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TITLE:

SEPARATION OF MICROALGAE PIGMENTS THROUGH TLC (THIN LAYER CHROMATOGRAPHY) FOR BIOCOLOR USING DIFFERENT SOLVENT

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ABSTRACT

This study is based on separation of microalgae pigments through Thin Layer Chromatography (TLC) by using different types of solvent, which could determine their function as bio colourant and this method is commonly used to test pigments and several solvent solutions were explored to improve pigment separation efficiency. Differences in mobile phase with distinct polarity were evaluated for their effect on pigment separation and based on the pigments, retention factors (Rf value) are obtainable. By comparing the Rf value and the appearances of pigment separation, types of pigment could be identified. For example, pigments such as carotenoids, chlorophyll, and lutein could appear based on the types of chosen solvent (non-polar or polar) and methods used homogenization or boiling is better to prepare the extract sample. The Rf values for homogenization process are 0.82 (orange pigment), 0.70 (yellow pigment) and 0.65 (green pigment). Meanwhile, for boiling process, the Rf values are 0.80 (orange pigment), 0.68 (yellow pigment) and 0.58 (green pigment). These outcomes highlight the need of optimising solvent selection for effective bio colourant extraction.

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CHAPTER ONE
BACKGROUND

1.1 Introduction

Algae can be distinguished by their sizes, either it is microalgae or macroalgae and their colours (green, brown, and red algae) (Vo et al., 2024). Microalgae are small aquatic organisms (5-50 μm) that capable of photosynthesis. It is a photosynthetic eukaryotic microorganism that have growth several times faster than agricultural crops (Rivera-Sánchez et al., 2025). These microalgae have high in nutrients including lipids, protein, carbohydrates, vitamins, minerals, and bioactive substances such as carotenoids, phycocyanin, polyunsaturated fatty acids and etc (Liu et al., 2025). Typically, the main primary photosynthetic pigments found in algae including chlorophylls, carotenoids and phycobiliproteins (Somasundaram & Sathya, 2017). Based on biologist, they have classified algae into Chlorophyta, Rhodophyta, and Phaeophyta depending on their colour (Chen et al., 2023).

Pigments produced from microorganisms have been proven to be an effective alternative for producing natural food colours precisely contribute to the benefits of extracting and producing natural colours from them (Bandyopadhyay et al., 2019). Over the years, synthetic food colouring is increasingly used in food application instead of natural colours to meet several standards of attributes. For example, enhancing the appearances, high saturation, colour stability and consistency. (Dey & Nagababu, 2022). Synthetic dyes can be classified as azo dyes, triphenylmethane dyes, xanthene dyes, indigoid dyes, and quinoline dyes. In food sectors, azo dyes are an organic compound molecule having N—NN- and make it as a common type of synthetic dye. It is synthesised through a conventional method (Khanum & Shoukat Ali, 2024). Due to the growing of industrialization, synthetic chemistry, and dyeing industry grew in tandem. There are a broad spectrum of colour palettes and hues that were created in large quantities with a specific formulation (Mutaf-Kılıc et al., 2023). In textile industry, dyeing process including adding colour to materials using synthetic dyes that are developed chemically, which produced a bright, stunning, and long-lasting tones (Hevira et al., 2024). Compared to natural dyes, majority of it do not pose to any risks, however