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Research Article

***In-vitro* antioxidant activity of hydroalcoholic extract of leaves of *Hydnocarpus pentandrus* (Buch. - Ham) Oken**

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ABSTRACT

Cellular damage can arise from the reactions of free radicals with membrane lipids, nucleic acids, proteins, enzymes, and other micro molecules. Free radical-induced cell damage seems to be a primary factor in the aging process and degenerative diseases, including but not limited to cancer, heart disease, cataracts, liver disorders, diabetes mellitus, inflammation, and renal failure. Naturally, the body produces free radicals, and antioxidants scavenge them to shield the body from harmful consequences. This dynamic equilibrium exists between the two. It's possible that there aren't enough antioxidants in the body under typical physiological circumstances to offset the production of free radicals. Thus, it stands to reason that adding antioxidants to our diet will help shield us from dangerous illnesses. Thus, the creation of "natural antioxidants" from plant material has drawn more attention from the food business and preventative medicine. Given the importance of antioxidant activity, a hydroalcoholic crude extract from the leaves of the Achariaceae family plant *Hydnocarpus pentandrus* (Buch. - Ham) Oken was made, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay, the Ferric Ion Reducing Assay (FRAP), the Nitric Oxide Assay, and the H₂O₂ Radical Scavenging Assay were used to measure the radical scavenging activity. The antioxidant activity of the hydroalcoholic extract was examined in comparison to the reference; in all four methods, solvent-based findings were observed. Higher antioxidant activity is shown by the FRAP and DPPH assays, which are followed by the nitric oxide, and H₂O₂ radical assays. DPPH > FRAP > H₂O₂ > Nitric oxide.

INTRODUCTION

Hydnocarpus pentandrus (Buch. - Ham) Oken belongs to Family Achariaceae. It is deciduous

tropical tree distributed throughout India and few other tropical countries. Traditionally boiled leaves were applied in sprains and bruises, local

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swellings, pains of body and in elephantiasis [1]. Five dihydroflavonoids were isolated and identified from the leaves of *Hydnocarpus pentandrus* [2]. The leaves are useful in cuts, wounds, bruises, ulcers, ophthalmia, sprains, diarrhoea and dysentery. The fruit paste of *Hydnocarpus pentandrus* is therapeutically used for bone fractures by tribes in western ghats of India [3]. The pharmacological properties of extracts of *Hydnocarpus pentandrus* leaves were screened for antidiabetic activity [4]. In the present study we investigated radical scavenging potential of hydroalcoholic extract of leaves of *Hydnocarpus pentandrus*.

Oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). They are continuously produced by the body's normal use of oxygen such as respiration and some cell-mediated immune functions. ROS include free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals ($OH^\bullet$) and non - free radical species such as hydrogen peroxide (H_2O_2) and single oxygen [5]. ROS are continuously produced during normal physiologic of membrane lipids, leading to the accumulation of lipid peroxides. ROS are also capable of damaging crucial biomolecules such as nucleic acid, lipids, proteins and carbohydrates and may cause DNA damage that can lead to mutations. If ROS are not effectively scavenged by cellular constituents, they lead to disease conditions. ROS have been implicated in more than 100 diseases [6]. Antioxidants can protect the human body from free radicals and ROS effects. They retard the progress of many chronic disease as well as lipid peroxidation [7]. Hence, a need for identifying alternative natural and safe sources of food antioxidants has been created, and the search for natural antioxidants, especially of plant origin, has notably increased in recent years. Antioxidants have been widely used as food additives to provide

protection against oxidative degradation of food [8].

MATERIAL & METHODS

The plant material of *Hydnocarpus pentandrus* for the present work was collected from Dapoli, Chandra Nagar, Maharashtra, India ($17^\circ44'36.3''N$ $73^\circ09'39.1''E$) & authenticated from the Blatter Herbarium, St. Xavier's College, Mumbai (Accession no.- R3060). The leaves were shade dried. Dried leaves were ground to coarse powder, extracted with hydro alcohol. The antioxidant activity of the plant extract and standard were assessed based on radical scavenging effect of the stable DPPH free radical according to method of [9]. The reducing power of the hydroalcoholic extract was determined according to the method of [10]. The ability of the extract to scavenge hydrogen peroxide was determined according to the method of [11]. Nitric oxide was generated from nitroprusside and measured by the Griess reaction [12].

Result & Discussion

DPPH Free radical scavenging activity

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples [13]. The assay is based on the increase of DPPH radical in hydroalcoholic extract which causes an absorbance drop at 517 nm. DPPH is known to abstract labile hydrogen [14]. DPPH being a stable free radical can accept an electron or hydrogen radical to become stable diamagnetic molecule. Due to its odd electron, the hydro-alcoholic solution of DPPH shows a strong absorption at 517nm, DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichiometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of compounds in plant extracts to acts as a free radical scavenger. Antioxidants molecules can quench DPPH free radicals and convert them to a colourless product. Figure 1.



shows the dose responses curve of DPPH radical scavenging activity of hydroalcoholic extract, compared with ascorbic acid, as standard.

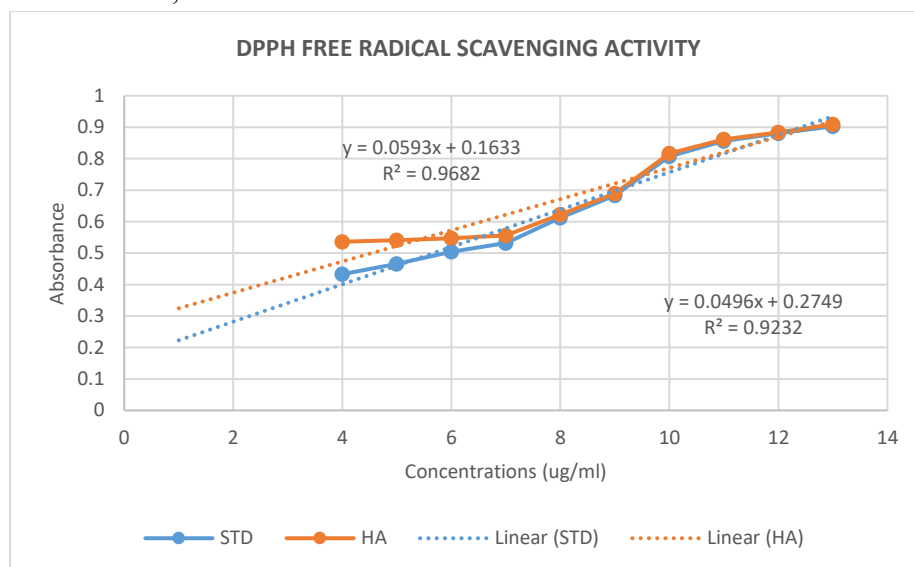


Figure 1: DPPH Free radical scavenging activity of hydroalcoholic extracts

Reducing power assay (FRAP)

The reducing power of the extracts was measured by the direct electron donation in the reduction of $[\text{Fe}(\text{CN})_6]^{3-}$ to $[\text{Fe}(\text{CN})_6]^{4-}$. The product was visualized by addition of free Fe^{3+} ions after the increase, by forming the intense Prussian blue colour complex and quantified by absorbance measurement at 700 nm [15]. The reducing capacity of hydroalcoholic extract might serve as a significant indicator of its potential antioxidant activity and reducing power of certain plant

extracts has been observed. The reducing power of certain plant extracts has been observed. The reducing properties are generally associated with the presence of atoms, which have been shown to exert antioxidant action by donating a hydrogen atom [16]. Figure 2 shows the reduction capabilities of plant extract compared to ascorbic acid. The reducing power of hydroalcoholic extract could reduce the Fe^{3+} ions, which had a great reducing activity than the standard of ascorbic acid.

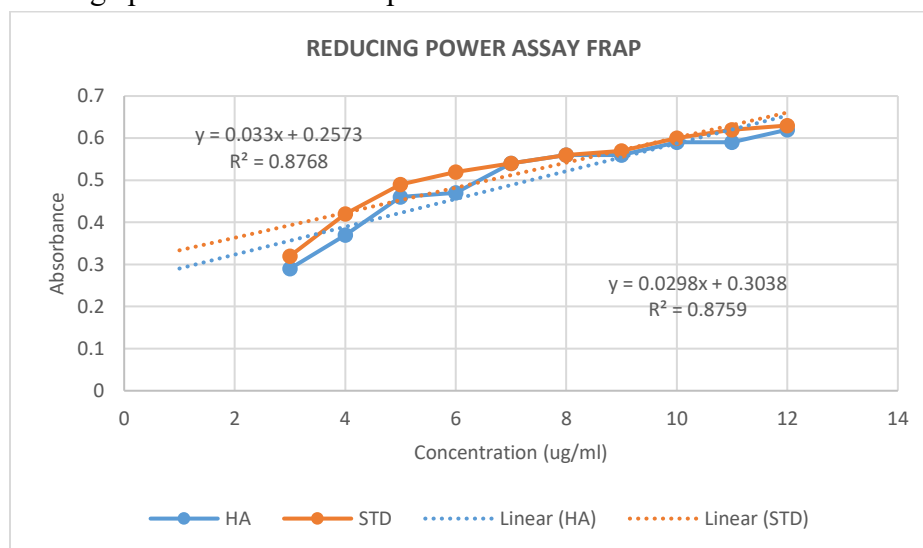


Figure 2: Reducing power assay of hydroalcoholic extracts

Hydrogen peroxide

Hydrogen peroxide itself is not very reactive but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells [17]. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to,

H_2O_2 thus neutralizing it to water. The hydroalcoholic extract was capable of scavenging hydrogen peroxide in a concentration-dependant manner. Figure 3 shows that hydroalcoholic shows less scavenging activity H_2O_2 than that of ascorbic acid.

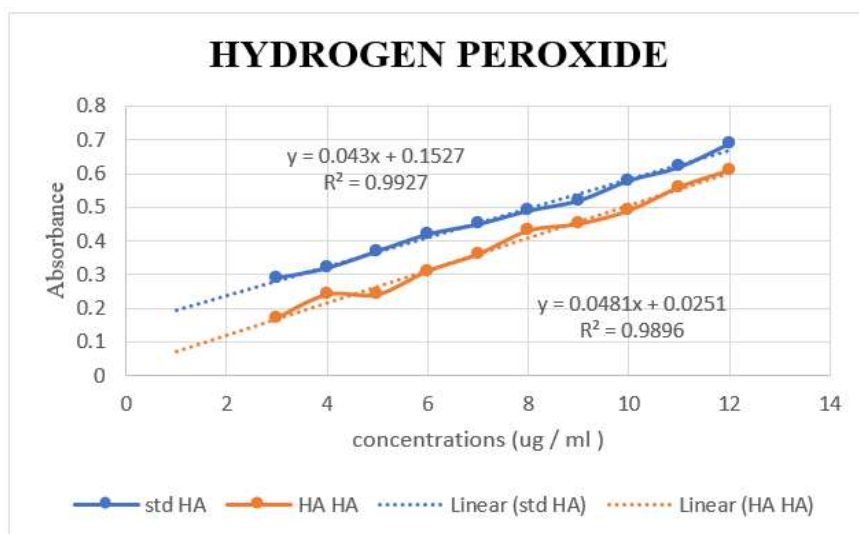


Figure 3: Hydrogen peroxide activity of hydroalcoholic extract

Nitric oxide scavenging activity

Nitric oxide is an important radical in biological systems, which serves many important biological functions as an intra and intercellular messenger, as a free radical, it is oxidised, reduced or complexed with other biomolecules, depending on

microenvironment [18]. These reactive nitrogen species are implicated in inflammation, cancer and other pathological conditions. Figure 4 shows moderately good nitric oxide scavenging activity. The percentage of absorbance were increased with increasing concentration of the extracts.

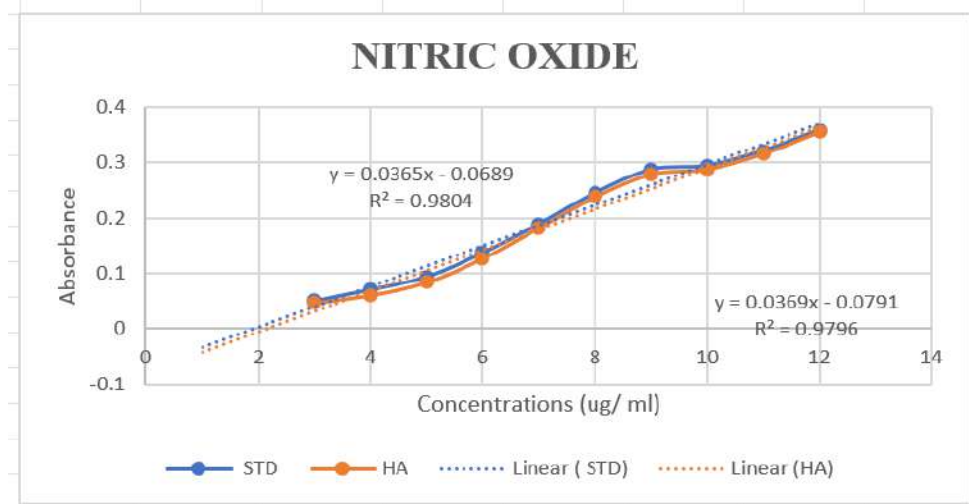


Figure 4: Nitric oxide scavenging activity of hydroalcoholic extract

CONCLUSION

The antioxidant activity of the hydroalcoholic extract was examined in comparison to the

reference; in all four methods, solvent-based findings were observed. higher antioxidant activity is shown by the FRAP and DPPH assays, which



are followed by the nitric oxide, and H_2O_2 radical assays. DPPH > FRAP > H_2O_2 > Nitric oxide. based on results obtained in the present study, it is concluded that hydroalcoholic extract of *Hydnocarpus pentandrus* leaves, exhibits high antioxidant and free radical scavenging activities. It also chelates iron and has reducing power. These in vitro assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

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