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DEVELOPMENT OF A VALIDATED HPTLC METHOD FOR QUANTIFICATION OF STIGMASTEROL IN MARINE ALGAE

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ABSTRACT

This study presents the report of HPTLC method which has been developed and validated for quantification of stigmasterol in marine algae. The separation was performed on HPTLC Silica gel 60 F_{254} glass plate 20 cm x 10 cm. Good separation was performed in mobile phase using n-hexane: ethyl acetate: acetic acid (60: 36: 4, v/v/v). Determination and quantitation were achieved by densitometric scanning at 550 nm in absorbance mode. This method gave samples spot at R_f = 0.78 corresponding to stigmasterol standard. The method was validated in terms of precision and repeatability. Linearity range for stigmasterol was 0.5-10 µg/spot with correlation coefficient, R^2=0.9746. The LOD and LOQ were found to be 0.61 µg and 2.04 µg respectively. The highest amount of stigmasterol content obtained from marine algae sample (track 6 and 16) were 1.90 % w/w and 1.94 % w/w respectively. HPTLC method was found to be a simple, precise, accurate and convenient method for rapid screening of active constituents present in ethanol and ethyl acetate extracts. It can be used for analysis and routine quality control of herbal materials and formulations containing marine algae.

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TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Marine algae can be found in a variety of forms, sizes, and colours. Some of them are filamentous and some of them are delicate and complex seaweeds. Nevertheless, although they have different forms, their internal structure composed of similar cells, with differentiation of stems, roots, and leaves. Taxonomy of marine algae commonly based on the pigments, growth pattern, holdfast, branching and many other. These photosynthetic organisms can be classified into three groups based on their pigments which are Rhodophyceae (red algae), Phaeophyceae (brown algae), and Chlorophyceae (green algae) (Devi, Manivannan, Thirumaran, Rajathi, & Anantharaman, 2011). Seaweeds contain numerous primary metabolites such as proteins, dietary fibers, essential fatty acids, vitamins and minerals (Foon, Ai, Kuppusamy, Yusoff, & Govindan, 2013). Chinese division often consumed the seaweeds as their traditional medicine to treat hyperlipidemia, sunstroke, and urinary disease (Nwosu, Morris, Lund, Stewart, Ross, & Mcdougall, 2011).

Marine algae able to grow in an extreme environment condition such as high concentration of light and oxygen. Both conditions could contribute to the increase in the formation of free radicals. However, they managed to grow better and have a lack of oxidative damage in their structures (López, Rico, Rivero, & Tangil, 2011). There