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Research Article

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Qualitative Analysis and Free Radicals Scavenging Ability of Marine Algae, *Chara baltica*

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Abstract

Qualitative analysis and free radical scavenging activity was evaluated for an algae, *Chara baltica*. The dichloromethane extract was prepared and subject to proton NMR and qualitative analysis reveals the presence of carbohydrates, carboxylic acids, flavonoids, phenols, proteins and amino acids, saponins and xantho-proteins. The free radical scavenging activity was carried out on α,α-diphenyl-β-picrylhydrazyl (DPPH) and hydroxyl radicals. The extract of *Chara baltica* showed the dose dependent activity on tested free radicals along with ascorbic acid. Further research is going on to identify the biological activities of *Chara baltica* with different extractions and isolation of bioactive compounds.

Keywords: Qualitative analysis, Dichloromethane extract, free radicals, proton NMR.

1. Introduction

Free radical scavenger are effective in protecting the body against damage by reactive ozygen species (such as superoxide radical, hydroxyl radical, peroxyl radical 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, and carcinogenesis) (Xiao-Juan Duan *et al.*, 2006; Mallikarjuna *et al.*, 2013; Dorman *et al.*, 2003; Dodge *et al.*, 1963; Dahle *et al.*, 1962; Chang *et al.*, 2001). There is a booming interest in natural free radical scavengers for treatment or prophylaxis of various oxidative stress-related diseases because of safety and toxicity problems of synthetic antioxidants such as butylated hydroxyanisol and butylated hydroxytoluene (Cakir *et al.*, 2003; Black *et al.*, 1995; Bandoniene *et al.*, 2002; Amarowicz *et al.*, 2000).

Many natural free radical scavengers have already been isolated from different kinds of plant such as roots, leaves, cereal crop, vegetables, herbs and spices. Moreover, natural free radical scavengers are not limited to terrestrial sources. Some of the seaweeds are considered to be a rich source of free radical scavengers, for instance, carotenoids, chlorophylls, tocopherols and isoprenoid derivatives (Mylarappa *et al.*, 2008; Fenton *et al.*, 1984; Droge *et al.*, 2002).

The present work was carried out to evaluate free radicals scavenging ability on α, α-diphenyl-β-picrylhydrazyl (DPPH) and hydroxyl, qualitative analysis and NMR study on dichloromethane (DCM) extract of algaee, *Chara baltica.*

2. Materials and methods

2.1 Collection of Algae material

The marine algae sample, *Chara baltica* (Fig. 1) was collected off the coasts of Korangi (16.81°N, 82.24°E), Kakinada, India during the month of December, 2014 and were identified by Dr. G. Mohan Narasimha Rao, Department of Botany, Andhra University, Visakhapatnam. A voucher specimen has been deposited in the Marine Organisms Museum in the AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India (Voucher No. AU-AG-029).

2.2 Extraction

The total algal material was cleansed from extraneous matter and soaked in methanol at the site of collection and brought to the laboratory. The material was exhaustively extracted with methanol

at room temperature. The combined extract was suspended in water and fractionated with dichloromethane (DCM). The organic layer was separated, dried on anhydrous sodium sulphate and evaporated under reduced pressure to obtain 3g of extract. The DCM extract was subject to proton NMR, phytochemical analysis and antioxidant activity.

Fig 1. *Chara baltica*

2.3 Chemical profile analysis of C. baltica

The presence or absence of various bioactive molecules were examined for DCM extract of *C. baltica* using standard chemical analysis tests.

2.3.1 Detection of Alkaloids

Solvent free extract (5mg) was stirred with few mL of dilute hydrochloric acid and filtered, the filtrate was used for testing of alkaloids by addition of two drops of Mayer's reagent. The presence of white creamy precipitate indicates the presence of alkaloids.

2.3.2 Detection of Carbohydrates

To 1mL of filtrate of extract dissolved in water as 1mg/ml, 1mL of Benedict's reagent was added. The solution was mixed well and boiled on water bath for 2min. A characteristic coloured precipitate indicated the presence of carbohydrates (Benedict's test).

2.3.3 Detection of Carboxylic acids

To the few mL of extract, few mL of sodium bicarbonate solution was added. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acids.

2.3.4 Detection of Coumarins

The extract (10mg) was diluted with distilled water and few mL of alcoholic 10% sodium hydroxide was added, the formation of yellowish colour indicates presence of coumarins..

2.3.5 Detection of Flavonoids

To the extract (10mg) was dissolved in alcohol (ethyl alcohol) and filtered, few drops of neutral ferric chloride solution was added to the filtrate. A blackish red colour indicates presence of flavonoids (Ferric chloride test).

2.3.6 Detection of Glycosides

Few mgs of extract was hydrolysed with Conc. HCl on water bath for 2h and filtered, to the hydrolysate added 3ml of chloroform and shaken well. 10% NaOH was added to the separated chloroform layer, formation of a pink colour indicated the presence of glycosides.

2.3.7 Detection of Phenol compounds

2.3.7.1 Ferric Chloride Test

The extract (10mg) was dissolved in 1 mL of distilled water. To this few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenol compounds.

2.3.7.2 Lead acetate Test

The extract (10mg) was dissolved in 1ml of distilled water. To this 3 mL of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compounds.

2.3.8 Detection of Proteins and Amino acids

To 1mL of filtrate (10mg/mL) added 2% of copper sulphate (2mL) and followed by 95% ethanol (1mL) and mixed well. Then, added excess of KOH pellets, formation of pink colour in the ethanolic layer indicates presence of proteins and amino acids (Biuret test).

2.3.9 Detection of Quinones

The extract (10mg) was diluted with distilled water and alcoholic potassium hydroxide solution was added. Quinines give coloration ranging from red to blue.

2.3.10 Detection of Resins

The extract (10mg) was diluted with distilled water, development of black precipitate indicate presence of resins.

2.3.11 Detection of Saponins

The presence of saponins were detected by foam test. 10mg of extract was dissolved in distilled water (5mL) and shaken well using graduated cylinder for 15min, formation of 2cms layer of foam indicated the presence of saponins.

2.3.12 Detection of Steroids

The extract (10mg) was dissolved in 2 mL of chloroform. To the solution added sulphuric acid was added carefully from sides of test tube. Formation of reddish brown ring at interface of two layers indicates presence of steroids.

2.3.13 Detection of Tannins

The extract (10mg) was dissolved in 1 mL of distilled water. To this few drops of neutral 5% ferric chloride solution were added. A blackish precipitate indicated the presence of tannins.

2.3.14 Detection of Terpenoids

To the extract (1mg), 2 mL of chloroform and 1 mL of concentrated sulphuric acid were added. Reddish brown colour indicates the presence of terpenoids (Salkowski's Test).

2.3.15 Detection of Xantho proteins

The extract (10mg) was diluted with distilled water and few drops of concentrated nitric acid and ammonia solution. Formation of reddish orange precipitate indicates the presence of xantho proteins.

2.4 Proton NMR analysis

The proton NMR of DCM extract was carried out using Bruker-Biopsin 400Hz at Andhra university, Visakhapatnam.

2.5 Antioxidant activity

2.5.1 DPPH radicals scavenging assay

The free radical scavenging activity of the DCM extract of marine algae, *Chara baltica* was examined in vitro using DPPH radical. 1.0 ml of various concentrations of extract (1 mg/10 ml) was mixed with 1.0ml of 0.8 mmol/L DPPH solution. The mixture was shaken vigorously and left to stand for 30 min and the absorbance (ABS) was measured at 517 nm against a reagent blank. Ascorbic acid was used as standard (Mallikarjuna Rao *et al.*, 2013).

2.5.2 Hydroxyl radicals scavenging assay

The hydroxyl radical scavenging activity is measured as per established method. It was studied by the competition between deoxyribose and the extract's antioxidant molecules for hydroxyl radicals generated from the Fe+2/ EDTA/H_2O_2 system (Mallikarjuna Rao *et al.*, 2013).

The inhibition percentage for scavenging DPPH radical was calculated according to the equation.

% decolorization=[1-(ABS_{sample}/$ABS_{control}$)]×100.

3. Results and Discussion

The qualitative phytochemical analysis of the *Chara baltica* DCM extract gave the presence and absence of tested compounds (Table 1). The extract gave positive result for presence of carbohydrates, carboxylic acids, flavonoids, phenolic compounds, proteins, saponoins and xantho proteins. The extract gave negative result for alkaloids, coumarins, glycosides, quinones, resins, steroids, tannins and terpenoids.

Table 1. Qualitative chemical analysis of dichloromethane extract of *Chara baltica*

Name of the Phytochemicals	***C. baltica* DCM extract**
Alkaloids	-
Carbohydrates	+
Carboxylic acids	+
Coumarins	-
Flavonoids	+
Glycosides	-
Phenols	+
Proteins and amino acids	+
Quinones	-
Resins	-
Saponins	+
Steroids	-
Tannins	-
Terpenoids	-
Xantho proteins	+

+ = Present; - = Absent

The NMR study the illustrates that the extract may contains alkyl, allylic, carbonyl, benzylic alkynyl, methoxy, vinylic groups (Fig. 2).

The DCM extract of *Chara baltica* and ascorbic acid at various concentrations (25, 50, 100, 200, 400 µg/ml) were found to have DPPH and hydroxyl radical scavenging activity in dose dependent manner. The amount of DCM extract of marine algae and ascorbic acid needed for 50% DPPH scavenging activity (IC_{50}) were found to be 94.16 and 34.16µg/mL respectively (Fig. 3). 50% scavenge (IC_{50}) on the hydroxyl radical were found to be 103.4 and 44.12µg/mL respectively (Fig. 4). The DCM extract showed significant percent of inhibition along with ascorbic acid. The DCM extract have more scavenging activity on DPPH free radical compared to hydroxyl radical. The

percentage of inhibition was increased with the concentration of extract.

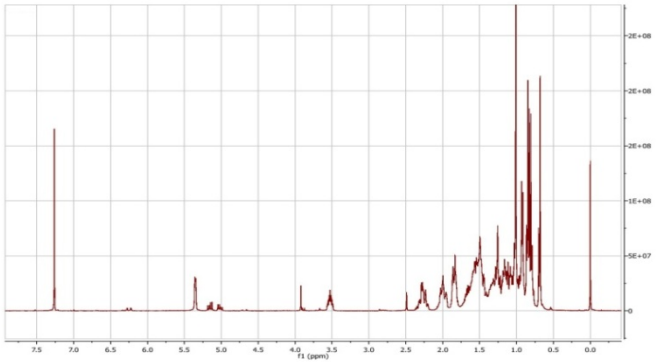

Fig 2. Proton NMR of dichloromethane extract of *Chara baltica*

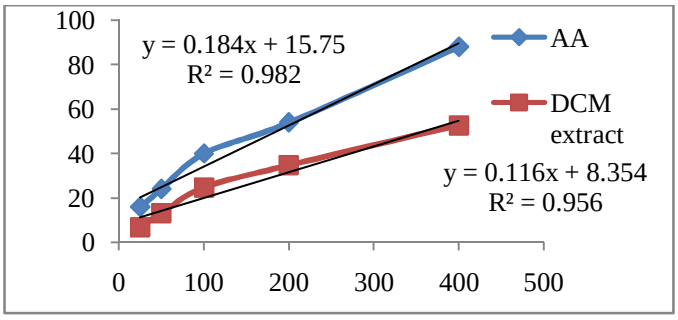

Fig 3. Percentage inhibition of *Chara baltica* on DPPH free radical

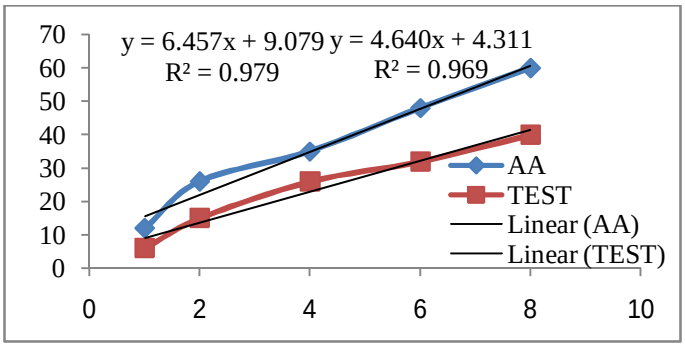

Fig 4. Percentage inhibition of *Chara baltica* on hydroxyl free radical

4. Conclusion

The results obtained in the present study indicates that the selected algae, *Chara baltica* have different bioactive molecules in it. The DCM extract of *Chara baltica* showed the free radical scavenging activity. Now a days, researchers are identifying the new biological compounds from natural sources such as medicinal plants, microorganisms because of different side effects are injuring humans on long term use of modern medicinal entities. The present study provide the evidence about the marine algae also contains the potent biological active compounds in the nature.

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