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In vitro antioxidant properties from tissue extract of Gastropods around Lower and Grand Anicut Reservoir, Tamil Nadu

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Abstract

Molluscs are species that have a wide range of uses in pharmacology. They are considered as an important natural source to derive many novel biological active compounds. Discovery of molluscan bioactive potential and their curious roles are still inadequate. The present study was evaluated on the antioxidant activity of ampullariidae (*Pila virens*) whole tissue extract at various concentrations (20-250µg/ml). The results of gastropods methanolic tissue extracts exhibited significant total antioxidant activity, DPPH, Hydroxyl radical scavenging activity, total reducing power, chelating ability on ferrous ions activity which predicted as 67.09%, 74.83%, 60.21% and 59.89%. respectively. These results concluded that, the tissue extract of *Pila virens* has novel antioxidant potential and it has to further characterize to improve the pharmacological active natural products.

Keywords: Molluscs, Gastropods, Methanolic extract, Antioxidant activity and Secondary metabolites.

INTRODUCTION

Molluscs are likely to be rich sources of biologically active secondary metabolites. Today, more than 60% of the anticancer drugs commercially available are of natural origin [1]. The knowledge of the physiological and biochemical features of freshwater organisms might contribute to the identification of natural products of biomedical importance. Many of these natural products have interesting biomedical potential, pharmaceutical relevance and diverse applications and also they provided the significant components of

pharmacologically important chemical bioactive substances.

The molluscs have received a considerable amount of research effort, reflecting both their ecological and economic importance, and now gaining importance in deriving drugs[2]. Reactions of free radicals, such as superoxide radical, hydroxyl radical, peroxyl radical and other reactive oxygen and nitrogen are associated with diseases such as atherosclerosis, dementia, and cancer. Antioxidants delay or prevent oxidative damage and thus they may be useful as therapeutics or food additives.

Natural products in simplest term are the chemical compounds, produced by living organisms. The cells of living organisms can be considered as micro chemical reactors producing large number of chemical compounds through metabolic reactions. These compounds can be categorized into primary and secondary metabolites. Primary metabolites such as proteins, carbohydrates *etc*. are required as nutrients

MATERIALS AND METHODS

Collection of samples and extraction

The freshwater gastropods *P.virens* were collected from the Grand and Lower Anaicut Reservoir. The collected fresh molluscs were preserved with ice and transported to the laboratory and identified by the standard literature of [9]. The shell removed fresh tissue samples were washed with sterile distilled water. The extraction method was followed by [10].

The freshly collected mollusc tissues each 25g in wet weight were soaked in methanol and ethanol separately and maintained for 3 days. The extracts were filtered through Whatman No.1 filter paper and the solvents were concentrated by rotary evaporator (VC100A Lark Rotavapor at 30°C) and freeze dried to give light yellow gummy mass which stored at 4°C for further analysis.

Reagents

Ammonium molybdate, Phosphate buffer, Ascorbic acid, Hydrogen peroxide solution, 1,1-diphenyl-2- picrylhydrazyl (DPPH), Sodium nitroprusside, Sulfanilic acid, Naphthyl ethylenediamine dihydrochloride, Sulfuric acid, Butylated Hydroxyltoluene, Potassium ferricyanide, were purchased from Sigma Chemical Co. (St. Louis, MO). TCA, FeCl3 and H2O2 were obtained from Merck Co. (Darmstadt, Germany). Other chemicals used were of analytical grade.

Antioxidant Assays

The antioxidant activity of the methanolic extract of *P. virens* were estimated in terms of total antioxidant activity, DPPH, Hydroxyl radical scavenging activity , total reducing power, chelating ability on ferrous ions followed by the method of Moovendhan (2016) with slight modification.

Statistical Analysis

The data were subjected to ONE-WAY ANOVA followed by Duncan's multiple range tests using statistics software package (SPSS, ver. 16) and the mean value of six replicates is presented.

RESULTS

The antioxidant activities of freshwater gastropod *P. virens* methanolic extracts were evaluated in various methods which formulate to have different levels of antioxidant activity at different concentrations (20, 40, 60, 80 and 250 µg/ml).

Total Antioxidant Activity

The *P. virens* methanolic extract showed the total antioxidant activity in the range of 19.78 to 67.09% at different concentrations 50 - 250 µg/ml. The maximum of 67.09% inhibition was observed at the concentration of 250 µg/ml of *P. virens* methanolic extract. It was observed that the total antioxidant activity was found increasing with increasing concentration. On comparison the standards L- ascorbic acid and BHA reported 80.12% and 88.05% of total antioxidant activity at the highest concentration of 250µg/ml respectively.

DPPH Radical Scavenging Activity

The effect of *P. virens* methanolic extract on oxidative damage induced by hydroxyl radical at different concentrations (0.1 – 2.0 µg/ml) was found between 20.06% and 74.83%. The maximum of 74.83% inhibition was observed at the highest concentration of 2.0 µg/ml of *P. virens* methanolic extract. The hydroxyl radical scavenging activity of L-ascorbic acid and BHA was 79.8 and 80.2% at 2.0 µg/ml. The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydroxyl donation.

Hydroxyl Radical Scavenging Activity

The effect of *P. virens methanolic* extract on oxidative damage induced by hydroxyl radical at different concentrations (0.1–2.0 µg/ml) was found between 9.08% and 60.21%. The maximum of 60.21% inhibition was observed at the highest concentration of 2.0 µg/ml of *P. virens* methanolic extract. The hydroxyl radical scavenging activity of L-ascorbic acid and BHA was 80.34 and 82.56% at 2.0µg/ml.

Superoxide Radical Scavenging Activity

The effect of *P. virens* methanolic extract on oxidative damage induced by superoxide radical at different concentrations (0.1 – 2.0 µg/ml) was found between 25.59% and 59.89%. The maximum of 59.89% inhibition was observed at the highest concentration of 2.0 µg/ml of *P. virens* methanolic extract. The hydroxyl radical scavenging activity of L-ascorbic acid and BHA was 82.45 and 85.29% at 2.0µg/ml.

Antioxidant Activity

Antioxidants play an important role in food industries for purposes of nutritional preservation and prevention of color and flavor deterioration. Analysis of antioxidant activity on *P. virens* methanolic extract showed higher ferrous chelating (67.09%) and hydroxyl radical scavenging activities (60.21%) compared to BHA (80.34 % and 82.56%). However, BHA showed higher reducing power (2.52%).

The ferrous ion (Fe^{2+}) is a pro-oxidant that interacts with hydrogen peroxide (Fenton reaction) to produce reactive oxygen species (ROS) and the hydroxyl (OH) free radical, which may initiate and/or accelerate lipid oxidation [11]. The complex formation of the ferrous ion is disrupted when chelating agents are present, resulting in decreased of color [4]. As for reducing power, the presence of antioxidants in causes the *P. virens* methanolic extract reduction of the ferricyanide complex to its ferrous form.

Validation Tests

Validation trials were run to determine the actual yield and antioxidant activity under the stated optimized conditions. Experimental results for optimized hydrolysis produced a 9.08% yield with an averaged antioxidant activity of 60.21%. Both values were less than predicted values which anticipated a yield of 19.78% and antioxidant activity of 67.09%. These lower results were likely due to losses occurring during the process of freeze drying as a consequence of small batch drying [12].

DISCUSSION

In the light of this, the aim of the present work was to purify and characterize methanolic extracted from the *P.virens* snails. Moreover the antioxidant property is derived from in the marine ecosystem only although present in the freshwater ecosystem. Nevertheless antitumor pharmacological studies were conducted with potentially promising *in vitro* cytotoxicity data generated with freshwater snail and human tumor cell lines were reported extremely scanty. In the scientific research, freshwater snails are used as model animals especially in molecular biology and immunology. In the *P. virens* methanolic extract is more excellent bioactive compound.

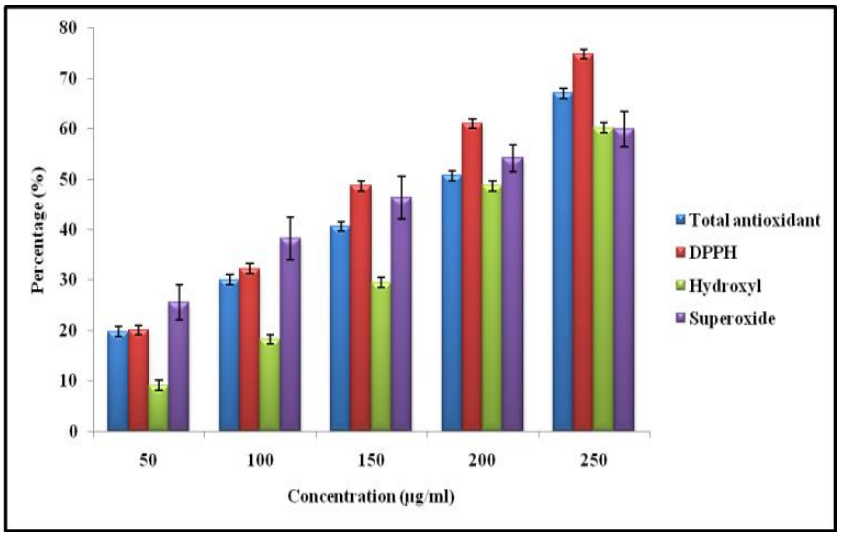

Fig. 1 Antioxidant activity in different concentration (50-250µg/ml)of *Pila virens* methanolic extracts

Table 1. *Pila virens* methanolic extract antioxidant activity in different concentration (50-250µg/ml)

	50µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml
Total antioxidant	19.78	30.04	40.64	50.72	67.09
DPPH	20.06	32.28	48.66	61.06	74.83
Hydroxyl	09.08	18.22	29.46	48.72	60.21
Superoxide	25.59	38.22	46.36	54.18	59.89

The secondary metabolites derived from number of molluscs possess antibiotic, anti-parasitic, antiviral and anti-cancer activities. Protein is a major biochemical constituent in all invertebrate and received highly attention due to their potential bioactive and functional properties. Many molluscs have evolved chemical defense mechanism for their *P.virens* methanolic extract and thus producing secondary metabolites which possess antioxidant activities.

Diphenylpicrylhydrazyl (DPPH) is stable nitrogen centered free radical which can be effectively scavenged by antioxidants [13]. DPPH is also considered as a good kinetic model for peroxylradicals [14]. The ability of protein to scavenge DPPH radical was determined by the decrease in it is absorbance in spectrophotometer. When, the solution of diphenylpicrylhydrazyl was mixed with that of a substance that can donate a hydrogen atom then this give rise to the reduced form (Diphenylpicrylhydrazine) which indicates the loss of this violet color [15].

The present, investigation shown that the partial purified from *P.virens* methanolic crude extract exhibited DPPH scavenging activity. Since the effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The reducing power ability of partial purified protein of *P.virens* methanolic extract greatly depends on the presence of reductions, which have exhibit antioxidant potential by breaking the free radical chain by donating a hydrogen atom [16]. Hydrogen peroxide is a weak oxidizing agent [17] and once inside the cell it can probably react with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radicals and this may be the origin of toxic effects [18].

The result of present study reveals that there is a strongest H_2O_2 scavenging activity was observed for protein at various concentrations when compared to be good scavenger of hydrogen peroxide. Nevertheless the maximum activity has observed in *P. virens* methanolic hydrogen peroxide free radicals. In the present study, *P. virens* methanolic extract at the concentration from 20 to 250µg/ml exhibited 67.09%, 74.83%, 60.21% and 59.89% respectively. The present result suggests that the P.virens methanolic crude extract might be potent agent for

scavenging assay. In the present study of P.virens extract at the various concentrations showed higher absorbance to indicating the tissue extract is the best source of antioxidant compounds. The chelating effects of various extracts on Fe^{2+} were determined by the formation of ferrozine –Fe^{2+} complexes.

Chelating agents are able to capture ferrous ion before ferrozine, thus hindering the formation of ferrozine – Fe^{2+}. Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, $O_2^{\bullet-}$ and $OH^{\bullet}$ quenching assays are most commonly used for the evaluation of antioxidant activities of extracts [19–21].

In this study methanolic extract of Pila virens and standard compounds have potential of antioxidant activity. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimating the metal chelating activity of the coexisting chelator. In this assay methanolic extracts of *Pila virens* and standard compounds are interfered with the formation of ferrous and ferrozine complex, demonstrating that they have chelating activity and are able to capture ferrous ion before ferrozine.

Spectrophotometer assessment of ferrozine–Fe^{2+} absorbance can accordingly be used to calculate ferrous ion chelating activity. The metal chelating capacity is important since it reduces the concentration of transition metals that may act as catalysts to generate the first few radicals to initiate the radical–mediated oxidative chain reactions in biological and food systems. Ion chelating agents also may inhibit the Fenton reaction and hydro peroxide decomposition [20]. extract can be a good antioxidant for removing hydrogen peroxide free.

CONCLUSION

In present study, antioxidant activity of purified crude methanolic extract of *P.virens* was investigated. Although radical scavenging and antioxidant activities, as determined by scavenging effect on the total antioxidant activity, DPPH, chelating effect on ferrous ions, Hydrogen peroxide scavenging activity, superoxide scavenging activity and reducing power. Consequently the present study concluded that *P.virens* methanolic extract possessed the potential antioxidant and anticancer activity and it could be used as natural accessible source for treating human diseases. The methanolic extract of *P.virens* proved to be a reservoir of bioactive constituents, which could be used in various diseases in future. However, isolation of individual compounds and their biological activities needs to be uncovered further to enhance its pharmacological importance and open new avenues in research. It could be concluded that, freshwater snails *P.virens* contains various bioactive compounds and may be recommended as a freshwater gastropods of pharmaceutical importance.

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