DEMONSTRATING THE STAINING ABILITY OF ZINGIBER OFFICINALE AND CURCUMA LONGA AS REPLACEMENT TO EOSIN Y IN EA50

BY

USMAN AHMED MUHAMMED
BMS2009030

DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
SCHOOL OF BASIC MEDICAL SCIENCES,
COLLEGE OF MEDICAL SCIENCES,
UNIVERSITY OF BENIN,
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BEING A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELORS DEGREE IN MEDICAL LABORATORY SCIENCE (BMLS) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SUPERVISED BY
DR. N.T. OMORODION

FEBRUARY, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **USMAN AHMED MUHAMMED** with the matriculation number **BMS2009030** under the supervision of **DR. N.T. OMORODION** in partial fulfillment for the award of Bachelor of Medical Laboratory Science (BMLS) degree.

DR. N.T. OMORODION	**DATE**
Supervisor	
DR (MRS) Z. OMORUYI	**DATE**
Ag. Head of Department	
External Examiner	**DATE**

DEDICATION

I dedicate this Project to my Family, for their endless love, support and encouragement.

ACKNOWLEDGEMENTS

I give thanks to almighty God, my creator who has granted me grace and strength to finish this project work within the limited time frame.

My profound gratitude goes to my supervisor DR. N.T. OMORODION for his genuine concern, support and guidance throughout the course of this study.

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ABSTRACT

The three major stains used in combination to stain cervical smears was discovered by Nicholas Papanicolaou and referred to as Pap stain. One of the stains is Eosin – Azure which is made up of Eosin Y, Light Green SF and Bismarck brown. Curcuma longa extract was applied in place of Eosin Y as a constituent of EA50. Cervical smears from premenopausal women were collected and stained with papanicolaou's stain as control, while modified papanicolaou's stain with *Curcuma longa* extract replacing Eosin Y in EA50 was also used to stain cervical smear. The staining ability of the modified stain was examined from the result. Superficial cells were well stained and demonstrated with the modified stain (T.A-extract) while the *Zingiber Officinale* extract (Z.A) stained the nuclei and cytoplasm of superficial cells poorly. The staining ability of the extract is 100% when compared to smears stained with Papanicolaou's stain. The major purpose of staining is to demonstrate cellular components to aid diagnosis. Most of the stains available are age long discoveries which require modifications for better application. Local alternative will help boost availability and affordability. *Curcuma Longa* is readily available and from our findings, it can be used in place of Eosin Y as a component of Eosin Azure. The dye component of herbal products and their staining ability should be researched to create cheaper and readily available local alternative to imported stains

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Staining is a crucial technique used in biology and medical sciences to visualize and identify different biological structures. However, the commonly used synthetic dye Eosin Y has been shown to have adverse effects on human health and the environment, leading to an increased need for safer and eco-friendly alternatives. Natural dyes extracted from plant sources have shown great potential as a safe and sustainable alternative to synthetic dyes. *Zingiber Officinale* (ginger) and *Curcuma Longa* (turmeric) are two plants that have been reported to possess staining properties, and their extracts could potentially serve as natural alternatives to Eosin Y. This project aims to demonstrate the staining ability of *Zingiber Officinale* and *Curcuma Longa* extracts as alternatives to Eosin Y for biological staining, evaluate their efficiency, safety, and eco-friendly, and compare them with Eosin Y.

Zingiber Officinale, commonly known as ginger, is a herbaceous plant that belongs to the Zingiberaceae family. Ginger has been traditionally used as a spice and a medicinal plant due to its anti-inflammatory, antioxidant, and anti-cancer properties (Bode and Dong, 2011). The staining properties of ginger have been reported in several studies, where it has been used to stain bacterial and fungal cells, as well as plant tissues (Shinde *et al.,* 2016; Tahmasbi *et al.,* 2018). The staining ability of ginger has been attributed to the presence of anthocyanins, which are water-soluble pigments that impart a red to blue color (Tahmasbi *et al.,* 2018).

Curcuma longa, commonly known as turmeric, is a member of the ginger family and is native to India and Southeast Asia. Turmeric has been used in Ayurvedic and traditional medicine for its anti-inflammatory, antioxidant, and antimicrobial properties (Aggarwal *et al.,* 2013). The active compound responsible for turmeric's medicinal properties is curcumin, a

polyphenolic compound that imparts the characteristic yellow color to turmeric(Aggarwal *et al.,* 2013). Turmeric has also been reported to possess staining properties and has been used to stain plant tissues, fungal cells, and bacterial cells (Rehman *et al.,* 2017; Kumar *et al.,* 2019). The staining ability of turmeric has been attributed to the presence of curcuminoids, which are water-soluble pigments that exhibit a yellow to orange color (Rehman *et al.,* 2017). The use of natural dyes such as ginger and turmeric extracts for biological staining has several advantages over synthetic dyes. Firstly, natural dyes are biodegradable and do not contribute to environmental pollution. Secondly, natural dyes are safe for human use and do not pose health risks compared to synthetic dyes, which have been shown to cause genetic damage and potentially lead to the development of cancer (Hu *et al.,* 2013). Additionally, natural dyes are readily available and cost-effective, making them an attractive alternative to synthetic dyes.

Therefore, this project aims to demonstrate the staining ability of *Zingiber Officinale* and *Curcuma longa* extracts as alternatives to Eosin Y for biological staining. The project will involve extracting the pigments from ginger and turmeric, optimizing the staining protocols, and comparing the staining efficiency of the natural extracts with Eosin Y. The project will also evaluate the safety and eco-friendliness of the natural extracts and compare them with Eosin Y. The results of this project will contribute to the development of safer and sustainable alternatives to synthetic dyes for biological staining, which can be adopted by researchers and the wider scientific community.

1.2 Statement of Problem

The use of eosin Y as a staining agent in histological and cytological preparations is widely accepted; however, there are concerns regarding its toxicity, environmental impact, and the need for safer, more sustainable alternatives. Recent studies have suggested that natural compounds derived from plants, such as Zingiber officinale (ginger) and Curcuma longa

(turmeric), possess staining properties that could serve as effective replacements for synthetic dyes (Tahmasbi *et al.,* 2018).

1.3 Justification

There is need for new local alternative to existing stains, not much have been done in the field of histopathology with regard to development of new and local herbal alternatives for a long time. Therefore, dye producing plants of Africa and Nigeria extractions need to be researched for cytopathological and histological staining abilities.

1.4 Aim

The aim of this study was to demonstrate the cytological staining ability of *Zingiber Officinale*

(Ginger) and *Curcuma Longa* (Turmeric) used in place of Eosin Y in EA50

1.5 Objective of the Study

1. To compare the cytological staining ability of *Zingiber Officinale* extract (Z-A) used in place of Eosin Y to papanicolaou stain.
2. To compare the cytological staining ability of *Curcuma Longa* extract *(*T-A) used in place of Eosin Y to papanicolaou stain.

1.6 Significance of the Study

This project will provide a safe, accessible, and cost-effective local alternative to the conventional stains. Furthermore, it can pave the way for future research on the application of natural extracts in staining techniques. Due to the wide application and consumption of the plant and also the dye producing nature of the plant necessitate the quest to examine if the staining ability of the dye matches Eosin Y.

CHAPTER TWO

LITERATURE REVIEW

2.1 Zingiber Officinale

Zingiber Officinale, commonly known as ginger, is a flowering plant that belongs to the *zingiberaceae* family. It is widely cultivated for its aromatic rhizomes, which are used as a spice, flavouring agent, and traditional medicine in various cultures around the world. Ginger has a long history of culinary and medicinal use, and its therapeutic properties have been recognized for centuries.

Zingiber Officinale is a perennial herbaceous plant that grows up to a meter in height. It has narrow, lance-shaped leaves that emerges from the underground rhizomes. The rhizomes are thick, knobby, and have a pale yellow color. The plant produces cone-like inflorescence's with yellow-green flowers that are often hidden among the leaves. The rhizomes of *Zingiber Officinale* are the most sought-after of the plant due to their distinctive flavor and aroma (Ali *et al.,* 2008)

Figure 2.1: An Image Depicting Zingiber Officinale Plant (Wikipedia, 2022)

Figure 2.2: An Image Depicting Zingiber Officinale Rhizome (Encyclopedia Britannica, 2019)

Figure 2.3: A Picture Showing The Image Of Zingiber Officinale (Singh, 2020)

2.1.1 Cultivation

Ginger is native to southeast Asia and is now cultivated in tropical and subtropical regions worldwide. It requires a warm and humid climate to grow successfully. The plant prefers well-drained soil enriched with organic matter. The cultivation of ginger involves planting sections of the rhizomes called “seed rhizomes” in the soil. After planting ,the rhizomes sprout shoots that develop into the above-ground foliage, while the underground rhizomes continue to grow and accumulate nutrients. Ginger plants take about 8-10 months to mature, at which point the rhizomes can be harvested (Parvathi *et al.,* 2020).

2.2.2 Uses of Zingiber Officinale

Zingiber Officinale, commonly known as ginger, is a versatile plant that `has been used for centuries in various traditional medicine systems, culinary practices, and industries.

1. *Medicinal uses:*

Ginger has a long history of medicinal use and is known for its therapeutic properties. Some common medicinal uses of *Zingiber Officinale* include:

i. **Digestive Health:** Ginger has been traditionally used to alleviate digestive issues such as indigestion, bloating and gastrointestinal discomfort (Hu ml *et al.,* 2011)

ii. **Nausea and Vomiting Relief:** Ginger is recognized for its antiemetic properties and is commonly used to reduce nausea and vomiting, including pregnancy-related morning sickness and chemotherapy-induced nausea (Keating A *et al.,* 2002)

iii. **Anti-Inflammatory Effects**: Ginger exhibits potent anti-inflammatory effects, which can help in managing conditions such as osteoarthritis and rheumatoid arthritis (Mozaffari *et al.,* 2016)

iv. **Immune System Support:** Ginger possesses immunodulatory properties that can enhance immune function and protect against certain infections (Mashhadi *et al.,* 2013)

2. Culinary Uses:

Ginger is widely used as a spice and flavoring agent in various cuisines around the world. Its pungent and slightly sweet flavor adds a distinctive taste to dishes. Fresh ginger is commonly grated, chopped, or sliced and added to Stir-fries, soups, curries, marinades, and beverages. It is also used in baking, particularly in gingerbread, cookies, and cakes. Dried ginger powder is another form in which ginger is used for culinary]purposes. Additionally, ginger is a key ingredient in many traditional beverages, such as ginger tea and ginger ale (Butt et al, 2011).

Some culinary uses of *Zingiber Officinale* include:

i. **Cooking:** Ginger adds a distinctive flavor and aroma to a wide range of dishes, including curries, stir-fries, soup, and sauces.

ii. **Baking:** Ginger is commonly used in baking, especially in desserts like gingerbread, ginger cookies, and cakes, providing a warm and spicy taste.

3. *Industrial Uses:*

Ginger also finds applications in various industries. Some industrial uses of *Zingiber Officinale* include:

i. **Perfumery:** Ginger oil is utilized in the production of perfumes and fragrances, adding a spicy and warm note to the compositions.

ii. Cosmetics: Ginger extracts and oil are incorporated into skincare and hair care products for their antioxidants and anti-inflammatory properties, offering potential benefits for the skin and hair.

2.1.3 Preparation of Zingibger Officinale

Preparation of *Zingiber Officinale* (ginger) involves several steps to ensure its optimal use and extraction of its beneficial components.

1.Selection of Fresh Ginger:

Choose fresh ginger rhizomes that are firm, smooth, and free from mold or blemishes. Look for ginger with a strong aroma, indicating its freshness and potency.

2. Cleaning and peeling:

Thoroughly wash the ginger rhizomes under running water to remove any dirt or debris. Use a vegetable brush to gently scrub the surface. Once cleaned, peel the ginger skin using a knife or a peeler. Removing the skin helps eliminate any texture.

3. Slicing o r grating:

Depending on the intended use, ginger can be sliced or grated. For thin slices, use a sharp knife and cut the ginger into desired thickness. For grating, use a fine grater to create a finely grated ginger. Grating ginger facilitates better extraction of its flavor and active compounds.

4. *Infusion or Decotion:*

Ginger can be prepared as an infusion or decotion, depending on the desired outcome.

i. **Infusion:** To prepare an infusion, place the sliced or grated ginger in a cup or teapot and pour hot water over it. Allow it to steep for about 5-10 minutes, depending on the desired strength. Strain the liquid and it is ready for consumption. Ginger infusion can be enjoyed as a soothing and aromatic tea.

ii. **Decoction:** For a decotion, add the sliced ginger to a pot of water and bring it to boil.

Reduce the heat and simmer for 15-20 minutes to extract the flavors and beneficial compounds. Strain the liquid and use it as desired. Decoctions are commonly used for making ginger-based soups, broths, or as an ingredient in various recipes.

5. Drying and powdering (Optional)

If desired, ginger can be dried and powdered for long-term storage and convenience. To dry ginger, slice it into thin pieces and spread them out in a well-ventilated area or use a dehydrator at a low temperature. Once completely dry, grind the ginger slices into a fine powder using a mortar and pestle or a spice grinder. Store the powdered ginger in an airtight container in a cool, dry place.

2.2 Curcuma Longa

Curcuma longa, commonly known as turmeric, is a flowering plant that belongs to the ginger family, Zingiberaceae, the rhizomes of which are used in cooking The plant is a perennial, rhizomatous, herbaceous plant native to the Indian subcontinent and Southeast Asia that requires temperatures between 20 and 30 c (68 and 86 f) and high annual rainfall to thrive. Plants are gathered each year for their rhizomes, some for propagation in the following season and some for consumption. The rhizomes are used fresh or boiled in water and dried, after which they are ground into a deep orange-yellow powder commonly used as a coloring and flavouring agents used in many Asian cuisines, especially for curries, as well as for the dyeing characteristics imparted by the principal constituent, curcumin. Natural dyes find use in the coloring of textiles, drugs, cosmetics (Wikimedia Foundation, 2023)

Turmeric powder has a warm, bitter, black pepper-like flavor and earthy, mustard-like aroma. Curcumin, a bright yellow chemical produced by the turmeric plant, is approved as a food additive by the World Health Organization, European Parliament, and United States Food and Drug Administration.

The ability of a dye to stain specific tissue structures is determined by certain factor, one of which is the acidity of the stain. Acidic structures1would be stained by basic dyes while basic structures would be stained by acidic dyes (Avwioro OG *et al.,*)

Figure 2.4: A picture showing plant of Curcuma Longa (i Naturalist, n.d)

Figure 2.5: A picture showing the Rhizome of Curcuma Longa (Wikipedia, 2019)

2.2.1 Appearance

Turmeric is a perennial herbaceous plant that reaches up to 1m (3 ft 3 in) tall. It has highly branched, yellow to orange, cylindrical, aromatic rhizomes.

The leaves are alternate and arranged in two rows. They are divided into leaf sheath, petiole, and leaf blade. From the leaf sheaths, a false stem is formed. The petiole is 50 to 115 cm (20-45 in) long. The simple leaf blades are usually 76 to 115cm (30-45 in) long and rarely up to 230 cm (7 ft 7 in). They have a width of 38 ton 45cm (15 to 17 in) and are oblong to elliptical, narrowing at the tip.

2.2.2 Cultivation

1. Environmental Requirements:

Curcuma longa thrives in tropical and subtropical regions, preferring temperatures between 20 and 30 degrees Celsius (68 to 86 degrees Fahrenheit). The plant requires a well-drained soil with a pH range of 5.5 to 7.5. It grows best in areas with an annual rainfall of 1500 to 2500 millimeters (59 to 98 inches), although it can be cultivated with irrigation facilities or during the rainy season.

2. Propagation:

Turmeric is primarily propagated through rhizomes, the underground stems of the plant. Select healthy rhizomes from a reputable source or your own stock. Cut the rhizomes into small sections, each containing at least two to three viable buds (eyes).

3. Planting:

Prepare the planting area by clearing weeds and debris. Dig furrows or trenches that are approximately 20 centimeters (8 inches) deep and spaced 30 to 45 centimeters (12 to 18 inches)

apart. Place the rhizome sections horizontally in the furrows, ensuring the buds face upward, and cover them with soil.

4. Field Management:

Maintain consistent soil moisture levels by regular watering, particularly during dry periods. However, avoid water logging, as excess moisture can lead to rhizome rot. Provide shade or mulch to retain moisture and suppress weed growth. Weed the field regularly to minimize competition for nutrients and space.

5. Fertilization:

Prior to planting, incorporate well-decomposed organic matter, such as compost or aged manure, into the soil. Additionally, apply balanced fertilizers with a higher potassium content during the active growth stage to support healthy rhizome development.

6. Pest and Disease Control:

Monitor the crop regularly for pests and diseases such as rhizome rot, root-knot nematodes, or leaf spot diseases. Use organic or appropriate chemical controls to manage these issues when necessary. Practicing crop rotation and maintaining good soil hygiene can also help prevent pest and disease problems.

7. Harvesting:

Turmeric plants typically mature within 7 to 10 months. Harvesting can commence when the leaves start turning yellow and drying. Carefully dig around the rhizomes using a garden fork or shovel, taking care to avoid damaging them. Gently remove the soil, cut off the foliage, and wash the rhizomes to remove any residual soil. Allow them to air-dry for several days before storage or further processing.

2.2.3 Uses of Curcuma Longa

Curcuma longa, commonly known as turmeric, is a perennial plant belonging to the ginger family (zingiberaceae). It is native to south Asia and has been used for centuries in traditional medicine and culinary practices. Curcuma longa contains a bioactive compound called curcumin, which is responsible for its vibrant yellow colour and numerous health benefits.

2.2.4 Medicinal Uses:

Turmeric has a long history of use in traditional medicine systems such as Ayurveda and traditional Chinese medicine. Some common medicinal uses of curcuma longa includes:

i. **Anti-inflammatory effects:** Curcumin, the main active compound in turmeric, possesses potent anti-inflammatory properties (Gupta *et al.*, 2013). It inhibits various inflammatory pathways and can help in managing chronic inflammatory conditions such as arthritis, inflammatory bowel disease, metabolic syndrome.

ii. **Antioxidant activity**: Curcumin exhibits strong antioxidant activity, which helps protect cells against oxidative stress and damage caused by free radicals (Hewlings SJ *et al.,*2017).It may contribute to overall cellular health and play a role in disease prevention.

iii. **Digestive Health:** Turmeric has been traditionally used to support digestive health. It stimulates the production of bile, which aids in digestion, and may help alleviate symptoms of indigestion, bloating and gas (Bundy *et al.,* 2004).

iv. **Liver Health:** Curcumin has been found to have neuroprotective properties, supporting liver health and function (Lee *et al.,*). it may help protect the liver from damage caused by toxins, alcohol, and certain medications.

2.2.5 Culinary Uses:

Turmeric is widely used as a spice in culinary practices, particularly in south Asian and middle eastern cuisines. Some culinary uses of curcuma longa includes

i. **Flavoring Agent:** Turmeric adds a distinct flavor and vibrant yellow color to dishes. It is commonly used in curries, rice dishes, soups, and sauces, importing a warm and earthy taste.

ii. **Food Preservation:** Turmeric has antimicrobial properties and has been traditionally used as a natural food preservative. Its active compounds may help inhibit the growth of certain bacteria, fungi and parasites.

2.2.6 Cosmetics and Skincare Uses:

Curcuma longa finds applications in the cosmetic industry due to its potential benefits for the skin. Some cosmetic uses of Curcuma longa includes:

i. **Skincare:** Turmeric is believed to possess anti-inflammatory, antioxidant and antimicrobial properties that may benefit the skin. It is used in skincare products such as cleansers, mask, and creams to promote a healthy complexion.

ii. **Wound Healing:** Curcumin has been studied for its potential to enhance wound healing. It may help in reducing inflammation, promoting tissue regeneration, and accelerating the healing process.

1. Neurological Health:

Curcumin has shown neuroprotective effects and may have potential be3nefits for brain health. It exhibits antioxidants and anti-inflammatory properties that could help in reducing neuroinflammation and oxidative stress, which are implicated in neurodegenerative diseases such as Alzheimer's and Parkinson’s (Kulkarni *et al.,* 2018)

2. Cardiovascular Health:

Studies have suggested that curcumin may have cardiovascular benefits. It has been found to improve endothelial function, reduce inflammation, and lower oxidative stress, which contribute to the prevention, management of cardiovascular diseases (Goyal *et al.,*) However, more clinical trials are needed to establish its efficacy in this context.

2.2.7 Preparation of Curcuma Longa

Preparation of Curcuma longa (turmeric) involves several steps to maximize its flavor, aroma, and extraction of its beneficial compounds.

1. Selection of Fresh Turmeric:

Choose fresh turmeric rhizomes that are firm, plump, and vibrant in color. Look for turmeric with a rich golden hue, indicating its freshness and potency. Avoid rhizomes that appear wrinkled, moldy, or discolored.

2. Cleaning and Peeling:

Thoroughly wash the turmeric rhizomes under running water to remove any dirt or debris. Use a vegetable brush to gently scrub the surface. Once cleaned, peel the turmeric skin using a knife or a peeler. Removing the skin helps eliminate any bitterness and facilitates further processing.

Turmeric can be prepared in various forms, depending on the intended use.

i. **Slicing:** Use a fine grater to create finely grated turmeric. Grated turmeric is commonly used in spice blends, marinades, and dressings, providing a concentrated flavor and aroma.

ii. **Grinding:** For powdered turmeric, grind the peeled turmeric rhizomes into a fine powder using a mortar and pestle, spice grinder, or a food processor. Grinding helps release the essential oils and active compounds present in turmeric.

3. Drying (Optional):

If desired, turmeric can be dried for long-term storage and convenience. After peeling and slicing the turmeric, spread the pieces out in a well-ventilated area or use a dehydrator at a low temperature. Once completely dry, grind the dried turmeric into a fine powder using a spice grinder or mortar and pestle. Store the powdered turmeric in an airtight container in a cool, dark place.

4. Extract preparations:

Turmeric extracts can also be prepared using solvents like alcohol or oil. These extracts are commonly used in traditional medicine or dietary supplements. To prepare an alcohol-based extract, mix the grated or powdered turmeric with carrier oil (such as coconut, olive, or sesame oil) and allow it to infuse for a specific p e r i o d .

2.3 Papanicolaou Stain (Pap Stain)

Papanicolaou stain is a multi-chromatic (multicolored) cytological staining technique developed by George Papanicolaou in 1964 (Gill *et al.,*2013) The Papanicolaou stain is one of the most widely used stains in cytology, where it is used to aid pathologist in making a diagnosis. Although most notable for its use in the detection of cervical cancer in the Pap test or Pap smear, it has reduced the incidence of cervical cancer by 70% especially in developed countries having well planned screening programs. The Papanicolaou is a polychromatic counterstaining method consisting of stains such as orange G6 (OG6) and modified Eosin azure (EA). It is also used to stain non-gynecological specimen preparations from a variety of bodily secretions and from small needle biopsies of organs and tissues (Kumar *et al.,*2013) Several specimens can be used to prepare the pap smear depending on the screening infection, including sputum, urine, cerebrospinal fluid, abdominal fluid, tumor biopsies, synovial fluid, fine needle aspirates, pleural fluids.

2.3.1 Applications of Papanicolaou Staining (Pap Stain)

1. Screening of thyroid cancer.
2. Screening for cell carcinomas.
3. Examination and characterization of benign tumors.
4. Identification of Candida species.
5. Identification of Chlamydia trachomatis
6. Used in the Pap smear (or Pap test).
7. Screening for cervical cancer.

2.3.2 Limitations of Papanicolaou Staining (Pap Stain)

1. It is only a screening test that must be followed up with more specialized diagnostic tests.
2. It has low sensitivity with limited accuracy.

2.3.3 Diagnostic Relevance of Papanicolaou Stain

Cervical cancer screening: Papanicolaou stain is widely employed as a screening tool for cervical cancer. By examining the cellular morphology of cervical cells, pathologists can identify any abnormal changes, such as dysplasia or carcinoma, indicating the presence of precancerous or cancerous conditions. Regular Pap smears have been instrumental in reducing the incidence and mortality rates of cervical cancer.

Detection of precancerous lesions: The Papanicolaou stain allows the identification of precancerous lesions known as cervical intraepithelial neoplasia (CIN). These abnormalities, ranging from mild (CIN1) to severe (CIN 3), Indicate cellular changes that have the potential to progress to invasive cancer if left untreated. Detecting and treating these precancerous lesions early can prevent the development of cervical cancer.

Identification of infectious agents: The Pap smear can also provide diagnostic information about the presence of infectious agents, such as human papilomavirus (HPV), which is the primary cause of cervical cancer. In addition, other infectious organisms, such as Candida or Trichomonas, can be detected through the examination of stained cervical cells.

Evaluation of endomentrial abnormalities: Although the Pap smear is primarily focused on cervical cytology, abnormal cells from the endometrium (the lining of the uterus) may be also be detected in some cases. While the pap smear is not as effective for evaluating endometrial abnormalities as it is for cervical abnormalities, it can provide initial clues for

further investigation and may warrant additional diagnostic procedures, such as endometrial biopsies.

Follow-up after treatment: The Papanicolaou stain is valuable in post-treatment monitoring of cervical abnormalities. It helps assess the effectiveness of interventions, such as surgical excision or ablative procedures, by examining the cellular changes in subsequent Pap smears. Regular follow-up Pap smears are essential to ensure that any residual or recurrent abnormalities are detected early.

2.4 Pap Smear

A Pap smear,also know as a pap test or cervical cytology, is a medical screening procedure performed to detect abnormal cells in the cervix, which is the lower part of the uterus that opens into the vaginal. The test is primarily used to identify early signs of cervical cancer or other cervical cancer or other cervical abnormalities, allowing for early intervention and treatment. During a Pap smear, a healthcare professional collects a small sample of cells from the cervix using a speculum to hold the vaginal open. The cells are then gently scraped or brushed from the cervix and placed on a glass slide or in a liquid solution. The sample is sent to a laboratory where it is examined under a microscope to identify any abnormal or pre-cancerous cells. Pap smears are typically recommended for individuals with a cervix stating at the age of 21, although guidelines may vary slightly depending on the country and healthcare provider. The Pap smear is a relatively simple and quick procedure and is generally well-tolerated by most individuals. It is an effective method for early detection and prevention of cervical cancer. If abnormal cells are detected, further diagnostic test such as colposcopy or a biopsy, may be recommended to determine the extent of the abnormality and guide further treatment if necessary.

2.5 Constituents of Eosin Azure (EA)

2.0.1 Eosin Y

Eosin Y is a red dye commonly used in histology and cytology staining procedures. It is a synthetic compound that belongs to the xanthene dye family.

2.5.1 Chemical Properties

Eosin Y has the chemical formula C20H6Br4O5 and a molecular weight of 879.86 g/mol. It is a brominated derivative of fluorescein, which gives it its vibrant red color. Eosin Y is soluble in water and alcohol but insoluble in ether and chloroform.

2.5.2 Uses of Eosin Y

1. Histology and Cytology Staining: Eosin Y is commonly used in histological and cytological staining procedures to stain the cytoplasm and extracellular structures of cells. It is often used as a counter stain to Haematoxylin, which stains cell nuclei.
2. Microscopy: Eosin Y can be used in microscopy to enhance the visibility of certain structures and tissues, aiding in their identification and analysis.
3. Medical Applications: Eosin Y has antimicrobial properties and can be used in some topical antiseptic solutions.
4. Dyeing: Eosin Y is used as a dye in various applications, including textiles, cosmetics, and ink manufacturing.

2.5.3 Staining Mechanism

Eosin Y is an acidic dye, meaning it carries a negative charge at neutral pH. In staining procedures, Eosin Y is often used in combination with a basic dye, such as Haematoxylin. The acidic Eosin Y stains the basic components of cells, such as cytoplasm, while the basic dye stains the acidic components, such as nuclei. This differential staining allows for improved visualization and differentiation of cellular structures.

2.5.4 Safety Precautions

Eosin Y is generally considered safe to handle and work with. However, as with any chemical substance, it is important to follow proper safety precautions. This may include wearing protective gloves, goggles, and a lab coat to prevent direct contact with the skin, eyes, or clothing. Additionally, Eosin Y should be used in a well-ventilated area to avoid inhalation of dust or fumes.

2.6 Light Green SF

Light Green SF is a green dye that belongs to the triarylmethane class of dyes. It is widely used in various applications, including microscopy, histology, and as a biological stain.

2.6.1 Chemical Properties

Light Green SF has the chemical formula C27H20N2O6S2 and a molecular weight of 532.58 g/mol. It is a synthetic dye that exhibits bright green coloration. Light Green SF is soluble in water and alcohol, and its solubility increases in acidic solutions.

2.6.2 Uses

1. Histology and Cytology Staining: Light Green SF is commonly used in histological and cytological staining procedures. It stains collagen, mucins, and other connective tissues, allowing for their differentiation and analysis.
2. Microscopy: Light Green SF can be used in microscopy to stain specific cellular structures and tissues, aiding in their visualization and identification.
3. Biological Staining: Light Green SF is used in various biological staining techniques to highlight specific cell types or structures.
4. Textile Dyeing: Light Green SF is employed as a dye in the textile industry, adding vibrant green color to fabrics and materials.

2.6.3 Staining Mechanism

Light Green SF is a basic dye, carrying a positive charge at neutral pH. It interacts with negatively charged components in cells and tissues, resulting in selective staining. In histology and cytology, Light Green SF stains collagen fibers and mucins due to their acidic nature. It is often used in combination with other dyes to achieve a desired staining pattern.

2.6.4 Safety Precautions

Light Green SF is generally considered safe to handle, but precautionary measures should be taken to ensure personal safety. It is advisable to wear gloves, goggles, and a lab coat when working with Light Green SF to avoid direct contact with the skin, eyes, or clothing. Proper ventilation should also be ensured to prevent the inhalation of dust or fumes.

2.7 Bismarck Brown

Bismarck Brown is a brown synthetic dye belonging to the azo dye class. It is widely used in various applications, including histology, microscopy, and as a biological stain.

2.7.1 Chemical Properties

Bismarck Brown has the chemical formula C27H24N6O7S2 and a molecular weight of 624.64 g/mol. It is a synthetic dye that exhibits a dark brown color. Bismarck Brown is soluble in water and alcohol.

2.7.2 Uses

1. Histology and Cytology Staining: Bismarck Brown is commonly used in histological and cytological staining procedures. It stains cell nuclei and other acidic components, aiding in their identification and analysis.
2. Microscopy: Bismarck Brown can be used in microscopy to stain specific cellular structures and tissues, enhancing their visibility and differentiation.
3. Biological Staining: Bismarck Brown is used in various biological staining techniques to highlight specific cell types or structures.
4. Textile Dyeing: Bismarck Brown is employed as a dye in the textile industry, imparting dark brown color to fabrics and materials.

2.7.3 Staining Mechanism

Bismarck Brown is a basic dye, carrying a positive charge at neutral pH. It interacts with negatively charged components in cells and tissues, resulting in selective staining. Bismarck

Brown stains acidic components, such as nuclei, due to their affinity for basic dyes. It is often used in combination with other dyes to achieve desired staining patterns.

2.7.4 Safety Precautions

Bismarck Brown should be handled with care to ensure personal safety. It is advisable to wear gloves, goggles, and a lab coat when working with Bismarck Brown to avoid direct contact with the skin, eyes, or clothing. Proper ventilation should also be ensured to prevent the inhalation of dust or fumes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Laboratory Equipment:

The following laboratory equipment's were used in this study:

Microscope: Equipped with a high-resolution objective lens and appropriate magnification options.

Centrifuge: Used for separating the plant extract from the solvent.

Glassware: Including glass slides, cover slips, and staining dishes for preparing and staining the cervical smears.

Pipettes: Used for precise measurement and transfer of reagents.

Water bath: Employed for temperature-controlled incubation during the staining process.

Digital camera: Used to capture microscopic images of stained samples for analysis.

3.2 Chemicals and Reagents

The following chemicals and reagents were used in this study:

1. Ethanol: Used as a solvent for extracting active compounds from *Zingiber Officinale* and Curcuma longa.
2. Eosin Y: Traditional staining dye used in Papanicolaou staining.
3. Acetic acid: Used in the preparation of fixative solution for cervical smears.
4. Haematoxylin: Counter stain for Papanicolaou staining.

5. Distilled water: Used for preparing reagents and dilutions.
6. Xylene: Clearing agent used for deparaffinization of cervical smears.
7. Ethanol series: Solutions of different ethanol concentrations for dehydration of cervical smears.
8. DPX mountant: Used for mounting the stained cervical smears onto glass slides.

3.3 Plant Harvesting and Preparation

Fresh rhizomes of *Zingiber Officinale* (ginger) and Curcuma longa (turmeric) were obtained from a reliable source. 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.

3.4 Ethanol Extract

The ethanol extract was prepared by macerating 100 grams of 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 hours with intermittent shaking. Afterward, the extract was filtered using a Buchner funnel and Whatman filter paper to obtain a clear liquid extract.

3.5 Cervical Smears

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

3.6 Design

Case control experimental design was adopted. A total of 30 cervical smears were stained, ten were stained with Papanicolaou stain to serve as control and another ten were stained with Papanicolaou stained containing curcuma longa in place of Eosin Y and another ten were stained with Papanicolaou stain containing *Zingiber Officinale* extract in place of Eosin Y.

3.7 Methodology

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

3.8 Extraction of Curcuma Longa (turmeric)

100grams of the powder was dissolved into 500mls of 95% ethanol and left for 24hrs. The top fluid was poured into another jar gently and allowed to stand for 2 hours. The fluid portion was gently filtered and the sediments was excluded using filter paper. The filtered stain was then kept in an airtight container.

3.9 Extraction of Zingiber officinale (Ginger)

100grams of the powder was dissolved into 500mls of 95% ethanol and left for 24hrs. The top fluid was poured into another jar gently and allowed to stand for 2 hours. The fluid portion was gently filtered and the sediments was excluded using filter paper. The filtered stain was then kept in an airtight container.

3.10 Preparation of Turmeric Solution (Turmeric Azure)

Eosin azure contains the following

1. Eosin Y
2. Light Green
3. Bismark Brown
4. Phosphotungstic Acid

3.11 Preparation of Working Solution

Standard solution of 200ml was done following the standard procedure and the right proportion of the various chemicals, weighed and dissolved with the solvent. In place of Eosin Y, turmeric was the solvent of choice.

3.12 Preparation of Zingiber officinale (Zingiber-Azure)

Eosin azure contains the following

1. Eosin Y
2. Light Green
3. Bismark Brown
4. Phosphotungstic Acid

3.13 Preparation Of Working Solution

Standard solution of 200ml was done following the standard procedure and the right proportion of the various chemicals, weighed and dissolved with the solvent. In place of Eosin Y, zingiber was the solvent of choice.

3.14 Photomicrograph (presentation)

Representative smears stained with ZA and TA will be observed and photomicrograph will be done and presented as plates.

3.15 Ethical Consideration

Ethical approval for this study was obtained from Edo State Ministry of Agriculture and Security Research and Ethical Committee..

CHAPTER FOUR

RESULT

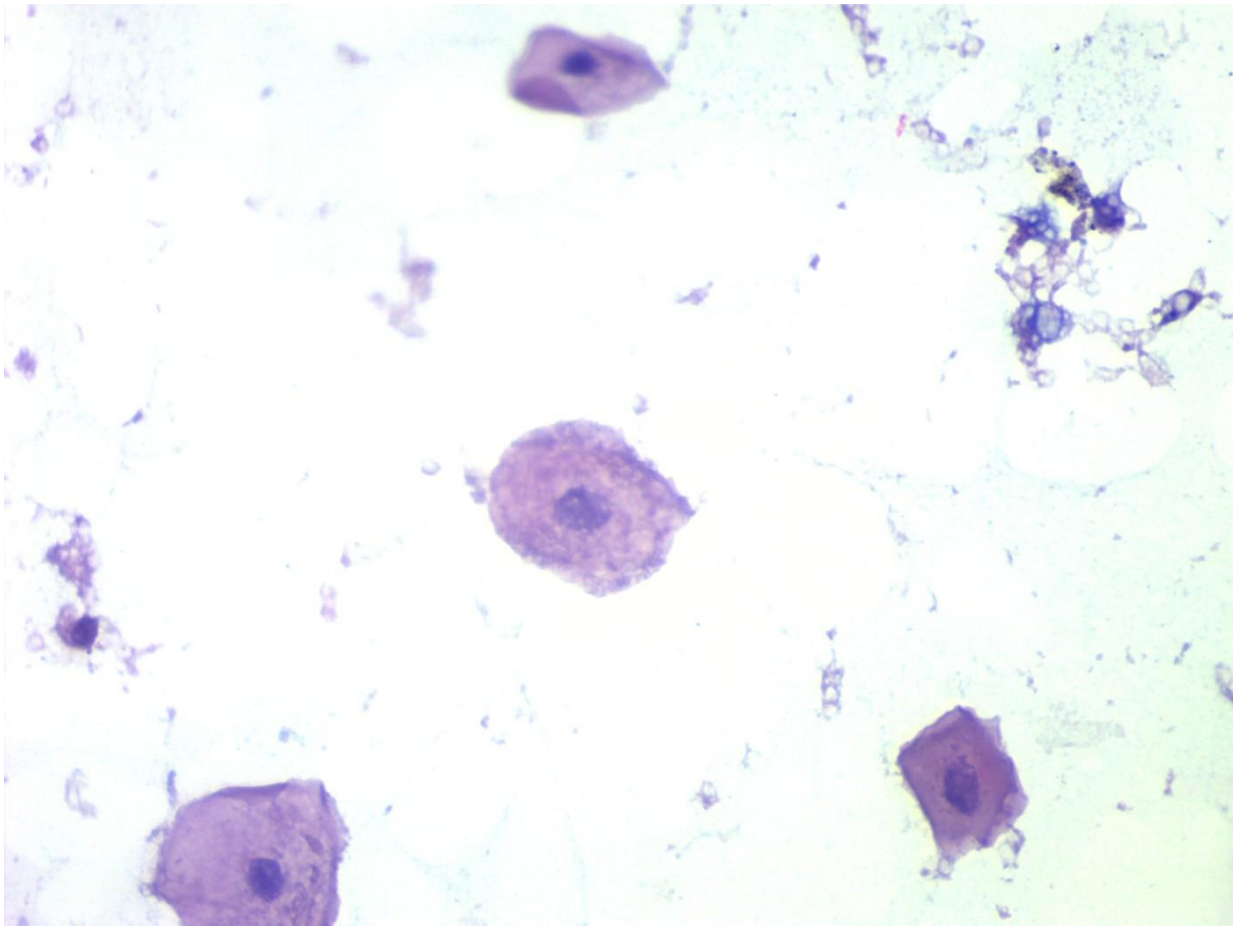

PLATE 4.1: Control slides with Cervical smears showing intermediate epithelial cells with well stained nuclei and well stained cytoplasm on a clean background. Papanicolaou's stain x400

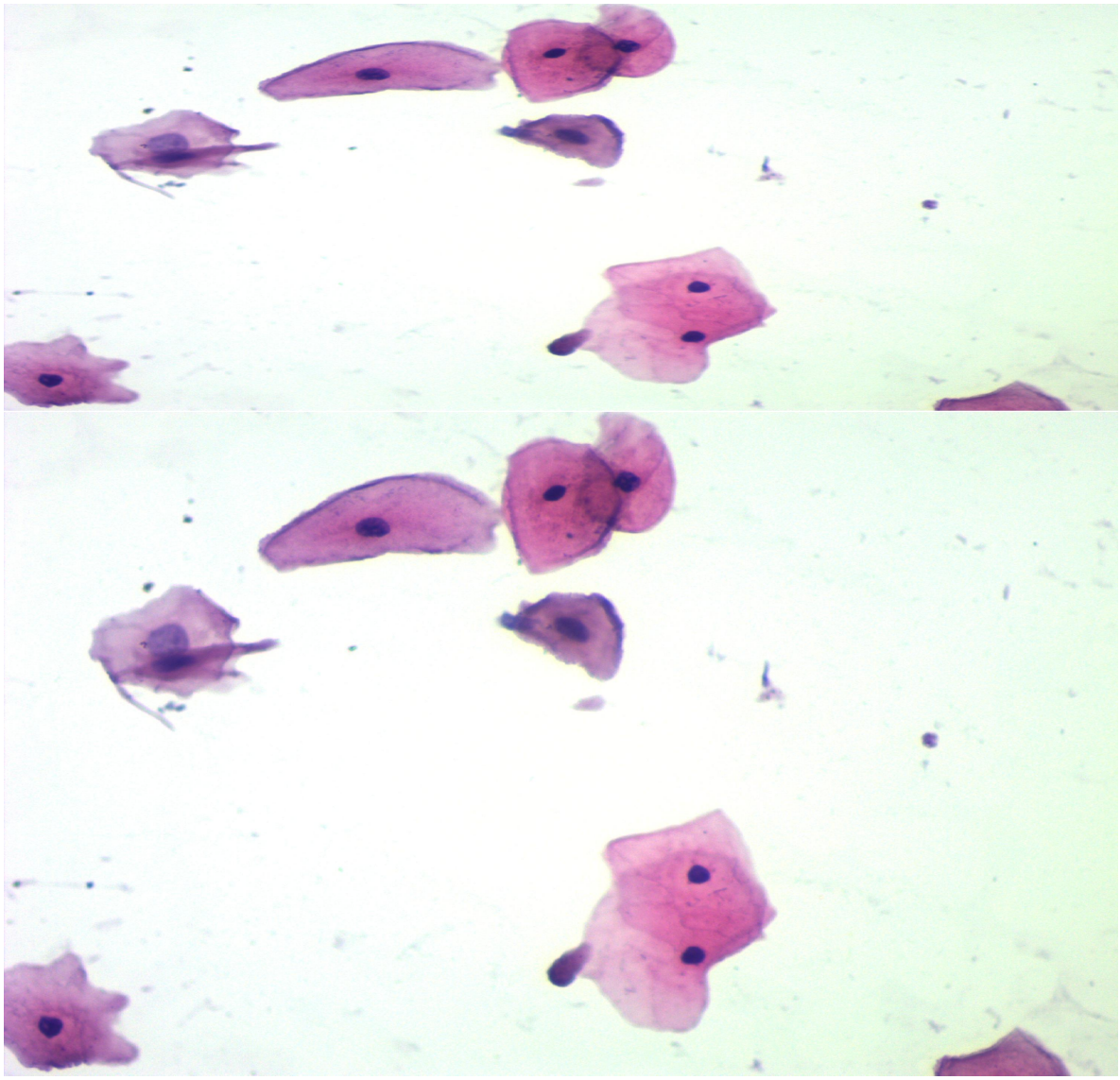

PLATE 4.2: Control slide with Cervical Smear showing superficial epithelial cells with well stained nuclei and well stained cytoplasm on a clean background. Papanicolaou's stain x400

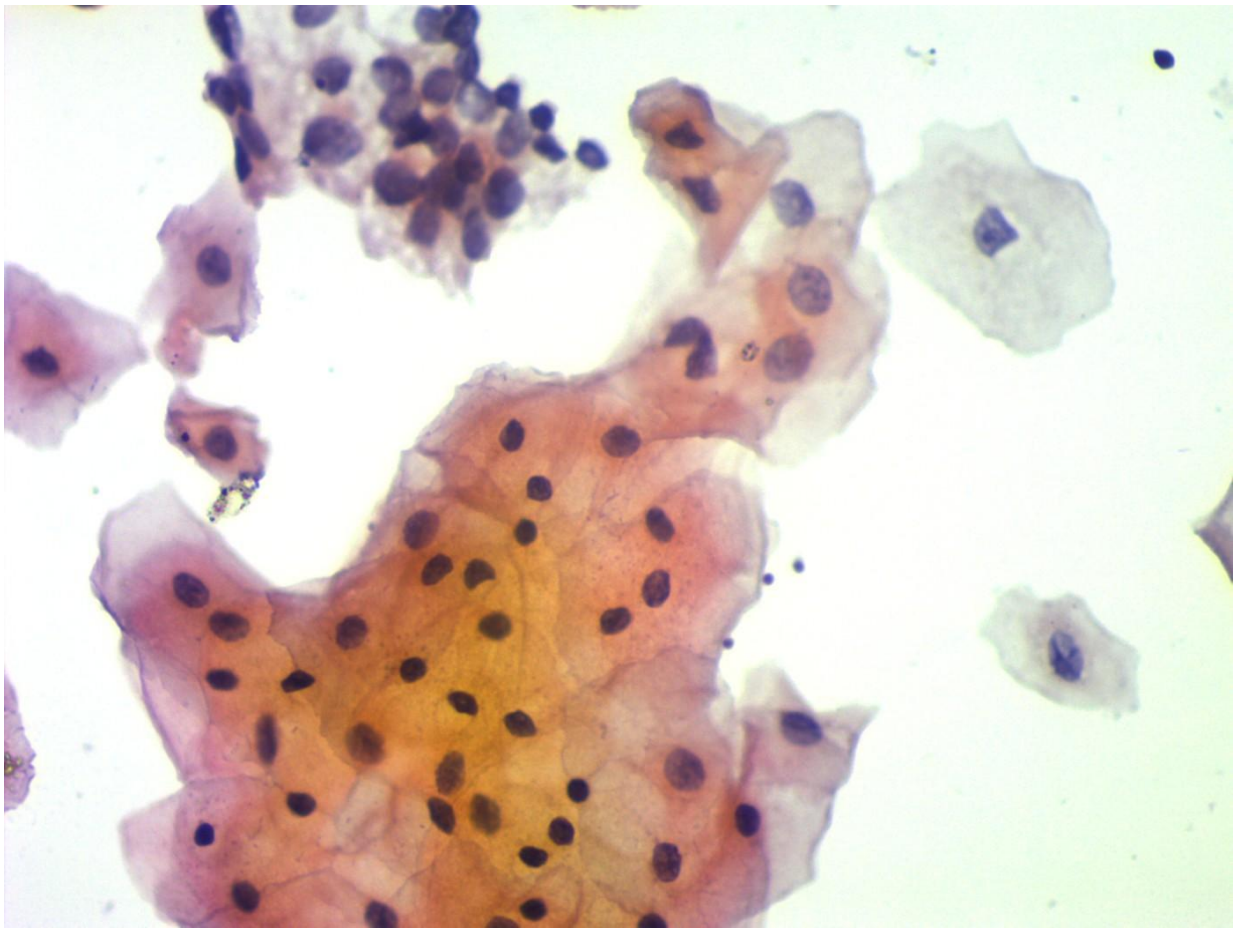

PLATE 4.3: Cervical Smear showing superficial epithelial cells (thick arrow) with well stained nuclei and well stained cytoplasm. The intermediate epithelial cells (thin arrow) have well stained nuclei but moderately stained cytoplasm on a clean background. T-A Extract x400

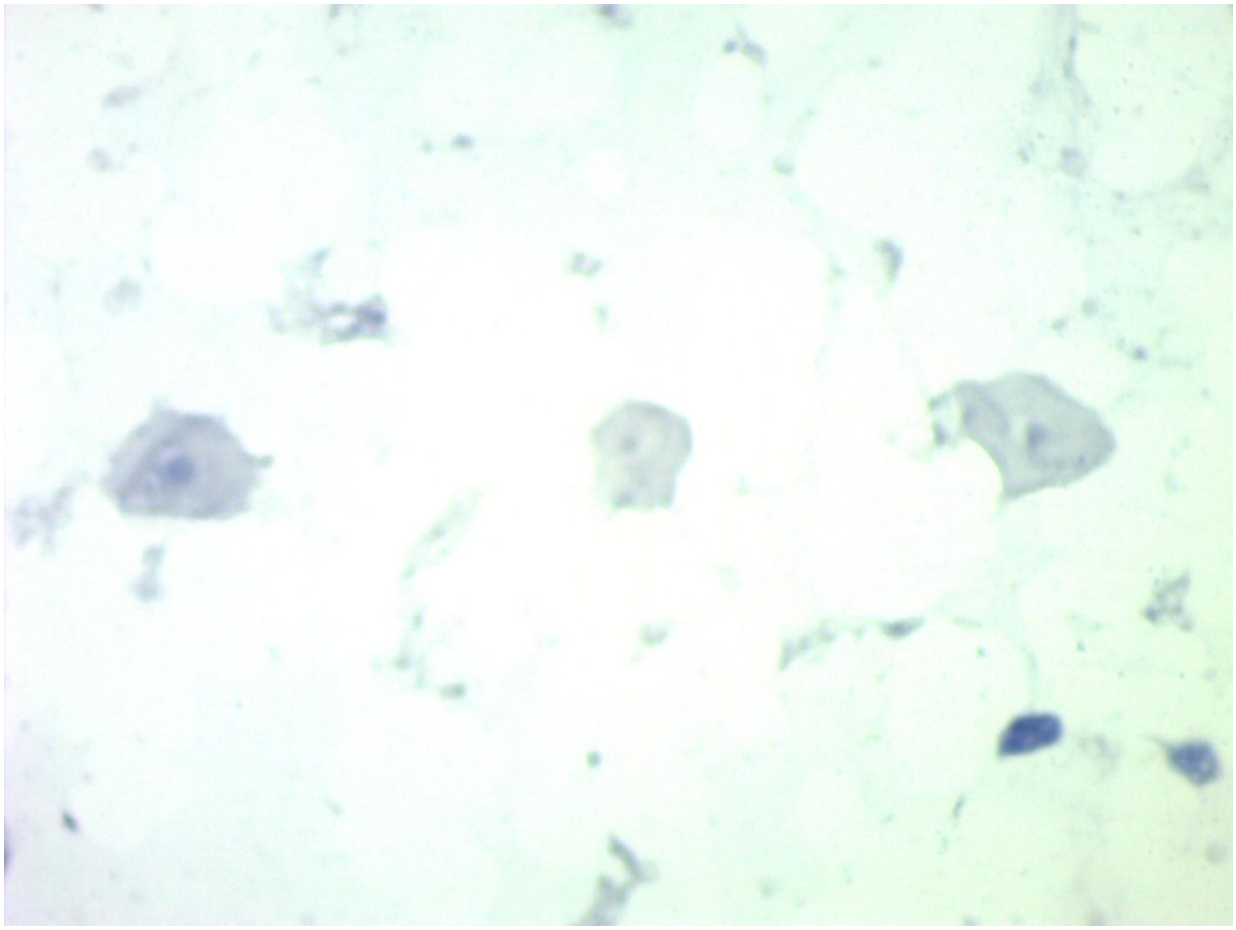

PLATE 4.4: cervical smear shows superficial epithelial cells (thick arrow) with poorly stained nuclei and poorly stained cytoplasm. The intermediate epithelial cells (thin arrow) have poorly stained nuclei and poorly stained cytoplasm on a clean background. Z-A EXTRACT X400

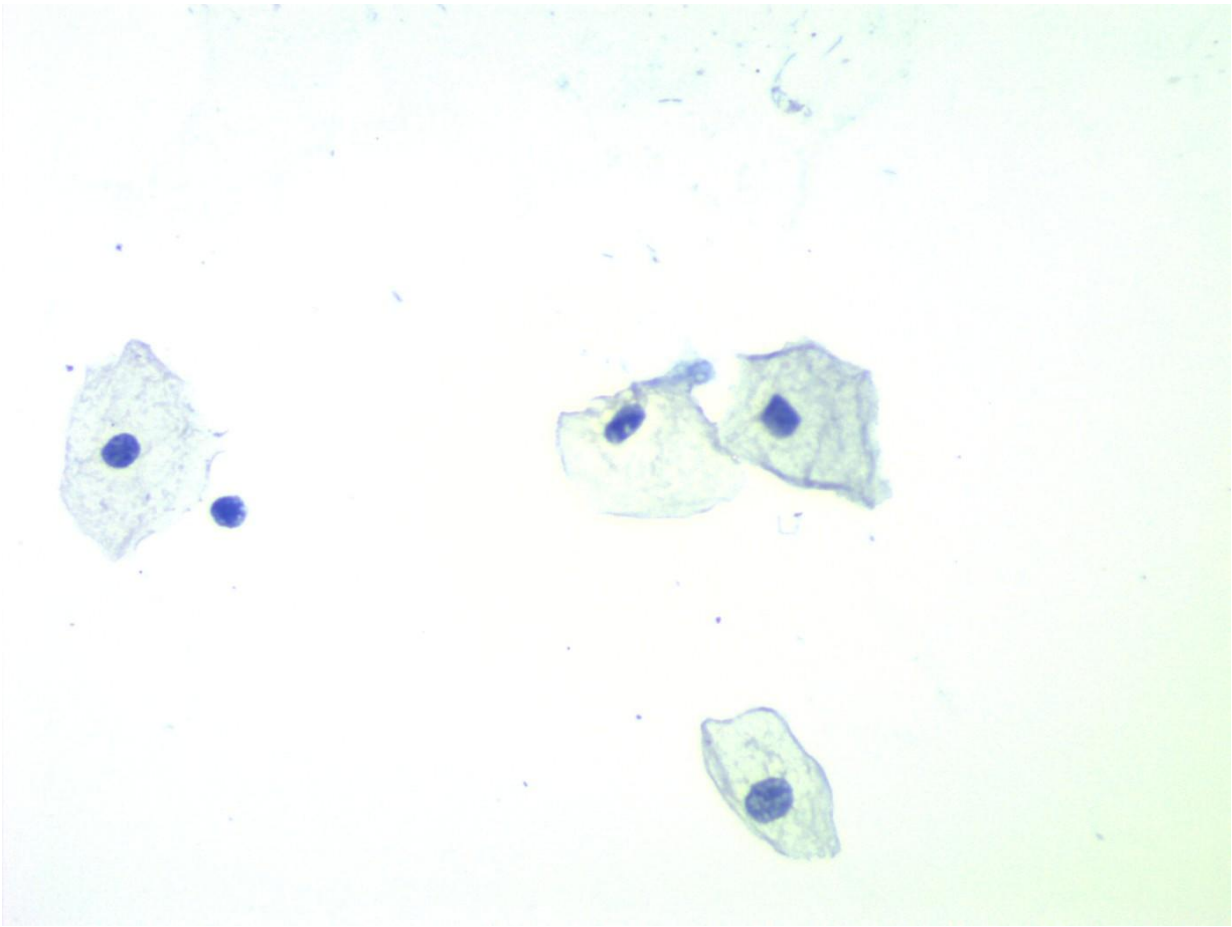

PLATE 4.5: Cervical Smear showing intermediate epithelial cells with well stained nuclei and moderate staining of cytoplasm on a clean background. T-A extract x400

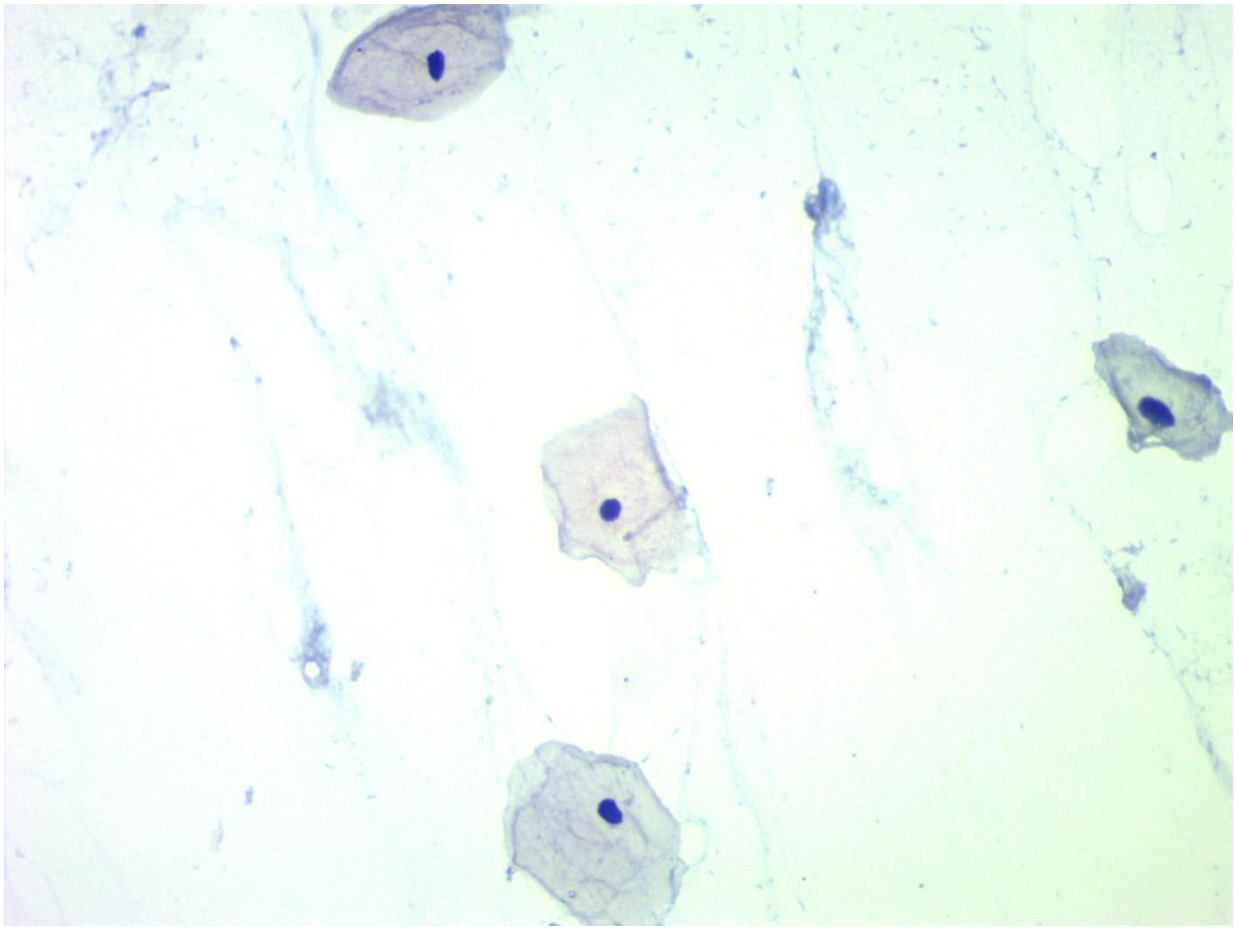

PLATE 4.6: Cervical smear shows superficial epithelial cells (thick arrow) with well stained nuclei but moderately stained cytoplasm. The intermediate epithelial cells (thin arrow) have well stained nuclei but moderately stained cytoplasm on a clean background. Z-A Extract X400

CHAPTER FIVE

DICUSSION, AND CONCLUSION

5.1 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 visualisation. 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). This study was carried out to determine the staining ability of the extracts of *Zingiber Officinale* and *Curcuma longa* in view of being used as replacements for eosin in EA50 stain.

The visualization of the control slides are shown in Plates 4.1 and 4.2. Plate 4.1 reveals that the use of Papanicolaou's stain led to proper staining of nuclei and cytoplasm of epithelial cells against a clean background. Plate 4.2, on the other hand, shows that superficial cells stained with Papanicolaou's stain had well-stained nuclei and cytoplasms. The cell colours 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 visualisation of cells upon staining with *C. longa* extract is shown in Plates 4.3 and 4.5. It is seen from plate 4.3 that staining of superficial cells with *C. longa* extract produced proper staining of the nuclei and cytoplasm. However, in the case of intermediate epithelial cells (Plates 4.3 and 4.5), only nuclei were well-stained, while cytoplasms were moderately stained

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 results reported by Rubina *et al.* (2020). 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.

Staining was also done using *Z. officinale* extract and the subsequent visualisation of the cells are shown in Plate 4.4 and 4.6. From Plate 4.4, it is seen that there was poor staining of nuclei, superficial and intermediate epithelial cells against a clean background. This results contrast with that of Sudhakaran *et al.* (2018) where *Z. officinale* extract provided good stains for cells with better intensity and crispness. However, Plate 4.6 reveals that *Z. officinale* extract produced well-stained nuclei and moderately stained cytoplasm in both superficial and intermediate epithelial cells against clean backgrounds. This aligns with the results of the study by Sudhakaran *et al.* (2018). A possible reason for the variation in results for Plate 4.4 and 4.6 is the variance in concentrations of *Z. officinale* extract which affects the staining ability as described by Lichius (2022).

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

5.2 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.

5.3 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 optimise 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 visualisation 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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APPENDIX A

PREPARATION OF TA-EXTRACT

A standard solutrion of turmeric-azure was prepared following the standard protocol of preparing EA50. 200ml volume was prepared and the following was weighed and mixed together.

1. 0.9g of Light Green SF was weighed and dissolved in 90ml of 95% alcohol.
2. 0.1g of Bismarck Brown was weighed and dissolved in 20ml of 95% alcohol.
3. 0.5g of turmeric powder was weighed and dissolved in 90ml of 95% alcohol.
4. 0.2g of Phosphotungstic acid was added.
5. 1 dropn of Lithium carbornate was added.

The solution was mixed together by shaking and was allowed to stay for 24hrs.

It was filtered and ready for use.

PREPARATION OF ZA-EXTRACT

A standard solutrion of *Zingiber Officinal*e-azure was prepared following the standard protocol of preparing EA50. 200ml volume was prepared and the following was weighed and mixed together.

A standard solutrion of *Zingiber Officinal*e-azure was prepared following the standard protocol of preparing EA50. A 200ml volume was prepared and the following was weighed and mixed together.

1. 0.9g of Light Green SF was weighed and dissolved in 90ml of 95% alcohol.
2. 0.1g of Bismarck Brown was weighed and dissolved in 20ml of 95% alcohol.
3. 0.5g of Zingiber Officinale powder was weighed and dissolved in 90ml of 95% alcohol.

4. 0.2g of Phosphotungstic acid was added.

5. 1 dropn of Lithium carbornate was added.

The solution was mixed together by shaking and was allowed to stay for 24hrs.

It was filtered and ready for use.