The Effectiveness of Lerak Fruit (*Sapindus Rarak* DC) As an Alternative to Xylol in the Hematoxylin-Eosin Staining Deparaffinization Process

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Abstract. Deparaffinization is the initial stage in the staining process which aims to remove paraffin from the tissue so that it can absorb the dye properly. The solution used at this stage is xylol because it is non-polar. However, in its use xylol has disadvantages, one of which is toxic. Lerak contains saponin compounds. Saponin in lerak can function as a surfactant so that it can dissolve paraffin. The purpose of this study was to observe the quality of restosigmoid, mammary, endometrial, uterine, renal, lymph node, and uterine leiomyoma tissue preparations, which were deparaffinized using xylol and lerak. This study was conducted at the Anatomical Pathology Laboratory of Dr. Soetomo Hospital Surabaya in March - May 2023. The type of research used was laboratory experimental with post-test and control group design. The samples used were 144 preparations from 6 different tissues deparaffinized with xylol and 1%, 3%, 5%, 7%, & 9% lerak solution. The results of preparations deparaffinized with xylol based on the Kruskal wallis test showed an average (mean rank = 22.50). The results of preparations deparaffinized with lerak based on the Kruskal wallis test showed that the highest mean rank value was occupied by 1% lerak with an average (mean rank = 18.50). If based on the type of tissue, 5% lerak is effective as an alternative to xylol in lymph node tissue. It was concluded that lerak is not yet effective as an alternative to xylol in the deparaffinization process.

Keywords: Deparaffinization, Xylol, Lerak.

1 Introduction

Histotechnics is a supporting examination in establishing the diagnosis of a disease, one of which is cancer [1]. Histotechnics is a technique for presenting histological tissue from certain specimens through a series of processes to become a presentation that can be analyzed [2]. Several specimens including human or animal tissue will be processed through this technique to produce microscopic specimens that have been stained with hematoxylin and eosin (HE) staining [3]. Staining in histotechnics aims to give color to transparent cell and tissue components, such as structure, morphology, presence and prevalence of a cell and tissue so that they can be seen microscopically. The stain that is often used is hematoxylin eosin (HE) staining [4].

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Deparaffinization is one of the stages in the Hematoxylin Eosin staining process. Deparaffinization aims to remove paraffin from the tissue, so that the tissue can absorb the dye well. The solution used at this stage is an aromatic hydrocarbon solution that has non-polar properties such as xylol [5].

Xylol is a flammable, toxic, organic solvent and the only available solvent used for tissue deparaffinization prior to staining with hematoxilin eosin (HE) [6]. The advantage of xylol is that it can make tissues quickly become transparent. The disadvantages of xylol are that it is relatively expensive, toxic, harmful to humans and can cause shrinkage of the tissue if it is soaked for too long so xylol needs a cheaper and harmless alternative [7].

Dish soap and lerak fruit have the same function, which can be a surfactant that can dissolve dirt or fat. The combination of saponins from star fruit leaf extract combined with citric acid (citrun) at a concentration of 3% can have cleaning solution properties and can be used as a deparaffinizing agent in the hematoxilin eosin staining process [8].

Lerak fruit can be used as a substitute for xylol because one of the properties of paraffin is hydrophobic (insoluble in water) so paraffin must be dissolved in a non-polar solvent. Xylol is included in the same non-polar solution as lerak, one of which is non-polar can dissolve substances that are hydrophobic. This is because lerak fruit contains saponin compounds. The chemical structure of saponins consists of polar and non-polar compounds [9]. The content of hydrophilic and lipophilic saponins makes lerak fruit a surfactant so that it can dissolve paraffin. Surfactant in lerak fruit will bind fat in this case paraffin from the tissue. The existence of this bond makes the paraffin dissolve in the lerak fruit solution [10]. So this study aims to see the effectiveness of lerak fruit as an alternative solution to xylol in the deparaffination process.

2 Methods

This research is a laboratory experiment (true experiment) with post only control group design. This research was conducted at the Anatomical Pathology Laboratory of Dr. Soetomo Hospital Surabaya. The samples used in this study were 144 tissue preparations obtained from tissue blocks of restosigmoid, mammary, uterine endometrium, kidney, lymph nodes and uterine leiomyoma.

Then as many as 120 preparations were deparaffinized using 1%, 3%, 5%, 7%, 9% lerak solution heated at 75° C for 5 minutes. Tissue processing procedures were carried out in accordance with procedures at the Anatomical Pathology Laboratory of Rsud Dr. Soetomo. The quality of the preparation was read descriptively and assessed based on the color score of the cell nucleus, cytoplasm, color uniformity, color sharpness and color clarity.

3 Result

The results of HE staining can be seen in the cell nucleus, cytoplasm, color uniformity, color sharpness, and stain brightness, which are assessed by intensity, or the strength

of color absorption. The rating of HE painting based on its intensity can be seen in the Figure 1 and Table 1 in below.

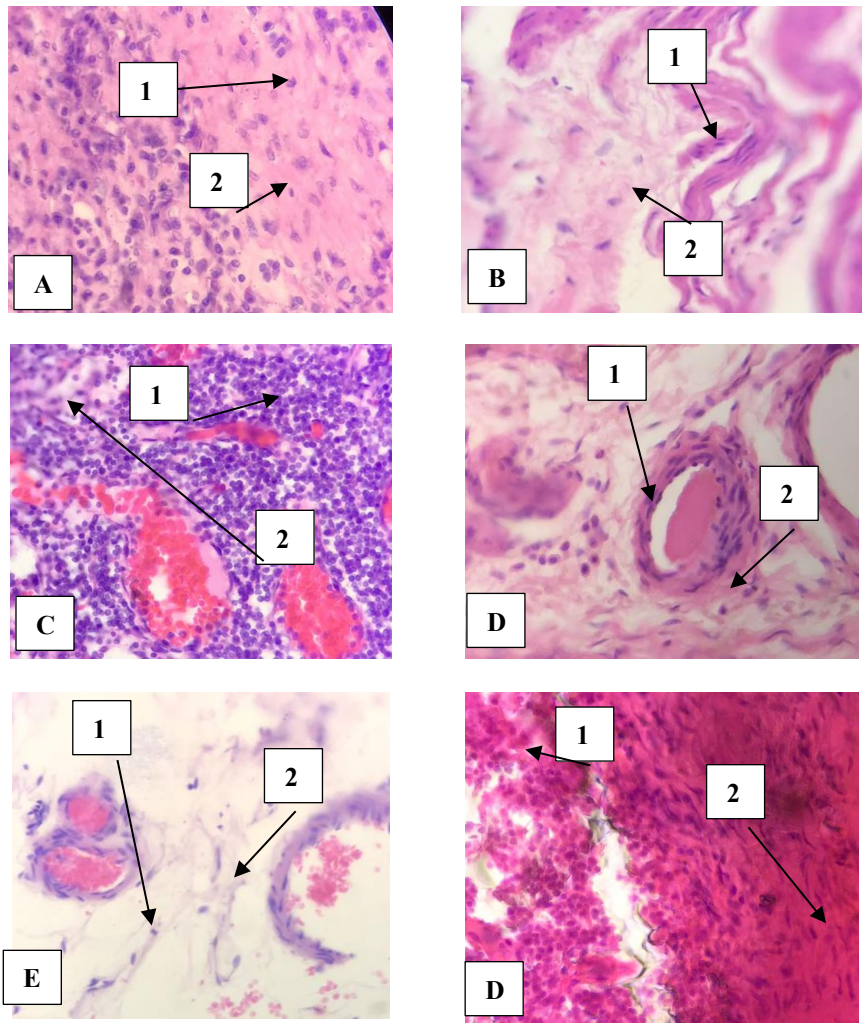

Fig. 1. HE painting using xylol (A), lerak 1% (B), lerak 3% (C), lerak 5% (D), lerak 7% (E), lerak 9% (F), cell nucleus (1), cytoplasm (2).

3.1 Data Analysis

All data obtained in this research will be analyzed statistically with the SPSS application using non-parametric test analysis. This is because the data was not normally distributed, so a different test was carried out using a non-parametric test, namely the Kruskall-Wallis test to determine whether there was a significant difference between

the results of HE staining using xylol and lerak fruit solution (*Sapindus rarak* DC) concentrations of 1%, 3%, 5%, 7%, and 9% (Table 1).

Table 1. Kruskal-Wallis Test Results.

	Total skor					
	Rectosig-moid	Mammae	Uterine endome-trium	Kidney	Lymph gland	Uterine Leio-myoma
Chi-Square	23.000	20.569	23.000	22.939	16.677	20.327
Df	5	5	5	5	5	5
Asymp. Sig	0.000	0.001	0.000	0.000	0.005	0.001

From the results of the Kruskall-Wallis test, a decision can be drawn in accordance with the hypothesis. The results of the Kruskall-Wallis test can be seen in Table 2.

Table 2. Kruskal-Wallis Test Results

Histology tissue	P-value (Sig p<0.05)	Conclusion
Restosigmoid	p=0.000	There is a difference
Mammae	p=0.001	There is a difference
Uterine endometrium	p=0.000	There is a difference
Kidney	p=0.000	There is a difference
Lymph gland	p=0.005	There is a difference
Uterine Leiomyoma	p=0.001	There is a difference

4 Discussion

The concept of using lerak in the research as a deparaffinizing agent is because the saponin content of lerak can function as a surfactant so that it can dissolve paraffin [11]. Lerak fruit is a form of local wisdom that has many useful chemical compounds, one of which is a saponin compound. Saponins in Lerak fruit give a bitter taste due to triterpenoid aglycones formed from hydrolysis. Aglycones convert lerak saponins into non-polar compounds. If lerak fruit reacts with water to form a stable froth, this indicates that the saponins in lerak fruit contain polar compounds. Because of its polar and nonpolar compounds, lerak fruit saponin is classified as a surfactant, so it can be used as an alternative to artificial surfactants in making soap and other hygiene products [12].

The results obtained after microscopic observation of the xylol control obtained good results with a score of 4 for mammary tissue and kidney tissue. Xylol can make tissues transparent, allowing better evaluation of the imaging results of each xylol deparaffinized tissue [13].

In Hematoxylin Eosin painting using 1%, 3%, 5%, 7%, and 9% lerak solution as deparaffin agent, the color intensity was reduced compared to the control (xylol). The color intensity of the nucleus is less blue, the color of the cytoplasm is less bright, the color is less uniform, less bright, less sharp. The low intensity score of the quality of the coloring results is due to the solution used is not a pure saponin extract from lerak but a solution of lerak without being extracted [14].

Then the quality of HE staining results using 1% lerak as a deparaffinizing agent on restosigmoid tissue preparations and uterine endometrium, decreased color quality intensity with a mean rank (10.50). Whereas in mammary tissue, kidney, lymph nodes and uterine leiomyoma are better when compared to other tissues, namely with a mean rank (16.50). Each tissue has a different affinity to absorb dyes. Affinity is the attraction between color and tissue . Then another factor is the lower concentration of lerak used, so that saponins work less strongly as surfactants in the lerak solution, so that paraffin dissolves in the tissue without damaging the tissue. While the possible causal factor that causes the low score obtained from the restosigmoid and uterine endometrial tissue blocks is inadequate fixation [15].

The quality of HE staining results deparaffinized using lerak at concentrations of 1%, 3%, 5%, 7% & 9% has different results. This is caused by several factors. Among them is the difference in concentration, the working power of saponin as a surfactant from lerak will also be different. The quality of HE staining results deparaffinized using lerak at a concentration of 1% has a better staining intensity compared to concentrations of 3%, 5%, 7% & 9%. While at higher concentrations the quality of HE staining results decreased in color intensity. Increasing the concentration of saponins can reduce the surface tension of the solution, but after reaching a certain concentration the surface tension tends to remain even though the concentration of saponins is increased, at the time of micelle formation it is called the critical micelle concentration. After reaching CMC, the surface tension is constant and saturated. Reducing surface tension can improve the wetting and cleaning ability of soap [16].

Timeliness does not guarantee that the microscopic quality of the preparation is obtained good results because timeliness is influenced by the size and type of tissue itself, so that the use of time can change according to needs. Meanwhile, the cytoplasm stained with eosin is less bright due to incomplete deparaffinization and insufficient fixation, which makes the cytoplasm pale [17].

Based on the results, it can be seen that preparations deparaffinized using lerak with a concentration of 5% in lymph node tissue have the same quality results as xylol as a deparaffinizing agent. This is because there are differences in the affinity of each tissue in absorbing dyes. In addition, temperature also greatly affects the results in this study [18].

The microscopic results of a preparation said to be unfavorable can be due to several factors of HE staining as the main color. Inadequate staining of the nucleus refers to the insufficiency of hematoxylin staining to stain the cell nucleus due to insufficient

fixation or autolysis, incomplete deparaffinization, improper staining time, too strong or excessive staining process, or too thin sections and incorrect pH value [19].

From 5 variations of lerak concentration, it can be seen that the quality of HE staining results deparaffinized using lerak at a concentration of 1% is closest to xylol. the closest to xylol is lerak concentration 1%. The best temperature in this study is the temperature of 70º C- 75º C. Good temperature for dissolving paraffin is a temperature of 90º C to 94º C will help dissolve paraffin in the tissue, so that the surfactant properties of lerak can prevent paraffin from reattaching to tissue preparations and can help the deparaffination process [20]. The temperature of the lerak solution used in this study is around 70º C - 75º C. This is because at a temperature of 70º C - 75º C the paraffin in the tissue has passed its melting point [21]. This temperature is based on the results of trials conducted by researchers on 10 tissue preparations. based on the results of the trial showed that lerak with a concentration of 1% at a temperature of 70º C- 75º C can already dissolve paraffin. Whereas if the temperature is 90º C-95º C can damage the tissue.

However, lerak with a concentration of 1% also has a pretty good quality of staining results on certain tissues. So that lerak is not yet effective to be an alternative to xylol as a deparaffinizing agent, but 1% lerak can be used if at any time in certain places you run out of xylol. However, when viewed from the results of the Mann Whitney U test of lerak at a concentration of 5% in the lymph node tissue, the results show $p>0.05$, which means that there is no significant difference from the use of xylol and 5% lerak [22]. This is due to temperature factors and also differences in tissue types in absorbing dyes, this is in accordance with research conducted which states that each cell and tissue has its own color [23]. In addition, another influencing factor is the saponin content of lerak fruit. Saponins can function as soap or surfactant. So that the higher the surfactant can usually damage cells and tissues [24]. this is in line with research conducted which states that excess surfactant power can cause cell and tissue damage [25].

5 Conclusion

The purpose of this study was to observe the quality of restosigmoid, mammary, endometrial, uterine, renal, lymph node, and uterine leiomyoma tissue preparations, which were deparaffinized using xylol and lerak. The finding of our study showed that Lerak (*Sapindus rarak* DC) solution cannot be used as an alternative to deparaffination in histological preparations stained with hematoxylin-eosin. Suggestions for further research could be to use other natural products such as olive oil.

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