

## Extraction, purification, and bioevaluation of phycoerythrin from *Halymenia refugium* against pathogenic bacteria and A549 lung cancer cell line

Sowmiya Kadalmani<sup>1</sup>, Sirajunnisa Abdul Razack<sup>2</sup>, Geethalakshmi Ramakrishnan<sup>1</sup> & Renganathan Sahadevan<sup>1\*</sup>

<sup>1</sup>Centre of Biotechnology, Alagappa College of Technology, Anna University, Chennai-600 025, Tamil Nadu, India

<sup>2</sup>Department of Biotechnology, Acharya Institute of Technology, Soladevanahalli-560 107, Karnataka, India

Received 13 April 2025; revised 22 April 2025

### Supplementary Data

Table S1 — Free radical scavenging potential analysis

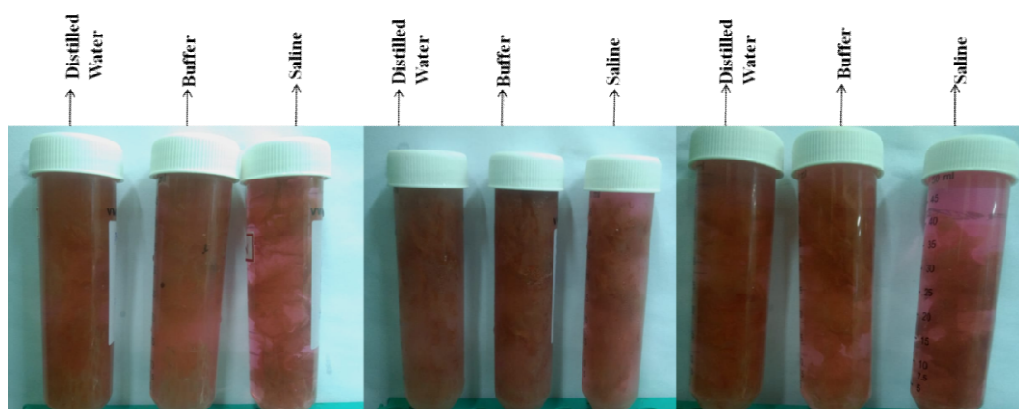
| DPPH                  |                 |                  |                       |                 |                  |
|-----------------------|-----------------|------------------|-----------------------|-----------------|------------------|
| Concentration (µg/mL) | % of inhibition | IC <sub>50</sub> | Concentration (µg/mL) | % of inhibition | IC <sub>50</sub> |
| Ascorbic acid         |                 |                  | Phycoerythrin         |                 |                  |
| 20                    | 34.25           | 41.94            | 20                    | 31.94           | 45.01            |
| 40                    | 52.31           |                  | 40                    | 45.83           |                  |
| 60                    | 64.81           |                  | 60                    | 66.66           |                  |
| 80                    | 83.79           |                  | 80                    | 77.31           |                  |
| 100                   | 95.83           |                  | 100                   | 91.20           |                  |
| ABTS                  |                 |                  |                       |                 |                  |
| Concentration (µg/mL) | % of inhibition | IC <sub>50</sub> | Concentration (µg/mL) | % of inhibition | IC <sub>50</sub> |
| Ascorbic acid         |                 |                  | Phycoerythrin         |                 |                  |
| 20                    | 37.18           | 44.85            | 20                    | 23.10           | 63.07            |
| 40                    | 48.73           |                  | 40                    | 32.49           |                  |
| 60                    | 67.14           |                  | 60                    | 44.40           |                  |
| 80                    | 81.58           |                  | 80                    | 59.20           |                  |
| 100                   | 92.41           |                  | 100                   | 81.58           |                  |
| Phosphomolybdate      |                 |                  |                       |                 |                  |
| Concentration (µg/mL) | % of inhibition | IC <sub>50</sub> | Concentration (µg/mL) | % of inhibition | IC <sub>50</sub> |
| Ascorbic acid         |                 |                  | Phycoerythrin         |                 |                  |
| 20                    | 21.73           | 49.32            | 20                    | 25.92           | 61.19            |
| 40                    | 36.87           |                  | 40                    | 31.40           |                  |
| 60                    | 47.82           |                  | 60                    | 49.75           |                  |
| 80                    | 58.61           |                  | 80                    | 55.23           |                  |
| 100                   | 88.27           |                  | 100                   | 86.15           |                  |

Table S2 — *In vitro* anti diabetic assay of phycoerythrin

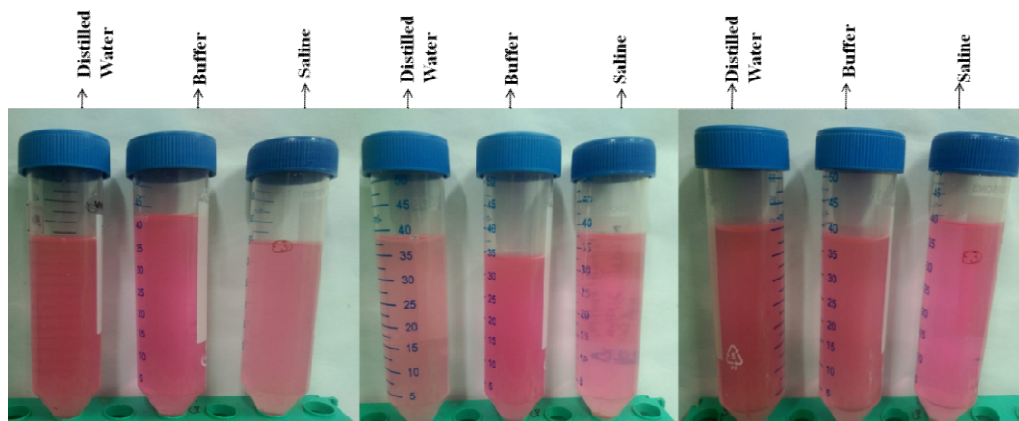
| $\alpha$ -Amylase inhibitory assay    |                 |                  |                                    |                 |                  |
|---------------------------------------|-----------------|------------------|------------------------------------|-----------------|------------------|
| Concentration ( $\mu\text{g/mL}$ )    | % of inhibition | $\text{IC}_{50}$ | Concentration ( $\mu\text{g/mL}$ ) | % of inhibition | $\text{IC}_{50}$ |
|                                       | Acarbose        |                  |                                    | Phycoerythrin   |                  |
| 20                                    | 32.07           |                  | 20                                 | 33.96           |                  |
| 40                                    | 58.49           |                  | 40                                 | 54.33           |                  |
| 60                                    | 73.20           | 35.14            | 60                                 | 66.41           | 30.81            |
| 80                                    | 87.92           |                  | 80                                 | 81.13           |                  |
| 100                                   | 95.84           |                  | 100                                | 88.30           |                  |
| $\beta$ -Glucosidase inhibitory assay |                 |                  |                                    |                 |                  |
| Concentration ( $\mu\text{g/mL}$ )    | % of inhibition | $\text{IC}_{50}$ | Concentration ( $\mu\text{g/mL}$ ) | % of inhibition | $\text{IC}_{50}$ |
|                                       | Acarbose        |                  |                                    | Phycoerythrin   |                  |
| 20                                    | 37.02           |                  | 20                                 | 33.19           |                  |
| 40                                    | 50.63           |                  | 40                                 | 57.87           |                  |
| 60                                    | 58.29           | 42.13            | 60                                 | 67.65           | 36.34            |
| 80                                    | 70.63           |                  | 80                                 | 84.25           |                  |
| 100                                   | 86.80           |                  | 100                                | 91.06           |                  |

Table S3 — *In vitro* Anti-Inflammatory Activity Evaluation of the phycoerythrin

| Protein Denaturation Assay         |                   |                  |                                    |                 |                  |
|------------------------------------|-------------------|------------------|------------------------------------|-----------------|------------------|
| Concentration ( $\mu\text{g/mL}$ ) | % of inhibition   | $\text{IC}_{50}$ | Concentration ( $\mu\text{g/mL}$ ) | % of inhibition | $\text{IC}_{50}$ |
|                                    | Diclofenac sodium |                  |                                    | Phycoerythrin   |                  |
| 20                                 | 35.23             |                  | 20                                 | 25.05           |                  |
| 40                                 | 46.23             |                  | 40                                 | 44.67           |                  |
| 60                                 | 59.67             | 46.22            | 60                                 | 67.07           | 44.62            |
| 80                                 | 66.32             |                  | 80                                 | 89.34           |                  |
| 100                                | 80.92             |                  | 100                                | 94.09           |                  |
| Egg Albumin Denaturation Assay     |                   |                  |                                    |                 |                  |
| Concentration ( $\mu\text{g/mL}$ ) | % of inhibition   | $\text{IC}_{50}$ | Concentration ( $\mu\text{g/mL}$ ) | % of inhibition | $\text{IC}_{50}$ |
|                                    | Diclofenac sodium |                  |                                    | Phycoerythrin   |                  |
| 20                                 | 26.46             |                  | 20                                 | 28.72           |                  |
| 40                                 | 38.73             |                  | 40                                 | 46.24           |                  |
| 60                                 | 53.03             | 53.51            | 60                                 | 62.81           | 47.31            |
| 80                                 | 71.27             |                  | 80                                 | 70.32           |                  |
| 100                                | 84.62             |                  | 100                                | 85.57           |                  |



After filtration

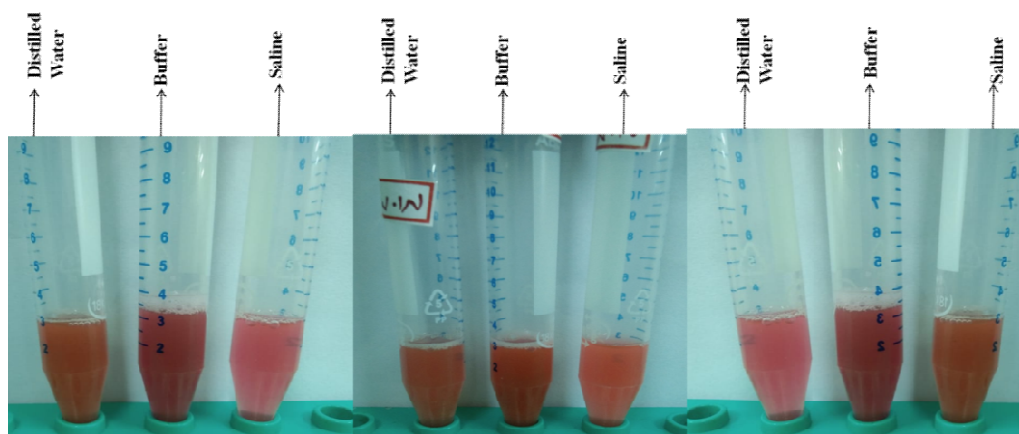


Freeze thaw method

Water bath sonication

Ultra sound sonication

After precipitation



Freeze thaw method

Water bath sonication

Ultra sound sonication

Fig. S1 — Extraction of phycoerythrin

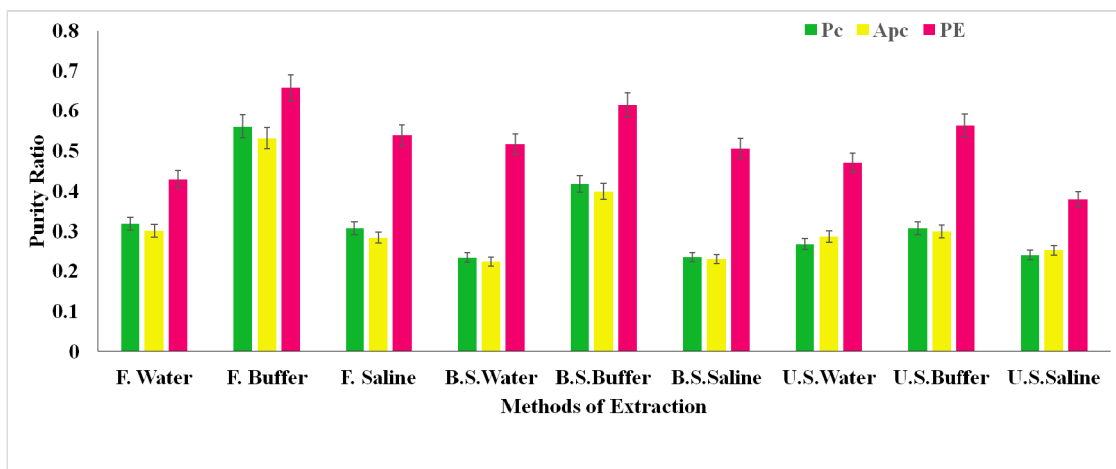


Fig. S2 — Purity ratio after Precipitation

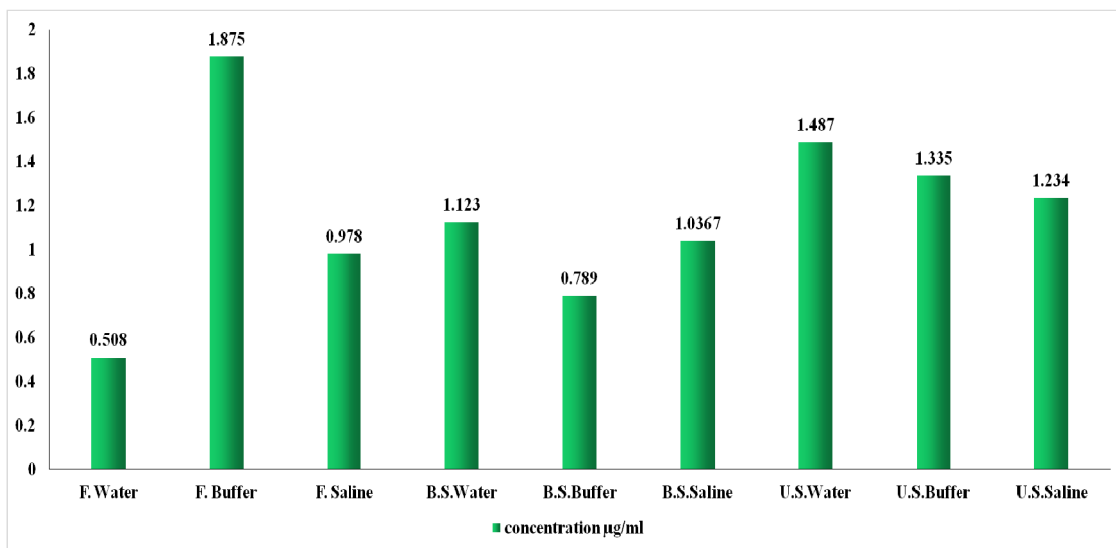


Fig. S3 — Concentration of the protein by Bradford

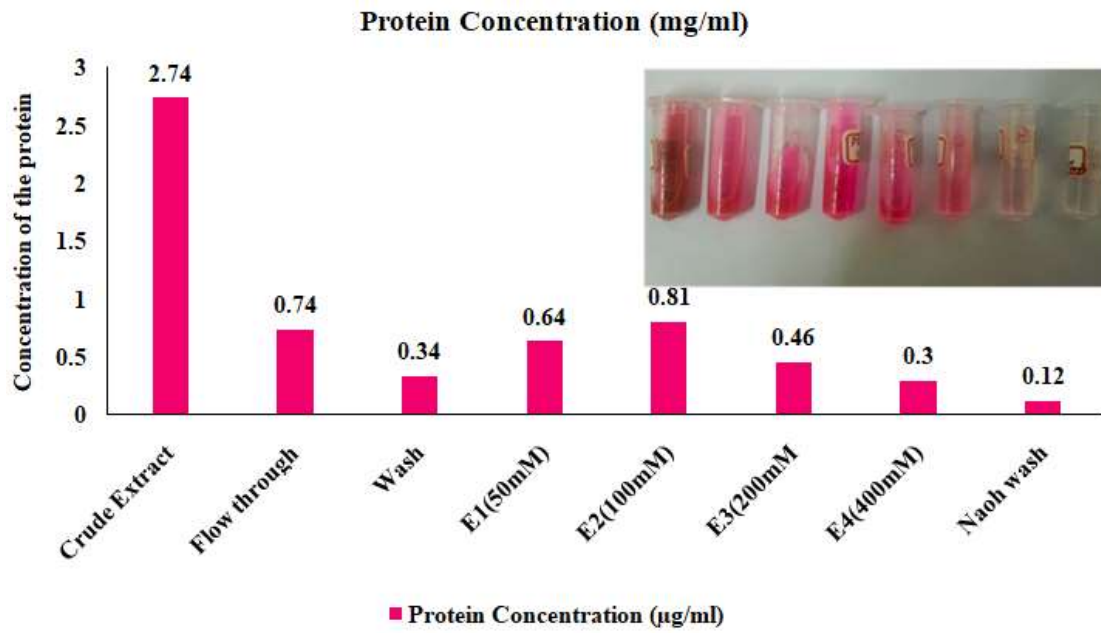


Fig. S4 — Ion Exchange chromatography