *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) were obtained from a reliable sourced from New Benin market and authenticated by the herbarium at the Edo University, Uzairue. The rhizomes were thoroughly washed to remove dirt and debris, air- dried, and ground to a fine powder using a grinding mill. The powdered plant material was stored in airtight containers until further use.

Ethanol Extract

The ethanol extract was prepared by macerating 100g the powdered plant material in 500 ml of 95% ethanol. The mixture was placed in a tightly sealed container and allowed to stand for 72 h with intermittent shaking. Afterward, the extract was filtered using a Buchner funnel and Whatman filter paper to obtain a clear liquid extract.

Cervical Smears

Cervical smears were obtained from patient samples following the standard protocol for Papanicolaou staining. The smears were fixed in equal volume of 50% ethanol and 50% acetic acid mixture for 10 minutes. After fixation, the smears were washed with distilled water to remove excess fixatives.

Research Design

A total of 30 cervical smears were stained, 10 were stained with Papanicolaou stain to serves control and another 10 were stained with Papanicolaou stained containing *Curcuma longa* in place of Eosin Y and another 10 were stained with Papanicolaou stain containing *Zingiber officinale* extract in place of Eosin Y.

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Methodology

The staining procedure using *Zingiber officinale* and *Curcuma longa* extracts as a replacement for Eosin Y in EA50 is as follows:

Extractionof *Curcumalonga* (turmeric)

Hundred-gram (100 g) of the powder was dissolved into 500 ml of 95% ethanol and left for 24 hr. The top fluid was poured into another jar gently and allowed to stand for 2 h. The fluid portion was gently filtered, and the sediments was excluded using filter paper. The filtered stain was then kept in an airtight container.

Extraction of *Zingiber officinale* (Ginger)

100 g ofthepowderwasdissolvedinto500 ml of95% ethanol and left for24 hitherto fluid was poured into another jar gently and allowed to stand for 2 h. The fluid portion was gently filtered, and the sediments were excluded using filter paper. The filtered stain was then kept in an airtight container.

Preparation of Turmeric Staining Solution (Turmeric Azure)

Eosinazure containsthe following: EosinY, LightGreen, BismarkBrown and Phosphotungstic Acid

Preparation of Working Solution

Standard solution of turmeric (200 ml) was done following the standard procedure and the right proportion of the ingredients weighed and dissolved in the solvent. In place ofEosin Y, turmericas the staining solution.

Preparation of *Zingiber officinale* (Zingiber-Azure)

Eosinazure containsthe following: EosinY, LightGreen, Bismark Brown, Phosphotungstic Acid

Preparation of Working Solution

Standard solution of ginger (200 ml) was done following the standard procedure and the right proportion of the various chemicals, weighed and dissolved in the solvent. In place of Eosin Y, zingiber staining solution was used.

Photomicrograph (presentation)

Representative smears stained with ZA and TA were observed and photomicrographs were taken and presented as plates.

RESULT

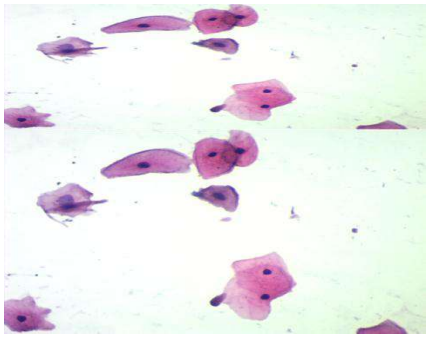

Plate 1: Control slides with cervical smears showing intermediate epithelial cells withwell stained nuclei and well stained cytoplasm on a clean background. Papanicolaou's stain, magnification x 400

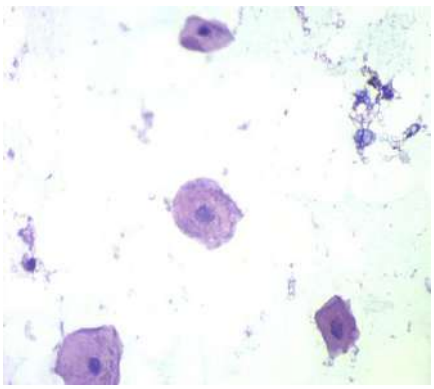

Plate 2: Control slide with cervical smear showing superficial epithelial cells with well stained nuclei and well stained cytoplasm on a clean background. Papanicolaou's stain, magnification x 400

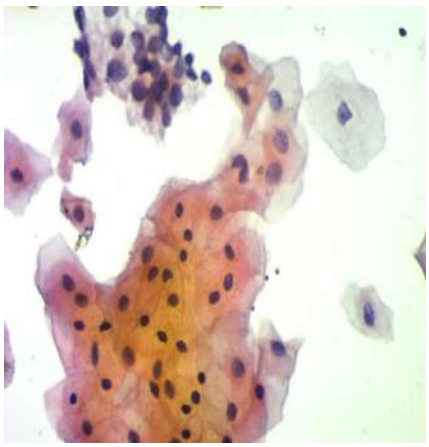

Plate3: Cervical smear showing superficial epithelial cells (thick arrow) with well stained nuclei and well stained cytoplasm.The intermediate epithelial cells (thin arrow) have well stained nuclei but moderately stained cytoplasm on a clean background

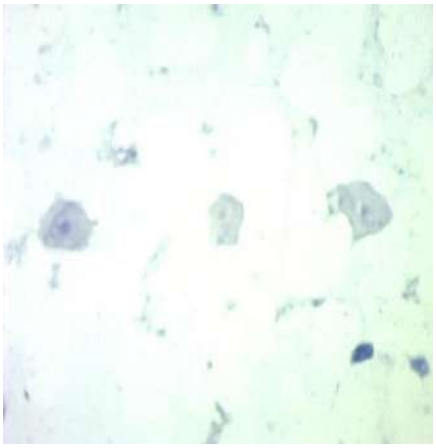

Plate 4: cervical smear shows superficial epithelial cells (thick arrow) with poorly stained nuclei and poorly stained cytoplasm.The intermediate epithelial cells (thin arrow) have poorly stained nuclei and poorly stained cytoplasm on a clean background. T-A Extract x400

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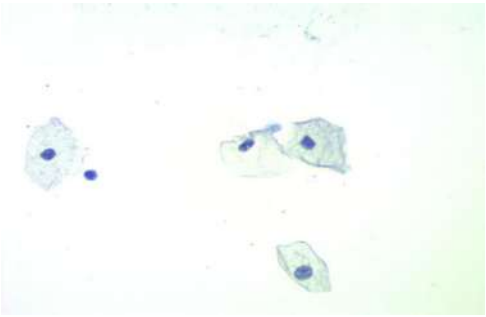

Plate 5 and 6: Cervical smear showing intermediate epithelial cells with well stained nuclei and moderate staining of cytoplasm on a clean background, cervical smear shows superficial epithelial cells (thick arrow) with well stained nuclei but moderately stained cytoplasm.

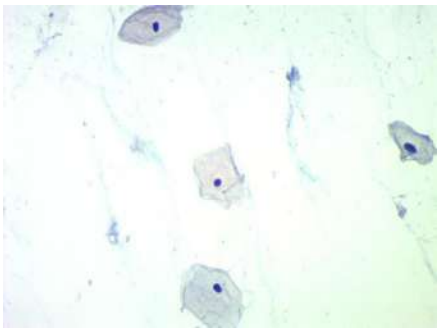

Plate 6:

The intermediate epithelial cells (thin arrow) have well stained nuclei but moderately stained cytoplasm on a clean background. Z-A Extract stain, magnification X40

DISCUSSION

Papanicolaou smear, which is also referred to as Pap smear, is an important screening test for cervical cancer. The process entails the collection of cervical cells which are then examined for cellular abnormalities, and this aids in early detection and prevention of cervical cancer (Bora *et al.,* 2017). The EA50 stain is used in Pap staining for its ability to highlight the structures of the cell such as those present in the nucleus, and by this, it enhanced cellular visualization. The use of the technique allows pathologists to correctly identify cells which are abnormal, and promote rapid medical interventions, thereby decreasing the risk of cervical cancer (Chankong*et al.,* 2014).

The visualization use of Papanicolaou's stain for the control slides showed proper staining of nuclei and cytoplasm of epithelial cells against a clean background. On the other hand, superficial cells stained with Papanicolaou's stain had well-stained nuclei and cytoplasm. The cell colors were found to be in the range of pink to blue which is normal (Chantziantoniou *et al.,* 2017). Intermediate and superficial epithelia are two of the four types of cervical epithelial cells, the others being basal and parabasal cells. Their presence in smears is indicative of normal healthy status of the individual (William *et al.,* 2019).

The visualization of cells upon staining superficial cells with *C. longa* extract showed proper staining of the nuclei and cytoplasm against a clean background. The use of *C. longa* extract resulted to blue staining of the nuclei which allowed these cell parts to be easily visible and identified, similar to the findings of Rubina *et al.* (2020), thereby corroborating our present results. However, the moderate visibility of the cytoplasm recorded is in line with the results of Sudhakaran*et al.* (2018) where eosin was found to be better for staining than *C. longa* extract

However, in the case of intermediate epithelial cells (Plates4.3and4.5), only nuclei were well-stained, while cytoplasm were moderately stained.

Staining was also done using *Z. officinal* extract and the subsequent visualization of the cells. It is seen that there was poor staining of nuclei, superficial and intermediate epithelial cells against a clean background. This result differs from that reported by Sudhakaran et al. (2018), where Z. officinale extract provided better staining with greater intensity and clarity. In their study, the extract resulted in well-stained nuclei and moderately stained cytoplasm in both superficial and intermediate epithelial cells, with clean backgrounds. This finding is consistent with Sudhakaran et al.'s results. The variation observed in Plates 4 and 6 may be due to differences in the concentrations of Z. officinale extract, which can impact staining effectiveness, as discussed by Lichius (2022).

Based on the results obtained, extracts from *C. longa* were found to be more effective than that of *Z. officinael* in the staining of cervical smears. However, eosin used in EA50 stain of the Papanicolaou test was more effective than both extracts.

CONCLUSION

This study was carried out to examine the staining ability of the extracts of *Zingiber officinale* and *Curcuma longa* and their potentials to be used as replacements for eosin in EA50 stain. The results obtained showed that good staining of cell nuclei and moderate staining of cytoplasm was obtained from all samples using *C. longa* extracts. The smears stained using *Z. officinale* produced mixed results as poor staining of nuclei and cytoplasm was recorded for one of the samples, while the other showed proper staining of nuclei and moderate staining of cytoplasm. Overall, *C. longa* provided better staining results compared to *Z. officinale.* However, neither produced results as good as those obtained using eosin, hence, none could be used as a replacement for eosin.

Recommendations

Based on the results obtained from the study, the following recommendations are made:

The emphasis should be on improving the staining methods for ginger and turmeric extracts. To optimize the staining process, experiment with different concentrations, application periods, and pre-treatment approaches. This might potentially improve the effectiveness of the extracts and bridge the staining gap between them and eosin.

Examination of the viability of a combined staining method. This would entail utilizing eosin as a basic stain and ginger/turmeric extracts as supplemental stains. Such a strategy might capitalize on the benefits of both eosin and the extracts, perhaps reaching a staining profile that competes with eosin alone.

Comprehensive examination of stained cervical cells utilizing modern microscopy and image analysis techniques. It is feasible to identify which staining approach improves visualization and cellular differentiation by focusing on certain cellular components, providing insights into prospective improvements in the ginger and turmeric extract staining procedures.

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