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EVALUATING ANTIOXIDANT PROPERTIES OF HOMEOPATHIC FUCUS VESICULOSUS IN DPPH AND NITRIC OXIDE RADICAL SCAVENGING ASSAYS

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ABSTRACT

The body has a complex antioxidant defence grid that relies on endogenous enzymatic and non-enzymatic antioxidants. Antioxidant represents the ability to inhibit all molecules having high red potential which makes them destructive for body structures. These molecules act against free radicals to resist their damaging effects to vital biomolecules and ultimately body tissues. The body's defense against oxidative stress relies on a complex network of endogenous enzymatic and non-enzymatic antioxidants. These antioxidants play a crucial role in neutralizing free radicals, which can cause significant damage to vital biomolecules and tissues. This study evaluates the antioxidant activity of the homeopathic remedy Fucus vesiculosus using two established assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) and Nitric Oxide Radical Scavenging assays. In the DPPH assay, Fucus vesiculosus exhibited significant free radical scavenging activity, with a maximum inhibition of 95.37% at a concentration of 80 µl. In the Nitric Oxide Scavenging assay, the remedy demonstrated a moderate scavenging effect, with a maximum inhibition of 32.94% at the same concentration. These results suggest that Fucus vesiculosus possesses notable antioxidant properties, which could contribute to its therapeutic efficacy in homeopathic practice. Further studies are recommended to explore its potential in mitigating oxidative stress-related conditions.

KEYWORDS: Antioxidant capacity, Free radical invasion, Fucus Vesiculosus

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INTRODUCTION:

Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy; however, research has not shown antioxidant supplements to be beneficial in preventing diseases. Examples of antioxidants include vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin. Free radicals are uncharged molecules they can damage the cell of organism by damaging the DNA. Free radicals also caused the oxidative stress by chain reactions. Antioxidants are those substances which inhibit the process of oxidation Antioxidants are those compounds which scavengers the free radicals [1].

Free radicals are notorious substance produced in the cell, which damages normal healthy cells, causing cellular dysfunction and cellular death [2]. Antioxidants are well known for their various benefits to human health in concerns of improving quality of sleep, preventing neurodegenerative changes, lowering blood pressure and reduced obesity, improves eve vision, protects liver toxification, supports immune system, anti- aging effects and protects renal toxification. [3]. Free radicals need to be controlled, as they are responsible for aging, chronic diseases such as heart disease, parkinsonism and even cancer. Vitamins such as A, C and E are very rich in antioxidants and so are bioflavonoids [4]. Homeopathic mother tinctures having potential to prevent oxidation. Homeopathic mother tinctures of plant origin carry the hydro-alcoholic extracts of medicinal plant [1]. Antioxidants were discovered long ago in 1900 but its medical application has only recently drawn attention [2].

Fucus vesiculosus plants are loaded with plant compounds and its functions. Hahnemann, in chapter IV, which talks about the pharmaco-dynamic of the substances mentions: "We are not able to find out this immaterial force that is latent in the close essence of the medicine just with the reason's efforts. Just by the experience (experimentation) we can clearly notice the phenomena it provokes when it acts in a healthy organism". The experimentation in vitro can complement the knowledge of the homeopathic medicine pathogeneses itself, as well as its action in the several organic alterations. [8]. When free radicals are present in large numbers, they go on rampage and damage the normal healthy cells, laying the foundation of sickness. Antioxidants have the ability to trap free radicals as well as it contains host of properties that make them great for a good and healthy life. Therefore, we need to evaluate in an experimental basis the anti-oxidative property of Homoeopathic mother tinctures.

MATERIALS AND METHODS:

Antioxidant activity should not be concluded based on a single antioxidant test model. Many authors have stressed the need to perform more than one type of antioxidant activity measurement to take into account the various mechanisms of antioxidant action [9] [10]. The study will be conducted in Trichy Research Institute of Biotechnology. DPPH scavenging activity: Antioxidant activity was estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition assay The use of free radical 2,2-diphenyl 1-picryl hydrazyl (DPPH) is the preferred method to measure antioxidant activity as it is a

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simple and rapid and cost effective method. Moreover, it is not specific for particular antioxidant component, measure total antioxidant capacity of extract mother tincture [1].

RESULTS:

DPPH scavenging activity

The antioxidant activity of the Fucus vesiculosus mother tincture was assessed using the DPPH radical scavenging assay. Briefly, 2 ml of DPPH solution was added to both standard and test tubes. Varying concentrations (5-80 µl) of the test sample and standard solutions were introduced to their respective tubes. The tubes were incubated at room temperature for 30 minutes to allow for the reaction. After incubation, the color change was observed, and the absorbance of each tube was measured at 520 nm using a spectrophotometer. The percentage of inhibition at each concentration was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

Table 1: DPPH scavenging activity

Concentration	OD Value	Percentage of Inhibition
Blank	0.606	
SGC 5 µl	0.325	46.36
SGC 10 µl	0.131	78.38
SGC 20 µl	0.051	91.58
SGC 40 µl	0.035	94.22
SGC 80 µl	0.028	95.37
Ascorbic Acid 5 µl	0.035	94.22
Ascorbic Acid 10 µl	0.032	94.71
Ascorbic Acid 20 µl	0.019	96.86
Ascorbic Acid 40 µl	0.008	98.67
Ascorbic Acid 80 µl	0.004	99.33

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Nitric oxide generation and assay of Nitric oxide scavenging method:

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO (15, 16), which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO (17, 16). SNP (10mM) in phosphate buffer saline (PBS) was mixed with different concentration of extract (5 -80µl) of the drug and incubated at 25°C for 180 minutes. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dichloride and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dichloride was read at 546 nm and referred to the absorbance of ascorbic Acid, used as a positive control treated in the same way with Griess reagent. Scavenging effect (%) = [(control OD−sample OD)/ (control OD)] ×100

Table 2: Nitric oxide scavenging method

Concentration	OD Value	Scavenging effect Percentage
Blank	0.425	
SGC 5 µl	0.358	15.76
SGC 10 µl	0.344	19.05
SGC 20 µl	0.321	24.47
SGC 40 µl	0.290	31.76
SGC 80 µl	0.285	32.94
Ascorbic Acid 5 µl	0.334	21.41
Ascorbic Acid 10 µl	0.317	25.41
Ascorbic Acid 20 µl	0.289	32
Ascorbic Acid 40 µl	0.274	35.52
Ascorbic Acid 80 µl	0.248	41.64

DISCUSSION:

The antioxidant activity of Fucus vesiculosus mother tincture was evaluated through two distinct assays: the DPPH radical scavenging assay and the nitric oxide (NO) scavenging assay. These tests provide

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insights into the tincture's ability to neutralize free radicals and inhibit oxidative stress.

In the DPPH radical scavenging assay, the Fucus vesiculosus tincture demonstrated a concentration-dependent increase in antioxidant activity. At the highest concentration tested (80 µl), the tincture exhibited a remarkable 95.37% inhibition of DPPH radicals, comparable to the activity of ascorbic acid, which achieved a 99.33% inhibition at the same concentration. This significant scavenging effect indicates that Fucus vesiculosus possesses potent free radical scavenging capabilities, which may be attributed to its rich phytochemical content. The nitric oxide scavenging assay further supported these findings, although the observed scavenging effect was less pronounced compared to the DPPH assay. The tincture's maximum scavenging effect of 32.94% at 80 µl was lower than the 41.64% scavenging effect achieved by ascorbic acid at the same concentration. This difference suggests that while Fucus vesiculosus is effective in scavenging nitric oxide, its activity is somewhat lower than that of ascorbic acid, a known potent antioxidant.

These results collectively highlight the antioxidant potential of Fucus vesiculosus, particularly in scavenging DPPH radicals, which are commonly used to assess the radical scavenging ability of antioxidants. The tincture's performance in the NO scavenging assay, though less pronounced, still underscores its potential as a beneficial antioxidant agent. Further studies could explore the specific compounds responsible for these effects and their mechanisms of action to fully elucidate the therapeutic potential of Fucus vesiculosus in combating oxidative stress and related disorders.

CONCLUSION:

The study demonstrates that Fucus vesiculosus mother tincture exhibits significant antioxidant activity, as evidenced by its performance in both the DPPH radical scavenging assay and the nitric oxide scavenging assay. The tincture effectively neutralizes DPPH radicals, with a maximum inhibition of 95.37% at the highest concentration tested, closely paralleling the antioxidant activity of ascorbic acid. Although the tincture's nitric oxide scavenging ability was somewhat less potent, achieving a maximum scavenging effect of 32.94% compared to 41.64% for ascorbic acid, it still indicates a notable capacity to mitigate oxidative stress. These findings support the potential of Fucus vesiculosus as a valuable source of natural antioxidants, which could have implications for its use in preventing or managing oxidative stress-related conditions. Further research is warranted to isolate and identify the specific bioactive compounds responsible for these antioxidant effects and to explore their therapeutic potential in greater detail.

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