UNIVERSITI TEKNOLOGI MARA

ENHANCEMENT OF NATURAL DYE FROM FRUIT FLESH EXTRACT USING PHOSPHOTUNGSTIC ACID AND FERROUS SULPHATE AS MORDANT

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AUTHOR'S DECLARATION

I declare that the task done in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Papanicolaou stain is one of the most commonly used stain in cytology and also known as a gold standard stain. Eosin Azure 50 (EA50), which is one of the components in Papanicolaou stain protocol, is a synthetic dye used to give eosinophilic colour to the cytoplasm of the cells. The usage of natural dye usually needs an enhancement by a chemical known as mordant for the dye to stay on the cell. Thus, the objective of this research was to identify which mordant that able to enhance the staining abilities of *Hylocereus polyrhizus* and *Rubus idaues* to be used as alternative to EA50 in Papanicolaou stain protocol. In this study, natural dyes of *H. polyrhizus* and *R. idaeus* with the addition of phosphotungstic acid and ferrous sulphate as mordant were used to enhance the staining of buccal cells. Aqueous extraction of the fruits flesh was performed. Three types of natural dyes were prepared – a pure aqueous extract without mordant, natural dye with phosphotungstic acid mordant and natural dye with ferrous sulphate mordant. All of the extracted dyes had their pH measured and the concentrations were determined spectrophotometrically. Papanicolaou stain protocol was used. Qualitative analysis was done by having 10 respondents reviewing the staining outcome, and the results were analysed by using weighted kappa test. Intensities of the staining were measured by using MIPAR (Materials Image Processing and Automated Reconstruction) to calculate the pixels. Statistical analysis was performed using One-way ANOVA to determine the differences in pH, concentration and staining intensities among the dyes and Papanicolaou stain. Paired sample T-test was used to compare between non-mordanted and mordanted natural dyes. The results showed that non-mordanted and mordanted natural dyes have poor agreement with Papanicolaou stain. The pH, concentrations, and staining intensities of the extracted dyes were significantly lower than Papanicolaou stain. Hence, addition of both mordant does not enhance the staining intensities of the extracted natural dyes. In conclusion, all the fruits flesh extracts have the ability to stain the cytoplasm of buccal cells, but their colour intensities were incomparable with Papanicolaou stain as the gold standard.

Keywords: *Hylocereus polyrhizus* (red dragon fruit), *Rubus idaeus* (raspberry), phosphotungstic acid mordant, ferrous sulphate mordant, Papanicolaou stain