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PHYTOCHEMICAL SCREENING AND *IN VITRO* BIOLOGICAL ACTIVITIES OF *Acmella paniculata* FLOWER AND LEAF EXTRACTS BY CITRIC ACID MONOHYDRATE/GLYCEROL DEEP EUTECTIC SOLVENT

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ABSTRACT

Subang nenek, scientifically known as *Acmella paniculata*, is a flowering herb rich in various bioactive compounds. Traditionally, this plant has been conventionally extracted using organic solvents that are harmful to the environment. Recently, a green extraction method has used deep eutectic solvent (DES) as a solvent extractor. However, the potential of this extraction approach with DES requires further investigation. Therefore, the study aimed to characterise the physicochemical properties of a DES consisting of citric acid monohydrate, as the hydrogen bond acceptor (HBA) and glycerol, as the hydrogen bond donor (HBD) and to assess its efficiency through the phytochemical content and biological activities of *A. paniculata* flower and leaf extracts. The DES was created by mixing the HBA and HBD components, forming CA/Gly. The CA/Gly's physicochemical analysis included examination of functional groups, physical homogeneity, viscosity, pH, density, surface tension and solubility. A method of maceration-centrifugation was done, using CA/Gly to extract *A. paniculata*. A total of 0.02 g *A. paniculata* for flower and leaf parts was mixed with three concentrations of CA/Gly (50%, 60% and 70% v/v in water), respectively. Phytochemical screenings, total phenolic content (TPC) and total flavonoid content (TFC) were performed on the plant extracts. Further bioactivities were conducted to assess the plant extracts' antibacterial, antioxidant and anti-inflammatory properties. The FTIR and POM results confirmed that hydrogen bonding between the carboxyl and hydroxyl groups in CA/Gly prevents crystallisation which creates a homogenous eutectic solvent. Other physicochemical properties of DES remained stable across the three concentrations tested (50%, 60% and 70% CA/Gly), indicating strong intermolecular bonding. The phytochemical test showed that flavonoids, phenolics, terpenoids and steroids were present for all extracts. Leaf extracts exhibited the highest total phenolic content (1.81±0.015 mg GAE/g to 2.16±0.098 mg GAE/g) while flower extracts exhibited the highest total flavonoid content (20.48 ±0.0036 mg QUE/g to 25.69±0.036 mg QUE/g). Both plant parts extracted with 50% and 60% CA/Gly had the highest value of TPC and TFC. The antibacterial efficacy against four pathogens (*Escherichia coli, Salmonella* Typhimurium, *Bacillus subtilis* and *Staphylococcus aureus*) demonstrated susceptibility to most CA/Gly-*A. paniculata* extracts with inhibition zones ≥15 mm. The most notable antibacterial reaction was observed in 60% and 70% CA/Gly-*A. paniculata* extracts. The antioxidant activity in flower extracts (75.24±1.05% to 77.49±0.90%) showed higher free radical scavenging activity (RSA) than leaf extracts (65.49±0.76% to 69.73±0.76%). A slight trend of increased CA/Gly concentration led to an increased RSA value, especially in 60% and 70% CA/Gly-*A. paniculata* extracts. The anti-inflammatory activity was also stronger in flower extracts than in leaf extracts, with up to 95.21±0.65% protein denaturation inhibition compared to 89.46±0.85%, respectively. Both 70% and 60% CA/Gly-*A. paniculata* extracts contributed in high anti-inflammation activity. In conclusion, the physicochemical behaviour of CA/Gly was successfully characterised, confirming its role as a eutectic solvent. The phytochemical results showed that CA/Gly effectively extracted phytocompounds from *A. paniculata*. The biological activity results indicated that CA/Gly exhibited strong antibacterial properties, while the flower part of *A. paniculata* showed prominent antioxidant and anti-inflammatory activities. The combination effects between CA/Gly and *A. paniculata* had augmented the bioactivities, making 60% CA/Gly-flower extract the optimal dose in plant extraction.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Plants have been utilized in traditional medicines for generations because of their beneficial bioactive compounds. The World Health Organisation (WHO) had estimated that more than 80% of the population in developing countries depend on traditional medicine for human health (Tran *et al.*, 2020). Furthermore, about one-third of the best-selling pharmaceutical products are derived from natural sources, such as plants and microorganisms (Elkordy *et al.*, 2021). In recent years, natural products have become pivotal therapeutic agents, especially in modern healthcare markets.

Hence, a crucial factor to note is the choice of plant material, as different plant species have varying phytoconstituents that respond differently to certain solvents. In this study, a local plant in Malaysia, known as *Acmella paniculata* was selected. This plant is relatively small in size and abundantly thrives in damp/moist environments or tropical rainforests (Rahim *et al.*, 2021). Locals would chew the flower and/or leaf parts to treat toothaches, mouth ulcers and other oral health issues (Shivananda *et al.*, 2023). Evidently, it has been documented that *A. paniculata* contains bioactive compounds with medicinal properties such as polyphenols (Rahim *et al.*, 2021).

This revelation highlights the increasing interest in extracting valuable metabolites from plant samples through various methods and solvent systems. The combination of solvent-method in plant extraction systems could possibly affect the effectiveness of the assay in extracting the plant compounds. The choice of a solvent is equally crucial, especially in solid-liquid extractions (maceration), as it essentially determines the interaction of plant compounds. The polarity of solvent can directly influence the extracted solutes because of the bonding interactions and the structure of chemical compounds (Lefebvre *et al.*, 2021). Thus, the choice of solvent is essential in the extraction of bioactive compounds from plant matrices.

The plant parts of *A. paniculata* have been utilised in conventional methods such as Soxhlet and maceration with organic solvents (Al Ragib *et al.*, 2020; Purushothaman *et al.*, 2018; Rani *et al.*, 2019). Although many studies documented plant compounds using organic solvents, minimal attention has been given to the solubility of compounds