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Assessment of Antioxidant Activity in Polyclinum Madrasensis Extracts by Different Methods

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Abstract:- **Antioxidants act on free radical species by inhibiting their formation, converting them into less harmful molecules and thus prevent chain reactions that are injurious to cells. Therefore the need for the search of antioxidants from natural origin has been greatly felt in the recent years. The present study aimed to investigate the antioxidant activity in Polyclinum madrasensis extracts by methods like DPPH, Hydroxyl, ABTS, superoxide radical scavenging activity, and reducing power using in vitro models. In DPPH and ABTS radical scavenging activity methanol extract (146.26, 142.16) showed higher percentage of inhibition. Whereas, ethanol extract was found to be higher in hydroxyl (119.14) and reducing power. In superoxide radical scavenging activity ethyl acetate extract (113.46) was higher.**

Key Words: Polyclinum madrasensis, DPPH, Hydroxyl, ABTS, superoxide and reducing power.

I. INTRODUCTION

Free radicals are unstable and electrically charged highly toxic molecules that target and break the cell membranes resulting in serious damage to the cellular components, nucleic acids, proteins and enzymes. This charge by free radicals on cells and tissues is known as oxidative stress which brings about the loss of the structure and function of cells leading to their destruction. In normal cases, the natural antioxidant defence system of the cells efficiently manages this threat. An antioxidant is a molecule that inhibits the oxidation of other molecules and protects the body cells and tissues from damages caused by free radicals and ROS. They are released from the diet that we take and enter the cells through the blood stream and prevent damage to cells by cleaning up. They include enzymatic antioxidants like SOD, CAT, peroxidase that catalytically remove free radicals as well as scavengers of ROS and reactive nitrogen species (RNS) for example GSH, Vitamin C and E. Zn is a trace element and a cofactor of many enzymes including the cytoplasmic antioxidant Cu-Zn SOD, isoenzyme of SOD whereas selenium is a cofactor of glutathione peroxidise. Vitamin C is a water soluble free radical scavenger and Vitamin E is a standard lipid soluble antioxidant that functions as chain breaker during lipid peroxidation in cell membranes.

In addition to this, carotenoids like beta-carotene, lycopene, lutein also function as antioxidants. Flavonoids present in colouring pigments have potent antioxidant capacity. Antioxidants act on free radical species by inhibiting their formation, converting them into less harmful molecules and thus prevent chain reactions that are injurious to cells. The protection of the body cells depends on the balance between the production of free radicals and their scavenging by the antioxidants. An imbalance leads to oxidative stress which upsets the functions of cells resulting in pathological conditions including cardiovascular dysfunction, neurodegenerative diseases, gastroduodenal pathogenesis, metabolic dysfunction of almost all vital organs, cancer and premature aging. [1]

Nearly 100 disorders like rheumatoid arthritis, hemorrhagic shock, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastrointestinal ulcerogenesis, AIDS, Alzheimers, Parkinson's, atherosclerosis, cancer, Down's syndrome, ischemic reperfusion injury in different tissues including heart, liver, brain, kidney and gastro intestinal tract have been reported as ROS mediated [2]. The role played by ROS in stress induced gastric ulcer and inflammatory bowel diseases have been well established, as well as their involvement in the process of aging. Studies on marine organisms showed that they produce several kinds of antioxidant substances such as Xanthophyll, β-carotene, astaxanthin and lycopene compounds [3]-[5]. Such natural antioxidants are safer and hence studies on the role of antioxidant dietary supplements during oxidative stress and pathogenesis of chronic diseases are an area of research that can stimulate the identification of drug leads with therapeutic potential. In order to address this problem, search for newer antioxidants from marine resources has been attempted. Ethyl acetate extracts from marine bacteria associated with sponge Stylotella sp. by CUPRAC radical test showed potential antioxidant activities [6]. In vitro antioxidant effect of the ethanol extract of Didemnum psammathodes indicated that it may be due to hydroxyl groups existing in the phenols and flavonoids [7]. Studies on the antioxidant efficacy of the different extracts

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of the colonial ascidian Eudistoma viride by DPPH method revealed that the ethanolic extract has promising potential against free radical induced oxidative damage [8]. Antioxidant potential of colonial ascidians Eudistoma viride and Didemnum psammathodes which showed efficient scavenging activity indicating that these ascidians represent promising biological resource with far reaching implication in biomedical research [9].

MATERIALS AND METHODS

Collection of Sample

Polyclinum madrasensis was collected from Tuticorin harbor basin in the month of October by SCUBA diving. It was washed several times with sea water dried under shade and homogenized to get a coarse powder.

DPPH radical scavenging activity

The study of free radical in the antioxidant component is assayed by DPPH radical scavenging activity [10]. This method is based on the reduction of DPPH in methanol solution in presence of a hydrogen donating antioxidant resulting in the formation of the non-radical form DPPH-H. DPPH in methanol (0.1 mM) was prepared. To 3 ml of various solvent extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) of Polyclinum madrasensis at different concentrations (50, 100, 200, 400, 800 µg/ml), 1 ml of DPPH in methanol was added. After vigorous shaking the mixture was kept undisturbed at room temperature for 30 min. The absorbance was measured at 517 nm in a UV-VIS spectrophotometer. Ascorbic acid was used as the standard. A reagent blank was also run simultaneously.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured as per the modified method [11]. 1mM EDTA, Ascorbic acid 10 mM FeCl3, H2O2 and deoxyribose, were prepared in distilled de ionized water as stock solutions. The hydroxyl radical scavenging activity was estimated by serially adding 0.1ml EDTA, 0.01ml of FeCl3, 0.1ml H2O2, 0.36 ml of deoxyribose, 1.0 ml of solvent extracts at different concentration (50, 100, 200, 400, 800 µg/ml), 0.33 ml of phosphate buffer (50 mM, pH 7.9) and 0.1 ml of ascorbic acid. The reaction mixture was incubated for 1h at 37 °C. 1 ml of the incubated mixture was mixed with 1ml of 10% TCA and 1ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA). The pink chromogen developed was measured at 532 nm. Along with the analysis, a reagent blank and standard was also run. Percentage inhibition of deoxyribose degradation is taken as the hydroxyl radical scavenging activity of the extract.

ABTS+ cation radical scavenging activity

Modified method was adopted for ABTS assay [12]. To ABTS solution (7 mM), potassium per sulphate (2.45 mM) was added and the mixture was allowed to stand in the dark at room temperature for 12-16 h in order to produce ABTS radical cation (ABTS+). To 100 µl of extract at different concentration (50, 100, 200,400, 800 µg/ml), 3.9 ml of ABTS+ solution diluted with ethanol was added. The absorbance was measured at 734 nm using a UV-VIS spectrophotometer exactly after 6 min. Trolox was used as standard. The results are expressed as trolox equivalent antioxidant capacity (TEAC).

Superoxide radical scavenging activity

To measure the superoxide anion scavenging activity modified method was adopted [13]. To 3 ml of Tris-HCl buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM),1 ml extract at different concentration (50, 100, 200,400, 800 µg/ml) and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0) were added in order to generate superoxide anion radicals. Initiation of the reaction was done by adding 0.5 ml PMS solution (0.12 mM). The mixture was incubated at 25°C for 5 minutes. The absorbance was measured at 560 nm against a reagent blank and standard ascorbic acid.

Ferric reducing power

The reducing power of the extract was done as per Kumar and Hemalatha, 2011 [14]. 1ml of extract at different concentration (50, 100, 200,400, 800 µg/ml) was mixed with 5 ml sodium phosphate buffer (0.2 M, pH 6.6) and 5 ml potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 minutes and 5 ml trichloro acetic acid (10%) was added. It was centrifuged in a refrigerator centrifuge at 5 °C and 980 g for 10 minutes. 5 ml of supernatant was diluted with 5 ml of distilled water and ferric chloride. The absorbance was read at 700 nm. A reagent blank and standard ascorbic acid was used.

Statistical analysis

All experiments were carried out in triplicate and the results are expressed as mean of these three independent analysis ± standard deviation (n=3). The free radical scavenging activity was calculated using the formula, % scavenging activity = $\{(A_{Control}-A_{Test})/(A_{Control})\times 100\}$where $A_{Control}$ is the absorbance of control, ATest is the absorbance of the extracts.

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RESULTS AND DISSCUSSION

DPPH radical scavenging activity

DPPH radical scavenging activity was higher in methanol, ethanol and ethyl acetate compared to standard ascorbic acid. The radical scavenging effect was found to increase with increasing concentrations. Polyclinum madrasensis extracts and standard showed maximum percentage inhibition in the following order - methanol (146.26), ethanol (136.15), ethyl acetate (109.36), benzene (92.13) petroleum ether (84.26), and standard (96.16) with IC50 values of 48.92, 45.13, 36.94, 31.66, 29.74 and 32.92 µg/ml respectively (Fig. 1). Studies on ascidians like Styela clava, Ecteinascidia venui, Didemnum psammathodes, Eudistoma viride also exhibits similar observations of antioxidant activity by DPPH method [15]-[18], [7] and [8].

Hydroxyl radical scavenging activity

Hydroxyl scavenging activity was higher in ethanol and methanol compared to standard. The hydroxyl radical scavenging effect was found to increase with increasing concentrations. Polyclinum madrasensis extracts and standard showed maximum percentage inhibition in the following order - ethanol (119.14), methanol (103.13), ethyl acetate (93.16), petroleum ether (93.16), benzene (78.16) and standard (94.56), with IC50 values of 34.88, 32.16, 31.65, 30.16, 29.16 and 29.96 respectively (Fig. 2). Studies on Ulva pertusa and Styela clava indicated that sulphated polysaccharides and oligosaccharides might be responsible for scavenging the hydroxyl radicals and the mechanism might be either by suppressing the generation or by scavenging those generated [19].

ABTS+ cation radical scavenging activity

The methanol and ethanol extract exhibited high antioxidant activity. The cation radical scavenging effect was found to increase with increasing concentrations. Polyclinum madrasensis extracts and standard showed maximum percentage inhibition in the following order - methanol (142.16), ethanol (131.16), ethyl acetate (104.16), petroleum ether (103.13), benzene (89.32) and standard (98.27) with IC50 values of 43.18, 39.03, 34.87, 33.67, 29.18 and 31.92 (µg/ml) respectively (Fig. 3). To determine the activity of natural antioxidants ABTS assay is a simple indirect method [20].

Superoxide radical scavenging activity

Ethyl acetate extract exhibited high antioxidant activity. The effect was found to increase with increasing concentrations. Polyclinum madrasensis extracts and standard showed maximum percentage inhibition in the following order - ethyl acetate (113.46), ethanol (108.26), methanol (101.44), benzene (99.26), petroleum ether (92.84) and standard (106.26) with IC50 values of 36.95, 34.57, 31.89, 30.93, 28.63 and 33.43 respectively (Fig. 4). Superoxide anions are the most common free radical in vivo and are generated in a variety of biological systems, either by auto-oxidation processes or by enzymes. The concentration of superoxide anions increases under conditions of oxidative stress and related situations [21]. Similarly, superoxide anions produce other kinds of cell damaging free radicals and oxidizing agents [22]. In the present study, the results indicate that Polyclinum madrasensis extract can effectively scavenge superoxide anions which may be due to the presence of potent antioxidants.

Ferric reducing power

The radical scavenging effect was found to increase with increasing concentrations. Higher reducing power is indicated by higher absorbance. Ethanol and methanol extracts exhibited higher reducing ability among the solvent tested. In comparison with ascorbic acid the extracts possess potential reducing power and hence antioxidant activity (Fig. 5). Studies on halophilic bacterial bionts revealed that reduction of ferric/ferricyanide complex to ferrous form [23].

In the present study, it was observed that the antioxidant substances present in Polyclinum madrasensis are non-polar in nature as organic solvent fraction, particularly ethanol and methanol fractions exhibit higher antioxidant activity. GC-MS analysis of Polyclinum madrasensis has shown the presence of triterpene compounds and palmitic acid with antioxidant properties [24]. The results indicate that the ascidian extracts can be a good source of natural antioxidant. The presence of these antioxidant components may assist the sedentary ascidians to overcome the oxidative stress arising as a result of desiccation when exposed to the sun during at low tides. Further investigations are suggested for isolating and identifying the compounds responsible.

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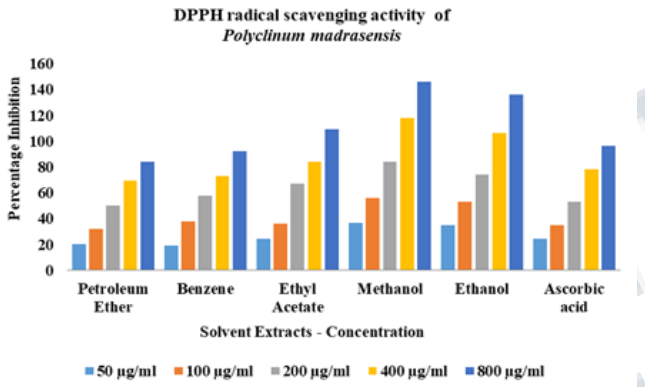

Fig. 1 DPPH radical scavenging activity of Polyclinum madrasensis

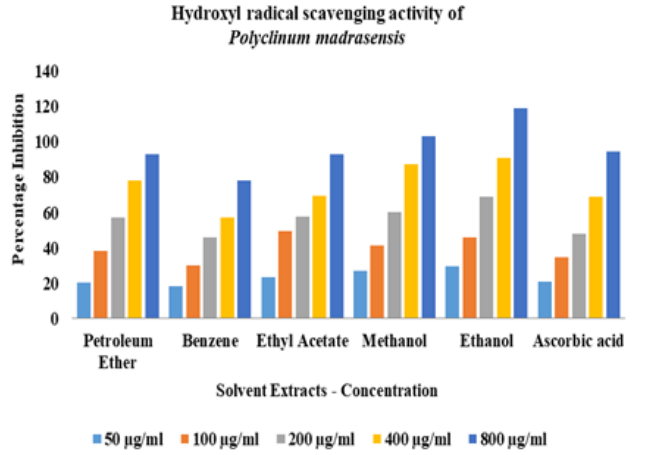

Fig. 2 Hydroxyl radical scavenging activity of Polyclinum madrasensis

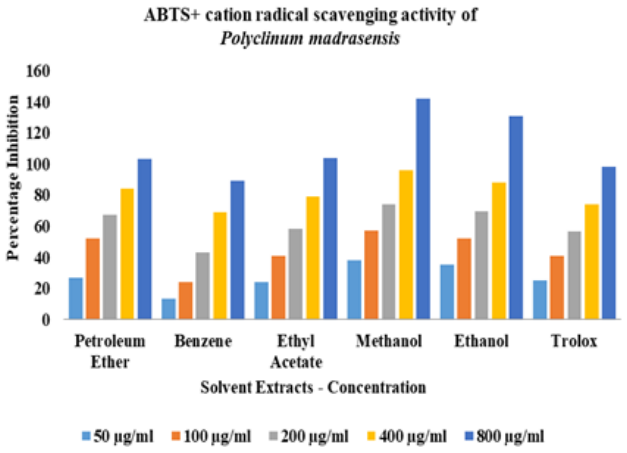

Fig. 3 ABTS+ cation radical scavenging activity of Polyclinum madrasensis

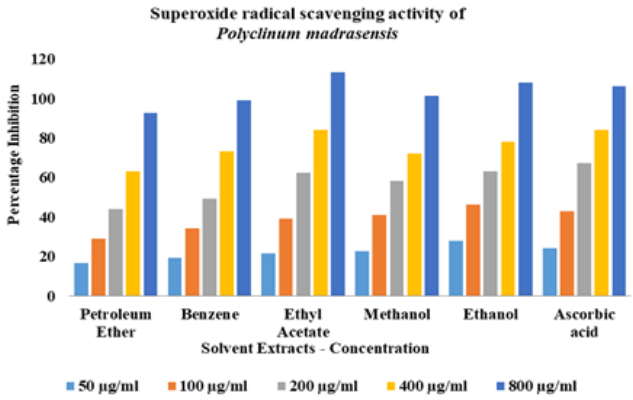

Fig. 4 Superoxide radical scavenging activity of Polyclinum madrasensis

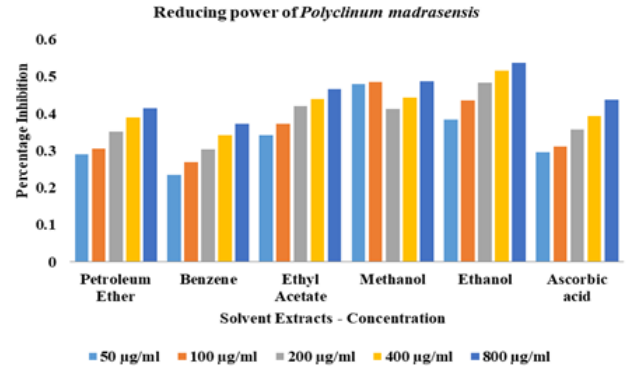

Fig. 5 Ferric reducing power of Polyclinum madrasensis