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Natural Dyes from Red Dragon Fruit (*Hylocercus costaricensis*) on Plant Chromosome Staining

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Abstract: Efficacy of a dye extracted from red dragon fruit (*Hylocercus costaricensis*) was studied in chromosome staining of onion root tip with a solvent of the appropriate concentration and pH by using ethanol and acetic acid solvents at concentrations of 30%, 45% and 60%, the ratios of 1: 1, 2: 1, 5: 1 and 10: 1 for 12 and 24 hours, pH 1.5, 2.5, 3.5 and 6.5. It was found that acetic acid at a concentration of 60% at the ratio of 10: 1 for 24 hour and the pH 1.5 gave the best results in dyeing. Staining was found in areas of the nucleus and chromosomes at different stages of mitosis cell division. This experiment would reduce the use of chemicals in the laboratory, reduce the contamination of chemicals in the environment. It reduced the cost on purchasing of chemicals to use in the laboratory as well.

1. INTRODUCTION

Studies on the chromosomes of living organisms are very important. This is especially true of cell division, because the effects of cell division disorders can change the structure of plants. (Lakprayoon *et al*., 2010). The study of chromosomes can be studied under a microscope, but the plant cells studied are transparent and visually blurry. This makes chromosome dyeing very important so that the chromosomes are clearly visible and can be studied in cellular genetics.

Currently, laboratory colors are relatively expensive colors, difficult to obtain, accessible only to school laboratories with sufficient budgets and synthetic colors from chemicals, which pose a risk and harm to preparers and users. Therefore, natural dyes in both fruits and vegetables are extracted to replace synthetic colors (Lakprayoon *et al*., 2010). Most natural coloring substances are in the form of anthocyanin, flavonoid, carotenoid, quinine, alkaloid and betalain, extracted from different parts of the plant, where extracts capable of dyeing chromosomes are among the plants that provide pink, red, purple and blue, where red dragon fruit is one of the plant groups that gives its color (Kaewpuk, 2015).

Research has been conducted using natural plants to extract various chromosome dyes, including black glutinous rice (Supanuam *et al*.,2010, Phakpaknam *et al*.,2021), mangosteen rinds (Somboon *et al*., 2019), white mulberry (Sikhruadong *et al*.,2009, Lakprayoon *et al*., 2010), and Karonda fruit (Boonphan *et al*.,2019).

The red dragon fruit is the fruit of a tree with the scientific name *Hylocercus costaricensis*, which abroad calls this fruit as dragonfruit, a plant in the cactaceae family, and contains anthocyanins as a protector from the destruction of ultraviolet rays and has an antioxidant effect. The red dragon fruit is spherical, with a multi-seeded texture (berry) that has a sepal, inside the fruit, when dissected, it has a milky white, pink, red or magenta flesh, the seeds are small in black, resembling sesame seeds. The red dragon fruit contains betalain, a red and purple giver in beetroot (Chinmueang *et al*., 2008). Red dragon fruit is the fruit of the Thai economy as well. (Chairueangyot 2020)

According to this study, red dragon fruits were used. These can be found locally or in the market, were extracted for dyes for chromosome staining of plant roots as a way to reduce the cost of purchasing chemicals and to increase the value for fruit in the market, reducing the toxicity of chemicals on the environment. Therefore, there was an idea to extract natural dyes by using extracts from red dragon fruits.

There is not much research on extracting natural colors from red dragon fruit. The study of color extraction from red dragon fruit is very interesting. Therefore, the red dragon fruit was selected to study the chromosome dyeing performance of the plant roots by choosing the right group of solvents to extract colorants along with studying the best potential of hydrogen ion (pH) range in order to publish knowledge and be able to further research.

2. MATERIALS AND METHODS

2.1 Extraction of colored substances from red dragon fruit

Blend the pulp of red dragon fruit and mix it with solvent in a ratio of 1:1 (weight (g): volume (ml)) using solvents is ethanol and acetic acid concentrations of 30%, 45% and 60% at room temperature for 12 and 24 hours, when the specified time is reached, filtered with a thin white cloth and re-filtrated with filter paper.

2.2 Study the extraction result of colorants in solvents at different concentrations.

Part 1: Study the efficacy of chromosome staining of onion root tips using natural dyes extracted from red dragon fruit in no 2.1. Then select the best solvent concentration and time result from the experiment and adjust the ratio to 2:1, 5:1 and 10:1.

2.3 Detection of chromosome staining

Each natural dye sample obtained from several treatments was used for onion root chromosome staining. All metaphase chromosomes were accomplished by the squash technique (Chaiyasut, 1989; Campiranon, 2003).

Part 2: Study on chromosome staining of onion roots by squash technique of natural colors extracted from red dragon fruit best from adjusting the ratio to 2:1, 5:1 and 10:1 in part 1, adjusting the level of the potential of hydrogen ion (pH) from 1-7, and then taking it for microscopic viewing. Observe coloring and compare chromosome dyeing in different cell divisions with chemical dyes using aceto-orcein dyes and take pictures.

3. RESULTS

3.1 Extraction of colored substances from red dragon fruit

The extraction of colored substances from red dragon fruit using two solvents, ethanol and acetic acid at concentrations of 30%, 45% and 60%, a ratio of 1:1 over a period of 12 and 24 hours, found that group 1 ethanol solvents for 12 hours and group 2 ethanol solvents for 24 hours didn't detect coloring. Group 3 acetic solvents at 12 hours, a faded infection was found but the nucleus wasn't clearly visible. Group 4 acetic solvents at 24 hours, coloring was observed in the nucleus area and no coloring was detected in the cytoplasm area (Figure 1 and Table 1), thus concluding that extraction using a 60% concentration of acetic acid over a 24-hour period produced the best results (figure 1:4(c)) when adjusted to an additional ratio of 2:1, 5:1 and 10:1 in order to best extract the color. It was found that the optimal ratio for extracting natural dyes from red dragon fruit was 10:1 (Figure 2).

3.2 The extraction result of colorants in solvents at different potential of hydrogen ion (pH) levels

The red dragon fruit, the ratio of 10:1 was adjusted to the potential of hydrogen ion (pH) in the range 1-7, i.e., 1.5, 2.5, 3.5 and 6.5, and then studied the staining result by using the squash technique. The best result of the dye extract on the chromosomes was 1.5 (Figure 3).

When examined the effectiveness of dyeing plant chromosomes with natural extracted colors from red dragon fruit compared to synthetic chemical dyes from aceto-orcein. It was found that it could be dyed chromosome at the tip of the onion roots and showed the peculiarities of each cell division stage in the interphase, prophase, prometaphase, metaphase, anaphase, and telophase clearly and similarly (Figure 4).

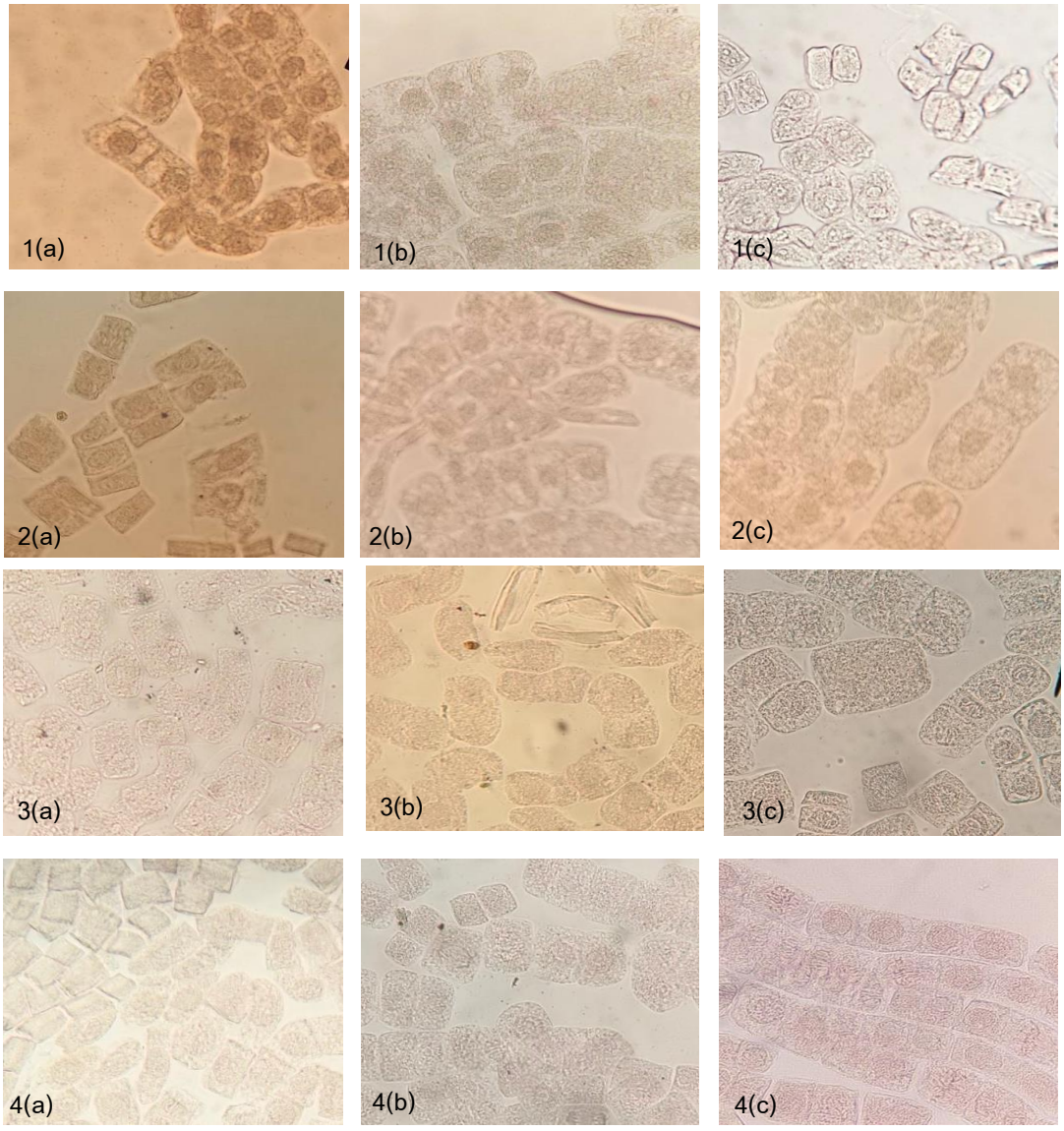

Figure 1: Compares extraction using ethanol and acetic acid concentration levels of 30%, 45% and 60% are stored at room temperature for 12 hours and 24 hours at a ratio of 1:1; 1(a). Ethanol 30% at 12 h.,1(b). Ethanol 45% at 12 h.,1(c). Ethanol 60% at 12 h; 2(a). Ethanol 30% at 24 h. ,2(b). Ethanol 45% at 24 h.,2(c). Ethanol 60% at 24 h; 3(a). Acetic acid 30% at 12 h. ,3(b). Acetic acid 45% at 12 h. ,3(c). Acetic acid 60% at 12 h; 4(a). Acetic acid 30% at 24 h, 4(b). Acetic acid 45% at 24 h. and 4(c). Acetic acid 60% at 24 h.

Table 1: Compares chromosome coloring results in nucleus and cytoplasm of red dragon fruit extract using solvents and different times in a ratio of 1:1

Coloring Style / Example	Chromosome dyeing in the nucleus	Cytoplasm dyeing
Ethanol 30% at 12 h	-	-
Ethanol 45% at 12 h	-	-
Ethanol 60% at 12 h	-	-
Ethanol 30% at 24 h	-	-
Ethanol 45% at 24 h	-	-
Ethanol 60% at 24 h	-	-
Acetic acid 30% at 12 h	-	-
Acetic acid 45% at 12 h	-	-
Acetic acid 60% at 12 h	+	-
Acetic acid 30% at 24 h	-	-
Acetic acid 45% at 24 h	-	-
Acetic acid 60% at 24 h	+++	-

Note: - means no color; + means slightly colored; ++ means medium stick; +++ means stick to a clear color

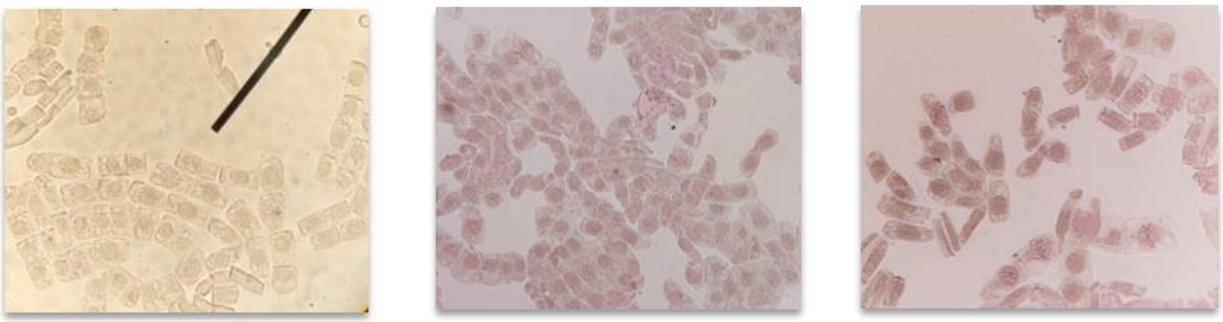

Figure 2: Compares extraction using 60% concentration of acetic acid over a 24-hour period with a ratio of 2:1, 5:1 and 10:1

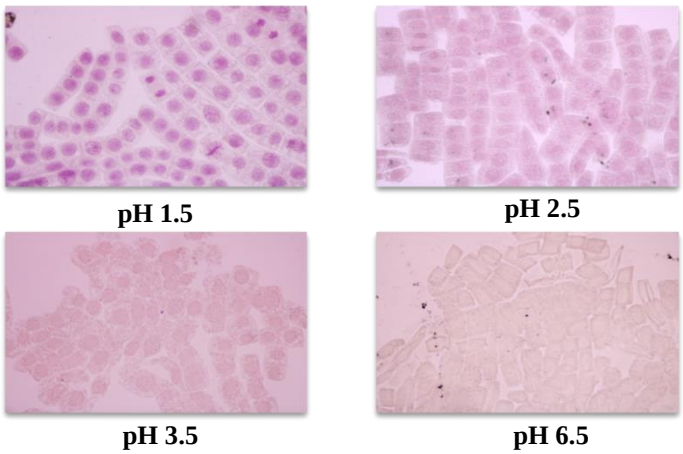

Figure 3: Compares extraction using 60% acetic acid concentration with a ratio of 10:1 with the potential of hydrogen ion (pH) of 1.5, 2.5, 3.5 and 6.5

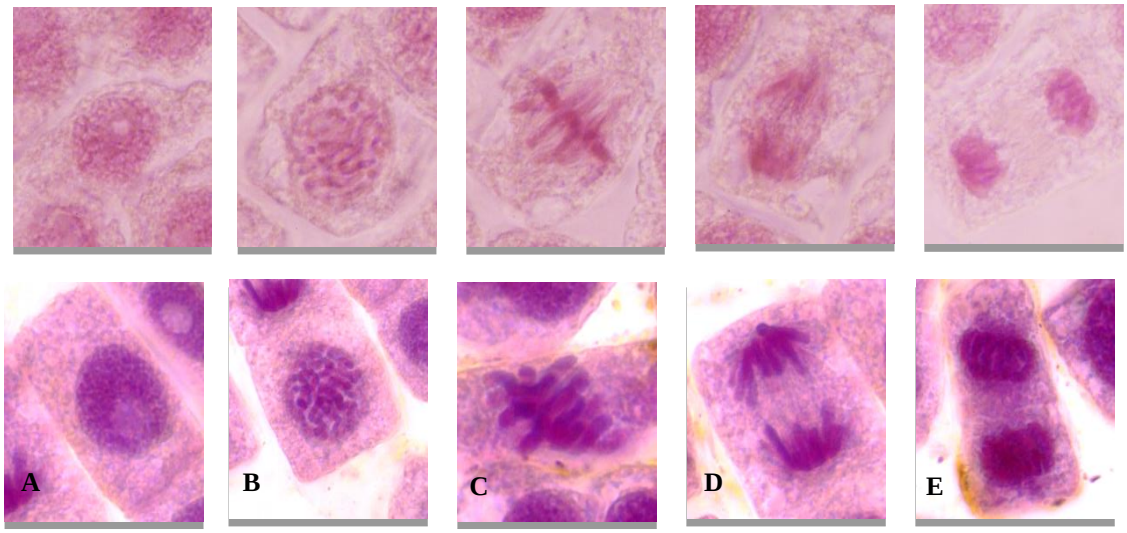

Figure 4: Compares the color performance from the red dragon fruit (pictured above) and aceto-orcein dyes (pictured below). A) Interphase B) Prophase C) Metaphase D) Anaphase and E) Telophase

4. DISCUSSION

Extracting colored substances from red dragon fruit using two solvents: ethanol and acetic acid at concentrations of 30%, 45% and 60%, ratio 1:1 (weight(g): volume(ml)) over a period of 12 and 24 hours found that extraction using 60% acetic acid concentrations over 24 hours produced the best results, which is close to Supanuam *et al*., (2010) reports, comparison of chromosome dyeing of white crinum asiaticum using a 45% concentration of acetic acid solvent to extract dyes from black glutinous rice, Sikhruadong *et al*., (2009) used a 45% concentration of acetic acid solvent to extract dyes from mulberry, and Somboon *et al*., (2019), which used a 45% concentration of acetic acid solvents to extract dyes from mangosteen rinds, but it took 72 hours to extract the substance (Table 2).

When adjusted, the ratio is further adjusted to 2:1, 5:1 and 10:1 to be able to extract the best coloring agents. It was found that the optimal ratio was 10:1, unlike the study of Sikhruadong *et al*., (2009), whose ratio to extract dyes from mulberry was appropriate, 2:1, and Boonphan *et al*., (2019), which used a ratio of 1:1 from extracts of Karonda fruit (Table 2).

Table 2: Compares results from experiments on chromosome dye extraction from other plants

Plant	Solvent	Concentration (%)	Extraction time (hours)	Ratio	pH	References
Red dragon fruit	Acetic acid	60	24	10:1	1.5	Current study
Black glutinous rice	Acetic acid	45	-	-	1-3	Supanuam *et al.* (2010)
	Butanol	45	-	-	1-2	
	Ethanol	50	24	1:1	-	Phakpaknam *et al.* (2021)
	Methanol	50	24	1:1	-	
Mangosteen rinds	Acetic acid	45	72	-	2.42	Somboon *et al.* (2019)
White mulberry	Acetic acid	45	24	2:1	-	Sikhruadong *et al.* (2009)
	Hydrochloric	0.1	24	1:1	-	Lakprayoon *et al.* (2010)
Karonda fruit	Alcohol	95	1	1:1	-	Boonphan *et al.* (2019)

When using red dragon fruit, the ratio of 10:1 to adjust the potential of hydrogen ion (pH) in the range of 1-7 was 1.5, 2.5, 2.5, 3.5 and 6.5, found that the pH caused the extract to be dyed in the best chromosome coloring were 1.5, similar to the reports of Supanuam *et al*., (2010), Sikhruadong et al. (2009), and Somboon *et al*., (2019), which used colored substances extracted from Lac, black glutinous rice, mulberry and mangosteen rinds, respectively. It could be dyed in plant chromosomes and observed mitosis cell division behavior, whereby dyes must be pH in the range of 1-3 (Table 2). Therefore, the use of dyes extracted from red dragon fruit with acetic solvents. The 60% concentration, the ratio of 10:1, pH 1.5 was the most suitable dye because the dye is clearly attached to the chromosome color. It could be used to study the mitosis cell division process of onion roots where biology is taught, which required studying the basics of chromosomes. It was found that the colorants in the anthocyanin group, flavonoid phenolic compounds, give magenta. It was found in plants, vegetables and fruits that are dark red, such as red dragon fruit, which had the ability to dye to study chromosomes. It was also inexpensive, uncomplicated to find, easy to extract, safe for the environment and could be used as a substitute for synthetic colors. (Lakprayoon *et al*., 2010)

5. CONCLUSION

Extraction of colored substances from red dragon fruit with acetic acid solvent concentration 60%, ratio 10:1 (weight (g) : volume (ml)): pH of 1.5 could be dyed and the behavior of the chromosomes was clearly observed, effective in dyeing chromosomes similar to aceto-orcein dyes. It could save budgets and reduce the use of chemicals in chromosome dyeing.

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