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Sustainable approach in phlorotannin recovery from macroalgae

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In this present study, alcohol/salt liquid biphasic system was used to extract phlorotannin from brown macroalgae. Liquid biphasic system is a new green technology that integrated with various processes into one-step, by concentrating, separating and purifying the bioproduct in a unit operation. The solvent used is non-toxic and there is potential for solvent recovery which is beneficial to the environment. Phlorotannin is a bioactive compound that has gained much attention due to its health beneficial effect. Therefore, the isolation of phlorotannin is lucrative as it contains various biological activities that are capable to be utilised into food and pharmaceutical application. By using 2-propanol/ammonium sulphate system, the highest recovery of phlorotannin was 76.1% and 91.67% with purification factor of 2.49 and 1.59 from *Padina australis* and *Sargassum binderi*, respectively. A recycling study was performed and the salt phase of system was recycled where maximum salt recovery of 41.04% and 72.39% could be obtained from systems containing *P. australis* and *S. binderi*, respectively. Similar recovery of phlorotannin was observed after performing two cycles of the system, this concludes that the system has good recyclability and eco-friendly.

[**Keywords:** Liquid biphasic system; Phlorotannin recovery; Macroalgae; *Padina australis*; *Sargassum binderi*]

Phlorotannin is a type of polyphenolic compounds exists in macroalgae (commonly known as seaweed), especially brown macroalgae (Phaeophyta). It was synthesized to defence against stress conditions, for example grazing, UV radiation and bacterial infection (1,2) or acts as the secondary defence metabolites during the development of algal cell walls (3). This type of bioactive compound has attracted huge interest from community due to the variation of their bioactivities, such as antidiabetic, antioxidant, anti-bacterial, and anti-inflammatory (4). Stern et al. (5) have stated that the precipitation of protein could be occurred through the interaction of phlorotannin and protein. Protein precipitation is significant in downstream processing to isolate the protein from all possible contaminants in blood. Phlorotannins are made up of oligomers and polymers of phloroglucinols (1,3,5-trihydroxybenzene). Different structure of phlorotannins may formed via various polymerization degrees (6). Natural bioactive compounds or metabolites in algae are often extracted due to their non-toxicity properties and potential health benefits. The conventional methods are solid–liquid extraction, supercritical fluid extraction, pressurised liquid extraction and centrifugal partition extraction (3). Conventional methods are time-consuming and often required high volume of toxic organic solvent during the extraction process. Therefore, liquid biphasic system (LBS) is introduced in extracting and purifying the components or metabolites from plant and aquaculture at the same time.

LBS is a better alternative compared to conventional methods as higher yield and purified product could be obtained through this method. There are several types of LBS, for example polymer/salt, alcohol/salt or polymer/polymer as the phase forming components (7,8). Over decades, this technique has been studied intensively as a separation technology in separating and purifying biological products such as enzyme, proteins and metabolites from biological sources without denaturing the targeted product. It was reported that LBS is capable to extract biomolecules in a short mass transfer period with high efficiency and selectivity using lower cost (9,10). However, parameters such as types of phase forming components, pH, temperature or addition of inorganic salt may affect the extraction and purification process accordingly depending on the targeted product. Apart from that, the LBS formed by alcohol/salt has been proved to extract out and purify high value product (11,12) like laccase from processed *Hericium erinaceus* (Bull.:Fr) Pers. Fruiting bodies (13), γ-cyclodextrin by *Bacillus cereus* cyclodextrin glycosyltransferase (14) and fucoxanthin from *Isochrysis galbana* and *Phaeodactylum tricornutum* (15) successfully.

Until now, most of the reported studies are related to solvent extraction and enzyme-assisted extraction to recover phlorotannin. This is the first report on phlorotannin recovery using LBS, which no study has done before. In this present study, we were focussing on the feasibility of alcohol/salt LBS technique in extraction/purification of phlorotannin from *Padina australis* and *Sargassum binderi*. Ammonium sulphate has been chosen to be the type of salt phase due to the versatility of ammonium sulphate forming two phases with other alcohols. Besides, it is a cheaper choice compared to the usage of polymer and copolymer as the bottom phase in the system. Ammonium sulphate is also known for its functional usage in

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