Comparative Analysis of *In Vitro* Photo-Protective Effects on *Clitoria ternatea* Ethanolic Extract

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In this study, *Clitoria ternatea* flowers (CT) underwent four distinct ethanolic extraction procedures: maceration, soxhlet, ultrasonication-assisted extraction (UAE), and enzyme-assisted extraction (EAE). Analysis of the extracts centred on their yield percentage, sun protective factor (SPF), total phenolic content (TPC), and total flavonoid content (TFC). A comparison of the four extraction techniques revealed a significantly greater amount of CT extract in terms of yield (UAE: 15.40 ± 0.04 %; EAE: 11.97 ± 0.03 %), TPC (UAE: 35.53 ± 0.08 GAE µg/ml; EAE: 35.77 ± 0.73 GAE µg/ml), and TFC (UAE: 118.67 ± 0.78 QE µg/ml; EAE: 21.44 ± 0.02 QE µg/ml) when utilizing the non-conventional methods of UAE and EAE, as opposed to the conventional methods of Soxhlet and maceration. FTIR spectroscopy analysis confirmed the presence of phenolic compounds and flavonoids in all samples. These findings indicate that while UAE resulted in a higher content of flavonoids, EAE was more efficient in extracting phenolic compounds. Based on these results, UAE and EAE serve as potential eco-friendly, highly efficient and preferable 'green' or non-conventional extraction methodologies for obtaining sun-protective phytochemicals from CT.

Keywords: Ultrasonicated-assisted extraction; enzyme-assisted extraction; conventional, non-conventional

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The *Clitoria ternatea* (CT), also known as the Fabaceae flower or referred to locally as *bunga telang*, is a species from the Fabaceae family. Renowned for its vibrant deep blue petals, this plant is extensively utilized as a natural dye in food and beverage preparation. It has a longstanding history in traditional medicine, serving as a remedy to enhance cognitive function and alleviate symptoms associated with various ailments such as fever, inflammation, pain and diabetes [1]. In ayurvedic medicine, CT is used to treat a range of neurological conditions, including depression, fever and infertility [2, 3]. The therapeutic potential of CT has received international recognition, and is backed by scientific validation which attributes it with an array of biological activities. These include antioxidant, anti-diabetic, antitumor, antimicrobial, and anti-inflammatory properties [4, 5].

Recently, the potential of CT extracts for sun protection has garnered significant attention in research circles. Prior studies have investigated the sun-protective properties of CT extracts, utilizing various solvents such as water [6-8], ethanol [9], and a blend of water with ethanol or methanol [10, 11]. It is important to highlight that thus far, maceration has been the sole extraction technique reported in the exploration of CT's sun-protective capabilities. However, the maceration method is known to present several limitations, including low extraction yields, diminished efficiency and the necessity of large volumes of solvents, which could potentially pose health risks [12]. The efficacy of this method further decreases in the presence of substantial amounts of polysaccharides attached to the cell wall [13, 14]. In light of these challenges, this preliminary investigation aimed to delve into alternative, more sustainable and efficient 'green' or non-conventional extraction methods. The goal was to enhance the yield of CT extraction and optimize its sun-protective effects, contributing to the development of eco-friendlier and more effective solutions in this domain.

Microwave-assisted extraction (MAE) and ultra sonic-assisted extraction (UAE) represent innovative, non-traditional approaches that have demonstrated promising results in extracting CT. However, when considering the application of MAE in future studies, it is important to consider its requirement for specialized equipment, its lower selectivity, and the potential for undesirable reactions at elevated temperatures [16-18]. Given these considerations, UAE emerges as a more practical and viable option, and is the focus of this study. Utilizing ultrasonic energy in a commonly-available ultrasonic bath to disrupt plant cell walls [19-21], UAE allows for the use of reduced solvent quantities and facilitates the completion of the