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Antioxidant Properties of Acerola (*Malpighia Emarginata* Dc.) and Acerola squash

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Abstract: ***Antioxidant activity (DPPH radical activity, reducing power, SOA activity, total phenolic content and total flavonoid content) were evaluated in Indian variety of acerola and its squash. The average scavenging DPPH radical activity, reducing power assay and super oxide anion radical activity of acerola fruit ranges from 89.12 ± 0.42% inhibition, 3.047±0.01absorption, 71.110±1.68%, and acerola squash was reported relatively less antioxidant properties. Total phenolic and total flavonid content of fruit is high and reported 809.143±37.792 µg of PE (pyrocatechol equivalent) and 47.947±0.358 µg RE (Rutin equivalent) respectively. Hence acerola squash serves as functional fruit beverage and available in off season.***

Keywords: Acerola, Ascorbic acid, DPPH radical activity, Super oxide anion radical activity

1. Introduction

Oxidative stress is initiated by reactive oxygen species (ROS), such as superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and singlet oxygen (O_2). At normal physiological concentrations ROS are required for cellular activities. They play a positive role in energy production, phagocytosis and regulation of cell growth, intercellular signaling and synthesis of biologically important compounds. However, at higher concentrations, ROS can be toxic leading to the development in over a hundred of diseases which range from arthritis and connective tissue disorders to carcinogenesis, CVD, physical injury, infection and acquired immunodeficiency syndrome (Goody *et al.* 2008, Goomes *et al.* 2002) The most effective way to eliminate ROS which causes the oxidative stress is with the help of antioxidants.

Currently, there is a major concern of consumers about the importance of choosing healthy food as a means of prevention of diseases and improving the quality of life. Fruits are included in this type of food, since they are widely accepted by consumers and they are important sources of antioxidant compounds, vitamins and other nutrients. Acerola (*Malpighia emarginata* DC.) fruit also known as Barbados cherry, as any other minor non-conventional fruit plants, leaves doubt on its origin. It was introduced in Brazil about 50 years back, in the state of Sao Paulo, brought from Puerto Rico (Dinizi *et al.*2003) Various studies have highlighted acerola fruit as one of the best natural sources of vitamin C, surpassing fruits like guava, cashew, orange and lemon, which are excellent sources of this vitamin (Santos *et al.* 2009). About 100 g of juice possesses 50 to 100 times more of this vitamin than that of an equal quantity of lemon or orange juice (Glucin *et al.* 2004). Recently, much attention has been paid to their content in carotenoids and bioflavonoids for their antioxidant properties (Mezadri *et al.* 2008)

The association between the consumption of fruit and vegetables and a decreased risk of cardiovascular disease and cancer is supported by considerable epidemiological evidence. This beneficial effect is due to the action of antioxidant compounds, which are capable of neutralizing free radicals and reduce oxidative damage in the body (WHO, 2003).For this reason, the interest in the evaluation of antioxidant activity of fruits and vegetables has substantially increased. Therefore, the aim of this work was to evaluate the antioxidant Properties of Acerola and its squash.

2. Materials and Methods

2.1 Procurement and Preparation of the samples

The acerola fruits evaluated in this study were harvested during the September - October 2012, from different trees in the botanical garden of Acharya N G Ranga Agricultural University, Hyderabad, India. The fruits were picked up in the early hours of the day, when they were in ripening state, which is when their peel presents a reddish color. Arriving at the laboratory, the fruits were selected (uniform size, characteristic color, absence of physical flaws, mechanical damage or visible microbial infection), in order to form a homogeneous sample for several determinations.

2.2 Squash

The Barbados cherry fruits at stage of maturity were selected, cleaned with chlorine (2 ppm of active Cl2) water and triturated in a blender, thus obtaining the pulp is strained and used for preparation of squash preparation. The strained Juice is measured and mix with syrup (sugar 600gms $^+$ water 30ml + acid heating just to dissolve 1gms), and addition of preservative (1.0gm of sodium benzoate/liter squash) and then Bottling and Storage in refrigerator at 4^0C (Srivastava. and Sanjeeva 2004).

Sample preparation: Extract was prepared by using 50g fruit and 50 ml of squash by adding equal quantity of acetone, methanol and water (250ml). The extracts were centrifuged, filtered and kept in amber colored screw cap bottles at -20^0C until further analysis.

The filtered extract was used for analysis of Scavenging DPPH radicals (Dorman *et al.,* 2004). Reducing power

assay (Oyaizu, 1986), super oxide anion radical scavenging activity (Nishimiki *et al.,* 1972), Total phenolic compounds (Folin-Ciocalteu reagent according to the method of Slinkard and Slingleton, 1997) and Total flavonoid content (Meda *et al.,* 2005).

Scavenging DPPH radicals: The DPPH free radical concentration was calculated using the following equation:

DPPH scavenging effect (%) $= (A_0 - A_1 / A_0) X 100$

Where, A_0 was the absorbance of the negative control or blank and A_1 was the absorbance of reaction mixture or standards.

Superoxide anion radical scavenging activity: The following formula was used to calculate the percentage inhibition of superoxide anion generation.

Superoxide anion scavenging activity (%) $= (A_0 - A_1/A_0) \times 100$

Where A_0 is the absorbance of the negative control consisting of all the reaction agents except the extract); A_1 is the absorbance of reaction mixture or standards.

Total phenolic compounds: The total phenol content in the extract was determined as microgram of PE (Pyrocatechol equivalent) according to the equation that was obtained from standard pyrocatechol graph as:

Absorbance = 0.0021 × total phenols [µg pyrocatechol equivalent]- 0.0092

Total flavonoid content: The total concentration of flavonoids in the extracts was determined as microgram of RE (Rutin equivalent) according to the formula that was obtained from standard rutin graph as

Absorbance = 0.0144 × total flavonoid [µg rutin equivalent] + 0.0556

Statistical analysis: The results obtained were subjected to statistical analysis with the window STAT programme. Mean and standard deviation for three parallel replicates were calculated.

3. Result and Discussion

3.1 Scavenging DPPH Radicals:

The scavenging activity of DPPH radicals studied in acerola and acerol squash is shown in the Table 1. The free radical scavenging activity slightly decreased in squash, (88.18±0.145%) compared to fresh fruit (89.117±0.42%). Blueberry juice shows a very strong radical-scavenging activity even considering that the juice samples were diluted before being analysed. Blanching of fruit greatly increased the radical-scavenging activity of the juice, in relation to the higher recovery of anthocyanin pigments and total cinnamates (Rossia *et al.,* 2003)

Reducing power assay: Reducing power assay measures the electron-donating capacity of an antioxidant. The reduction of the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) is measured by the intensity of the resultant blue-green solution which absorbs at 700 nm, and an increased absorbance is indicative of higher reducing power (Nagendran *et al.,* 2005).The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity. The reducing properties are generally associated with antioxidant action of reductones. The results of reducing power activity assay of Acerola fruit and acerola squash are summarized in the table 1. High absorbance indicates high reducing power. High Reducing power was observed in raw fruit (3.047±0.015) where as lower reducing power was observed in acerola squash (1.870±0.98). This may be reduction in reductones such as ascorbic acid content in squash as compared to fresh fruit.

Superoxide anion radical scavenging activity: Results of Superoxide anion radical scavenging activity of fresh fruit and squash made with acerola is presented in Table 1 Super oxides are produced from molecular oxygen due to oxidative enzyme of body as well as via non-enzymatic reaction such as autoxidation by catecholamines. Although Superoxide anion radical scavenging activity cannot directly initiate lipid oxidation, super oxide anion radicals are potential precursors of damaging oxygen species and thus the study of the scavenging of this radical is important (Jayasri *et al.,*2009). The Superoxide anion radical scavenging activity increased in squash (97.333 ±1.335%) as compared to the fresh fruit (71.11±1.681%). A study by Sunil *et al*. (2011) reported that overall cellular activities of SOD, POX, CAT, APX and GR decreased during storage of ber fruits, which explains their reduced ability to scavenge the toxic oxygen species accumulated during storage.

3.2 Total phenolic content

The Folin-Ciocalteu method (Slinkard, and Singleton 1999) was used to measure total amount of phenolic compounds in given sample. The number of –OH or potentially oxidizable groups in each sample creates a color change (Barbara *et al.,* 2005). The antioxidant activity of phenolic compounds is mainly attributed for their redox actions, neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. The results of total phenolic content of acerola fruit are given in table 1. The total phenolic content in acerola was found to be 809.14 ± 37.80 µg of PE (pyrocatechol equivalent) where as squash had 168±1.10 µg of PE. Verdramini and Trugo (Vendramini *et al.,* 2004) reported that the phenolic compounds detected in acerola may be classified in two categories; phenolic anthocyanin pigments and non-anthocyanin phenolics. The pigments detected were a 3,5-diglycosilated malvidin, a 3-monoglycosilated cyanidin and pelargonidin. Nonanthocyanin phenolic compounds identified in acerola were pcoumaric acid, ferulic acid, caffeic acid, chlorogenic acid, kaempferol and quercetin.

3.3 Total flavonoid content

The protective effect of flavonoids is due to several mechanisms such as free radicals trapping, enzymes inhibition and metallic ions chelation. These properties depend on the structure of the flavonoids and the degree of substitution and saturation. Fruits and vegetables are rich source of flavonoids (Ioannou *et al.,* 2012).

The results of total flavonoids content of acerola are mentioned in Table 1. Total flavonoids content in Raw acerola was 47.947±0.358 µg of rutin equivalent (R E) but it decreased in acerola squash (3.20±0.08 µg R.E). Addie *et al.* (2002) reported that the levels of flavonoids and chlorogenic acid in the juice were reduced to between 50% (chlorogenic acid) and 3% (catechins) and most of the antioxidants were retained in the pomace. Hence, the acaerola squash has lower total flavonoid content than fresh fruit.

4. Conclusion

Acerola is a fruit with a high content of phytochemicals with proven antioxidant activities. Acerola fruit presents high in vitro antioxidant activity, demonstrated with DPPH, reducing power, superoxide anion radical activity and total phenolics Among them, phenolic compounds are the main contributors to the antioxidant activity. These results therefore indicate that acerola fruit is a potent antioxidant food and might have potential value as a functional food ingredient.

5. Future Scope

Acerola fruits are rich in antioxidant properties Hence further *inviva* study of antioxidant activity of acerol is required to explore its potentiality and usage in health and functional food.

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Table 1: Antioxidant properties of acerola and acerola squash

Method of Antioxidant Properties	Acerola	Acerola squash
DPPH (% inhibition)	89.12±0.42	88.18±0.145
Reducing power (absorbance)	3.047±0.015	1.870±0.98
Super oxide anion radical activity (%)	71.11±1.68	97.333 ±1.335
Total phenolics (µg PE)	809.14± 37.80	168±1.10
Total flavonoid content (µg PE)	47.947±0.358	3.20±0.08

Values are represented in Mean ± SD from triplicate observations