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ANTIOXIDANT AND TOTAL PHENOL CONTENTS OF SELECTED ACIDIC FRUITS CONSUMED IN JAFFNA PENINSULA

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ABSTRACT

*The aim of the study was to determine the antioxidant content and total phenol content of acidic fruits such as sour orange (Citrus spp), lime (Citrus latifolia), sweet orange (*Citrus sinensis*), pineapple (*Ananascomosus*), pomegranate (Purnica granatum), strawberry (Fragaria spp), kiwi fruit (Actinidia deliciosa), gooseberry (Phyllanthus emblica) and wood apple (Feronia elephantum) available in Jaffna. Total phenolics were measured using the Folin Cio-calteu reagent with gallic acid as standard. The antioxidant contents of leafy vegetables were assayed by both phosphomolybdenum assay and reducing power assay with standards of ascorbic acid and butylated hydroxyl toluene respectively. Based on the phosphomolybdenum assay among the acidic fruits, highest antioxidant content was observed in straw berry [1875.88 (±0.30^{a}) mg/100g Dry Weight], and the lowest antioxidant content was detected in lime [69.843 (±0.42^{i}) mg/100g Dry Weight]. Based on the reducing power assay, highest antioxidant content was found in strawberry [175.16 (±0.23^{a}) mg/100g dry sample], and lowest antioxidant content was found in lime [7.948(±0.78^{i}) mg/100g Dry Weight]. Highest total phenol content was found in gooseberry [925.29 (±0.5^{a}) mg/100g Dry Weight] and lowest total phenol content was detected in lime [21.388 (±0.26^{i}) mg/100g Dry Weight]. From this study, highest antioxidant content and total phenol contents were found in strawberry and gooseberry while lowest amounts were found in lime. The present study shows that acidic fruits contain a lot of antioxidants and total phenols to support human health.*

Key words: ***Antioxidant, total phenol, ascorbic acid, gallic acid, butylated hydroxyl toluene***

1. Introduction

Oxidative stress has been defined as a disturbance in the equilibrium status of pro-oxidant/antioxidant systems in intact cells resulting in oxidative damage to lipids, proteins, carbohydrates, and nucleic acids, contributing to pathological dysfunction in the organism (Sies,1985). Antioxidants present in the diets can prevent the oxidation of cellular materials. Due to that oxidation of the cellular membrane and other easily oxidisable elements and cellular materials can be damaged and which can cause several illnesses. Consumption of antioxidants can prevent or decrease the diseases such as atherosclerosis, diabetes, neurodegenerative diseases, ageing and cancer (Kuo*et al*., 1997;McCord *et al*., 2000; Sun*et al*., 2002; Yang*et al*.,2001). It is always advisable to consume naturally occurring antioxidants rather than good for medication. Food samples such as berries, beans, red cabbage and grapes, etc, are recommended as good sources of antioxidants. Much attention has been focused on the activity of the natural antioxidants present in fruits, because potentially these components can reduce the level of oxidative stress.

Analysis of antioxidant contents and total phenol contents of acidic fruits are important for healthy life. The aim of our study is determination of antioxidant and total phenol contents of fruits which are commonly consumed in Jaffna Peninsula.

2. Literature Review

Oxygen is essential element for life to perform biological functions such as catabolism of fats, proteins and carbohydrates in order to generate energy for growth and other activities. However, a parallel role of oxygen as a toxic agent for living tissues has also been discovered. Oxygen, though not dangerous by itself, is involved in generation of various kinds of "reactive oxygen species" (ROS). ROS, formed during metabolism or through the action of ionizing radiation, can interact with bio-molecules and ultimately lead to an onset of degenerative diseases such as cancer, cardiovascular diseases (CVD) and other illnesses. To protective against the destructive action of free radicals, mature has created an antioxidant defence system composed of a group of compounds and enzymes potent enough to remove free radical before they cause tissue damage. Some antioxidants are produced in the body, while others must be sequestered from the diet or through supplementation. Most of fruits and vegetables have high antioxidant content. There are thousands of naturally occurring and synthetic antioxidants known. These antioxidants belong to different classes of compounds, such as carotenoides, poly phenolics, polyamines, gallic acid derivatives, tannins, and catechins. Examples include phytic acid, lipoic acid, bilirubin, melatonin, quercetin, carnosal, carnosic acid, hydroxyl tyrosal, rutin, butilated hydroxyl anisole, and butylated

hydroxyl toluene, vitamin E and C are among the most effective antioxidants with preventive effects against heart diseases and cancers.

3. Materials and methods

3.1 Materials

Nine different acidic fruits such as sour orange (*Citrus spp*), lime (*Citrus latifolia*), sweet orange (*Citrus sinensis*), pineapple (*Ananascomosus*), pomegranate (*Purnicagranatum*), strawberry (*Fragaria spp*), kiwifruit (*Actinidiadeliciosa*), gooseberry(*Phyllanthusemblica*)and wood apple (*Feronia elephantum*) were selected for this study and were purchased from local market, home garden and super markets.

All other chemicals used were of analytical grade and were obtained from standard sources.

3.2 Preparation of extracts

Each fresh sample (1g) was weighed and ground with a chilled mortar and pestle with 10ml of 0.05M TrisHCl, 3mM $MgCl_2.7H_2O$and 1mM EDTA buffer solution (pH 7.0). The extract was centrifuged at 4°C for 20 min at 3000 rpm. Supernatant was used to determine the antioxidant content (phosphomolybdenum assay method) and total phenol content (Padmaja*et al*., 2011).

3.3 Determination of total phenol content

To extract (0.2ml), Folin Ciocalteu reagent (1ml) and 0.8ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30min. Absorbance was measured at 765nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 100gram of extract (Singleton and Rossi, 1965).

3.4 Determination of antioxidant content by Phosphomolybdenum method

Extract (0.1ml) was mixed with ammonium molybdate solution (3ml) and mixed well. Tubes were incubated at 95ºC for 90 min. The mixture was cooled to room temperature, and absorbance of the solution was measured at 695 nm. The antioxidant content was expressed as Ascorbic Acid Equivalents (AAE) in milligrams per 100gram of the extract (Prieto *et al*., 1999).

3.5 Determination of antioxidant content by ferric reducing power assay method

Preparation of ethanol extract

Fresh sample (2g) was weighed and ground with a chilled mortar and pestle with of 99% ethanol (20ml). Finally, extract was filtered through filter paper (Whatman No 1).

Ferric reducing power assay method

Extract (1ml), 2.5ml of phosphate buffer (pH 6.6) and1% potassium ferricyanide (2.5ml) were incubated at 50°C for 30min and 10% trichloroacetic acid (2.5ml) was added to the mixture and centrifuged at 3000rpm for 10min. Supernatant (2.5ml) was diluted with distilled water (2.5ml) and shaken with freshly prepared 0.1% ferric chloride (0.5 ml). Absorbance was measured at 700nm. Antioxidant content was expressed as butylated hydroxyl toluene (BHT) equivalents in mg per 100gram sample (Ferreira *et al*., 2007).

4. Results and Discussion

As the phenolic content and antioxidant content are expressed in terms of gram per dry weight, moisture content of the samples was determined. Table 1 shows the moisture content, antioxidant contents of acidic fruits by phosphomolybdenum assay and reducing power assay) and total phenol contents of the acidic fruits. Moisture content of acidic fruits ranged from 68.45 to 92.20%. The similar results were found by Bastin S and Henken K, 1994. Among the acidic fruits, highest moisture content was found in orange [92.20 (±0.18)%, followed with strawberry [91.84 (±0.31)%], lime [89.62 (±0.65)%], sour orange [87.05 (±0.24)%], pine apple [85.24 (±0.23)%], goose berry [84.67 (±0.19)%], kiwi fruit [84.58 (±0.11)%] and Pomegranate [75.97 (±0.37)%] and lowest moisture content was detected in Wood apple [68.45 (±0.44)%]. The high moisture content of fruits makes them to aid the digestion of food. Their shelf life is very short because the high moisture facilitates bacterial action resulting into spoilage.

The phosphomolybdenum method is based on the reduction of molybdenum by the antioxidants and the formation of a green molybdenum (V) complex, which has absorption at 695 nm. The reduction of Mo (VI) to Mo (V) by administration of reference chemicals; ascorbic acid, suggested the presence of effective antioxidants. Therefore, the results were correlated with ascorbic acid content of acidic fruits. Antioxidant contents of acidic fruits (Phosphomolybdenum assay) significantly ($p< 0.05$) differed from each other. Among the acidic fruits, highest antioxidant content was observed in straw berry [1875.88 ($\pm 0.30^{a}$)mg/100g Dry Weight], followed with goose berry [1572.85 ($\pm 0.12^{b}$) mg/100g Dry Weight], pineapple [1077.18 ($\pm 0.09^{c}$)mg/100g Dry Weight], kiwi fruit [670.2 ($\pm 0.85^{d}$) mg/100g Dry Weight), pomegranate [598.64 ($\pm 0.29^{e}$)mg/100g Dry Weight), Orange[445.6 ($\pm 0.13^{f}$) mg/100g Dry Weight], wood apple[340.03 ($\pm 0.53^{g}$)) mg/100g Dry Weight] and sour orange [310.89

(±054[h])mg/100g Dry Weight] and lowest antioxidant content was obtained in lime [69.843 (±0.42[i]) mg/100g Dry Weight].

The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow colour of the test solution changes to green and blue depending on the reducing power of test specimen. Greater absorbance at 700 nm indicated greater reducing power. In this method, antioxidant contents were expressed in BHT equivalent. BHT was used as the standard instead of vitamin E. Therefore, the results were correlated with vitamin E content of spices. BHT is the fat soluble antioxidant. Because it is not dissolved in tris buffer extract. Therefore, ethanol was used to prepare the standards and extracts in this method. Antioxidant contents of leafy vegetables based on reducing power assay differed significantly ($p< 0.05$) from each other. In reducing power assay, highest antioxidant content was found in found in strawberry [175.16 (±0.23[a]) mg/100g Dry Weight] followed with Goose berry [169.56 (±0.38[b]) mg/100g Dry Weight], Orange[80.838 (±0.48[c]) mg/100g Dry Weight],Wood apple[67.01 (±0.46[d]) mg/100g Dry Weight], pomegranate [53.79 (±0.19[e]) mg/100g Dry Weight], pineapple[34.76 (±0.23[f]) mg/100g Dry Weight],kiwi fruit [21.61 (±0.06[g]) mg/100g Dry Weight] and sour orange [10.93 (±0.33[h]) mg/100g Dry Weight] and lowest antioxidant content was detected inlime [7.948(±0.78[i]) mg/100g Dry Weight] among acidic fruits.

The plant phenolic compounds are responsible for effective free radical scavenging and antioxidant activities (Velioglu*et al*., 1998). Total phenol contents of acidic fruits significantly ($p< 0.05$) differed from each other. Whereas, highest total phenol content was found in gooseberry[925.29 (±0.5[a])mg/100g Dry Weight] followed with strawberry [126.47 (±0.93[b]) mg/100g Dry Weight], Orange[64.687 (±0.70[c]) mg/100g Dry Weight],Wood apple[37.29 (±0.24[d])) mg/100g Dry Weight], pomegranate [29.41 (±0.57[e]) mg/100g Dry Weight), sour orange[28.472 (±0.17[f]) mg/100g Dry Weight], pineapple [27.80 (±0.68[g]) mg/100g Dry Weight] and kiwi fruit [23.40 (±0.07[h]) mg/100g Dry Weight] and lowest total phenol content was detected in lime[21.388 (±0.26[i]) mg/100g Dry Weight] among acidic fruits.

The antioxidant activity obtained by three methods *viz* phosphomolybdenum assay, ferric reducing assay and total phenolic content determination, are almost same as shown in table 1, though the value obtained by phosphomolybdenum assay was highest among the three methods and least values were observed in ferric reducing power assay method. A comparison of the values of the antioxidant activity observed in the present investigation with the data in the literature was problematic due to large variability and lack of standardization of the assay methods. Therefore, for discussion the ranking order of the antioxidant capacity of fruits is used. The highest antioxidant activity was observed in straw berry followed with goose berry, pineapple, kiwi fruit,

pomegranate, orange, wood apple, sour orange and lowest antioxidant content was obtained in lime. Berries are an important dietary source of vitamin C, minerals and amino acids and also contain phenolic compounds and tannins, phyllembelic acid, phyllemblin, rutin, curcuminoides and emblicol and vitamin C content alone is about 2% (Yokozava*et al*., 2007). Thus, the highest antioxidant activity observed for berries in present investigation may be due to high content of vitamin C and other compounds which have antioxidant activity.

It is also known that majority of antioxidant capacity of fruits are contributed by polyphenols, vitamin C, vitamin E, Maillard reaction products, β- carotene and lycopene. The differences in the antioxidant activities among the fruits could be attributed to their differences in phenolic contents and compositions and other non- phenolic antioxidants present in the samples (Hassanien, M.A.R., 2008)

5. Conclusion

The present study demonstrated that acidic fruits showed differences in their antioxidant content and total phenol content. From that observation it can be concluded that strawberry, goose berry and pineapple are good sources of natural antioxidants.

***Table 1**: Moisture, antioxidant and total phenol contents of acidic fruits.*

Acidic Fruits	**Moisture content (%)**	**Antioxidant content (mg/100g dry sample)**		**Total phenols (mg/100gdry sample) (gallic acid equivalent)**
		Phosphomolybdenum Assay (Ascorbic acid equivalent)	**Reducing Power Assay (Butylated hydroxyl toluene equivalent)**	
Orange	92.20 (±0.18)	445.6 (±0.13^{f})	80.838 (±0.48^{c})	64.687 (±0.70^{c})
Lime	89.62 (±0.65)	69.843 (±0.42^{i})	7.948(±0.78^{i})	21.388 (±0.26^{i})
Sour Orange	87.05 (±0.24)	310.89 (±054^{h})	10.93 (±0.33^{h})	28.472 (±0.17^{f})
Pine apple	85.24 (±0.23)	1077.18 (±0.09^{c})	34.76 (±0.23^{f})	27.80 (±0.68^{g})
Straw berry	91.84 (±0.31)	1875.88 (±0.30^{a})	175.16 (±0.23^{a})	126.47 (±0.93^{b})
Pomegranate(local)	75.97 (±0.37)	598.64 (±0.29^{e})	53.79 (±0.19^{e})	29.41 (±0.57^{e})
Kiwi fruit	84.58 (±0.11)	670.2 (±0.85^{d})	21.61 (±0.06^{g})	23.40 (±0.07^{h})
Goose berry(Nelli)	84.67 (±0.19)	1572.85 (±0.12^{b})	169.56 (±0.38^{b})	925.29 (±0.5^{a})
Wood apple(ripe)	68.45 (±0.44)	340.03 (±0.53^{g})	67.01 (±0.46^{d})	37.29 (±0.24^{d})

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