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DETECTING THE PRESENCE OF REBAUDIOSIDE A IN TWO VARIETIES OF *STEVIA REBAUDIANA* (MORITA & PARAGUAY) IN VARIOUS PARTS OF PLANT VIA THIN LAYER CHROMATOGRAPHY (TLC)

NAJWA BINTI BAHAROM

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ABSTRACT

Stevia rebaudiana is a species which is characterized by high level of steviol glycosides in its tissues, especially in leaves. These glycosides are a source of natural zero-calorie sweetener which is considered sweeter than sugar (sucrose). There are two popular varieties in this species, namely Morita and Paraguay. In this study, it is suggested that Morita variety leaves contain higher level of rebaudioside A compared to Paraguay variety. Apart from leaves, there are other potential parts of plant that contain steviol glycosides such as stem and root. This allows optimizing usage of other parts of plant in order to maximize the production of rebaudioside A. Thus, extraction was made with water, methanol and ethanol as the extraction solvents. After extraction has completed, Thin-Layer Chromatography (TLC) analysis was performed to detect the compound that exists in the crude extract plant. With some modification of mobile phase, ethyl acetate: ethanol: methanol with ratio 20: 10: 2 was used to both varieties. For Morita variety, the R_f values of roots, leaves and stems with water extraction were 0.42, 0.53 and 0.51 respectively. While, the R_f values for ethanol extraction were 0.26, 0.64 and 0.60 and the R_f values for methanol extraction were 0.61, 0.67 and 0.50. For Paraguay variety, the R_f values of roots, leaves and stems with water extraction were 0.36, 0.51 and 0.56 respectively. While, the Rf values for ethanol extraction were 0.73, 0.64 and 0.57 respectively and lastly, the Rf values for methanol extraction were 0.70, 0.53 and 0.52 respectively. From the results, it showed that methanol extracts gave distinctive spots with better colour intensity whereas other solvents (water and ethanol) exhibited smear lines with fade colour on TLC plate. This can be suggested that methanol extract interacted compatibly with the mobile phase mixture through the chromatography process. The comparison of colour between these two varieties was made and it can be suggested that most of Morita's extracts in every parts of plant showed higher intensity than Paraguay's extracts. Among the parts of plant, leaves extract of Morita showed the best intensity spots. This can be initially indicated that leaves of Morita contained more rebaudioside A compared to other parts of plant.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Diabetes in Malaysia is growing very fast from year to year, thus people are looking for drug free intervention drugs to overcome diabetis. *Stevia rebaudiana* is a brilliant plant that completely can replace artificial sweetener. It is a shrub perennial herb plant native of Paraguay and Brazil and also known as "Sweet Weed", "Honey-Leaf", "Sweet-Leaf" and "Sweet-Herb". The species leaves are estimated to be 300 times sweeter than sugar cane (Chalapathi et al., 1997). *S. rebaudiana* plant is one of 154 members of the genus Stevia and one of two species that produce sweet steviol glycosides (Madan et al., 2010). It consists about nine active constituents of steviol glycosides which are stevioside, rebaudioside A-F, steviolbioside rubusoside, and dulcoside A. Each of them contributes their own percentage of sweet flavor to the stevia leaf.

Although steviol glycosides cannot be patented, increasing concerns about managing appropriate caloric intake as well as consumer demand for more sugar-substitute options provided the commercial motivation. Therefore, researchers and market players try to overcome the technical and regulatory hurdles for