



**UNIVERSITI
TEKNOLOGI
MAR**

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Kampus Bukit Besi

TITLE:

**SEPARATION OF MICROALGAE PIGMENT
USING THIN LAYER CHROMATOGRAPHY AS BIOCOLOURANT**

SUPERVISOR:

DR. AHMAD ROZAIMEE BIN MUSTAFFA

**SCHOOL OF CHEMICAL
ENGINEERING COLLEGE OF ENGINEERING**

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ABSTRACT

The increasing concerns over the health risks associated with synthetic food colorants have driven the demand for natural alternatives. Microalgae, known for their diverse pigment composition, present a promising source of natural colorants for food applications. This study aims to extract, identify, and evaluate pigments from microalgae using Thin-Layer Chromatography (TLC) as an analytical technique. The research focuses on the separation and characterization of major pigments such as chlorophylls, carotenoids, and fucoxanthin, assessing their potential as safe and sustainable food colorants. TLC, combined with bioautography and spectroscopic analysis, provides a reliable method for pigment identification while enabling an assessment of their antioxidant and bioactive properties. The findings from this study contribute to the growing body of research supporting the use of natural pigments as an alternative to synthetic dyes, promoting healthier and environmentally friendly food production. The results also emphasize the efficiency of TLC in analysing bioactive compounds, reinforcing its application in food science and natural product research.

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CHAPTER 1

BACKGROUND

1.1 Introduction

Green algae or microalgae are rich in various pigments like chlorophylls, carotenoids, and phycobiliproteins, which are important for their function in photosynthesis and can be used for their antioxidant properties in conferring health benefits. The identification of these is very essential for the elucidation of their biochemical functions, besides pharmacological, food, and cosmetic applications. (Ranga Rao et al., 2017). TLC is an analytical technique through which the different components in the mixture can be separated and identified. Simplicity, low cost, and speed of performance are various reasons for its applicability in the analysis of pigments extracted from microalgae. For this purpose, methanol is used very frequently as an extraction solvent because it is a good solvent for most polar pigments. (Palanisamy et al., 2021).

Extraction Efficiency of Methanol Various studies have indicated the efficacy of methanol as an extracting solvent for pigments of different microalgae. For example, the brown seaweed *Sargassum Binderi* of Malaysia was subjected to solvent extraction using methanol:chloroform:water (4:2:1, v/v/v) which successfully separated carotenoids and chlorophylls. Further isolation revealed fucoxanthin as one of the major carotenoids with high antioxidant properties. Mohamad et al., 2014. Other studies concerned with the bioactive compounds from some seaweeds, such as *Caulerpa racemosa*, *Sargassum* sp., and *Gracilaria verrucosa*, employed different solvents, one of which included methanol. In this respect, methanol extracts exhibited high levels of bioactive compounds. Among others, *C. racemosa* gave the highest extractive yield and total phenolic content (Putri et al., 2017). TLC separation of these pigments involves applying the methanol extract.

1.2 Literature Review

1.2.1 Green Microalgae

Chlorophyta, often called green algae, is a heterogeneous group of photoautotrophic organisms that possess a high concentration of bioactive compounds that provide a wide range of health benefits, mainly related to bone health. The group is typified by the existence of chlorophylls a and b, sulfated polysaccharides, phenolic acids, and minerals such as calcium, magnesium, and phosphorus, all of which play a crucial role in improving bone mineral density