

Total phenolic content and In vitro antioxidant activity of four indigenous Citrus fruits of North East India

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Abstract - The interest has increased in the consumption of Citrus fruits, it is not only due to their sweet refreshing properties but also because of their other important properties like medicinal, antioxidant and nutritional values. Four different indigenous varieties of Citrus namely *Citrus grandis* var Robab, *Citrus grandis* var Khaloi, *Citrus sinensis* var Valencia, and *Citrus sinensis* var Sohniangriang have been collected from native places of Assam and Meghalaya of North East India. Direct juices have been extracted from ripen citrus fruits for different biochemical estimations. Total phenolic content and antioxidant scavenging activity have been found to be in very good and significant amount. For scavenging activity of the juices different concentrations of juices were taken and the scavenging activity was found to be increasing in significant amount with increase in the juice concentration. Total acidity (in terms of citric acid content) and pH were also determined.

Keywords - total phenolic, antioxidant activity, citrus, indigenous, Rutaceae, catechol.

1 INTRODUCTION

Genus Citrus belong to family Rutaceae and sub family Auratioidae. It is believed that most of the species under genus Citrus are native to tropical and subtropical regions of South East Asia, particularly India. North East India and South East Asia are considered to be the centre of origin and diversity of Citrus species (Borthakur, 1992). Among this only few like orange, lime and lemon are cultivated on commercial basis; the rest are grown as back ward crop but with good demand in local market. Citrus fruits not only sustain a major industry but also sustain rural economy being source of extra income for farmers. Citrus fruits are helpful to reduce the risk of pregnant women to have children with birth disease (Economos, 1999). Due to the hesperidin content as well as diosmine and other flavonoids, the citrus fruit reinforces the stability of the capillary vessels and improves venous blood flow (Rio *et al.*, 1997; Economos, 1999; Roger, 2002). They are useful in cases of swollen legs, edema, varicose veins, hemorrhoids, thrombosis and emboli (Roger, 2002). Many of the diseases are mainly linked to oxidative stress due to free radicals (Gutteridge, 1995). Antioxidants have been reported to prevent the occurrence of diseases, cancer and aging.

Reactive oxygen species, which consist of free radicals such as superoxide anion, hydroxyl radicals and non-radical species such as hydrogen peroxide, singlet oxygen are different forms of activated oxygen (Halliwell and Gutteridge, 1999, Yildirim *et al.*, 2000, Glucin *et al.*, 2002b). Over the last decades, many other virtues and medicinal benefits of citrus fruits have been discovered besides the anti-scurvy property (Rapisarada *et al.*, 1999). The protection that citrus fruits provide against these diseases has been attributed to the various antioxidant phytonutrients contained in citrus species (Okwu and Emenike, 2006; Rapisarada *et al.*, 1999).

The present study is focused on the evaluation of antioxidant activity and total phenolic content of ripen juice of four indigenous cultivars of N.E India.

2 Materials and methods

Citrus fruits namely *Citrus grandis* var Robab, *Citrus grandis* var Khaloi, *Citrus sinensis* var Valencia, *Citrus sinensis* var Sohniangriang for the present study were collected from native places of Assam and Meghalaya of North East India.

2.1 Determination of Edible and juice percent of whole fruit and edible part: The fruits were washed thoroughly in water and dried. Weight was taken for the samples both for total fruit and without the peel. The juices were extracted by cutting the fruits in half and carefully squeezing to extract juices. The collected juices were filtered through 4 fold muslin cloth.

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2.2 Estimation of total acidity and pH: This was determined by Khosa *et al*, 2011 method. pH of 10% juices was determined using pH meter. Total acidity of the juices was determined by titration method. 10% fruit juices were prepared in distilled water. 10mL of the 10% juices were titrated against 0.1N sodium hydroxide using phenolphthalein as indicator. The colour change from colourless to pale pink was recorded as end point. Total acidity was calculated in terms of citric acid by using the formula-

$$\text{Acidity (g/100mL)} = \text{Normality of the juice} \times \text{Equivalent weight of citric acid}$$

The values were expressed as mg/ml.

2.3 Estimation of Total phenolic content: The total phenolic content in the fruit juices was estimated by Folin-Ciocalteu method (Thimmaiah S, 1999). 250 μ L juice was mixed with distilled water to make final volume up to 3mL. 0.5mL Folin- Ciocalteu was added. 20% sodium carbonate was added and tubes were kept in boiling water for 1hr. Optical density was recorded at 650 nm. The values were expressed in terms of catechol. The result was expressed as mg/mL juice.

2.4 Antioxidant activity by DPPH free radical scavenging assay: The radical scavenging activity was determined by Kekuda *et al*, 2010 method on the basis of scavenging ability of the fruit juices on the DPPH free radical. Different concentration of juices were prepared (from 12.5 μ L to 100 μ L) in methanol. After incubation at room temperature for 30 min, optical density was measured at 517nm and the scavenging activity was calculated by the formula-

$$\text{Scavenging activity (\%)} = [(A-B)/A] \times 100$$

Where A = absorbance of DPPH and B = absorbance of fruit juice and DPPH combination.

3 RESULTS AND DISCUSSION

Direct ripen juices of citrus have shown a good amount of antioxidant scavenging activity and total phenolic content. The results have been shown in table 3.1 and table 3.2.

Out of all the fruits taken into account the maximum edible part percent is recorded in *Citrus grandis* var Khaloi as 59.47 % followed by 51.79% for *Citrus sinensis* var Valencia, 44.91% for *Citrus sinensis* var Sohniangriang and 43.54% for *Citrus grandis* var Robab.

The pH range from 2.24 to 3.68. Highest acidity was recorded in *Citrus sinensis* var Sohniangriang (2.24) followed by *Citrus grandis* var Khaloi (3.12), *Citrus grandis* var Robab (3.24) and *Citrus sinensis* var Valencia (3.68).

Total acidity (mg/ml citric acid) was recorded maximum in *Citrus sinensis* var Sohniangriang as 54.76 followed by *Citrus grandis* var Robab (18.25), *Citrus sinensis* var Valencia (16.33) and *Citrus grandis* var Khaloi (10.57).

Citrus grandis var Robab had maximum scavenging activity which is found to be 96.66% for 100 μ L followed by *Citrus grandis* var Khaloi (95.67%), *Citrus sinensis* var Valencia (89.97%) and *Citrus sinensis* var Sohniangriang (77.79%).

For total phenolic the maximum content value (mg/mL) is recorded highest in *Citrus sinensis* var Valencia as 0.00375 followed by *Citrus grandis* var Khaloi (0.00345), *Citrus grandis* var Robab (0.0032) and *Citrus sinensis* var Sohniangriang (0.00315). So citrus fruits can be considered as one of the important food which can be consumed for medical purpose because of its strong antioxidant scavenging activity and total phenolic content.

4 CONCLUSIONS

Citrus fruits are used globally. They have a strong flavor and rich in their total phenolic content. They also have a strong antioxidant activity. Most of the Citrus varieties are indigenous to North East India. So it is place of great research.

Table3.1 Values of different biochemical estimations

Species	% whole fruit	% edible part	pH	Total acidity (mg/mL)	Total phenolic (mg/mL)
<i>Citrus grandis</i> var Robab	30.500	43.54	3.24	18.25	18.25
<i>Citrus grandis</i> var Khaloi	38.500	59.47	3.12	10.57	10.57
<i>Citrus sinensis</i> var Valencia	44.615	51.79	3.68	16.33	16.33
<i>Citrus sinensis</i> var Sohniangriang	25.360	44.91	2.24	54.76	54.76

Table3.2 DPPH free radical scavenging activity

Concentration (μL)	<i>Citrus grandis</i> var Robab	<i>Citrus grandis</i> var Khaloi	<i>Citrus sinensis</i> var Valencia	<i>Citrus sinensis</i> var Sohniangriang
100	96.66	95.67	89.97	77.78
50	91.19	90.01	82.62	55.55
25	88.38	84.50	66.56	32.54
12.25	81.02	76.00	49.18	30.15

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