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IN VITRO ANTIOXIDANT ACTIVITY FROM ETHANOLIC EXTRACT OF GREEN ALGA, *MOUGEOTIASCALARIS*

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ABSTRACT

Objective: Many diseases are associated with oxidative stress caused by free radicals. The present study is carried out to perform the *in vitro* antioxidant activity of ethanolic extract from green alga, *Mougeotiascalaris.*

Methods: the ethanolicextractof *Mougeotiascalaris* was tested for total antioxidant assay, DPPH(1, 1-diphenyl-2-picrylhydrazyl) assay, reducing power assay, nitric oxide scavenging activity and hydrogen peroxide scavenging.

Results: the total antioxidant assay was observed as 44.71 ± 0.51 %. The ethanolic extract of *Mougeotiascalaris* showed 69.86 ± 1.43% in DPPH assay, reducing power assay of 81.21 ± 1.43%, nitric oxide scavenging activity of 59.28 ± 1.94%and the hydrogen peroxide scavenging activity of 75.68± 1.29%.

Conclusion: the present study revealed that *Mougeotiascalaris*can be act as natural antioxidant source for food preservation and protection from degenerative diseases.

Key words: Green alga, *Mougeotiascalaris*, Antioxidant activity, DPPH, reducing power assay.

INTRODUCTION

Natural antioxidants are found in some vegetables, fruits and a variety of other foods including microalgae. Specifically, many researchers reported the finding of various antioxidants present in seaweeds, for example polysaccharides, dietary fibers, minerals, proteins, amino acids, vitamins, polyphenols and carotenoids.

A free radical is a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals are in the form of reactive oxygen and nitrogen species, these can occur, due to oxidative stress brought about by the imbalance of the bodily antioxidant defense system and free-radical formation [1]. Oxidative stress has been linked to cancer, aging, ischemic injury, inflammation and neurodegenerative diseases. Reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, peroxylradical and nitric oxide radical, attack biological molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury associated with aging, atherosclerosis carcinogenesis [2]. The most commonly used synthetic antioxidants are butylatedhydoxyanisole (BHA), butylatedhydroxytoluene (BHT), and Propylgallate (PG).These synthetic antioxidants have side effects such as liver damage and are suspected to be mutagenic and neurotoxic [3]. Therefore, algal species as alternative materials to extract natural antioxidative compounds have attracted much attention. Previous literature reported the potential antioxidant compounds such as some pigments (i.e. fucoxanthin, astaxanthin, carotenoid) and polyphenols (i.e. phenolic acid, flavonoid, tannins), that are widely distributed in seaweeds and are known to exhibit higher antioxidativeactivities [4], [5].The intake of dietary antioxidant phytochemicals leads to protection against non-communicable diseases i.e. cancer, cardiovascular diseases and cataract [6].

The microalgae represent an almost untapped resource of natural antioxidants, due to their enormous biodiversity, much more diverse than higher plants.*Mougeotia*, members of order Zygnemales, are fresh water green filamentous alga, which show high incidence throughout the year in the fresh water bodies. Green microalgae are widely used in the life science as the source of compounds with diverse structural forms and biological activities. Algae have been historically and exceptionally rich source of pharmacologically active metabolites with

antineoplastic, antimicrobial and antiviral effects[7, 8]. Green micro algae like *Scenedesmus*and*Chlorella* contain rich source of active metabolites with anticarcinogeniceffects. Extracts from many microalgal strains including *Chaetoceroscalcitrans, Scenedesmus-quadricauda, Chlorella vulgaris, Nannochloropsisoculata* and *Tetraselmistetrathele* show inhibitory activity against lipid peroxidation of linoleic acid. Among the microalgae tested, *I. galbana* and *C. calcitrans* exhibit the highest antioxidant activity in the ferric thiocyanate and thiobarbituric acid assays [9].

Based on the above facts, we now report the effect of the ethanolic extract of green alga, *Mougeotiascalaris* for its potential antioxidant property and discuss it by measuring total antioxidant activity, DPPH radical scavenging activity, reducing power assay, nitric oxide scavenging activity and hydrogen peroxide scavenging activity.

MATERIALS AND METHODS

Instruments

- UNICO 7200 series UV- Visible spectrophotometer was used for all spectrophotometric studies. Evaporator was used for evaporate algal extract.
- Centrifuge was used for centrifugation.

Preparation of algal extracts:

Mougeotiascalaris was collected from intertidal regions of Damietta, Egypt by the hand picking method. One gram of powdered *Mougeotiascalaris*was extracted for 24 h in 10 ml of ethanol at room temperature under dark condition. The previous extraction process was repeated twice and filtered through whatmannNo.1 filter paper. The collected filtrate was evaporatedand then used for antioxidant assays.

Total antioxidant activity:

Total antioxidant activity of ethanol extract of *Mougeotiascalaris*was determined byphosphomoly-bdenummethod [10]. 5 mg of crude extract of *Mougeotiascalaris*were mixed with 1 ml methanol. Different concentrations of the algal extract (20-100 µg/ml) were prepared. Then each 1 ml of total antioxidant capacity (TAC)reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to each sample concentration separately. The tubes containing sample were capped and incubated in water bath at 95°C for 90 mins. After the samples were cooled to room temperature, the absorbance was measured at 695 nm using a Spectrophotometer against blank. The antioxidant activity is expressed as an equivalent of standard ascorbic acid in µg/ml.

DPPH Radical Scavenging Activity:

The free radical scavenging capacity of crude extract from *Mougeotiascalaris*wasdeterminedaccording to the method[11] usingDPPH. DPPH solution was prepared in 95% methanol (0.004% w/v). Thecrude extract was mixed with 95% methanol separately to prepare the stock solution (10mg/100 mL). Different concentrations of the algal extract (20 -100 µg/ml) were prepared in different test tubes.

Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubescontaining samples (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml) and after 10minutes, the absorbance was measured at 517 nm using a UV- Visible Spectrophotometer. Ascorbic acid was used as a reference standard. Theability to scavenge the DPPH radical was calculated using the following formula

$$\text{DPPH Scavenging activity (\%)} = \frac{\text{A control} - \text{A Test}}{\text{Control}} \times 100$$

Reducing power scavenging assay:

Reducing power scavenging assayofethanolic extract of *Mougeotiascalaris*was determined according to the method [12].Different concentrations of the *Mougeotiascalaris* extract (20 -100 µg/ml) were taken separately and mixed with 2.5 ml phosphatebuffer (0.2M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN_6)$] (1%), then the mixturewas incubated at 50°C for 20 minutes. To this, 2.5 ml of trichloroacetic acid (10%) was addedand centrifuged at 3000 rpm for 10 minutes. Finally, 2.5 ml of the supernatant solution wasmixed with 2.5 ml of distilled water and 0.5 ml $Fecl_3$ (0.1%) and the absorbance wasmeasured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer asblank solution. Increased absorbance of the reaction mixture indicates stronger reducingpower. The reducing power was calculated using the following formula

$$\text{\% increase in Reducing Power} = \frac{\text{A test}}{\text{A Blank}} - 1 \times 100$$

Nitric oxide scavenging activity:

Nitric oxide scavenging activity of ethanolic extract of *Mougeotiascalaris*was determined according to the method [13]. Sodiumnitroprusside (10 mm, 2 ml) in phosphate buffer saline was incubated with crude extract from *Mougeotiascalaris*in different concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml)at room temperature for 30 minutes. After 30 minutes, 0.5 ml of the incubated solution wasadded with 1 ml of Griess reagent and the absorbance was measured at 546 nm. Ascorbicacid is used as a standard. The nitric oxide radicals scavenging activity was calculatedaccording to the following equation

% of Inhibition = $(A_0-A_1) / A_0 \times 100$)
Where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance inthe presence of active compound (with extract).

Hydrogen peroxide scavenging activity:

H_2O_2 scavenging ability of crude ethanolic extract of *Mougeotiascalaris*wasdeterminedaccording to the method [14]. A solution of H_2O_2(40mM) was prepared in phosphate buffer(pH 7.4). The crude extract at different concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml) in 3.4ml phosphate buffer wereaddedto a H_2O_2 solution (0.6ml, 40mM). The absorbance value of the reaction mixture wasrecorded at 230nm. Blank solution was the phosphate buffer without H_2O_2. Thepercentage of H_2O_2 scavenging of ethanolic extract and standard compounds were calculatedaccording to the following equation

% Scavenged $[H_2O_2] = [(A_{Control}-A_{Sample}) /A_{Control}] \times 100$

Where $A_{Control}$ is the absorbance of the control, and A_{Sample} is the absorbance in the presenceofethanolic extract.

Statistical analysis:

All experiments were performed in triplicate (n=3) and the results were expressed as mean ±Standard Error Mean. Statistical analysis was carried out with SPSS version 23.

RESULTS AND DISCUSSION

Total antioxidant capacity

The total antioxidant capacity of the microalgae, *Mougeotiascalaris*has been expressed as an ascorbic acid equivalent. The total antioxidant capacity of the ethanolicextract of *Mougeotiascalaris*was 44.71 ± 0.51 % when compared to standard 82.29 ±0.54%.

The antioxidant activity of ethanol extract of *Mougeotiascalaris*was determined by phosphomolybdenum method. The assay is based on the reduction of Mo (VI) – Mo (V) by the extract and subsequent formation of a green phosphate / Mo (V) complex at acidic pH.The total antioxidant capacity of the ethanolic extract of *Mougeotiascalaris*was 44.71 ± 0.51 % when compared to standard 82.29 ± 0.54%. The antioxidative activity of water-ethanol extracts from *Spirulinaplatensis*biomass increases proportional to the concentration of ethanol used for the antioxidative activity of extraction 10% ethanol extract was 38 mg AA/g of active substance, 70% extract increases it twice. The increased of activity is not linear but most actively increased in the range of 55-70% of ethanol concentration whereas for lower range (10-55%), the increase is much slower. Ganesan*et al* [15] have also noticed a higher total antioxidant capacity in the methanolic extract of several brown and green seaweeds. The antioxidant activity was ranged between 31.2 and 75.9%, *Spirulinaplatensis*(75.9%), *Oscillatoria*sp (75.6%), *Anabaena flous-aqua* (73.6%) and *Nostocmuscorum*(72.8%) [16].

DPPH Radical Scavenging Activity

The free radical scavenging activity of ethanolic extract of *Mougeotiascalaris*was assessed by DPPH assay. The scavenging effect increases with the concentration of standard and samples(Fig. 1). TheDPPH assay of ethanolic extract shows 69.86 ± 1.43% in 100 µg/ml and the standard was found to be 72.32 ± 1.24%. Where the lower value of IC_{50}53.83µg/ml indicates the higher antioxidant activity.

DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger. DPPH assay was used to test the ability of the antioxidative compounds functioning as proton scavengers or hydrogen donors [17]. This assay has been extensively used for screening antioxidants such as polyphenols and anthocyanins from marine algae [18].

DPPH shows a strong absorption band at 517 nm in visible spectroscopy because of the odd electron. As this electron becomes paired off in the presence of a free radical scavenger, the absorption diminishes, and the resulting decolonization is stoichiometric with respect to the number of electrons taken up [19], [20].
The EC_{50} was determined to quantify the radical scavenging effects (Table 1). The lowest value of EC_{50} indicates strongest ability of the extract as DPPH scavengers. The highest radical-scavenging power observed with DPPH assay in water fraction of the extracellular substance belonged to *Chlorella vulgaris* (109.02±8.25), *Anabaena cylindrical*(84.91±4.89), *Nostocmuscorum* (45.24±2.78), and *Nostoc*sp. (41.81±2.56) were performed by earlier workers [16].

Reducing power scavenging assay

The present study showed the reducing power capacity of ethanolic extract of *Mougeotiascalaris*which is compared to standard ascorbic acid (Fig. 2). The reducing power scavenging effect increases with the concentration of standard and algal extract. The ethanolic extract of *Mougeotiascalaris*was found to be 81.21 ± 1.43% in 100 µg/ml and the standard was found to be 90.43±2.18%. The IC_{50} value of ethanolic extract of *Mougeotiascalaris*was56.15µg/ml.

The reducing ability of a compound greatly depends on the presence of reductones, which exhibits antioxidative potential by breaking the free radical chain by donating a hydrogen atom [21] The presence of reductants (i.e. antioxidants) causes the reduction of the Fe3+/ferricyanide complex to the ferrous form.

Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, the amount of Fe2+ can be monitored. Higher absorbance indicated higher reducing power [22]. The reducing capacity proved the potential phenolic compounds present in the sample because it act as a reductones that inhibit lipid peroxidation by donating the hydrogen atoms thereby it terminates the activity of the free radical chain reactions. [23]

Nitric oxide scavenging activity

The ethanolic extract of *Mougeotiascalaris*decreased the amount of nitrite generated fromthe decomposition of sodium nitroprussidein vitro. In nitric acid scavenging assay, the ethanolic extract of *Mougeotiascalaris*was found to be 59.28 ± 1.94%in 100 µg/ml and the standard was found to be 74.36 ± 1.58%. TheIC_{50} value of ethanolic extract of *Mougeotiascalaris*was62.36µg/ml (Fig. 3).

Nitric oxide radicals play an important role in inducing inflammatory response and their toxicity multiplies only when they react with O^{2-} radicals to form peroxynitrite, which damages biomolecules like proteins, lipids and nucleic acids [24]. Nitric oxide is generated when sodium nitroprusside reacts with oxygen to form nitrite. Algae inhibit nitrite formation by competing with oxygen to react with nitric oxide directly. These compounds alter the structure and function of many cellular components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage [25].Thus *Mougeotiascalaris*might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite.

Hydrogen peroxide scavenging activity

In the present study, ethanolic extract of *Mougeotiascalaris* was determined by hydrogen peroxide scavenging assay and it was compared with the standard ascorbic acid which is shown in Fig. 4. The activity increases with the sample concentration.The scavenging capacity of the ethanolic extract of*Mougeotiascalaris* wasfound to be 75.68± 1.29% in 100 µg/ml when compared to the standard (81.86 ± 1.51%).The IC_{50} value of ethanolic extract of *Mougeotiascalaris* was 55.61µg/ml.

Hydrogen peroxide can be formed in vivo by an antioxidant enzyme such as superoxide dismutase. It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells. Addition of hydrogen peroxide into living cells can lead to metal iondependent hydroxyl radicals mediated oxidative DNA damage [26]. Scavenging of H_2O_2 by extracts of *Mougeotiascalaris*may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. Acetone extract of *Desmococcusolivaceous* and methanolic extract of *Chlorococcumhumicola* showed relatively low H_2O_2 scavenging activities [27].

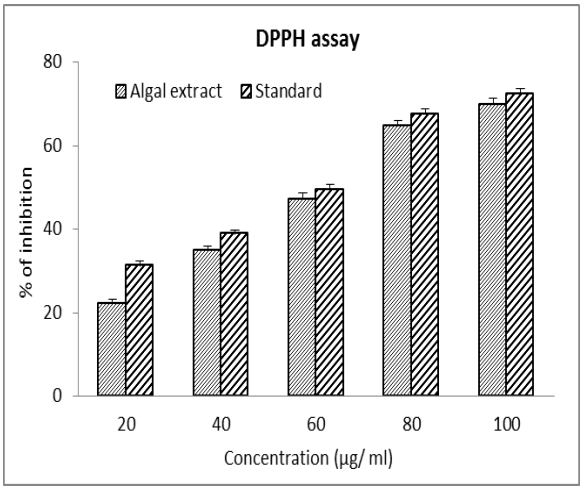

Fig 1: DPPH assay of ethanolic extract from*Mougeotiascalaris.*

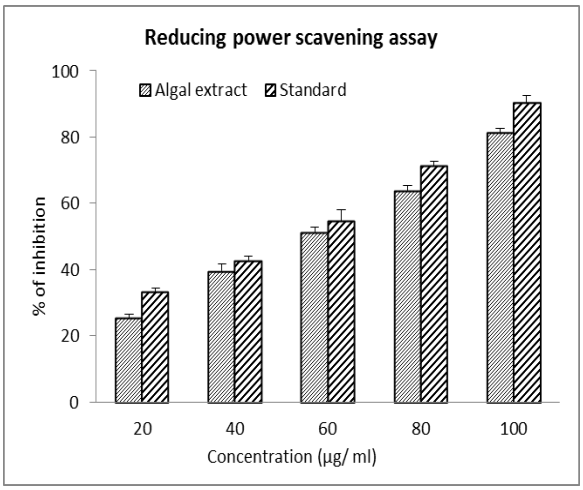

Fig 2: Reducing power scavenging assay of ethanol extract from *Mougeotiascalaris*

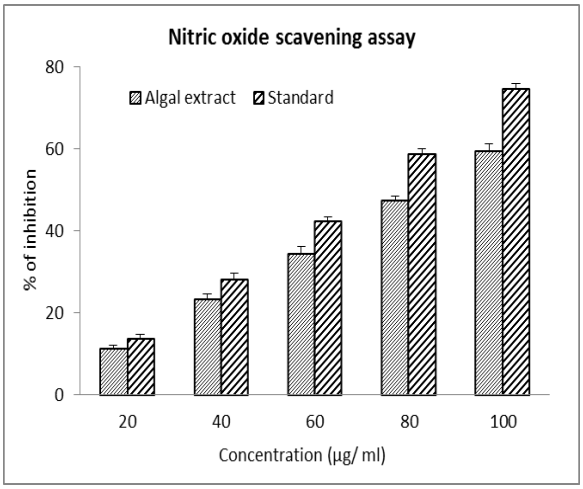

Fig 3: Nitric acid scavenging assay of ethanolic extract from*Mougeotiascalaris*

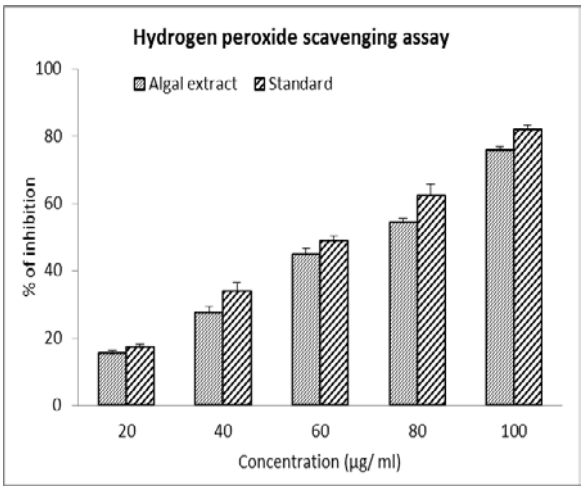

Fig 4: Hydrogen peroxide scavenging assay of ethanol extract from *Mougeotiascalaris*

CONCLUSION

In the present study, the ethanol extracts of green alga, *Mougeotiascalaris* at varying concentrations were shown as a potential DPPH radical scavenging, reducing power scavenging, Nitric acid scavenging and Hydrogen peroxide scavenging. Further investigation of antioxidant activity of individual isolated compounds from *Mougeotiascalaris*is needed.

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