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Screening of Curcuma Longa Linn. (Turmeric) Extract As Biological Stain for Microscopic Urine Elements

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Abstract: The demand for the use of natural dyes continues to increase all over the world. Natural dyes can be derived from different natural sources including plants. Application of natural dyes in food and textile industries is very pronounced but knowledge on staining biological specimens like urine is very limited. Currently, only synthetic dyes are available for staining urine sediments but empirical studies suggest that they are hazardous to health and to the environment. This study assessed the potential ability of the extract of turmeric rhizome as a suitable stain for microscopic urine sediments. Extraction and quantification of the natural product was evaluated using sohxlet apparatus. Results of the study were analyzed utilizing the statistical tool two-way Analysis of Variance (ANOVA) to determine the effect of certain factors to the efficacy of the plant dyes and Tukey's multiple comparison tests to determine the effect of each parameter on the different microscopic urine elements. The results of the study revealed that turmeric extract can be used as a stain for microscopic urine sediments such as urates, phosphates, crystals, red blood cells (RBCs), white blood cells (WBCs), hyphal elements and epithelial cells. However, urinary casts were not optimally stained due to its high refractile nature. The efficiency of the staining result to urine elements is affected by varying concentrations and pH except for casts. On the other hand, there were noted changes in the staining affinity of elements when they were subjected to varying length of staining time except for red blood cells which were best observed when incubated for 5 minutes.

Keywords: Curcuma longa Linn. ;Turmeric; microscopic urine elements.

1. INTRODUCTION

The art of using dye is considered a pre-historic practice. It was an old technique used by man dated back during the dawn of ancient civilizations [1]. A dye is a highly coloured substance that can impart colour to infinite materials like textiles, paper, wood, varnishes, leather, ink, fur, foodstuff, cosmetics, medicine, toothpaste, and other materials [2]. Dyes are materials that can be formulated into stains.

Stains are widely used in different areas in the laboratory. In histopathology, stains have been used to augment accurate descriptions and characteristic structure of tissues, which is essential for diagnosis [3]. In urine microscopy, staining enhances the qualitative appearance of urine

elements by changing their refractive index [4]. The evaluation of urine elements is part of urine analysis which is used to evaluate the state of a patient's renal and genitourinary system [5].
Nevertheless, microscopic examination of urine sediment is time-consuming and offers limited precision and wide inter-observer variability [6] because unstained urine sediments appear to have a refractive index similar to that of urine [4]. Sometimes, a confirmation by an expert in clinical microscopy is needed and this is somewhat difficult in far-flung rural areas in developing countries [7].
Urinary stains are sometimes utilized to visualize and identify elements better. These make the appearance of red blood cells, white blood cells, epithelial cells, bacteria and other microscopic urine elements more visible. Common urinary stains include Sternheimer-Malbin stain, Oil red O and Sudan III, Gram stain, Hansel stain and Prussian blue stain. Although there are various stains that have been widely used in the clinical laboratory for many years, these are synthetic in nature. Recent studies emphasized synthetic dyes as toxic, carcinogenic and hazardous [8].
Moreover, many developing countries can no longer manage the high cost of synthetic dyes. Therefore, the use of cheaper, naturally occurring dyes from plants is being viewed as an alternative to synthetic dyes**.**
A local study by Chambal [9] focused on formulating a natural stain from Sibukao extract. The study reported decoction and reflux distillation to extract the dye. The efficacy of the extracted dye was compared to KOVA stain. The study reported that the said extract can be a promising stain for urine sediments.
Interestingly, there is an increasing demand for the use of natural dyes worldwide that is about 10,000 tons or equivalent to one percent of the consumption of synthetic dyes [10]. Some of the sources of natural dyes include madder root (*Rubiatinctorum L.)* [11], *Serratulatinctoria*L. (saw-wort)[12], *Hibiscus sabdariffa*[3], *and* allepey cultivar of *Curcuma longa* [13].
Curcuma longa Linn. (Turmeric) belongs to Zingiberaceae Family along with its other members such as ginger, cardamom, and galangal. It is also known as Haldi in Hindi [14], Kurkum in Arabic, Yu chin in Chinese, Indian saffron in English, Ukon in Japanese, Kitrinoriza in Greek, Safran in German, Geelwortel in Dutch, Azafranarabe in Spanish, and Dilau in Tagalog [15]. It is widely distributed throughout the tropics, particularly in Southeast Asia and is cultivated on large scale in India, China, Indonesia, Jamaica and Peru and in other countries with temperate climate [14].
Turmeric plants are leafy and have flower bracts that are ovoid, pale green with comma-like bracts tinged in pink. The flowers appear pale yellow and its rhizomes are thick and cylindrical in structure. These plants may grow to a height of three to five feet [16]and have a characteristic aromatic odour and distinct warm, bitter taste [13].
Chemical components of turmeric include volatiles and non-volatiles. The chemical constituents of the volatile oils include ar-turmerone, zingiberene, turmerone and curlone [14] ,while the major non-volatile phenolic compounds found in it is collectively known as curcuminoids.
Curcuminoids, the yellow pigment in turmeric rhizomes, have been identified as the most bioactive principle and were characterized as a group of bis-α, β-unsaturated β-diketone polyphenols; namely, curcumin, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). Because of turmeric's active colouring compounds, this study assessed its potential ability as a source of natural dye in staining microscopic urine elements as well as determines the appropriate parameters for staining of urine elements in terms of dye concentration, pH and staining time. To date, there is limited knowledge on the use and application of natural dyes in urine microscopy[10, 12].Formulation of a natural source of urinary stain can be very beneficial

for academic and research purposes. In a classroom setting, some students find it hard to distinguish one urinary element from another leading to confusion and variability in microscopic identification of urine sediments. Oftentimes, academicians devote longer time to demonstrate such elements. This is very tedious on the part of the professor. Therefore, a natural stain may be of great help in better visualization of microscopic elements. This in turn, may facilitate better understanding and appreciation from the students. This can also make identification of urine sediments less labour-intensive on the observer.

2. MATERIALS AND METHODS

1.1 Preparation of Plant Extract

The rhizomes were cut into small pieces, peeled, and dried at 50°C for 72 hours in an oven [13, 16]. After drying, these were milled to powder. Then, the yellowish powdered material was treated with 70% ethyl alcohol in a Sohxlet extractor for 72 hours [16]. The extract was filtered and concentrated in vacuo at 60°C [13].

1.2 Preparation of Turmeric Staining Solution

1.2.1 Dye Concentration

Crude extract of turmeric was transferred into a volumetric flask with different weights of the solute and were added with distilled water as solvent. The concentration range from 1%, 2%, and 3%weight/volume concentration [9].

1.2.2 pH

After the preparation of the concentration of the dye, the pH was determined using digital pH meter. Drop by drop, 1N NaOH and 1N HCl were added to the extract until the desired pH (5, 6, 7, and 8) was obtained [9].

1.3 Collection and Processing of Urine Sample

Forty random (40) urine samples were collected from a tertiary hospital-based laboratory in Batangas City. Thirty-two (32) test tubes for every urine sample were prepared and were labeled accordingly. Each of the forty-urine samples was poured into a test tube and was tested chemically using urine test strips for its specific gravity, pH, glucose, and protein content. The samples were centrifuged at 1,500 revolutions per minute for 3-5 minutes. The supernatant was removed leaving a small amount of urine in the tube. For the unstained sediment, 1-2 drops of sediment were placed on a glass slide with cover slip and examined microscopically under low power and high power, respectively.

In another tube, 1-2 drops of stain (Sedi-stain) were added to the suspended sediment and incubated at room temperature for 1-2 minutes. A drop of the stained sediment was transferred on a glass slide with cover slip and examined in the same manner with the unstained sediment.

1.4 Determination of Appropriate Parameters for Staining of Urine Elements

1.4.1 Dye concentration (%)

One to two drops of the extract with varying concentrations (1%, 2%, 3%) were added to tubes a, b, c and were incubated at room temperature for 1-2 minutes. A drop of the stained sediment was transferred on a glass slide with cover slip and examined microscopically under low power and high power, respectively.

1.4.2 pH

A drop or two of the extract with varying pH (5, 6, 7, and 8) were added to labeled tubes at room temperature for 1-2 minutes. A drop of the stained sediment was transferred on a glass slide with cover slip and examined microscopically.

1.4.3 Staining time in minutes (1, 5, 10)

Tubes with urine sediments were added with 1-2 drops of the extract and were allowed to stand at room temperature for 1 minute, 5 minutes, and 10 minutes. Then, a drop of the stained sediment was transferred on a glass slide with cover slip and examined microscopically.

1.5 Analysis

Three registered medical technologists, who served as independent evaluators, assessed the efficiency of turmeric as a stain. They were guided by the criteria of Tejada [17] with modifications as tabulated in Table 1.

In addition, all data were subjected to statistical analyses using SPSS 16.0.Two-way ANOVA and Tukey's multiple comparison tests were used to evaluate the data and demonstrate differences in the staining efficiency of the different parameters such as concentration, pH and staining time on the urine elements. Mean values were considered significant at $p < 0.05$.

Table 1.Criteria used for the assessment of the staining efficiency of turmeric [17]

Urine element	**(Optimal stain)(3)**	**(Moderate stain)(2)**	**(Insufficient stain)(1)**	**(Unsuitable stain)(0)**
Epithelial cell	Nuclei and cytoplasm stain yellow; distinct features of nucleus and cytoplasm are highly distinguishable	Nuclei and cytoplasm stain light yellow; distinct features of nucleus and cytoplasm are distinguishable	Nuclei and cytoplasm stain faint yellow; distinct features of nucleus and cytoplasm are hardly distinguishable	Nuclei and cytoplasm are not distinguishable due to overstained background
RBC	Cell stains yellow; central pallor is highly distinguishable	Cell stains light yellow; central pallor is distinguishable	Cell stains faint yellow; central pallor is less distinguishable	Cell is not distinguishable due to overstained background
WBC	Cell stains yellow; granules are highly visible	Cell stains light yellow; granules are visible	Cell stains faint yellow; granules are hardly visible	Cell and granules are not distinguishable due to overstained background
Hyphal element	Stains yellow and highly distinguishable	Stains light yellow and distinguishable	Stains faint yellow and hardly distinguishable	Not distinguishable due to overstained background
Crystal	Stains yellow and highly distinguishable	Structure stains light yellow and distinguishable	Structure stains faint yellow and hardly distinguishable	Not distinguishable due to overstained background
Cast	Stains yellow and highly distinguishable	Stains light yellow and distinguishable	Stains faint yellow and hardly distinguishable	Not distinguishable due to overstained background
Urates/ Phosphates	Stains yellow and highly distinguishable	Stains light yellow and distinguishable	Stains faint yellow and hardly distinguishable	Neither visible nor distinguishable due to overstained background

3. RESULTS AND DISCUSSION

The rhizome was dried at 50°C for 72 hours in an oven and was powderized using a grinder. Figure 1 (a) depicted the powered turmeric while Figure 1 (b) was the picture of turmeric extract. After grinding, 87.7 grams of yellowish brown powdered turmeric was obtained. A reddish brown extract was produced after it has been treated with 70% ethyl alcohol using Soxhlet apparatus for 72 hours. This was concentrated in vacuo at 60°C yielding 2.85 grams crude extract (3.25% yield). In a study by Azwanida [18], the same extraction technique was used to remove lypodial materials from powdered *Clitorea ternate* flowers but a different solvent was used. Petroleum ether was used in place of ethyl alcohol at 60°-80°C, resulting in a lower yield of 2.2% w/w.

Figure 1. (a) powdered turmeric (b) turmeric extract

The pH of the varying concentrations of the crude extract was measured revealing pH8.15, 8.43, and 9.01 at 1%, 2%, and 3%concentrations, respectively.

Table 2 showed the assessment result of the three evaluators in the staining efficiency of turmeric at varying concentration for the different urine elements. At 1% concentration, the staining reaction of crystal was scored 1.66 which was interpreted as insufficiently stained since it made the crystal faint yellow and hardly distinguishable. At 2% concentration, it was moderately stained with an average score of 2.0. Its structure stained light yellow and was distinguishable. On the other hand, it was optimally stained at 3% with a high score of 3.00 showing highly distinguishable, yellow stained appearance.

This revealed that crystal was stained better at higher concentration. This could be due to the fact that one of the driving forces for crystal formation was urinary supersaturation which could be dependent on the concentration of stone forming ions present in the urine [19].

As for epithelial cell, the obtained average score of 2.33 revealed that it was moderately stained showing light yellow nucleus and cytoplasm at 1% concentration. It was also optimally stained at 2% and 3% concentrations with yellow nucleus and cytoplasm emphasizing distinct features of these structures that were distinguishable. Both concentrations had an average score of 3.00 which showed that epithelial cells took up the extract easily which could be due to flattened appearance thereby creating a large surface area making the cell more permeable to stains.

RBC was stained moderately at 1% and 2% concentrations with average scores of 2.0 and 2.66, respectively, showing light yellow and distinguishable central pallor. On the other hand, they were optimally stained at 3% showing yellow cells with central pallor that was highly distinguishable. At 1% and 2% concentrations, WBCs were moderately stained with 2.33 and 2.66 average scores, respectively. These elements were stained light yellow and appeared to have visible granules. At 3% concentration, they were optimally stained showing highly visible granules with yellow appearance.

Yeast cells were moderately stained with average scores of 2.0, 2.33, and 2.0 at 1%, 2%, and 3% concentrations, respectively. They appeared light yellow with distinguishable features. Hyphal elements were scored 2.66. Hence, they were moderately stained at 1% concentration while they were optimally stained at 2% and 3% concentrations, both with average score of 3.00.
Urates and phosphates were moderately stained at 1% and 3% concentrations, both of which garnered an average score of 2.00. Conversely, they were optimally stained at 2% concentration and were scored 3.00. Lastly, casts were rated 1.0 in 1%, 2% and 3% concentrations; thus, interpreted as insufficiently stained.
In summary, turmeric extract can stain the urine elements using varying concentrations. This could be due to the component of turmeric which has a strong dye-binding property which was proven when its ability to act as a counter stain in histopathology study. It revealed that its staining reaction was similar to that of eosin in the hematoxylin and eosin technique except for its yellow coloration. With this, turmeric extract can be a promising histological dye due to its availability. Therefore, it could serve as a useful alternative to eosin in developing countries [13].
Moderate staining was either observed in yeast cells while crystals, epithelial cells, white blood cells, red blood cells, hyphal elements, urates, and phosphates were moderately or optimally stained. This implied that these elements can take up turmeric extract and thus, can be very miscible with stains. However, casts did not take up the stain efficiently. Therefore, were not sufficiently stained. This could be due to the high content of gelled Tamm-Horsfall protein in the matrix which was usually responsible to their very low refractive index making them appear colorless, homogenous and transparent [20].

Table 2. Assessment result of the staining efficiency of varying concentrations of turmeric extract

Urine element	**Average score (1%)**	**Interpretation**	**Average score (2%)**	**Interpretation**	**Average score (3%)**	**Interpretation**
Crystals	1.66	I	2.00	M	3.00	O
Epithelial cells	2.33	M	3.00	O	3.00	O
RBC	2.00	M	2.66	M	3.00	O
WBC	2.33	M	2.66	M	3.00	O
Yeast cells	2.00	M	2.33	M	2.00	M
Hypal elements	2.66	M	3.00	O	3.00	O
Urates/Phosphates	2.00	M	3.00	O	2.00	M
Casts	1.00	I	1.00	I	1.00	I

Note: 3.00- Optimal stain (O); 2.00-2.99 – Moderate stain (M); 1.00-1.99 – Insufficient stain (I); 0.00-0.99 – Unsuitable stain (U)

Table 3 presented the comparative results of the effects of varying concentrations of turmeric extract on the staining reaction of urine elements. Crystals showed different staining reactions on the given concentrations. Thus, making them more distinguishable on higher concentrations such as 2% and 3%. This could be due to their true geometrically formed structures or its amorphous material content making them easily permeable to stains in moderate or high concentrations [20]. In this case, most of the crystals identified in the research were acidic in nature. Hence, they would be highly precipitated in higher pH; requiring higher concentration of stains [4].

Epithelial cells also showed significant reactions in 1% vs 2% and 1% vs 3% concentrations which meant that high concentrations of dye can be used to stain and demonstrate these microscopic elements. This could be due to its large surface area causing the stain to penetrate the cytoplasm easily [21].
RBC appeared differently in 1% and 3% concentration which meant that these two were the preferred concentrations when staining these elements. This could be due to the nature of these elements since they lack characteristic structures, variations in size and close resemblance to other urine sediments. These are also the most difficult to recognize among all the urine elements [20].
Urates and phosphates showed significant staining reactions in 1% vs 2% and 2% vs 3%. They may appear yellowish to reddish brown granules making them more difficult to take up stain easily thereby requiring either lower or higher concentrations of staining solutions [20].

Table 3. Multiple comparison of the effects of varying concentrations of turmeric extract on urine elements

Urine elements	**p-value**	**Interpretation**
Crystals		
1% vs. 2%	0.5215	Not significant
1% vs. 3%	0.0002	Significant
2% vs. 3%	0.0053	Significant
Epithelial cells		
1% vs. 2%	0.0830	Significant
1% vs. 3%	0.0830	Significant
2% vs. 3%	> 0.9999	Not Significant
RBCs		
1% vs. 2%	0.0830	Not Significant
1% vs. 3%	0.0053	Significant
2% vs. 3%	0.5215	Not Significant
WBCs		
1% vs. 2%	0.5215	Not Significant
1% vs. 3%	0.0830	Not Significant
2% vs. 3%	0.5215	Not Significant
Yeast cells		
1% vs. 2%	0.5215	Not Significant
1% vs. 3%	> 0.9999	Not Significant
2% vs. 3%	0.5215	Not Significant
Hypal elements		
1% vs. 2%	0.5215	Not Significant
1% vs. 3%	0.5215	Not Significant
2% vs. 3%	> 0.9999	Not Significant
Urates/phosphates		
1% vs. 2%	0.0053	Significant
1% vs. 3%	> 0.9999	Not Significant
2% vs. 3%	0.0053	Significant
Casts		
1% vs. 2%	> 0.9999	Not Significant

1% vs. 3%	> 0.9999	Not Significant
2% vs. 3%	> 0.9999	Not Significant

Table 4 presented the rating given by the three evaluators on the staining efficiency of turmeric at varying pH levels. At pH 5 using 1% and 3% dye concentrations, crystals were graded as moderately stained and they were optimally stained at 2% concentration. At pH 6, they were insufficiently stained at 1% concentration and moderately stained at 2% and 3% concentrations, respectively. At pH 7 & 8, the only concentration that showed moderate staining reaction is at 2%. This meant that a lower pH level, the extract favored the integration of crystals since majority of the crystals observed in the study were acidic in nature. However, the extract was unable to produce an optimum result in staining.

Epithelial cells were optimally stained at pH 5 in 1% and 2% dye concentrations. They were moderately stained at pH 6 in 1% and 2% concentrations while optimally stained at 2% concentration. These elements were optimally stained using 2% concentration at pH 7 and 8. These findings showed that epithelial cells were optimally stained at an acidic pH even at lower concentrations.

RBC showed optimum staining capacity at 3% concentration at pH 5 and at 2% and 3% concentrations at pH 7 and pH 8. It showed moderate staining at pH 6 at 2% and 3% concentrations.

WBC showed the strongest staining reactions in pH 6 using 3% concentration and in 2% concentration both for pH 7 and pH 8. This meant that they were stained at either moderate or high concentrations in slightly acidic, neutral, or alkaline pH

Yeast cells showed moderate staining in all concentrations at pH 5, in2% and 3% concentrations at pH 6 and pH7 and in 2% concentration at pH 8.

Hyphal elements showed optimum staining reaction at pH 5, pH 6 and pH 7 in 2% and 3% concentrations. At pH 8, the only concentration that showed optimum staining was at 2% concentration.

At pH 5, urates and phosphates were optimally stained at 1% and 2% concentrations. It showed moderate staining at 2% and 3%concentrations at pH 6. It also exhibited optimum reactions at pH 7 and pH 8 at 2% concentration.

Casts were insufficiently stained in all the concentrations and all pH level. This only showed that these elements did not take up the plant dye well regardless of the varying concentrations and pH levels.

In summary, the initial assessment of the evaluators regarding the staining capacity of the extract implied that since most of the crystals identified in the experiment are found in acidic environment, they took up stains better in an acidic pH rather than alkaline pH because crystals' solubility is usually affected by changes in pH levels. For that reason, acidic crystals easily disintegrate at alkaline pH [4].

Epithelial cells were optimally stained even at low concentrations in all pH levels. This could be due to the cells' permeability to stain due to their larger surface area and bigger cytoplasm [21].

RBCs were optimally stained at pH 5, pH 7 and pH 8 using either 2% or 3% concentrations but did not show optimum results using 1% concentration. This could be due to the fact that they will only undergo distortion and become less intact in urine pH levels greater than pH 8 [20]. Majority of the urine pH used in this experiment did not exceed pH 8, hence, favored the staining of RBC.

WBC showed optimum staining at pH 6, pH 7, and pH 8. Urine pH does not necessarily affect appearance of WBC thereby causing them to maintain their cellular integrity and granules. Moreover, most of WBC found in the urine was granulocytes. These could have contributed to their susceptibility to staining since granules take up stain easily. For example, in hematology, Romanowsky stains differentially stain leukocyte granules which helped to demonstrate the characteristic morphology of the cells for identification [22].

Yeast cells were not optimally stained in any of the given pH however, showed moderate staining reactions at pH 5, pH 6, pH 7 and pH 8. Yeast cells were susceptible to staining since their cell wall were composed of polysaccharides, which could had contributed to their staining affinity and glycoproteins, which could had made it less impenetrable [23].

Hyphal elements showed optimal staining reactions at pH 5, pH 6, pH 7, and pH 8using either moderate or high concentrations. Hyphal elements, compared to yeast cells contained high contents of chitin which may be responsible to its affinity to dyes [24].

Urates and phosphates were optimally stained at pH 5 and pH 7. Urates may be found in acidic and neutral urine while phosphates may be found in alkaline urine [4]. Most of the urine samples used in the research were acidic hence, it was presumed that urates were commonly identified and observed. Thus, an acidic and neutral pH would have favored the staining affinity of turmeric towards these elements. Casts remained unstained in the different pH levels of turmeric used in this experiment. This could be due to the high protein content of casts making the matrix impenetrable [20].

Table 4. Assessment result of the staining efficiency of turmeric extract at varying pH levels

pH	Urine elements	1%	Interpretation	2%	Interpretation	3%	Interpretation
5	**Crystals**	2.00	M	3.00	O	2.66	M
	Epithelial cells	3.00	O	3.00	O	2.66	M
	RBC	2.00	M	2.00	M	3.00	O
	WBC	2.00	M	2.66	M	2.00	M
	Yeast cells	2.00	M	2.00	M	2.00	M
	Hypal	2.33	M	3.00	O	3.00	O
	Urates / Phosphates	3.00	O	3.00	O	2.00	M
	Casts	1.33	I	1.66	I	1.00	I
6	**Crystals**	1.66	I	2.00	M	2.66	M
	Epithelial cells	2.33	M	3.00	O	2.66	M
	RBC	1.33	I	2.66	M	2.00	M
	WBC	1.00	I	2.00	M	3.00	O
	Yeast cells	1.33	I	2.00	M	2.00	M
	Hypal	1.00	I	3.00	O	3.00	O
	Urates / Phosphates	1.00	I	2.00	M	2.66	M
	Casts	1.00	I	1.66	I	1.00	I
7	**Crystals**	1.33	I	2.33	M	1.00	I

	Epithelial cells	1.00	I	3.00	O	0.66	U
	RBC	1.00	I	3.00	O	3.00	O
	WBC	1.00	I	3.00	O	3.00	O
	Yeast cells	1.00	I	2.00	M	2.00	M
	Hypal	1.00	I	3.00	O	3.00	O
	Urates / Phosphates	1.00	I	3.00	O	2.33	M
	Casts	1.00	I	1.33	I	0.66	U
8	**Crystals**	1.00	I	2.33	M	1.00	I
	Epithelial cells	1.66	I	3.00	O	0.66	U
	RBC	1.33	I	3.00	O	3.00	O
	WBC	1.66	I	2.00	M	3.00	O
	Yeast cells	1.00	I	2.00	M	1.66	I
	Hypal	1.00	I	3.00	O	0.44	U
	Urates / Phosphates	1.66	I	2.00	M	2.33	M
	Casts	1.00	I	1.33	I	0.66	U

Note : 3.00- Optimal stain (O); 2.00-2.99 – Moderate stain (M); 1.00-1.99 – Insufficient stain (I); 0.00-0.99 – Unsuitable stain (U)

Table 5 showed the comparative effect of varying concentrations and pH on the staining affinity of turmeric to the urine elements. At pH 5, 7, and 8, 1% and 2% concentrations had a significant effect on crystals while it showed no significant effect at pH 6. When it was stained using 1% and 3% concentrations, it showed significant effect at pH 7 with p-value of 0.0007. At pH 8, a significant effect was seen at 2% versus 3% concentrations. This only showed that higher concentrations of stains also required higher pH so that crystals will be stained better.

For epithelial cells, significant reactions were seen at pH 5 in 1% versus 3% and 2% versus 3% concentrations. At pH 6, 2% versus 3% concentration was significantly different, while at pH 7 in 1% versus 2% and 1% versus 3% concentrations showed significant reactions.

At pH 8, 1% versus 2% and 2% versus 3% concentrations showed significant difference. This proved that varying concentrations and pH of stains may affect the appearance of epithelial cells. Although identification of epithelial cells was of rare difficulty, sometimes changes in its appearance and number such as clumping, folding, or abundance may cause obscurity in faster identification and may cause disintegration in the urine [4]. Thus, requiring variations in pH and concentrations of stains to be used for a better visualization.

RBCs showed different staining affinity at pH 5 at 1% versus 2% and 1% versus 3% concentrations. They also showed significant reactions at pH 7 at 1% versus 2% and 1% versus 3% concentrations, and at pH 8 at 1%versus 2% concentrations and 2% versus 3% concentrations. These elements were noted to be variable in nature. Hence, their lack of characteristic could have contributed to differences in the staining affinity.

Staining of WBC showed significant differences in 1% versus 2% and 1% versus 3% concentrations at pH 5. It also showed significant reactions in 1% versus 2%, 1% versus 3% and 2% versus 3% concentrations at pH 6. At pH 7, it showed significant difference 1% versus 2%

and 1% versus 3% concentrations. Lastly, significant reactions were seen at pH 8 in 1% versus 2% and 2% versus 3% concentrations. This showed that WBCs may appear variable depending on the pH used. This could be due to the abundant granules found on the cytoplasm which made it appear variable.

Yeast cells appeared significantly different at pH 7 in 1% versus 2% and 1% versus 3% concentrations. They also showed significant reaction in pH 8 in 1% versus 2% concentration. This implied that the staining affinity of yeast cells were different in either neutral or alkaline pH. This could have been influenced by their cellular components making them appear more complex in higher pH.

As for hyphal elements, there were significant reactions seen at pH 7 in 1% versus 2% and 1% versus 3% concentrations and at pH 8 in 1% versus 2% and 2% versus 3% concentrations. Hyphal elements, just like yeast cells, contained proteins and polysaccharides in their cell wall. This could have made them appear differently in terms of staining.

There was a significant staining reactions for urates and phosphates at pH 6 in 1% versus 2% concentration, at pH 7 in 1% versus 2% and 1% versus 3% concentrations and at pH 8 in 1% versus 2% and 2% versus 3% concentrations. These elements were highly granular, highly soluble in pH changes and are crystalline in nature. Thus, could have been factors in their differences in staining appearance.

Casts showed no significant reactions in the varying pH and concentrations. This only showed that their staining affinity was not affected by any of these factors at all.

In summary, crystals were best stained at pH 6 since most of the crystals identified were rarely seen in alkaline pH.Thus, crystallization is better in acidic urine. Furthermore, their inhibition in diluted urine was increased at high pH value causing the crystals to take up the stain in acidic pH [25].

Epithelial cells were stained in a different manner and intensity in all pH levels. No optimum pH was preferred for best results. Although these elements were easy to distinguish, identify and stain because of their prominent nucleus and cytoplasm, their ability to take up the stain can also be affected by changes in pH levels.

The preferred pH for staining RBC is pH 6 because no significant difference in their appearance was noted. Different staining affinity of red blood cells may be due to their lack of characteristic structures and close resemblance to other structures making it more difficult to penetrate the RBC structure affected by pH and that presence of crystals was frequently associated with concentrated specimens [4].

The staining efficiency of turmeric on WBCs was affected by factors such as pH and concentrations which could be due to the nature of WBCs having granules that exhibited Brownian movement hence making them less impenetrable [20].

Yeast cells can be best visualized at pH 5 and pH 6. The staining reaction of yeast cells showed that their cell wall may be easily miscible with stains or dyes due to their high polysaccharide and protein component [20]. However, changes in local environment, pH, nutrients, and oxygen also initiated changes in their cell wall causing them to appear differently in increasing pH [26].

Hyphal elements produced better staining results at pH 5 and pH 6. These elements had double walled structure that was rich in chitin, a highly indestructible material found in wall of fungal elements. This may have contributed to a lesser staining affinity to alkaline stains favoring greater staining affinity in acidic pH [20].

Both amorphous urates and amorphous phosphates showed the same staining reactions at pH 5. These two may flourished in acidic, neutral, or alkaline urine. Their staining affinity was affected

by variations in pH which was particularly supported by the fact that their solubility properties were greatly influenced by pH changes.
Casts were not easily stained by the extract in all the pH levels used because they were typically transparent and can be easily missed even in an unstained sample because of their pure protein precipitate property [21].

Table 5. Multiple comparison of the effects of varying pH levels of turmeric extract on urine

pH	**Urine elements**	**1% vs 2% (p-value)**	**Interpretation**	**1% vs 3% (p-value)**	**Interpretation**	**2% vs 3% (p-value)**	**Interpretation**
5	**Crystals**	0.0415	Significant	0.5174	Not Significant	0.9933	Not Significant
	Epithelial Cells	> 0.9999	Not Significant	0.0415	Significant	0.0415	Significant
	RBC	0.0415	Significant	0.0415	Significant	> 0.9999	Not Significant
	WBC	0.5174	Not Significant	0.5174	Not Significant	< 0.0001	Significant
	Yeast Cells	> 0.9999	Not Significant	> 0.9999	Not Significant	> 0.9999	Not Significant
	Hyphal Elements	> 0.9999	Not Significant	> 0.9999	Not Significant	> 0.9999	Not Significant
	Urates/Phosphates	> 0.9999	Not Significant	> 0.9999	Not Significant	0.0415	Significant
	Casts	> 0.9999	Not Significant	> 0.9999	Not Significant	0.9933	Not Significant
6	**Crystals**	0.9933	Not Significant	0.5174	Not Significant	0.5174	Not Significant
	Epithelial Cells	0.5174	Not Significant	0.9933	Not Significant	0.0415	Significant
	RBC	0.5174	Not Significant	> 0.9999	Not Significant	0.5174	Not Significant
	WBC	0.0415	Significant	0.0415	Significant	< 0.000	Significant

						1	
	Yeast Cells	> 0.9999	Not Significant	> 0.9999	Not Significant	> 0.9999	Not Significant
	Hyphal Elements	> 0.9999	Not Significant	> 0.9999	Not Significant	> 0.9999	Not Significant
	Urates/Phosphates	0.0415	Significant	0.9933	Not Significant	0.9933	Not Significant
	Casts	> 0.9999	Not Significant	0.9933	Not Significant	0.9933	Not Significant
7	**Crystals**	0.0415	Significant	0.0007	Significant	0.9933	Not Significant
	Epithelial Cells	< 0.0001	Significant	< 0.0001	Significant	0.9933	Not Significant
	RBC	< 0.0001	Significant	< 0.0001	Significant	0.9933	Not Significant
	WBC	< 0.0001	Significant	< 0.0001	Significant	> 0.9999	Not Significant
	Yeast Cells	0.0415	Significant	0.0415	Significant	> 0.9999	Not Significant
	Hyphal Elements	< 0.000	Significant	< 0.000	Significant	> 0.9999	Not Significant
	Urates/Phosphates	< 0.000	Significant	0.0007	Significant	0.5174	Not Significant
	Casts	> 0.9999	Not Significant	> 0.9999	Not Significant	> 0.9999	Not Significant
8	**Crystals**	0.0007	Significant	> 0.9999	Not Significant	0.0007	Significant
	Epithelial Cells	< 0.0001	Significant	> 0.9999	Not Significant	< 0.0001	Significant
	RBC	< 0.0001	Significant	0.9933	Not Significant	< 0.0001	Significant
	WBC	0.0007	Significant	0.5174	Not Significant	< 0.000	Significant

						1	
	Yeast Cells	0.0415	Significant	0.5174	Not Significant	0.9933	NS
	Hyphal Elements	< 0.0001	Significant	0.5174	Not Significant	< 0.0001	Significant
	Urates/Phosphates	0.0415	Significant	0.5174	Not Significant	< 0.0001	Significant
	Casts	> 0.9999	Not Significant	0.9933	Not Significant	0.9933	NS

Table 6 showed the assessment result of the staining efficiency of turmeric extract at varying staining time. From this, it was shown that crystals were moderately stained in all the given concentrations at 1, 5, and 10-minute duration. This meant that their structure was distinguishable and was stained light yellow regardless of the length of staining time. However, no specific staining time was indicated for optimum staining reaction.

Epithelial cells were moderately stained at 1-minute and 10-minute duration. Additionally, they were optimally stained at 5-minute duration. This indicated that their nuclei and cytoplasm stained yellow with distinct and highly distinguishable features. In addition, epithelial cells were stained better in moderate staining time. This could be due to their large cytoplasm which could be under stained if shorter staining time was utilized and could be overstained if longer period was used [21].

RBCs were stained moderately at 1-minute duration while they were optimally stained at 5-minute and 10-minute duration. This could facilitate better penetration of the cells since they were anucleated and have characteristic central pallor. This was due to the composite property of the phospholipid bilayer and spectrin network resulting in the disc-shaped morphology of healthy RBCs. Thus, the membrane obtained their elastic and biological properties [27].

WBCs showed moderate staining reaction in all the given staining time which meant that these cells appeared light yellow with visible granules regardless of the length of staining. No specific staining time gave optimum results.

Yeast cells and hyphal elements showed moderate staining reaction in all the given length of staining time. These elements appeared light yellow with distinguishable features at 1-minute, 5-minute, and 10-minute duration.

Urates and phosphates appeared light yellow and were distinguishable in 1, 5 and 10-minute staining time. This meant that they took up the stain in moderate degree and that their staining affinity was the same for all the staining time indication. Optimum staining reaction was not noted in any of the abovementioned time.

Casts were evaluated to be insufficiently stained in the different staining time. This meant that they did not take up the stain. Thus, they appeared faint yellow with hardly distinguishable structures.

Table 6. Assessment result of the staining efficiency of turmeric extract at varying staining time

Time (mins	**Urine elements**	**1%**	**Interpretation**	**2%**	**Interpretation**	**3%**	**Interpretation**

)							
1	**Crystals**	2.00	M	2.33	M	2.00	M
	Epithelial Cells	2.00	M	2.33	M	2.00	M
	RBC	1.66	I	2.00	M	2.00	M
	WBC	2.33	M	2.66	M	2.00	M
	Yeast Cells	2.00	M	2.33	M	2.00	M
	Hyphal Elements	2.00	M	2.00	M	2.00	M
	Urates/Phosphates	2.00	M	2.00	M	2.00	M
	Casts	1.33	I	1.66	I	1.66	I
5	**Crystals**	2.33	M	2.66	M	2.33	M
	Epithelial Cells	2.00	M	2.66	M	3.00	O
	RBC	2.33	M	3.00	O	2.33	M
	WBC	2.33	M	2.66	M	2.33	M
	Yeast Cells	2.00	M	2.33	M	2.33	M
	Hyphal Elements	2.33	M	2.66	M	2.33	M
	Urates/Phosphates	2.33	M	2.66	M	2.33	M
	Casts	1.33	I	1.33	I	1.33	I
10	**Crystals**	2.33	M	2.66	M	2.33	M
	Epithelial Cells	2.33	M	2.33	M	2.00	M
	RBC	3.00	O	2.66	M	3.00	O
	WBC	2.33	M	2.33	M	2.33	M
	Yeast Cells	2.33	M	2.00	M	2.33	M
	Hyphal Elements	2.33	M	2.00	M	2.33	M

	Urates/Phosphates	2.33	M	2.00	M	2.00	M
	Casts	1.33	I	1.33	I	1.33	I

Note :3.00- Optimal stain (O); 2.00-2.99 – Moderate stain (M); 1.00-1.99 – Insufficient stain (I); 0.00-0.99 – Unsuitable stain

Table 7 showed the effects of the length of staining time from 1 minute, 5 minutes, and 10 minutes to the different urine elements. Staining time of crystals, epithelial cells, WBCs, yeast cells, hyphal elements, urates, phosphates, and casts did not show significant difference in all the varying concentrations and durations of staining time. This meant that the staining affinities of these microscopic elements were not affected by the length of staining time. Thus, their structures remained the same even if they were subjected to shorter or longer incubation time during staining.

As for RBCs, 1% and 3% concentrations presented significant reactions in 1-minute versus 5-minute staining time. It was shown that RBCs took up the stain in a different degree using lower or higher concentrations. This only implied that if either 1% or 3% concentrations was utilized for staining RBCs, incubation time should take at least 5 minutes prior to microscopy so that details of RBCs will be observed better. This could be due to the discoid appearance of RBCs requiring a moderate staining time so that their structure can be penetrated well [27].

Table 7. Multiple comparison of the effects of varying staining time using turmeric extract on urine elements

Concentration	**Urine elements**	**1 min vs 5 min**	**Interpretation**	**1 min vs 10 min**	**Interpretation**	**5 min vs 10 min**	**Interpretation**
1 %	**Crystals**	-0.3333	Not Significant	0.0	Not Significant	0.3333	Not Significant
	Epithelial Cells	0.0	Not Significant	-0.3333	Not Significant	-0.3333	Not Significant
	RBC	-1.333	Significant	-1.000	Not Significant	-0.3333	Not Significant
	WBC	0.0	Not Significant	0.0	Not Significant	0.0	Not Significant
	Yeast Cells	0.0	Not Significant	-0.3333	Not Significant	-0.3333	Not Significant
	Hyphal Elements	-0.3333	Not Significant	0.0	Not Significant	0.3333	Not Significant
	Urates/Phosphates	-0.33	Not Significant	0.0	Not Significant	0.3333	Not Significant

		33					
	Casts	0.0	Not Significant	0.0	Not Significant	0.0	Not Significant
2 %	**Crystals**	-0.3333	Not Significant	0.0	Not Significant	0.3333	Not Significant
	Epithelial Cells	-0.3333	Not Significant	0.3333	Not Significant	-0.3333	Not Significant
	RBC	-1.000	Not Significant	-1.000	Not Significant	-1.000	Not Significant
	WBC	0.0	Not Significant	0.3333	Not Significant	0.3333	Not Significant
	Yeast Cells	0.0	Not Significant	0.3333	Not Significant	0.3333	Not Significant
	Hyphal Elements	-0.6667	Not Significant	-0.3333	Not Significant	0.3333	Not Significant
	Urates/Phosphates	-0.6667	Not Significant	0.0	Not Significant	0.6667	Not Significant
	Casts	0.3333	Not Significant	0.3333	Not Significant	0.0	Not Significant
3 %	**Crystals**	-0.3333	Not Significant	0.3333	Not Significant	0.6667	Not Significant
	Epithelial Cells	-0.3333	Not Significant	0.3333	Not Significant	0.6667	Not Significant
	RBC	-1.333	Significant	-0.3333	Not Significant	0.6667	Not Significant
	WBC	-0.3333	Not Significant	0.3333	Not Significant	0.6667	Not Significant
	Yeast Cells	-0.3333	Not Significant	-0.3333	Not Significant	-0.3333	Not Significant
	Hyphal Elements	-0.3333	Not Significant	0.0	Not Significant	0.3333	Not Significant
	Urates/Phosphates	-0.3333	Not Significant	0.6667	Not Significant	1.000	Not Significant

	Casts	0.33 33	Not Significant	0.33 33	Not Significant	0.33 33	Not Significant

4. CONCLUSION

In conclusion, this study revealed that turmeric has the ability to stain microscopic urine elements due to high content of curcumin which is an active agent for colouring materials. However, casts remained insufficiently stained in varying concentrations of the plant extract owing to their high gelled-protein matrix making it less adhesive to stains. Crystals and epithelial cells were visualized better in 2% and 3% concentrations making them more visible in the urine. RBC yielded best staining results in 2% concentration while urates and phosphates were observed in their best forms in either 1% or 3% concentrations. Other urine elements such as WBC, yeast cells and hyphal elements did not show optimum staining reactions in any of the concentrations. Nevertheless, these elements were moderately stained in the different concentrations of the extract.

Crystals and RBC were best observed at pH 6 and showed optimum staining affinity. Yeast cells and hyphal elements both showed optimal staining reactions at pH 5 and pH 6 while urates and phosphates showed better staining results at pH 5. WBC and epithelial cells were not optimally stained in a specific pH but they were moderately stained in varying pH levels. Casts showed insufficient staining capacity in all the pH levels used.

Epithelial cells, RBC, WBC, yeast cells, hyphal elements, urates, phosphates and casts showed better staining results in higher concentration at 3% using either 5-minute or 10-minute staining time. Crystals showed optimum staining reaction using 5-minute and 10-minute staining time regardless of the concentrations used.

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