

Antioxidant activities of the unripen and ripen *Citrus aurantifolia* of Assam

Sony Kumari¹, Neelanjana Sarmah², A.K. Handique³

Asst. Prof., Department Of Biotechnology, University of Science & technology, Ri-bhoi, Meghalaya, India¹

P.G scholar, Department Of Biotechnology, University of Science & technology, Ri- bhoi, Meghalaya, India²

Professor, Department Of Biotechnology, Gauhati University, Guwahati, Assam, India³

Abstract: The in-vitro antioxidant activities of juices of both unripen and ripen *Citrus aurantifolia* (Assamese: kaji nemu) was determined. Juices were extracted from fresh ripen and unripen fruits by mechanical squeezing and were used for the present study. The antioxidant activity of juice was evaluated by using the free radical scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), total antioxidant capacity (TAC) by the phosphomolybdenum method. pH and total acidity was also determined. Unripen juices showed more antioxidant scavenging activity as compared to ripen juices. The lower antioxidant activity of ripe fruit juices could be due to the possible reduction in the ascorbic acid and total phenolic content during ripening. The total antioxidant capacity in terms of ascorbic acid was also determined. Comparison was done for both types of juices. Our study reveals that juices of unripen and ripen *Citrus aurantifolia* possess strong antioxidant activity to provide protection against free radicals induced damage to biomolecules.

Keywords: Antioxidant, phenolics, free radical, DPPH, total antioxidant

I. INTRODUCTION

The interest in citrus fruits has been increased for its different important roles like anti-oxidant, nutritive, antimicrobial, antioxidant and many more other properties. 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 consumption of fruit juices, beverages and hot drinks has been inversely associated with morbidity and mortality from degenerative diseases (Gillman *et al.*, 1995; Rimm *et al.*, 1996; Cohen *et al.*, 2000; Rodriguez – Bernaldo de Quiros and Costa, 2006). A number of health protective effects of phenolic compounds have been reported due to their antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antimicrobial and other biological possessions (Robinns, 2003; Morton *et al.*, 2000).

Lemon is an important medicinal plant of the family Rutaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (*viz.*, leaves, stem, root and flower) of Lemon against clinically significant bacterial strains has been reported (Kawaii *et al.*, 2000).

Antioxidants are the substances, compounds or nutrients in our foods which can prevent or slow oxidative damage to our bodies. These agents are able to remove the deleterious effects of free radicals within our body. Now adays, considerable interest is focused on the development and evaluation of natural antioxidants and radical scavengers from plant materials which are rich in polyphenolic compounds. Many of these substances prevent damage to cell membrane and other structures by neutralizing free radicals. Ascorbic acid is the most important antioxidant in citrus fruit juices and it protects the organism from oxidative stress (Fernandez- Lopez *et al.*, 2005; Ebrahimzadeh *et al.*, 2004). Flavanones, flavones and flavonols are three types of flavonoids which occur in Citrus fruit (Calabro *et al.*, 2004) . Many papers have reported antioxidants in juice and edible parts of lemons of different origin and from different varieties (Miller and Rice-Evans, 1997; Rapisarda *et al.*, 1999; Roberts and Gordon, 2003). Also Larrauri *et al.*, (1996) compared lime and orange peel fibre with a- tocopherol and BHA.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 2, Issue 9, September 2013

II. MATERIALS AND METHODS

A. Extraction of Juice

Fresh unripen and ripen samples of *Citrus aurantifolia* were collected from local market of Guwahati, Assam, India. The fruits were washed thoroughly in water and dried. The peel was removed carefully avoiding any cut or damage to the pulp. The juice was extracted by mechanical squeezing by cutting the fruits in half. The collected juice was filtered through 4 fold muslin cloth, centrifuged at 5000 rpm for 8 mins. Supernatant was collected and used for the present study.

B. Determination of pH

pH was determined immediately after juice extraction.

C. Determination of total acidity:

Total acidity of the juice was determined by titration method against sodium hydroxide using phenolphthalein indicator (Khosla *et al.*, 2011). Total acidity was determined immediately after extraction of the juices and the result was expressed as mg/mL juice.

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

D. Total antioxidant capacity (TAC) by phosphomolybdate method

Total antioxidant capacity by phosphomolybdenum assay was determined (Pietro *et al.*, 1999). The basic principle is the scavenging ability of fruit juices on the phosphomolybdenum reagent. Different concentration of juices were prepared (from 25 μ L to 200 μ L) in distilled water. 4.5 mL phosphomolybdate reagent was added. After incubation at 95°C temperature for 90 minutes, optical density was measured at 695nm and ascorbic acid (0.25mg/mL) was used as standard. Total antioxidant capacity was calculated by the formula-

$$\text{Total antioxidant capacity (\%)} = [(As-Ac)/(Aaa - Ac)] \times 100$$

Where, Ac = control absorbance, As = sample absorbance, Aaa = ascorbic acid absorbance.

Total antioxidant activity was also determined in terms of ascorbic acid (in mg/mL).

E. Antioxidant activity by DPPH free radical scavenging assay

The antioxidant activity on the basis of scavenging ability of the fruit juices on the DPPH free radical was determined (Kekuda *et al.*, 2010). 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.

III. RESULT AND DISCUSSION

A. pH record

The pH for both the ripen and unripe juices are given in the TABLE 1. It is recorded more in the ripen juice as 2.60 and in unripen it is found to be 2.38.

**International Journal of Innovative Research in Science,
Engineering and Technology**

(An ISO 3297: 2007 Certified Organization)

Vol. 2, Issue 9, September 2013

TABLE 1
pH RECORD

<i>C. aurantifolia</i>	pH
Unripen	2.38
Ripen	2.60

B. Total acidity content

Data for the total acidity are given in the Table 2. The juices are acidic and shows strong values in terms of citric acid content. Total acidity in terms of citric acid was found to be 44.61 mg/mL and 48.99 mg/mL for ripen and unripen juices respectively.

TABLE 2
TOTAL ACIDITY IN TERMS OF CITRIC ACID (mg/mL)

<i>C. aurantifolia</i>	Citric acid (mg/mL)
Unripen	48.99
Ripen	44.61

C. Total antioxidant capacity (%)

The total antioxidant activity is determined as percent activity as well as in terms of ascorbic acid content. Ascorbic acid is used as standard. The total antioxidant capacity (%) is recorded in the TABLE 3. Out of ripen and unripe juices for the present activity determination, unripe juice shows slight more activity than the ripen juice. The unripe juice shows a range of 3.45 to 28.23 % at 25 to 200 µL concentrations respectively. At the same concentration range the value for the ripe juice is found to be 2.08 to 23.56 % respectively. The total antioxidant content in terms of ascorbic acid (mg/mL) is also determined which is recorded in the TABLE 4. In terms of ascorbic acid concentration the value is recorded 0.178 and 0.127 mg/mL for unripe and ripen juices respectively.

TABLE 3
TOTAL ANTIOXIDANT CAPACITY (%)

Juice concentration (µL)	Unripen (% activity)	Ripen (% activity)
25.0	3.45	2.08
50.0	5.66	4.34
75.0	8.49	6.79
100.0	11.35	9.20
125.0	15.48	12.87
150.0	19.50	15.12
175.0	24.86	19.40
200.0	28.23	23.56

**International Journal of Innovative Research in Science,
Engineering and Technology**

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Vol. 2, Issue 9, September 2013

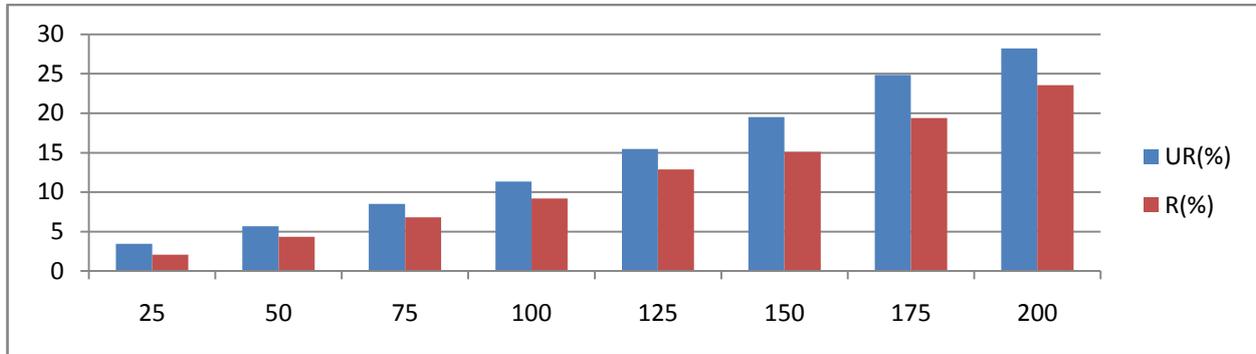


Fig. 1 Total antioxidant activity (%)

The total antioxidant activity is also shown in figure1. This histogram is representing the same value indicated by the TABLE 3. X- axis indicates the concentration of the juices taken for TAC determination and Y- axis is the percent activity at different concentrations. (UR=Unripen, R= Ripen).

TABLE 4
TOTAL ANTIOXIDANT CAPACITY IN TERMS OF ASCORBIC ACID (STANDARD)

<i>C. aurantifolia</i>	TAC in terms of ascorbic acid (mg/mL)
Unripen	0.178
Ripen	0.127

D. DPPH scavenging activity (%)

The free radical scavenging (%) activity for both the ripen and unripen juices is recorded in the TABLE 5. The activity is found to be in the range of 46.56 to 96.14 % for unripen juice concentrations 12.5 to 100 µL respectively. The activity (%) for the ripe juice at same concentration range is found to be in the range of 23.87 to 91.20 % respectively.

TABLE 5
DPPH FREE RADICAL SCAVENGING ACTIVITY (%)

Juice concentration (µL)	Unripen (% activity)	Ripen (% activity)
12.5	46.56	23.87
25.0	57.69	32.22
37.5	69.23	48.34
50.0	77.44	67.78
62.5	81.58	79.50
75.0	89.67	83.55
87.5	92.88	88.43
100.0	96.14	91.20

**International Journal of Innovative Research in Science,
Engineering and Technology**

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Vol. 2, Issue 9, September 2013

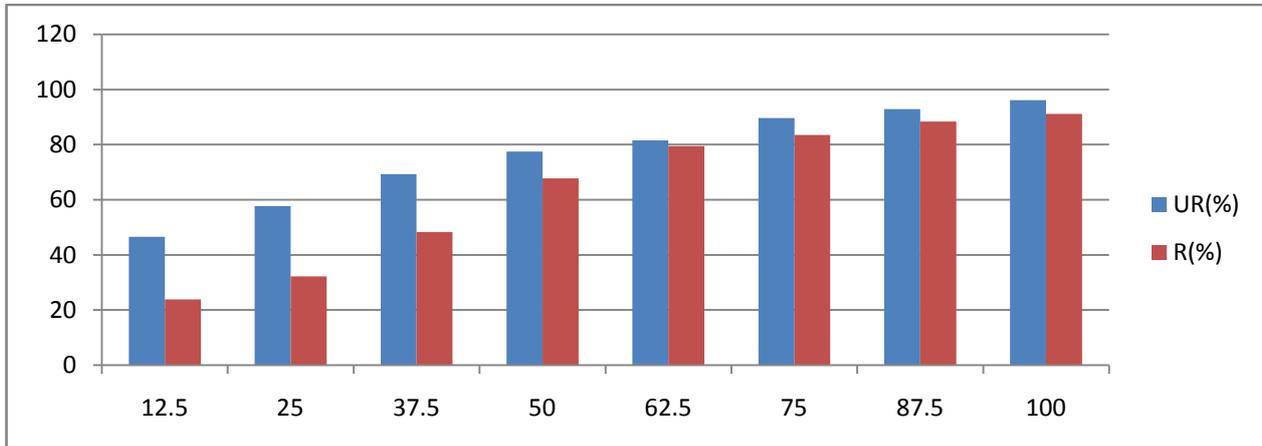


Fig. 2 DPPH scavenging activity (%)

Figure 2 represents the scavenging activity of the juices by DPPH. X- axis indicates the concentration of the juices taken for TAC determination and Y- axis is the percent activity at different concentrations. (UN=Unripen, R= Ripen).

IV. CONCLUSION

The present results have shown that Citrus fruits which are consumed for their nutritional value are also have a great antioxidant activity. Out of total antioxidant and DPPH scavenging activity, it has been recorded lemon shows a high scavenging activity against DPPH. Ripen juices shows 91.02 % and unripe shows 96.14% at 100 μL concentration of the juices. But TAC juices shows only 28.23% for the unripen and 23.56 % for ripen juice at 200 μL of the juices. Total antioxidant in terms of ascorbic acid is recorded as 0.178 mg/mL for unripen and 0.127 mg/mL for ripen juices. The pH for both the ripen is 2.60 and in unripe it is found to be 2.38. This indicates that unripe fruit is more acidic than the ripen fruit. Acidity in terms of citric acid content is found more in the unripen juice as 48.99 (mg/mL) and less in ripen juice as 44.61 mg/mL.

V. ACKNOWLEDGMENT

The authors express their profound gratitude for the assistance they received from management for facilitating the use of USTM laboratory.

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