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Original Research Article

Invitro Antioxidant Activity of Aqueous Extract of Water Apple (*Syzygium Aqueum*) Fruits

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Abstract

The Malaysian medicinal plant, Water Apple' (*Syzygium Aqueum*) can provide bioactive compounds that help to support people suffering from many diseases. The present study is undertaken to investigate the phytochemicals and invitro antioxidant activities from extract of Water Apple fruits. Determination of phytochemicals and invitro anti-oxidative capacity, established assay method 2, 2 - diphenyl – 1- picryl hydroxyl (DPPH) radical assay, nitric oxide and superoxide anion scavenging activity assays were used with reference to standard antioxidants ascorbic acid and butyl hydroxyl anisole (BHA).The percentage inhibition of radical scavenging activity increased with increase in concentrations of extract and when compared with standard antioxidants. The results show that the extract of fruits of Water Apple possesses significant antioxidant activity when compared to standard antioxidants. These findings provide a strong rationale to establish Water Apple fruits capability as an anti oxidant capacity. However, in-vivo antioxidant activity and mechanism of action is needed to be further studied.

Keywords: Antioxidant activity, Water Apple fruits, Butylated Hydroxy Anisole, DPPH radicals, Nitric Oxide radicals, Superoxide radicals.

INTRODUCTION

The associative function of consumption of fruits and vegetables has been shown to gives energy and support to prevention of chronic diseases. These benefits are often attributed to high antioxidant content of some plant foods [1]. These benefits are due to the results of antioxidant components of plant origin vitamins, phenols, flavonoids and Carotenoids [2, 3]. Fruits contain many antioxidant compounds which serve as radical scavengers [4]. Recent studies have shown the importance of antioxidant activity of compounds from fruits extracts [5-8].

Syzygium Aqueum, a species in the Myrtaceae family, commonly called 'water apple' is a native to Malaysia. Leaf extract of Water apple strongly suggest the protective activity against free radicals, antioxidant property, antibiotic and also potent antidiabetic [9, 10]. However role of water apple fruits as an antioxidant has not been studied till date. The present study investigated effective antioxidant activity of aqueous extract of Water Apple fruits using different *in vitro* model systems. This would reveal the antioxidant activity of aqueous extract of Water Apple in order to ascertain the scientific proof for its traditional use.

MATERIAL & METHODS

All the chemicals and reagents used Analytical grade were purchased from Chetana chemicals, Mysore and the Merck Co, and S.d. fine chem., Mumbai, India.

Sample Collection

The Water Apple (*S. Aqueum*) fruits were collected from authentic source, Karnataka, India. The collected fruits were cleaned thoroughly with double distilled water and dried under the shade. Once the drying process is complete, the dried fruits were grouned to powder, stored for further studies.

Aqueous Extract Preparation

Aqueous extract prepared according to Dinesha Ramadas *et al*., with minor modifications. Dissolving 5g of powdered Water Apple fruits (*S. Aqueum*) in 100ml of double distilled water, vortexed for 4 hours, centrifuged at 10000 rpm, supernatant collected, lyophilized and stored at -10^{O}C.

Phytochemical Analysis

The extract was tested for the presence of bioactive compounds by adopting standard procedures.

The proteins estimation was carried according to Bradford's method [11] using BSA as standard and absorbance was read at 535nm. Total phenols were determined according to the method of Folin Ciocalteu reaction [12] using Gallic acid as a standard and absorbance was read at 750 nm. Flavonoids estimation was done using Quercetin [13] as a standard; absorbance was measured at 415 nm. Total Sugars estimation was done according to Dubois method [14], the absorbance was read at 520 nm. The concentrations were calculated accordingly using standard graph.

Antioxidant Activity

DPPH Radical Scavenging Activity [15**]**

DPPH radical scavenging activity was assessed according to the method of Shimada *et al.*, [15]. The Aqueous extract of Water Apple at concentrations ranging from 20 to 200 µg mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M sodium acetate buffer pH 5.5. The resulting solutions then incubated at 37°C for 30 min. Ascorbic acid and BHA (20 to 100 µg) used as standards under the same assay conditions. Control was without any standards or extract. The % DPPH radical scavenging activity of extract is calculated from the decrease in absorbance at 517 nm and compared with control. The % DPPH radical scavenging activity is calculated using the following formula:

$$\% \text{ Inhibition of DPPH radical scavenging activity} = \frac{(\text{Abs of control} - \text{Abs of samples})}{\text{Abs of control}} \text{ X } 100$$

Nitric Oxide Radical Scavenging Activity [16]

Nitric oxide scavenging activity was determined according to the method reported by Marocci *et al.*, [18]. Aqueous extract of Water Apple, BHA and Ascorbic acid as standards in the range of 2 –20 µg were taken in respective tubes containing phosphate buffer saline, so that the volume in each tube was made up to 1ml. For controls, volume was made up to 3ml with phosphate buffer saline. Then 2ml of 10mM sodium nitroprusside added to all the tubes except the controls. Nitric oxide radicals were generated from the samples spontaneously during the incubation period of 150 min. 0.5ml of the solution taken from respective tubes. To this 1ml of 0.33% sulphanilamide added and allowed to stand for 5 min for completing diazotization, followed by the addition of 1ml of NED (0.1%) to each tube. Then incubate for 30 min at room temperature. The nitrite ions released were measured at 516nm and % Nitric Oxide radical scavenging activity was calculated using the following formula.

$$\% \text{ Inhibition of Nitric Oxide radical activity} = \frac{(\text{Abs of Control} - \text{Abs of Sample})}{\text{Abs of Control}} \text{ X } 100$$

Superoxide Scavenging Activity [17**]**

The Superoxide radical scavenging activity of aqueous extract of Water Apple was measured according to the method of Lee *et al.*, [17] with minor modifications. The reaction mixture (containing 100µl of 30mM EDTA (pH 7.4), 10µl of 30mM hypoxanthine in 50mM NaOH, and 200µl of 1.42mM nitro blue tetrazolium) with or without extract and standards (Ascorbic acid & BHA) at various concentrations ranging from 2-20µg. After the solution was pre-incubated at ambient temperature for 3min. 100µl of xanthine oxidase solution (0.5U/ml) was added to the mixture and incubated for 1 hour at 37°C, and the volume was made up to 3ml with 20mM phosphate buffer (pH 7.4). The solution was allowed to stand for 20min. Absorbance was measured at 560 nm against a control (without any inhibitor) to determine the quantity of formazan generated. Decreased absorbance indicates increased superoxide anion scavenging activity. The % inhibition was determined as below.

$$\% \text{ Super Oxide radical activity} = \frac{(\text{Abs of Control} - \text{Abs of Sample})}{\text{Abs of Control}} \text{ X } 100$$

Statistical Analysis

All data are expressed as mean ± standard deviation of five replicate (n=5). The significance of the experimental observation was checked by student's t-test and $P < 0.05$ was considered as statistically significant when compared to relevant controls.

RESULTS

The Aqueous extract of Water Apple are analyzed for phytochemicals and their antioxidant activity by DPPH radicals, Nitric Oxide radicals and Superoxide radical scavenging activity, where BHA & ascorbic acid used as standards at different concentrations. The results are expressed as percentage (%) inhibition exhibited by the test extract and the standard antioxidants.

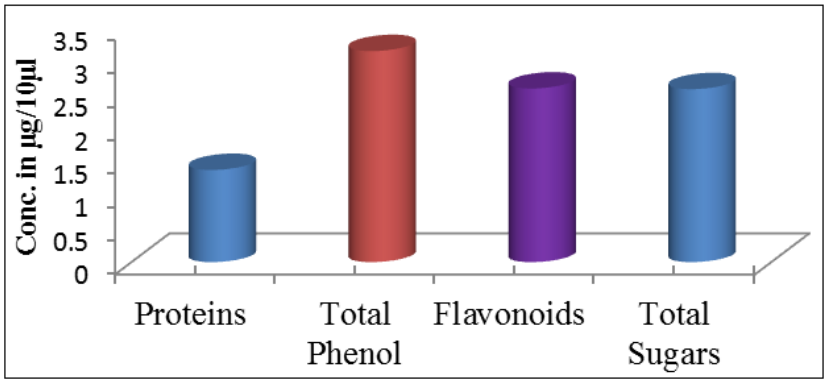

Fig-1: Phytochemicals in Aqueous extract of Water Apple fruits

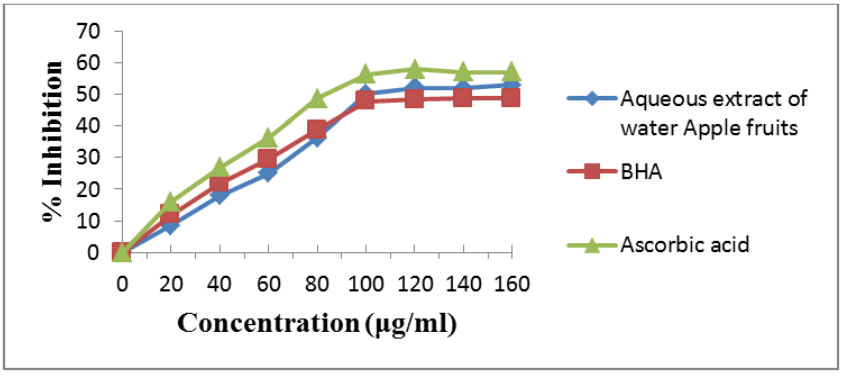

Fig-2: DPPH scavenging activity of Aqueous extract of Water Apple fruits & Standard antioxidants in different concentrations

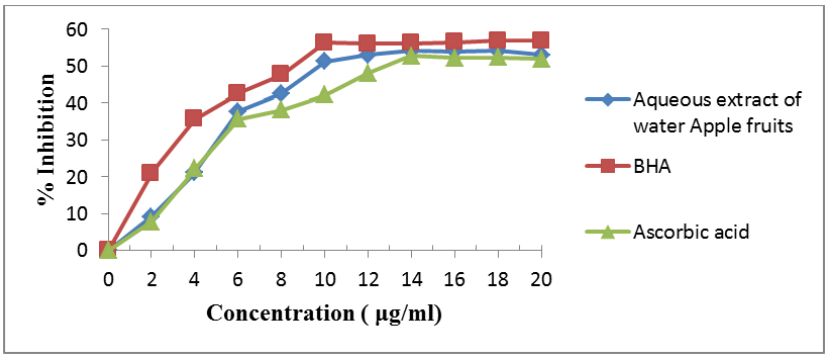

Fig-3: Nitric oxide scavenging activity of aqueous extract of Water Apple fruits & Standard antioxidants in different concentrations

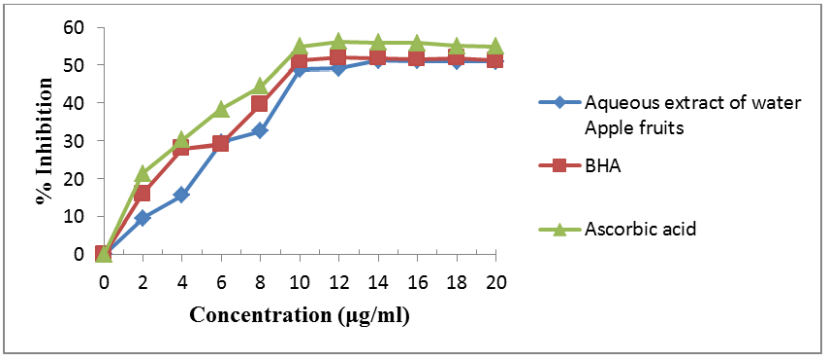

Fig-4: Superoxide radical scavenging activity of aqueous extract of Water Apple fruits & Standard antioxidants in different concentrations

DISCUSSIONS

Aqueous extract of Water Apple is subjected to phytochemical analysis using standard methods. The results are as shown in Fig-1, the phytochemicals like protein (1.24 ± 0.89µg/10µl), total phenols (2.9 ± 0.062µg/10µl), flavonoids (2.3± 0.18µg/10µl) and total sugars (2.59 ± 0.21µg/10µl) are present in water extract. It is found that, more total phenols are present in aqueous extract of Water Apple.

Fig-2, results show the percentage inhibition of DPPH radicals increased with increase in concentrations of aqueous extract of Water Apple. DPPH assay was based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, which denotes the extract has significant antioxidant potential. This observation has also been reported by other researcher [9].

Fig-3, results show nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH reacts with O_2 to form nitrite ion. Aqueous extract inhibited nitrite formation in concentration dependent manner. Antioxidant nature of the extract complete with oxygen to react with nitric oxide. It was observed that, percentage (%) inhibition was increased with the increase in concentration of aqueous extract of Water Apple with various concentrations.

The percentage inhibition of superoxide radical scavenging activity of aqueous extract of Water Apple was increased with increasing concentrations (Fig-4). In this method**,** superoxide anion plays an important role in plant tissues and it involved in the formation of other cell-damaging free radicals. Potent antioxidant capability of the extract is detected by the scavenging potential of the superoxide anion, with their electron donation.

CONCUSSION

Recent years have seen an exponential increase in research antioxidant properties of fruits and vegetables. The health promoting properties of fruits and vegetables may be due to its antioxidant properties and is also attributed to its multi therapeutic characteristics. The results concluded that, the extract of Water Apple fruits have rich total phenols and have good antioxidant activity, when compared to standards. Rich total phenols and other bioactive compounds show the antioxidant activities; it may improve immune functions, prevents heart diseases and certain cancers. Thus, fruits of Water Apple (*S. Aqueum*) extract might be useful in the preparation of medicine. However, in-vivo antioxidant activity and mechanism of action is needed to be further studied.

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