Estimation Of Antioxidant Assay In Snow-Capped Seabuckthorn Fruit Pulp Using DPPH [α, α-diphenyl-β-picrylhydrazyl] Reagent

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Abstract
Seabuckthorn has attracted worldwide engrossment because of its high medical and nutritional potential. Seabuckthorn berries are prosperous in vitamins, minerals, fatty acids, amino acids, carotenoids and antioxidants. Multifarious chemical substances are found in seabuckthorn which has a good effect on health. In this research, we used Thermo Scientific Multiskan Sky Cuvette Touch Drop Spectrophotometer to probe the presence of antioxidant assay in seabuckthorn fruit pulp using DPPH [α,α-diphenyl-β-picrylhydrazyl] reagent. The IC_{50} value for ascorbic acid and fruit pulp were calculated and observed that the IC_{50} values for ascorbic acid and fruit pulp sample were 19.07 µl and 12.82 µl, respectively. The pulp possessed remarkable antioxidant properties due to its ability to scavenge free radicals.

Keywords: Seabuckthorn, free radical scavenging, Vitamins, minerals, antioxidants, Multiskan sky Cuvette Touch Drop Spectrophotometer

1. INTRODUCTION

Bio-based products involved in therapeutic and remedial usance over the past few decades have resulted in the dwindling of human health. In the interest of the presence of various bioactive molecules in vegetables, fruits, and plant products, there are countless reports about their beneficial effect on human health [3]. These substances mainly include; flavonoids, Phenolic acid, strong antioxidants, minerals, vitamins and carotenoids. Utilization of such chemical compounds may be an important seminal to prevent or delay diseases caused by free radicals and veteran of oxidizing bio molecules that can cause many diseases. Biomolecules derived from natural plants are in high demand because of their frail side effects. Seabuckthorn is a thorny, deciduous Shrub or small tree that pertains to the family of Elaeagnaceae. Seabuckthorn is naturally found in China, Pakistan, India, Russia, Northern Europe, Canada and Mongolia. Research has shown that seabuckthorn has been used as a traditional medicine in China for over 1,000 years. The Berries of sea buckthorn are prosperous in vitamins, minerals, organic acids, amino acids, carotenoids and antioxidants. Many of the chemical components found in seabuckthorn have favourable effects on health and are necessary for the proper functioning of the human [18].

Seabuckthorn has attracted worldwide engrossment due to its medicinal and nutritional potential; it is cultivated in many-sided countries around the world. Due to its high nutritional properties, sea buckthorn is magnificently used to treat skin problems, memory loss, heart disease, cancer and many other ailments. Seabuckthorn berries are also used in the food industry to make jellies, juices, sauces, and drinks. People generally do not eat the berries raw because of the bitterness and sourness, the production of seabuckthorn beverage is a new product development opportunity for many because of the high concentration of chemical components and vitamin C. Seabuckthorn berries are highly moist, which reduces their shelf life, as they are vulnerable to mechanical damage and fungal infection during their storage and transportation [22]. Preservation through drying is an effective solution to reduce quality loss. Depending on the type of fruit, berries are dried in a number of ways: from inexpensive and easy processes such as sun-drying to expensive non-conventional methods such as microwave and freeze-drying. In addition, modified atmosphere is used for drying berries. The bioactive components in seabuckthorn vary according to fruit size, location, species, fruit maturity, method of extraction climate condition of the area and time of harvesting [18].The purpose of this research is to focus on antioxidants found in the pulp of seabuckthorn fruit that may provide health benefits when consumed regularly. This research will be of great importance to the development of future uses of seabuckthorn, thereby promoting the development both the pharmaceutical and food industries.

2. METHODS AND MATERIALS

2.1 Seabuckthorn fruit collection

Seabuckthorn fruit was used to determine the antioxidant content. Seabuckthorn fruits were taken from the growing region of Leh Ladakh from commercial plantations during November and it kept at 20◦C prior to analysis. Whereas the sample was tested in a National facility for biopharmaceuticals (A project sponsored by Dept. of Science and Technology, Govt. of India), GNKC, Matunga, Mumbai-19

2.2 Processing of sea buckthorn pulp

For processing a pulp, the Barries were defrosted and all the wood parts and non-healthy Barries were removed. Thenceforward pulpy Residue of the fruit and seeds were separated, later this pulpy Residue of the fruit has been crushed in a mixer grinder. Posterior grinding it was strained with the help of a sieve and hovered at 20°c temperature till further process.

2.3 Preparation of extract

For extract preparation, the fruit pulp was filtered with the help of filter paper and soaked in methanol for 24 hours at room temperature with intermittent stirring. The mixture was then filtered through filter paper and distilled water was added. Thereafter, the filtrate was dried in a rotatory vacuum evaporator at 40◦c to prepare the extract. Finally, the extract was lyophilized and stored at 4◦c until analysis for further experiments.

3. DPPH RADICAL SCAVENGING ACTIVITY

The DPPH [α,α-diphenyl-β-picrylhydrazyl] method is widely used to evaluate the free radical scavenging activity of antioxidants. After adding the antioxidant the decrease in absorbance of the DPPH solution was measured at 517 nm. In this experiment, ascorbic acid was used as an antioxidant. DPPH is a stable free radical with a purple colour that turns yellow when scavenged. This character has been used to show the free radical scavenging activity of the DPPH assay. The degree of discolouration indicates the purity antioxidant capacity of the extract in terms of hydrogen donating capacity. The great diversity of methods is evident from its different names. It is known many methods using DPPH for determination of: the radical scavenging activity or free radical scavenging activity (Kumazawa and Nakayama, 2001; Okawa et al., 2001; Pavlovet al., 2002; Yang et al., 2004; Bankeblia, 2005;Kaukovirta-Norja et al., 2005; Gülcin et al., 2010; Renet al., 2010; Uddin et al., 2010), the antioxidant activity (Schlesier et al., 2002; Molyneux, 2003;Potter at al., 2007; Butkhup and Samappito, 2008;Lachman et al., 2008; Belisario-Sanchez et al.,2009), the DPPH method/assay (Parkash, 2001; Kamkar et al., 2010), the DPPH scavenging assay (Gupta et al., 2007), the DPPH test/method (Kwon et al., 2003), the DPPH radical scavenging effect (Kim et al., 2002), the DPPH scavenging amount (Jing et al., 2008a; Jing et al., 2008b), the total antioxidant activity (Tarozzi et al., 2004;Singh et al., 2008) etc.

3.1 Preparation of DPPH reagent

To prepare a stock solution, in 100 ml of methanol stored in an Amber coloured Schott bottle, 400 mg of DPPH[α, α-diphenyl-β-picrylhydrazyl] reagent was dissolved and stored in a dark place, away from light.

3.2 DPPH Assay procedure

Various amounts of ascorbic acid (up to 100 ml) were made with 1000 µl of methanol and 1000 µl of DPPH solution was added. The reaction mixture was kept in the dark for 30 min. The exact procedure was repeated for triplicate sets of the ascorbic acid standard. Similarly, the same process was repeated for the food pulp sample. After 30 min incubation, samples were subjected to spectrophotometric determination for free radical scavenging activity at 517 nm using a Multiskan Sky Cuvette Touch Drop spectrophotometer. This set of triplicates was used as a control. The % free radical scavenging activity of the ascorbic acid standard and the fruit pulp sample was calculated using the following relation

% free radical scavenging activity= (Abs of control - Abs of sample/Abs of control) ×100

Where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the Absorbance of DPPH radical + plant extract.

The half-maximal inhibitory concentration (IC_{50}) values for ascorbic acid and fruit pulp were calculated using the following relation

$y= mx +c$

Where y is absorbance, m is slope and c is intercept, respectively.

4. RESULT

The discovery of antioxidant properties in a dilute extract of seabuckthorn fruit pulp using DPPH [α, α-diphenyl-β-picrylhydrazyl] radical scavenging activity is conveyed in this section. When the free radical DPPH interacts with an unpaired electron, the greatest absorption takes place at 517 nm. A free radical scavenger oxidant reacts to DPPH to form DPPHH, which has a lower absorbance than DPPH due to its lower hydrogen content. This radical variant causes

colourlessness when the number of electrons collected is increased compared to the DPPH-H state. Table-1 and Table-2 show the % of free radical scavenging activity of different amounts of ascorbic acid and fruit pulp.

Table1: Absorbance of the Ascorbic Acid Standard

Volume (µL)	Sample Absorbance	Control Absorbance	Control Absorbance-Sample Absorbance (A)	A/Control Absorbance (B)	Free Radical Scavenging Activity (B*100)
10	0.91	1.32	0.41	0.31	31.27
20	0.80	1.32	0.52	0.40	39.58
30	0.72	1.32	0.60	0.46	45.62
40	0.63	1.32	0.69	0.52	52.42
50	0.51	1.32	0.81	0.61	61.48
60	0.40	1.32	0.92	0.70	69.79
70	0.32	1.32	1.00	0.76	75.83
80	0.20	1.32	1.12	0.85	84.89
90	0.11	1.32	1.21	0.92	91.69
100	0.01	1.32	1.31	0.99	99.24

Table2: Absorbance of the Fruit Pulp Sample

Volume (µL)	Sample Absorbance	Control Absorbance	Control Absorbance-Sample Absorbance (A)	A/Control Absorbance (B)	Free Radical Scavenging Activity (B*100)
5	0.67	1.32	0.65	0.49	49.09
10	0.58	1.32	0.75	0.56	56.34
15	0.51	1.32	0.81	0.61	61.33
20	0.44	1.32	0.89	0.67	67.15
25	0.36	1.32	0.97	0.73	73.11

Table-1 and Table-2 display the results; figure-1 and figure-2 depict the plot. Where x is concentration, y is absorbance, c is intercept and m is slope respectively.

Figure 1: Graph for the free radical scavenging activity of Ascorbic Acid Standard

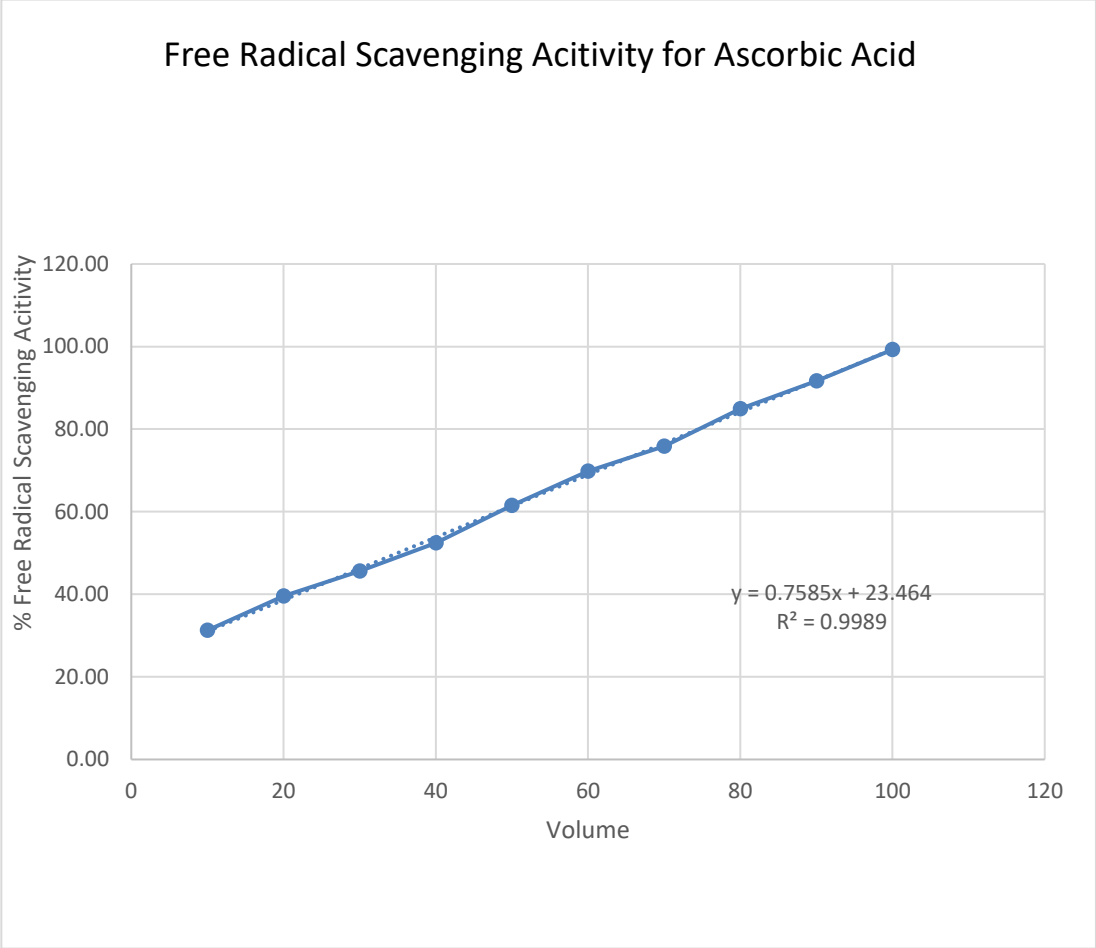
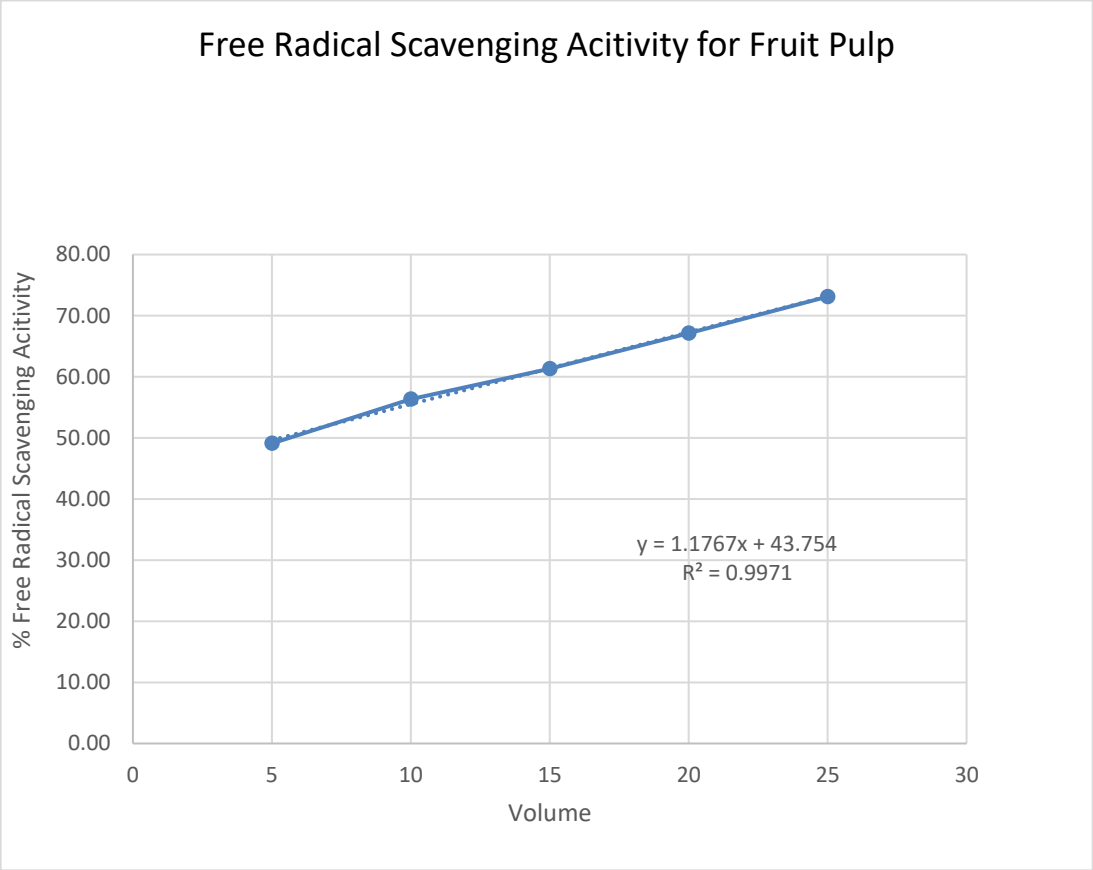


Figure 2: Graph for the free radical scavenging activity of Fruit pulp



From the above observation table, IC_{50} was calculated using the formulae:
Y = mx + c
Where "y" is 50, "m" and "c" are the constants as taken from the graphs above. The table-3 summarizes the calculation of the IC_{50} Values.

Table 3: IC_{50} Values and their calculation

SAMPLE	"y"	"m"	"c"	IC_{50}
ASCORBIC ACID	50	0.7585	23.464	19.07 µL
FRUIT PULP	50	1.1767	43.754	12.82µL

The IC_{50} values for the ascorbic acid standard and Fruit pulp sample were found to be 19.07 µl and 12.82 µl respectively. The Fruit pulp possessed remarkable anti-oxidant properties by its ability to scavenge free radicals.

4. DISCUSSION

In this antioxidant test, the results indicated that seabuckthorn has a strong ability to exert antioxidant action. The DPPH assay was used to test the antioxidant activity. seabuckthorn fruit pulp extracts have been studied for their ability to decolorize stable DPPH free radicals, providing information of the reactivity of compounds containing stable free radicals (Badami et al., 2003) [4]. The results of this study showed that seabuckthorn fruit pulp extract was effective in scavenging DPPH radicals. This suggests that the extract has scavenging properties and that it may act as free radical inhibitors. The results obtained from this assay supported the validity of the DPPH assay and reaffirmed the antioxidant capacity of seabuckthorn fruit pulp extract. Antioxidant activity shown by extracts of seabuckthorn fruit pulp provides a scientific validation.

The results showed that the pulp of seabuckthorn fruit contained high amounts of antioxidants. Seabuckthorn has antioxidant properties that can help restore human health by inhibiting oxidative damage like heart disease, memory loss, depression, skin problems even cancer. Sea buckthorn is known to improve circulation and heart function, according to several studies. We all know that nowadays cancer is a major problem in worldwide. Sea buckthorn may help fight cancer cells in the lab, early studies show. However, for the traditional use of these plants in traditional medicine systems, their efficacy needs further investigation. However, it has been observed that seabuckthorn fruit juice also exhibits good antioxidant capacity with some Phenolic compounds, but their contribution to the antioxidant effect is much less than that of ascorbic acid (Rosch et al.,2003) [21]. Similarly, Chauhan et al. (2007)[6] Showed Antioxidant and antibacterial activities of aqueous extract of Seabuckthorn seeds. The seed oil of Hippophae Rhamnodies Possesses

several strong antioxidative and antimicrobial Properties, which are due to the high content of tocopherols and Carotenoids present in the oil (Chen et al., 1990) [9]. Research in the development of formula food, pre-food and food additives of seabuckthorn should provide for conditions of great potential and markets.

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