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OPTIMIZATION OF SUPERCRITICAL EXTRACTION CONDITION OF *SENNA ALATA* AND EVALUATION OF BIOLOGICAL ACTIVITIES

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ABSTRACT

Supercritical fluid extraction (SFE) offer faster extraction process, decreased solvent usage and more selectivity on desired compounds. However, the major challenge for supercritical extraction is the optimization between the parameters (pressure, temperature etc) which could affect the yields and extraction of isolated compounds. Hence, the aim of this study is to optimize the pressure and temperature to obtain yield extracts from *S.alata* and to analyze its biological activities. In this present study, the influence of pressure (100, 200 and 300 bar) and temperature (40, 50 and 60˚C) on the *Senna alata* crude yield was investigated with fixed supercritical carbon dioxide (SC-CO_2) flow rate of 35 g/min. The parameters were optimized and modelled using response surface methodology (RSM) and central composite design (CCD). The analysis of variance (ANOVA) experimental design consists of 13 experimental runs with 5 replicates at the central points. Well-fitting quadratic model were successfully established for crude extract through backward elimination. The highest crude extract yield pointed out by RSM was pressure of 300 bar and temperature 40˚C respectively. Extraction yields based on SC-CO_2 varied in the range of 0.28 to 3.62%. The highest hyaluronidase inhibition activity and total flavonoids content obtained by *S.alata* crude extracts were 41.19% and 52.53% w/w, respectively. SC-CO_2 proves to be a great potential for extraction of yield, hyaluronidase inhibition activity and total flavonoids content for *S.alata*.

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CHAPTER ONE
INTRODUCTION

1.1 RESEARCH BACKGROUND

Extraction method and extracting solvent are important for quantity and quality of extracts. Hence appropriate extraction method for each plant should be applied to obtain highest amount of bioactive compounds. Sequential extraction using solvents such as petroleum ether, chloroform, methanol and ethanol has found to be effective against pathogenic bacteria (Chatterjee et al., 2012; Gritsanapan & Mangmeesri, 2009; Ehiowemwenguan et al., 2014; Hong & Lyu, 2011) as well as against a few fungi that causes dermatophytic disease such as *C. albicus, T. mentagrophyte, A. niger, D. congolensis, C.albicans* etc (Alalor et al., 2012; Ali-Emmanuel et al., 2003; Owoyale et al., 2005). However, recent studies have shown that supercritical fluid extraction (SFE) offers vast difference over solvent-based extraction techniques. Compared to conventional solvent method, extraction via supercritical fluid provides the following advantages: faster extraction process, more selectivity on desired compounds, decreased on solvent usage and lower costs for solvent disposal (Wright & DePhillipo, 2015; David & Selber, 1996). In addition, SFE requires very little to no dry-down time prior to the analysis and hence limits the thermal degradation (Capuzzo et al., 2013). There are many literatures about the natural materials extraction with SFE such as *Marchantia convoluta* (Chinese herb) (Xiao et al., 2007),

SFE of plant material is a growing topic of interest with solvents such as carbon dioxide (CO_2), propane, butane or ethylene. It allows the separation technique using supercritical fluid as the solvent. A substance is considered to be in supercritical condition when it is above its critical temperature and critical pressure. The main and commonly used solvent is CO_2. It is a cheap, eco-friendly, and generally recognized as a safe component. SFE using CO_2 is also attractive because of its high diffusivity and allows the extraction of easily oxidized compounds in natural products (David & Selber, 1996; Xiao et al., 2007; Wright & DePhillipo, 2015). However, conventional supercritical fluid carbon dioxide (SC-CO_2) suffers from low polarity which affect the efficiency of extracting the compound of interest.