

Mini Review

Isolation and Purification of Bioactive Protein(S) With Anti-Breast Cancer Property from *Gynura Procumbens* (Lour.) Merr Extract

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Gynura procumbens (Lour.) Merr is a medicinal plant found in several countries in South East Asia. In Malaysia, this plant is also known as Sambung Nyawa which means 'extending life'. *Gynura procumbens* (Family Asteraceae) is a small plant about 1 to 3 m in height. Its leaves are elliptic, succulent, glossy with purplish hairs and are arranged alternately on a hairy purple stem. *Gynura procumbens* is traditionally used to treat various types of illnesses such as kidney discomfort, cancer, fever, inflammation, and rheumatism [1].

Throughout the years, studies on *Gynura procumbens* have confirmed the therapeutic values of the plant. Several phytochemicals have been identified from the plant, namely chlorophyll, carotenoids, alkaloids, volatile oils [2], flavonoids, saponins, tannins, terpenoids [3], β -sitosterol, stigmasterol, kaempferol-3-O-rutinoside and quercetin [4,5]. *In-vivo* studies showed that extract of *Gynura procumbens* suppresses the elevated serum glucose levels in diabetic rats [6,7]. Besides, our previous study on the protein extracted from *Gynura procumbens* plant leaves has shown to inhibit the growth of human breast cancer cell line, MDA-MB-231 [8].

Isolation and purification of proteins are a significant step for identifying the bio-active proteins in the extract of *G. procumbens*. Due to the nature of the plant leaf extract which composition is rich in pigments and metabolites that are generally smaller in size as compared to the macromolecular proteins. Thorough cleaning of the extract is required to obtain a pure protein sample for analysis, therefore the protein extract was subjected to a series of cleaning up steps. The leaves were washed and air dried at room temperature, liquid nitrogen was used to make the dried leaves brittle and subsequently ground into powder form using a blender and the powdered leaves were reconstituted in 0.5M phosphate buffer,

pH 6.9. The mixture was vortexed and left at room temperature overnight. The mixture was centrifuged at 13,000 rpm for 30 minutes at 4 degrees Celsius. The supernatant was then subjected to ammonium sulphate precipitation. The precipitate was collected and constituted in deionized water and transferred to dialysis tubes with 1000 Da molecular mass cut-off.

The extract was dialyzed against distilled water for overnight. The next day, dialyzed extracts were collected and transferred for ultrafiltration. A few cycles of centrifugation were carried out and in each cycle deionized water was added to wash off impurities. The retained protein extracts were recovered and freeze dried to dry form. The dry proteins were reconstituted in deionized water and undergo the final step which is gel filtration. Gel filtration is a technique which separates macromolecules according to size, it is also known as size exclusion technique. In this technique, the matrix which consists of cross-linked copolymer of allyl dextran and N, N-methylenebisacrylamide with a mean particle size of 47 μ m was used.

The proteins were flushed with a mixture of 50 mM phosphate buffer pH 7.2 and 0.15 M sodium chloride at a flow rate of 0.5 ml/min. Detection wavelength was set at 280 nm. The proteins were eluted at 80 minutes onwards and the proteins were collected in five mL per fraction. A total of 38 fractions were collected for each run. All the separation techniques chosen above kept the separated proteins in intact form and therefore the activity of the proteins was preserved. This is critical as activity study on the separated proteins will be carried out in the subsequent study. The proteins collected in each fraction were subjected to further analysis, identification of each active protein was carried out on the LC/MS/MS analysis using the QT instrument.

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