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THE ASSESSMENT OF THE SPIROGYRA VARIANS AND CHARA VULGARIS COMPARATIVE AND COMBINED ANTIOXIDANT POTENTIAL

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ABSTRACT

Oxidative stress (OS) is a common mechanism of tissue destruction in vivo that has been implicated in the origin and impact of numerous diseases. The rising incidence of cancer can now be explained in two ways: first, by environmental contaminants; and second, by lifestyle choices including smoking, drinking, and eating. *Chara vulgaris* is a sophisticated kind of algae that is also known as sand grass, skunkweed, or stonewort. Alkaloids, steroids, flavonoids, tannins, and terpenoids are among the substances that *Spirogyra varians* produce. According to reports, Spirogyra neglecta (SN) has anti-inflammatory, antidiabetic, and antioxidant properties. We found the antioxidant potential of *Spirogyra varians* using DPPH free radical assay 15.2%, 26%, 33%. We observed the antioxidant potential of *Chara vulgaris* 23%, 35%, and 43%. Furthermore, we next examined the combined antioxidant potential of *Spirogyra varians* and *Chara vulgaris*. We found that the 23%, 41%, and 53%. Our results suggest that the combined antioxidant potential of *Spirogyra varians* and *Chara vulgaris* is more significant than the individual ones. Further, an in vivo approach is needed to clarify the mechanism of the combined antioxidant potential of both *Spirogyra varians* and *Chara vulgaris*.

INTRODUCTION

The emergence of cancer is significantly influenced by environmental factors. According to estimations, cancer affects between 1762450 and 606880 individuals in the United States each year. The cause and effect of many diseases have been linked to oxidative stress (OS), a ubiquitous mechanism of in vivo tissue damage. Nowadays, there are two ways to interpret the increasing prevalence of cancer: first, environmental pollutants; second, aspects of lifestyle including eating, drinking, and smoking [1-4].

The simplest living things with chlorophyll are algae, which lack the ability to differentiate into true leaves, stalks, or roots. Algae live in freshwater environments. Algae are essential on Earth. They create strong food chains and mostly produce in aquatic habitats. *Chara*, sometimes referred to as sand grass, skunkweed, or stonewort, is an advanced type of algae [5,6]. *Spirogyra varians* are filamentous freshwater green algae that contain high amounts of protein, carbohydrates, fats, sulfates, and dietary fiber. *Spirogyra* produces compounds such as alkaloids, steroids, flavonoids, tannins, and terpanoids. *Spirogyra* neglecta (SN) is reported to have antioxidant activity, antidiabetic and anti-inflammatory effects[7,8].

Material and methods

2, 2-diphenyl-1-picrylhydrazyl (DPHH), methanol, and aluminum foil were provided by the Department of Zoology, UST Bannu. *Spirogyra varians* and *Chara vulgaris* fraction and methanolic extract of the plant were at the lab of the Department of Zoology, UST Bannu, KP, Pakistan.

Plant material and preparation of crude extract

The *Spirogyra varians* and *Chara vulgaris* samples were collected from the district of North Waziristan, KP, Pakistan, during September 2024. The plants were washed with water and dried in a shed at room temperature for 20 days. After drying, the *Chara vulgaris* converted into a fine powder. This powder was thoroughly dissolved in 70% methanol and kept at room temperature for 72 hours with continuous stirring. The solution was then filtered through a Whatman No. 3 filter paper. Then it was kept at room temperature to allow the liquid content to evaporate. The resulting gummy methanolic extract was lyophilized and placed in a Falcon tube. For further usage, the lyophilized material was kept in storage.

Antioxidant assay

The antioxidant activity using DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging experiment was performed by following the published protocol with slight modification [2-4]. 100 µl was taken out from each sample solution containing 100 µg/ml, 500 µg/ml, and 1000 µg/ml. Now, 100 µl was mixed with 900 µl of DPPH solution. In the same process, the 500 µl and 1000 µl DPPH solutions were applied. An antioxidant activity of ascorbic acid solution was used in the same procedure. Due to their sensitivity to light, each of these test tubes was incubated at 25 °C for 30 minutes in the dark. The absorbance at 517 nm was subsequently determined using a spectrophotometer.

The ability of plant samples to scavenge DPPH free radicals was evaluated using the following equation.

The formula is given below

% DPPH free redials scavenging effect = (A1-A2 /)×100

Where A1= the absorbance of DPHH (control) and A2= the absorbance in the presence sample.

RESULT

The assessment of antioxidant potential of *Spirogyra varians*

The scavenging activity of a methanolic extract of *Spirogyra varians* at multiple concentrations was assessed in this antioxidant experiment in comparison with a control group that contained ascorbic acid.

We found that the scavenging capacity at the maximum concentration of *Spirogyra varians* was 15.2%, 26%, and 33% for various concentrations, such as 100µg/ml, 500µg/ml, and 1000µg/ml as compare to control group of ascorbic acid 60%, 71% and 87%. We noticed that the *Spirogyra varians* possesses a considerable antioxidant activity on various concentrations as shown in Fig 1.

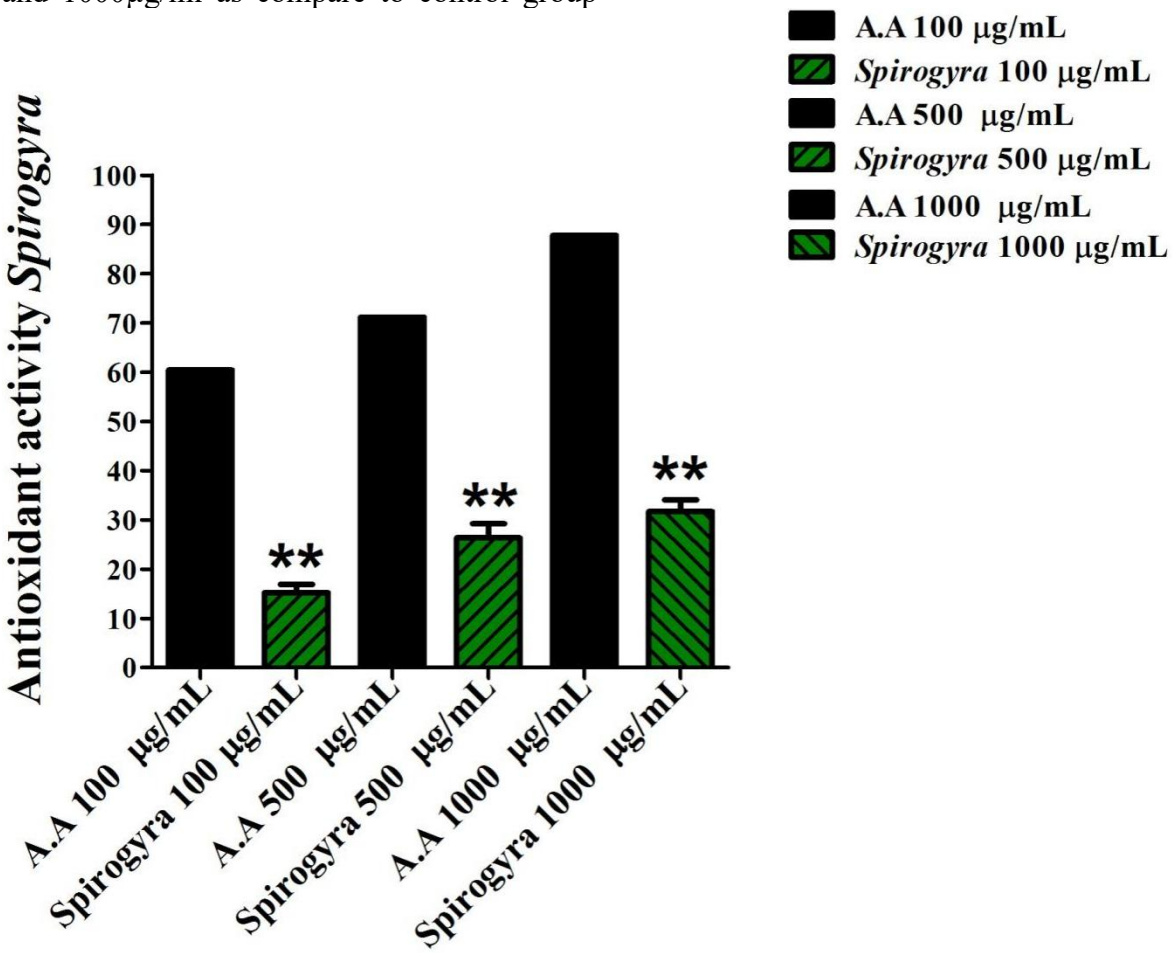

Figure 1. Represents antioxidant potential of Spirogyra varians at multiple concentrations

The evaluation of *Chara vulgaris* antioxidant potential

Next, we performed the antioxidant experiment to evaluate the scavenging effectiveness of a methanolic extract of *Chara vulgaris* at various concentrations compared to a control group that contained ascorbic acid. As shown in Figure 2, the antioxidant capacity of *Chara vulgaris* was 23%, 35%, and 43% at multiple concentrations such as 100, 500, 1000 ug/mL) as compared to 60%, 70%, and 87% of the control ascorbic acid as shown in Fig 2.

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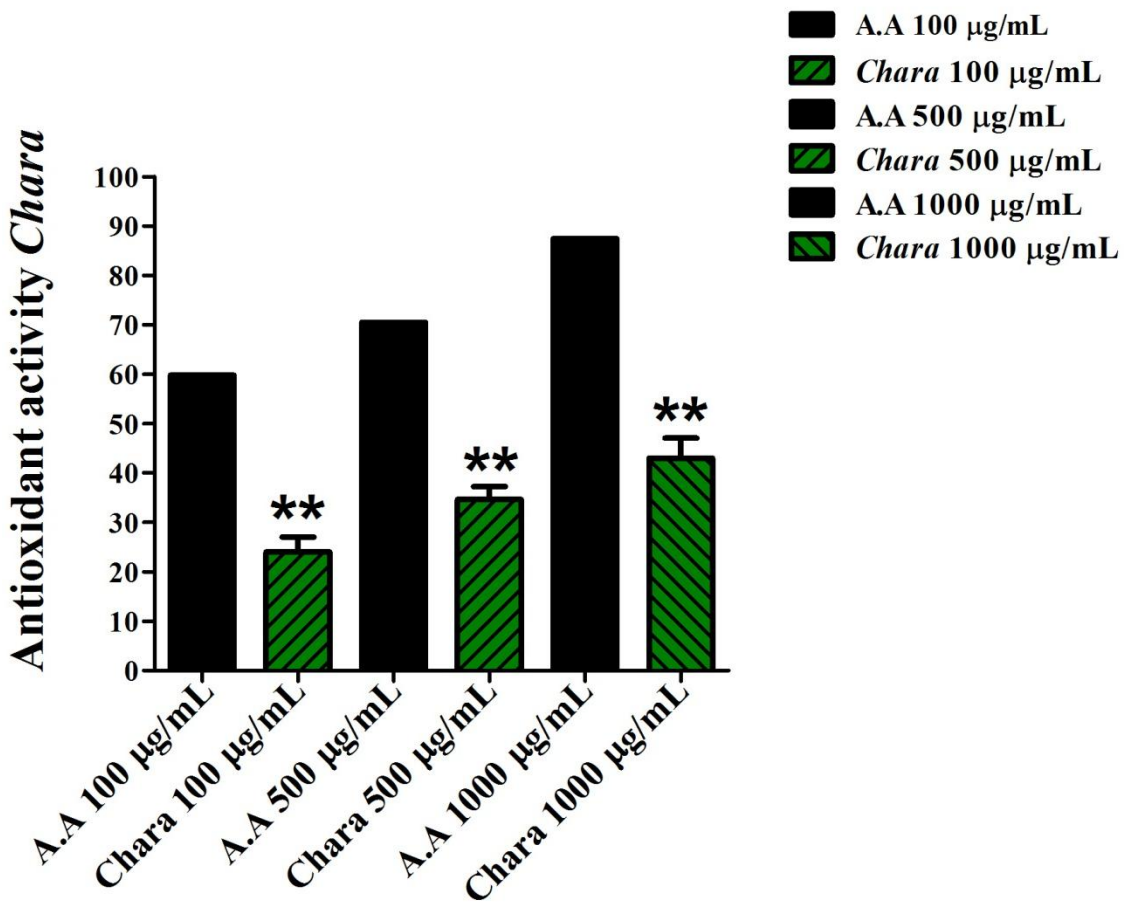

Figure 2. Represents antioxidant potential of *Chara vulgaris* at multiple concentrations

The combined antioxidant potential of *Spirogyra varians* and *Chara vulgaris*

We also analyzed the antioxidant effects of the compound of both plant extracts. We observed that the combined plant extract of *Spirogyra varians* and *Chara vulgaris* showed a substantial level of antioxidant capacity at various concentrations of 100 µg/ml, 500 µg/ml, and 1000 µg/ml, ranging from 23%, 41%, and 53%, with control group of ascorbic acid 61%,71, 88% respectively as shown in Fig 3.

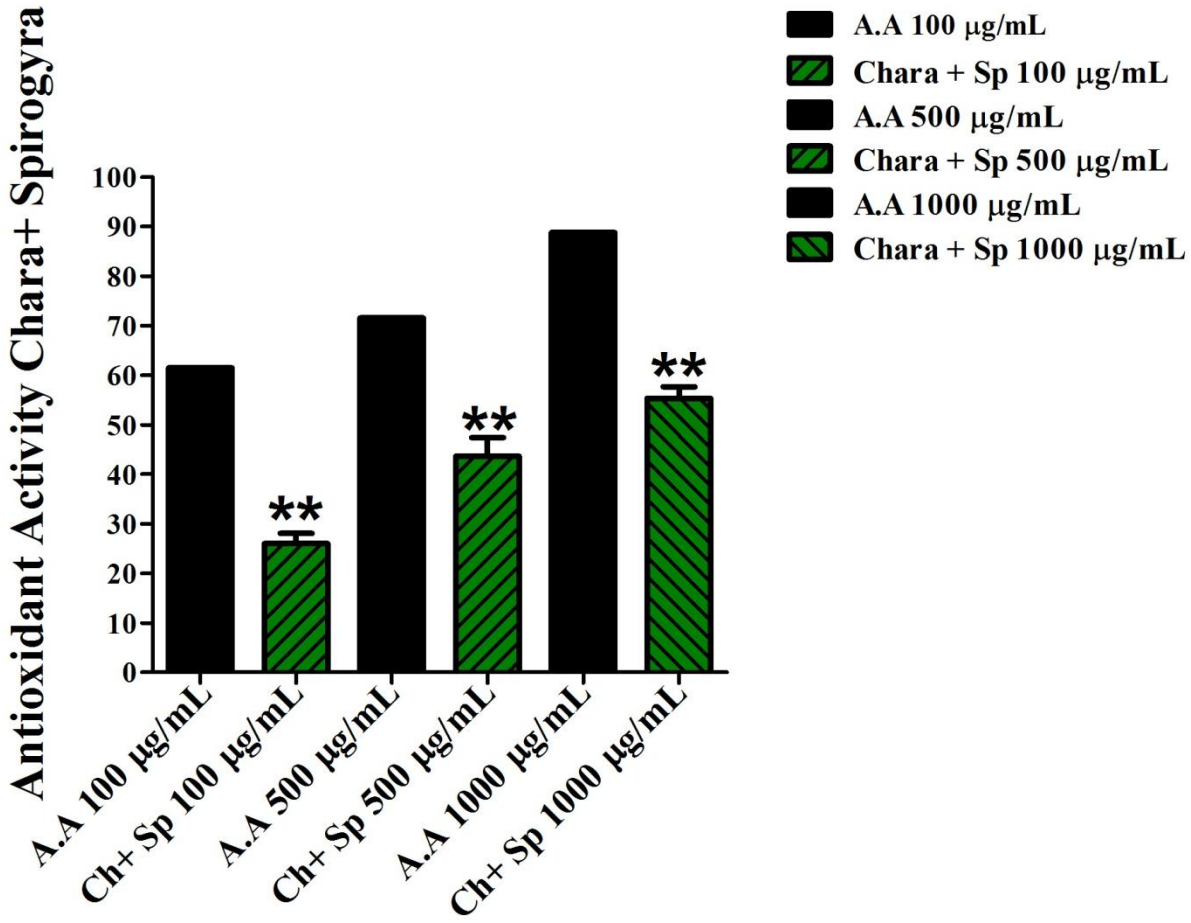

Figure 3. Represents antioxidant potential of *Spirogyra varians* and *Chara vulgaris* at multiple concentrations

Discussion

The redox reaction produces a variety of free radicals, such as hydrogen peroxide, superoxide anions, and hydroxyl radicals[9]. These are a type of reactive oxygen species (ROS) that can react with proteins, lipids, and DNA to cause cancer and other chronic diseases[10]. Plants with high antioxidant contents can block or inhibit the lipid and other sensitive molecular oxidation, which can cause chronic disorders including cancer[11]. Algae are a complex class of photosynthetic eukaryotes that include seaweed, which are microscopic and unicellular microalgae. The edible Chlorella species *C. vulgaris*, *Auxenochlorella pyrenoidosa* (formerly known as C. protothecoides), *Auxenochlorella protothecoides*, and *C. lobophora* are among the green microalgae that are rich in protein, fat, carbohydrates, fiber, chlorophyll, vitamins, and minerals [12]. Compounds including alkaloids, steroids, flavonoids, tannins, and terpenoids are produced by Spirogyra. It has been reported that algae possess cytotoxic, antiviral, antioxidant, antibacterial, and anti-inflammatory and antioxidant properties[7]. Here in we noticed that the antioxidant potential of *Spirogyra varians* was 15.2%, 26%, and 33% using various concentrations, such as 100µg/ml, 500µg/ml, and 1000µg/ml. Our results suggest that the *Spirogyra varians* shows antioxidant potential. Our results are supported by previous published reports.

The majority of the charophilous flora, of which *Chara hispida* is one of the larger species, have significant ecological and medicinal impacts. It is frequently found in North Africa (Atlas), Europe, and Western Asia. It is also visible all year round and is simple to spot. It habitat is permanent, moderately deep, oligomesotrophic to eutrophic, neutral to carbonate, stagnant or slow-flowing, phreatic, mildly murky, and infrequently contaminated waters on a variety of substrates under sunny conditions. Forest ponds, pools, bog ditches, gravel pits, canals, still areas, watercourse annexes, and man-made basins in parks and castles are just a few of the places where it can be found. It has been shown that the *Chara* has antioxidant potential[13]. We examined the antioxidant potential of *Chara vulgaris* and noticed that scavenging property was 23%, 35%, and 43% at multiple concentrations such as 100, 500, 1000 ug/mL) as compared to 60%, 70%, and 87% of the control ascorbic acid. Furthermore, we used and examined the combined antioxidant potential of *Spirogyra varians* and *Chara vulgaris*. We observed that that the combine antioxidant potential was 23%, 41%, and 53% compared with control group of ascorbic acid 61%, 71 and 88% respectively.

Our results demonstrates that both *Spirogyra varians* and *Chara vulgaris* showed antioxidant potential on individual or in combine form using DPPH scavenging experiments.

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Conflict of interest

All authors declared no conflict of interest.

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None

Author's contribution

Fahim Ullah Khan conceptualized the manuscript; Muhammad Hashim performed experiments. Fahim Ullah Khan wrote the manuscript. Saqib, Arif Ullah and Fardous Jamal, Hassan Iqbal, Saqib Ullah, Mahaz Ullah, Yasin Ullah, Muhammad Zakarya, Fazeelat Noureen, helped in the sample collection.

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