Potential of Mangosteen Peel Extract (*Garcinia mangostana*) as Alternative Dye to Eosine in Papanicolaou Buccal Smear Method

Vanesa Mariana[1], Ria Amelia[2]*, Siti Nurfajriah[3]

[1,2,3] Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Indonesia
*Correspondence author : ria.amelia@stikesmitrakeluarga.ac.id

Abstract

Introduction: Cytological staining using the *Papanicolaou* method combines hematoxylin and eosin staining. Eosin gives a red or orange color to acidophilic elements. Mangosteen peel extract has anthocyanin compounds, which turn orange in an acidic environment. This study aims to determine the effect of mangosteen peel extract as a substitute for eosin on buccal smear cytology using the *Papanicolaou* method.
Method: The location of this study was conducted at the STIKes Mitra Keluarga cytohistotechnology laboratory on February 13-March 3 2023 with an experimental research design. The sample is a buccal smear from 10 students. The sampling technique uses purposive sampling, and the mangosteen peel extract uses the maceration method.
Results: The results showed that dilutions of 1:1 (average: 2.8) and 1:5 (average: 2.85) yielded the lowest results. In contrast, the results were closest to control (average: 4) is retail of 1:3 (average: 3.4). The Normality Test obtains Sig. 0.0001, which shows <0.05, means that Ha is rejected or the data is not normally distributed. So, the non-parametric test was continued, namely the Kruskal-Wallis test. Kruskal Wallis test results sig. 0.0001 event <0.05, which means that Ho is rejected or there is an effect of mangosteen peel extract as an alternative dye to replace eosin in the *Papanicolaou* method on buccal smear cytology.
Conclusion: Mangosteen peel extract has the potential to stain the cytoplasm as an alternative dye to eosin in buccal smear cytology using the *Papanicolaou* method. The optimum concentration of mangosteen rind extract solution as a substitute for eosin is 1:3, with the highest average value of 3.4.

Keywords: Buccal smear, Mangosteen Peel Extract, Eosin, and *Papanicolaou*.

Introduction

Staining in cytology plays an essential role in producing quality preparations. This staining plays a role in giving color to cells or tissues so that a contrasting shape is observed under a microscope (Noor *et al*., 2020). Cytological staining can use the *Papanicolaou* method, a combination of hematoxylin and eosin staining. Hematoxylin will stain the cell nucleus, and eosin will stain the cytoplasm. Staining such as hematoxylin and eosin are synthetic dyes or chemicals (Kaban & Gigi, 2021).

Hematoxylin is a natural dye obtained from extracting American logs (*Haematoxylon campechianums*) with the chemical formula $C_{16}H_{14}O_6.xH_2O$. The alkaline nature of hematoxylin is capable of staining basophilic elements, making it useful for cytological and histological staining. Hematoxylin in cytological staining plays a role in coloring the cell nucleus, which is acidic so that it gives a blue color (Merck KGaA, 2021). Eosin is a dye for cytological synthesis that has acidic properties and is capable of coloring acidophilic elements with the chemical formula $C_20H_6Br_4Na_2O_5$.

Material Safety Data Sheet, according to regulation (EU) no. 1907/2006, states that eosin is a cytological solution that has an acute inhalation toxicity effect, which can give symptoms of irritation to the respiratory tract and severe eye irritation based on testing on rabbits. This threatens laboratory staff and users of eosin as a cytohistological dye (Smart Laboratory, 2019). Another weakness of synthetic dyes is that they are expensive, and the long storage time will damage the material. Based on the problem of the impact caused by synthetic dyes such as eosin, it is necessary to search for natural materials that have the potential as cytological stains to replace eosin, one of which is mangosteen rind. This is due to the mangosteen rind, which contains anthocyanins. When in a very acidic environment, it can give the maximum red color (Ernawati & Rahayu, 2017). This type of anthocyanin is pelargonidin, which appears freely in the form of a red pigment but gives an orange color to flowers and a red color to fruit.

This research refers to several previous studies. Research on the potency of red dragon fruit juice as an alternative stain for eosin in *Papanicolaou* staining by Permatasari *et al.* (2020) used chicken oral epithelial smear preparations from 7 treatments with varying concentrations of 1:1, 1:2, 1:3, 1:4, 1:5, pure or mother liquor and *Papanicolaou* solution as a comparison. Results 1:1 get the lowest value, while the results closest to the reference solution are 1:2, 1:3, 1:4, 1:5, and mother liquor or pure. However, this study stated that the maximum point of red

dragon fruit squeezing occurred at a shelf life of 4 days because the fruit had undergone a ripening and ripening process. Then, there will be a decrease in conditions followed by damage during aging (8 days of storage) (Permatasari *et al*., 2022).

As for research on the study of cytoplasmic staining on buccal smears using *Curcuma longa* (turmeric) extract as a substitute for eosin by Sankar *et al.* (2022) stated that the primary coloring pigment in *C. longa* which is acidic, gives a yellow color to the components of the cell cytoplasm. However, in this study, only centrifugation was carried out so that the supernatant was obtained, and pure extraction was not carried out (Sankar *et al*., 2022).

Based on the search for references, research has yet to be found regarding using natural dyes for mangosteen rind. This research will utilize mangosteen peel waste for cytological staining using the *Papanicolaou* method, so researchers are interested in conducting research on the potential test of mangosteen peel extract as an alternative dye to replace eosin on the *Papanicolaou* method on buccal smear cytology. Suppose the results of this study prove to be effective in substituting eosin. In that case, it is hoped that it can become a source of information for future researchers to develop the use of mangosteen rind as a cytological dye using the *Papanicolaou* method.

Methods

This type of research is descriptive quantitative. The research design used is experimental. The sampling method was carried out by purposive sampling. This research was conducted on 13 February-3 March 2023. Sampling in the form of buccal smears was carried out at STIKes Mitra Keluarga. Sample testing was carried out in the Pharmacognosy Laboratory (Room 214) and the Cytohistotechnology Laboratory (Room 305) STIKes Mitra Keluarga. The sample in this study was a buccal smear from STIKes Mitra Keluarga students. Buccal smear samples were taken from several 10 students. The samples will be stained with *Papanicolaou* as a control and mangosteen peel extract with concentrations of 1:1, 1:3, and 1:5, repeated twice to obtain a total sample of 80 preparations.

The test dye was obtained from 100 grams of powdered mangosteen rind. Mangosteen peel powder was dissolved in 495 ml of 96% ethanol and 5 ml of concentrated HCl. Leave it for one day, then strain it to get the extract. The extract is concentrated using a rotary vacuum evaporator until the volume is approximately one-fifth of the initial extract volume or until it becomes a paste. The extract results were made by making a soaking solution with a concentration of 1:1 (17 gr paste: 17 gr ethanol), 1:3 (8.5 gr paste: 25.5 gr ethanol), and 1:5 (5.6 gr paste: 28, 3 grams of ethanol). The density of the ethanol solution is 0.814 gr/ml. Ethanol is converted from grams to milliliters with the formula V = mass: density. The conversion calculation results are 1:1 (17 gr paste: 20.88 ml ethanol), 1:3 (8.5 gr paste: 31.32 ml ethanol), and 1:5 (5.6 gr paste: 34.76 ml ethanol).

The sample used is a buccal smear, which is scraped using a sterile spatula or wooden stick to obtain epithelial cells. The results of the smears were made preparations on the object glass. Preparations were stained with the *Papanicolaou* method. Eosin dye was used as a control, and mangosteen rind extract solution was used as a test dye. Buccal smear smear results were fixed with 95% alcohol for 3 minutes. The next step is rehydration with 90%, 80%, 70%, 60%, 50%, 40% and 30% alcohol for 3 minutes in each solution. Dip with distilled water—core staining stage with hematoxylin for 15 minutes. Run under running water for 1 minute. Blueing stage as much as three dips. Dehydration step with 30%, 40%, 50%, 60%, 70%, 80% and 90% alcohol for 2-3 minutes in each solution. G-6 (OG-6) orange staining stage for 1-2 minutes. Dip with 95% alcohol, as much as four dips. The glass objects to be immersed in the test dye solution and the control dye solution are separated. The slides for the control were soaked in an eosin dye solution for 3-5 minutes. The object glass for testing is immersed in the test solution (mangosteen peel extract 1:1, 1:3, and 1:5) for 3-5 minutes. Dip with 95% alcohol for four dips. They are clearing stage with alcohol-xylol for 3-5 minutes and xylol for 3-5 minutes. The object glass is dripped with mounting and then covered with a cover glass.

Staining results were observed microscopically to see cell shape and color intensity using a 100x10 magnification light microscope and oil immersion. The results of the observations will be processed and analyzed using Microsoft Excel 2019 and SPSS. The data obtained in tabular form are the results of observations, while the observed are the intensity of the core color, the intensity of the color of the cytoplasm, and the shape of the cell. The assessment of each point is in the form of ordinal data indicating the level of coloring quality. The meaning of ordinal data to be given is 1: unclear, 2: not clear, 3: clear, 4: very clear. After the data is obtained, it will be continued with the normality test. If the results are normally distributed, it will be tested with one-way ANOVA. If the data results are not normally distributed, then the Kruskal Wallis test will be continued. The value of the error rate of the data test results is 0.05, which means that the maximum data error rate is 5% and 95% of the data is correct.

Results and Discussions

Illustration of buccal smear preparations in test solutions 1:1, 1:3, and 1:5 with quality comparison results in **Figure 1. (A)** Researchers used the test solution, namely mangosteen peel extract as a substitute for eosin with a dilution of 1:1 with information on the quality of the cell shape The cytoplasm and nucleus are visible, the color intensity of the cytoplasm is clear with a thin orange color. In contrast, the nucleus is a faint grayish orange. **Figure 1. (B)** shows buccal smear epithelial cells using *the Papanicolaou* stain as a comparison/control. The quality of the comparison/control shows that the shape of the cytoplasm and epithelial cells is apparent, the intensity of the cytoplasmic color is apparent with a purplish pink color, the epithelial cell fragments are evident because the background of the epithelial cells in the preparation is visible faintly, the intensity of the nuclear color is apparent with a dark purple color.

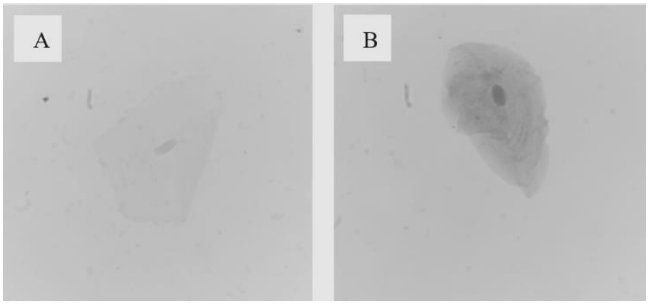

Figure 1. Buccal smear staining results, (A) mangosteen rind extract staining results at a dilution of 1:1, (B) buccal smear staining results using the *Papanicolaou* method

The results of the comparison of the quality of the preparations are in Figure 2. (A) the researcher used a test solution, namely mangosteen peel extract, as a substitute for eosin with a dilution of 1:3, with the description of the quality of the shape of the cyto-plasma and epithelial cells being evident, the intensity of the cytoplasm color being apparent with a slightly dark orange color, the epithelial cell fragments are evident because the background of the epithelial cells in the preparation is visible faintly, the intensity of the nuclear color is apparent with a grayish orange color.

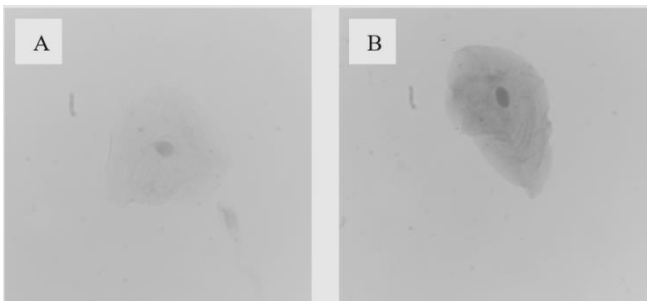

Figure 2. Buccal smear staining results, (A) staining results of 1:3 dilution of mangosteen peel extract preparations, (B) buccal smear staining results using the *Papanicolaou* method

The results of the comparison of the quality of the preparations are in **Figure 3. (A)** The researcher used a test solution, namely mangosteen peel extract, as a substitute for eosin with a dilution of 1:5 with a description of the quality of the cell shape, the presence of cytoplasm and nuclei was visible, the intensity of the color of the cytoplasm was clear with a thin orange color while in the nucleus faint grayish orange.

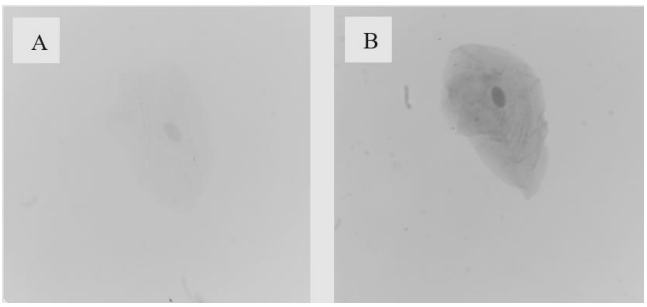

Figure 3. Buccal smear staining results, (A) staining results of 1:5 dilution of mangosteen peel extract, (B) buccal smear staining results *Papanicolaou* method

They were testing mangosteen peel extract as a substitute for eosin dye, which can color the cytoplasm so that it is orange. Testing was carried out on 80 buccal smear preparations, which had been assessed for quality based on a score of 1-4 for each preparation. The assessment data is searched for the sum, mean, and mode. The sum value of the 1:1 concentration test was 56, the 1:3 concentration was 68, the 1:5 concentration was 57, and the comparator (control) was 80. The mean value from the 1:1 concentration test was 2.8; a concentration of 1:3 was obtained at 3.4; a concentration of 1:5 was obtained at 2.85, and the comparator (control) was obtained at 4. The mode value of the test concentration 1:1 was obtained at 3, the concentration at 1:3 was obtained at 3, the concentration 1:5 was obtained at 3, and the comparator (control) was obtained at 4.

The results of the normality test and the non-parametric data test in this study were statistically tested, namely the regular distribution test, using the Kolmogorov-Smirnov test. Based on the **table 1**, a significant value is obtained, namely 0.0001. Sig. The table shows <0.05, which means Ha is rejected or the data is not normally distributed. So, the test cannot be continued with the one-way ANOVA test. This is because one-way ANOVA usually requires distributed data. So, the data will be continued with a non-parametric test, namely the Kruskal-Wallis test.

Table 1. Normal distribution test results using the Kolmogorov-Smirnov Test

Normality test

		Kolmogorov-Smirnov[a]		
	Treatment	Statistic	df	Sig.
Quality Value	1:1	0.327	20	0.0001
	1:3	0.387	20	0.0001
	1:5	0.420	20	0.0001
	Control	.	20	.

Testing continued with the Kruskal-Wallis test. Based on the Kruskal Wallis test on the data, the Asymp value was obtained. Sig. 0.0001. Asymp value. Sig. shows <0.05, which means Ho is rejected or there is an influence of mangosteen peel extract as an alternative dye to replace eosin in the *Papanicolaou* method on buccal smear cytology.

Table 2. Kruskal Wallis test results for each treatment of buccal smear preparations using the *Papanicolaou* method

Rank

	Treatment	N	Mean Rank
Quality Value	1:1	20	26.15
	1:3	20	44.00
	1:5	20	26.85
	Control	20	65.00
	Total	80	

Statistic test

	Quality Value
Kruskal-Wallis H	45.154
df	3
Asymp. Sig.	0.0001

Mangosteen rind extract contains anthocyanin compounds, which tend to give a red color in very acidic pH conditions (pH <3). If the anthocyanin is in an alkaline solution (pH >10), it will affect the anthocyanin pigment to a green color. The solvent used is ethanol-HCl. Research by Ernawati and Rahayu (2017) states that ethanol and HCl can extract the anthocyanin content of mangosteen rind. This is because anthocyanin compounds dissolve in polar solvents (Ernawati & Rahayu, 2017). The addition of polar HCl aims to provide an acidic atmosphere because anthocyanins are stable in an acidic environment. The pH test was carried out using a pH meter, and the measurement results obtained the pH of the extract, which was 2. However, the pH was measured once before mixing with the immersion solution. This can trigger the color results obtained by the extract needing to be more optimal (Lestari et al., 2014). Maceration is carried out for a day. Too extended maceration will damage the quality of the compound. According to research by Kurniawati (2020), soaking time for one day can extract mangosteen skin optimally. If the soaking time exceeds the optimum limit, it can cause the compound to decompose (Kurniawati, 2020).

The results of research with dilutions of 1:1, 1:3, and 1:5 obtained an average of 2.8 at a dilution of 1:1, 3.4 at a 1:3 dilution, 2.85 at 1:5 dilution, and four at control. The average value at the 1:1 dilution is the lowest; this is because during the process of making the test solution, the solution was already crusty, and the ethanol had evaporated so that the staining results often found cell shapes and the intensity of the cytoplasm color was less clear. The highest average value is a dilution of 1:3. This is because the observed staining results found that many cell shapes and cytoplasmic color intensity were clear, epithelial cell fragments were apparent. After all, the epithelial cell background was visible faintly, and the nuclear color intensity was evident. In line with the research of Permatasari *et al.* (2022), concentrations yield good results at dilutions of 1:2, 1:3, 1:4, and 1:5 concentrations and mother/pure solution, while at 1:1 dilution, the results are not good (Permatasari *et al*., 2022). The results of the study are by the usefulness of *Papanicolaou*: epithelial cells appear more transparent so that overlapping cells can be more easily distinguished, the color of acidophilic cells varies from red to orange, and basophilic cells are blue-green, stains that have partially or even wholly dried can be stained with entirely satisfactory, and the differential staining does not completely disappear (Chantziantoniou *et al*., 2017).

The *Papanicolaou* method consists of several stages, namely fixation, rehydration, blueing, dehydration, eosin, clearing, and mounting. These stages have different goals. Fixation was carried out using 95% alcohol to adhere the epithelial cells to the glass slide firmly. The rehydration stage uses alcohol with a decreasing concentration to introduce water into the cavities to facilitate the hematoxylin level. If the rehydration stage is not carried out correctly, it will affect the results of staining with hematoxylin. Hematoxylin will give a reddish-purple color to the cell nuclei and will turn blue after blueing (Beandrade *et al.*, 2020). The dehydration stage uses an alcohol solution for 1 minute with an increasing concentration to remove water from the preparation. If the immersion time for the dehydration stage is less than 1 minute, it will cause the water carried not to be lost to the maximum; if it is more than 1 minute, it can cause epithelial damage. Orange G-6 aims to counterstain cytoplasmic keratin to bright orange, making it easier to diagnose cancer cells. Eosin aims to dye the cytoplasm red or orange. The clarification stage using xylol aims to remove the solution used in the dehydration stage. The final stage is an attachment with Entelan, which aims to protect it from contamination by microbial growth. However, the final stage was not carried out, so it could be a factor of error during observation (Khristian & Inderiati, 2017; Dey, 2019).

Testing the quality of mangosteen peel extract compounds was influenced by irradiation, storage temperature, and storage time. Mangosteen peel extract is recommended to be stored in a dark bottle. This is because irradiation can affect the quality of the mangosteen peel extract. The anthocyanin contained can absorb light, causing damage to the anthocyanin structure, which results in the color changing to colorless. However, at the time of the study, mangosteen peel extract was not stored in dark bottles. The color results given by the mangosteen peel extract could be more optimal. Apart from being stored in a dark bottle, mangosteen peel extract also needs to be stored in the refrigerator. The refrigerator can help the extract be stable rather than stored in an open room. An increase in temperature can cause the decomposition (dissociation) of anthocyanin molecules, resulting in colorless compounds. According to Kurniawati (2020), storage time has a significantsmall effect. Storage must be in a dark bottle and at a low temperature so that it is not easily degraded. Based on research that has been carried out, it is necessary to pay attention to the storage and temperature of mangosteen peel extract to obtain maximum results.

Conclusions

The results of the buccal smear preparations were stained. However, not the same as the controls, with a significant value using the Kruskal Wallis test method of 0.0001 <0.05, which means that there is an effect of mangosteen peel

extract as an alternative dye for eosin in the Papanicolaou method on buccal smear cytology. Concentration results are good at 1:3 dilution with an average value of 3.4. Mangosteen peel extract with 1:3 dilution can replace eosin dye in the Papanicolaou method. However, further research still needs to be done regarding the resistance to staining levels of mangosteen peel extract.

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