Research on Antioxidant Properties of Several Marine Active Substances

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Abstract. Using DPPH free radical as an in vitro antioxidant model and using ascorbic acid (Vc) as a positive control, the four important marine activities of trehalose, carboxymethyl chitosan, glucosamine hydrochloride and corona bafil clam small molecule peptides were studied Antioxidant ability of the substance in vitro. The experimental results show that the four marine active substances have good antioxidant properties, and their antioxidant activity and concentration show a certain dose-effect relationship. The small molecular peptide of corrugated paphia is better at eliminating DPPH free radicals than the other two marine species. Active substance, when the concentration is 4 g/L, the elimination rate of DPPH radicals reaches 95.7447%.

1. Introduction
Free radicals are molecules, atoms, ions or groups with one or more unpaired electrons in the outer orbit. When excessive free radicals are generated and the body's ability to scavenge is reduced, free radicals can cause damage to many biomolecules [1-2].

Marine active antioxidants can scavenge endogenous and exogenous free radicals, which have strong antioxidant properties and low side effects, so they have gradually become a hotspot in the research of antioxidant active substances [3-4]. Based on the research on the antioxidant properties of trehalose, carboxymethyl chitosan, glucosamine hydrochloride, and paphia clam, four marine active substances have good antioxidant capacity. The research and application provided theoretical basis [5].

2. Experimental inquiry
Detection of the free amino nitrogen content: the name of DPPH is 1,1-diphenyl-2-trinitrophenylhydrazine. DPPH radical is a very stable nitrogen-centered radical. Its stability mainly comes from the resonance stabilization of three benzene rings and the space barrier prevents the unpaired electrons on the nitrogen atom sandwiched between them from performing their proper electron-pairing effect. The basic steps are as follows: take different types of marine active substances, add an equal volume of 0.1 mmol/L DPPH absolute ethanol solution, and mix them at room temperature for 30 minutes in the dark, and then centrifuge (4500 r/min, 15 min). Take the supernatant to measure the absorbance Ai at 517 nm; and use 4 mL of absolute ethanol and distilled water (both are 2 mL) as a blank control. The blank control group was 2 mL of sample solution plus 2 mL of absolute ethanol, and its absorbance at 517 nm was Aj. The blank control group was set up to remove...
the absorbance of the marine active substance itself. The model control group was 2 mL DPPH solution plus 2 mL absolute ethanol, and its absorbance at 517 nm was Ac. The absorbance of the positive control Vc was measured in parallel three times and averaged. Calculate the scavenging rate of DPPH free radicals according to the following formula:

\[
\text{Scavenging rate of samples to DPPH free radicals} = \left[1 - \frac{(A_i - A_j)}{A_c}\right] \times 100\% \quad (2-1)
\]

Where \(A_i\) is the DPPH radical absorbance at 517 nm; \(A_j\) is the Blank control group at 517 nm absorbance; \(A_c\) is the Model control group absorbance at 517 nm.

### 3. Results and analysis

The DPPH method is a simple and feasible method for evaluating the antioxidant activity of natural antioxidants, and is widely used at home and abroad. DPPH radical is a kind of stable organic radical. It has a characteristic purple-red group absorption peak in ethanol solution. When a radical scavenger is present, its absorption gradually disappears due to its single-electron pairing. The degree of discoloration is the number of received electrons has a quantitative relationship, so it can be quantitatively analyzed by spectrophotometry [3]. The results from experimental investigations are shown in the below Table 1~5.

#### Table 1. Different concentration samples per 50 mL liquid weighing sample quality.

| Concentration (g/L) | Ascorbic acid (g) | Trehalose (g) | Carboxymethyl chitosan (g) | Glucosamine Hydrochloride (g) | Paphia Undulate (g) |
|---------------------|------------------|---------------|---------------------------|-----------------------------|------------------|
| 0.1                 | 0.0056           | 0.0056        | 0.0056                    | 0.0051                      | 0.0050           |
| 0.5                 | 0.0259           | 0.0279        | 0.0256                    | 0.0267                      | 0.0273           |
| 1                   | 0.058            | 0.0566        | 0.0514                    | 0.0535                      | 0.0515           |
| 2                   | 0.1049           | 0.1146        | 0.1022                    | 0.1002                      | 0.1016           |
| 3                   | 0.1509           | 0.1600        | 0.1512                    | 0.1524                      | 0.1517           |
| 4                   | 0.2011           | 0.2252        | 0.2026                    | 0.2015                      | 0.2011           |
| 7                   | 0.3527           | 0.3860        | 0.3506                    | 0.3533                      | 0.3524           |

#### Table 2. Ascorbic acid (Vc) on DPPH free radical elimination rates.

| Concentration (g/L) | Absorbance \(A_i\) | Absorbance \(A_j\) | Absorbance \(A_c\) | Elimination rate (%) |
|---------------------|---------------------|---------------------|---------------------|----------------------|
| 0.5                 | 0.027               | 0.002               | 0.565               | 95.5752              |
| 1                   | 0.026               | 0.003               | 0.567               | 95.9436              |
| 2                   | 0.025               | 0.004               | 0.566               | 96.2898              |
| 3                   | 0.023               | 0.005               | 0.568               | 96.8310              |
| 4                   | 0.022               | 0.006               | 0.569               | 97.1880              |
Table 3. Trehalose on DPPH free radical elimination rates.

| Concentration (g/L) | Absorbance Ai | Absorbance Aj | Absorbance Ac | Elimination rate (%) |
|---------------------|---------------|---------------|---------------|---------------------|
| 0.5                 | 0.480         | 0.008         | 0.479         | 1.4614              |
| 1                   | 0.469         | 0.009         | 0.476         | 3.3613              |
| 2                   | 0.460         | 0.010         | 0.474         | 5.0633              |
| 3                   | 0.451         | 0.011         | 0.477         | 7.7757              |
| 4                   | 0.438         | 0.012         | 0.470         | 9.3617              |

Figure 1. Ascorbic acid (Vc) on DPPH free radical elimination rates.

Figure 2. Trehalose on DPPH free radical elimination rates.

Table 4. Carboxymethyl chitosan on DPPH free radical elimination rates.

| Concentration (g/L) | Absorbance Ai | Absorbance Aj | Absorbance Ac | Elimination rate (%) |
|---------------------|---------------|---------------|---------------|---------------------|
| 0.5                 | 0.400         | 0.002         | 0.483         | 17.5983             |
| 1                   | 0.381         | 0.005         | 0.474         | 20.6751             |
| 2                   | 0.370         | 0.006         | 0.478         | 23.8490             |
| 3                   | 0.351         | 0.007         | 0.480         | 28.3333             |
| 4                   | 0.330         | 0.008         | 0.475         | 32.2105             |

Table 5. Rates of glucosamine hydrochloride on DPPH free radical elimination.

| Concentration (g/L) | Absorbance Ai | Absorbance Aj | Absorbance Ac | Elimination rate (%) |
|---------------------|---------------|---------------|---------------|---------------------|
| 0.5                 | 0.415         | 0.000         | 0.490         | 15.3061             |
| 1                   | 0.398         | 0.001         | 0.475         | 16.4211             |
| 2                   | 0.380         | 0.002         | 0.480         | 21.2500             |
| 3                   | 0.365         | 0.003         | 0.488         | 25.8197             |
| 4                   | 0.341         | 0.004         | 0.487         | 30.8008             |
Figure 3. Carboxymethyl chitosan on DPPH free radical elimination rates.

Figure 4. Rates of glucosamine hydrochloride on DPPH free radical elimination.

Table 6. Undulate small peptides on DPPH free radical scavenging rates.

| Concentration (g/L) | Absorbance Ai | Absorbance Aj | Absorbance Ac | Elimination rate (%) |
|---------------------|---------------|---------------|---------------|----------------------|
| 0.5                 | 0.393         | 0.027         | 0.423         | 13.4752              |
| 1                   | 0.297         | 0.080         | 0.424         | 48.8208              |
| 2                   | 0.197         | 0.152         | 0.425         | 89.4118              |
| 3                   | 0.185         | 0.156         | 0.422         | 93.1280              |
| 4                   | 0.179         | 0.161         | 0.423         | 95.7447              |

Figure 5. Undulate small peptides on DPPH free radical scavenging rates.

From Table 1 to Table 6 and Figure 1 to Figure 5, it can be seen that as the concentrations of the four marine active substances increase, the absorbance Ai for eliminating DPPH radicals becomes smaller, and the smaller the absorbance Ai, the stronger the oxidation resistance; the absorbance Aj varies with concentration. Large and increased, in line with Lambert's law; the absorbance Ac
remained basically unchanged; the elimination rate of DPPH free radicals of the four marine active substances increased with increasing concentration.

According to the data of Table 1 to Table 6, the following Figure 6 is obtained:

**Figure 6.** Four kinds of Marine active substances eliminate DPPH free radical ability

It can be seen from Figure 1 that with the increase of the sample concentration, the elimination rate of DPPH radicals by the four marine active substances is higher. In the reaction system, the maximum elimination rate was basically reached when the Vc of the control group was 0.5 g/L, and the maximum elimination rate of the small-molecule peptide of corrugated bafil was close to the maximum elimination rate of Vc when it was 4 g/L. At this time, the elimination rate of trehalose was 9.3617%, the elimination rate of carboxymethyl chitosan was 32.2105%, and the elimination rate of glucosamine hydrochloride was 30.8008%, which were far below the maximum elimination rate of 97.1880% of Vc. On the other hand, the elimination rate of the remaining three marine active substances is not as obvious as the elimination rate of the small molecule peptide of corrugated bafi clam with increasing concentration. Therefore, the effect of small molecule peptide of corrugated bafi clam on eliminating DPPH radicals is better than the other three substance.

**4. Conclusions**

This study shows that the four marine active substances have certain anti-oxidation ability, and have a good elimination effect on DPPH free radicals. With the increase of the concentration, the elimination rate also increases, which is dose-effective in a certain concentration range. relationship. The small-molecule peptide of paphia nobilis has a stronger ability to eliminate DPPH free radicals than the other three marine active substances.

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