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Research Article

Comparative Nutritional Analysis and Antioxidant Activity of Fruit Juices of some *Citrus spp.*

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Abstract: Fruit juices of four species of *Citrus* genus i.e. *C. maxima, C. limon, C. sinensis, C. reticulata* were analyzed for their nutritional content (i.e. soluble sugar, total RNA & pentose sugar, free amino acids, soluble protein, total phenolic compounds and vitamin-C) and antioxidant potential. The content of all studied parameters varied from one species to another but no specific trend was observed. The juice of *C. reticulata* was found to be rich in total soluble sugar (15.43 mg/100 ml) and free amino acids (15.18 mg/100 ml) as compared to other species of *Citrus* while *C. sinensis* contained the highest amount (23.07 mg/100 ml) of total phenolic content. Further, *C. limon* contained the highest amount of total soluble proteins (180.67 mg/100 ml), total RNA & pentose sugar content (131.07 mg/100 ml), and Vitamin C (ascorbic acid) content (67.97 mg/100 ml). *C. sinensis* & *C. reticulata* showed good reducing power activity at the dose of 300 µg/ml while *C. limon* showed good DPPH radical scavenging at all concentration ranges in comparison to the standard.

Key words- ascorbic acid, soluble sugar, phenolic content, antioxidant activity and *Citrus*.

INTRODUCTION

Malnutrition problem is a matter of great concern in developing countries. Health profile of a community is greatly influenced by its nutritional status and life style. India is one of the developing countries of the world, where aforesaid problem is very common, especially in villages. Nutritionist have raised concern on the nutritive value of cooking food because density of the most nutrients like protein, carbohydrates, vitamins and mineral are very poor (Reis *et al.*, 1987). Fruits have been included in the human diet since prehistoric time and now in the western and developing countries, there is a habit to take fresh fruits after meal.

In India different kinds of seasonal fruits are available which are rich in food nutrients, vitamins and minerals and also popular to all aged people. Particularly some seasonal *Citrus* fruits are very delicious and nutritious. Fruits are major source of above mentioned food supplements. *Citrus* is primarily valued for the fruits, which is either eaten alone as fresh fruit, processed into juice, or added to dishes and beverages. All species have traditional medicinal value also (Goethesson, 1997; Whistler, 1992 & 1996).

The *Citrus* is rich source of Vitamin C. Its juice also contains carbohydrates, proteins, amino acids, phenolic compounds and minerals etc. Some times, after starvation of serious diseases, doctors suggest to take some *Citrus* fruits for vitamins, minerals and other necessary food supplements, which recover weak health condition by improving appetite quickly. Fruits and vegetables are rich of secondary metabolites such as phenolics which are now identified as natural antioxidant agents. Phenolic compounds have been shown to possess an antioxidant activity based on their (hydroxyl group) donation to free radicals (Karimi, *et al*., 2012, Parle & Chaturvedi, 2012). Therefore, present study was undertaken to analyze the comparative nutritive value and antioxidant potential of selected *Citrus* fruit species.

MATERIALS AND METHODS

The present study was conducted to analyze the nutritional and antioxidant potential of fruit juice of *Citrus maxima* (Chakotra -C), *Citrus limon* (Lemon -L), *Citrus sinensis* (Mosmi - M) and *Citrus reticulata* (Orange - O). Detailed methodology is given in following heads:

Materials

The fresh fruits of *Citrus* species were procured from the local market for the present study. The edible portion of the fruits was separated carefully and juice was extracted with the help of juicer. The fresh juice was used for all investigations. All the chemicals used in study were of analytical grade and of standard companies.

Sample preparation

5 ml Juice sample was placed in 40 ml of 80% ethanol and boiled till 10 ml remained. The mixture was centrifuged at 3000 rpm for 10 min. The supernatant was decanted and the residue was again dissolved in 20 ml ethanol and centrifuged at 3000 rpm for 5 min, then both the supernatants were combined. Meanwhile, the supernatant was dried over hot plate till about 6 ml remained and the final volume was made up to 20 ml with 20% ethanol. This aliquot was used for determination of total soluble sugar, phenolic contents and free amino acids in the juice samples.

Total soluble sugars content

The estimation of total soluble sugars was carried out as per the method given by Dubois et al. (1956), 1 ml ethanolic extract was treated with 1 ml of 5% phenol and 5 ml concentrated sulphuric acid. The mixture was allowed to cool down at room temperature and absorbance was recorded at 490 nm in UV/VIS spectrophotometer (Merk- UV-1), against a blank in which the diluted extract was replaced by 1 ml distilled water. D-Glucose was used as standard and the amount of sugar was expressed in mg/100 ml of fruit juice.

Total phenolic content

The total phenolic content in the fruit juices was estimated spectrophotometrically using method suggested by Bray & Thorpe (1954). 1 ml ethanolic extract was mixed with 1 ml folin-ciocalteau reagent followed by 2 ml of 20% sodium carbonate solution. The tubes were kept in a boiling water bath for 1 min and cooled under running water to room temperature. The contents were finally diluted to 25 ml and absorbance recorded at 650 nm after standing the tubes for 30 min. Total phenolic content in the juice sample was read from the reference curve prepared using m-cresol as a standard and expressed in mg/100 ml of the juice sample.

Total free amino acids contents

The amino acids in the fruit juices were determined by the method suggested by Lee & Takahashi (1966). Ethanolic extract (1 ml) was taken and ninhydrin reagent (1% ninhydrin in 0.5M Citrate buffer, 5.5 pH), glycerol and 0.5M citrate buffer in the ratio of 5:12:2 was added to it with vigorous shaking. The mixture was heated in a boiling water bath for 12 min and thereafter, the tubes cooled under running water to room temperature. Absorbance was recorded at 570 nm against blank prepared by adding 1 ml of 80% alcohol in place of extract. Glycine was used as a standard and the amount of free amino acids in the juice samples was estimated and expressed in mg /100 ml of the juice sample.

Total soluble protein content

Total soluble protein content was estimated with the method of Lowry et al. (1951), according to the method 5 ml juice sample was taken and then make up to 50 ml with phosphate buffer. From the above solution 2 ml was taken and 2 ml of 20% trichloroacetic acid was added to it. After 30 min the solution was centrifuge at 3000 rpm for 25 min and wash with acetone twice and again centrifuged. The solid was dissolved in 5 ml NaOH (0.1N). 1 ml of above solution was mixed with freshly prepared 5 ml alkaline copper sulphate reagent. After 1 min, 0.5 ml Foline's reagent was added to it, mixed and allowed to stand for 30 min. The absorbance of the mixture was recorded at 660 nm with a blank which contained 1 ml NaOH (0.1N) instead of the sample aliquot. BSA (bovine serum albumin) was used as a standard.

Total RNA and pentose sugar content

Total RNA and pentose sugar was estimated by the method reported by Sawhney & Singh (2005). The fresh juice sample was diluted to 5 times and 2 ml of the solution of was mixed with 2 ml of 5% $HClO_4$ and 3 ml of orcinol reagent. Then the samples were kept in a boiling water bath for 20 min. After cooling them, 7 ml n-butanol was added to each tube and the absorbance was measured at 665 nm against blank. Yeast RNA was used as a standard.

Total vitamin-C (ascorbic acid) content

Vitamin C content in fruit juice sample was determined by the method of Sawhney & Singh (2005) according to it the juices were filtered through muslin cloth and immediately diluted 10 folds with 6% metaphosphoric acid. Transferred 25 ml of the above and titrated with 6- dichlorophenol indophenol solution (52 mg of the sodium salt of dye and 42 mg of sodium carbonate dissolve in water and volume is made up to 500 ml).

Antioxidant activity

Reducing power activity

The reducing powers of all *Citrus* juices were determined according to the method of Oyaizu (1986). In different amount of fruit juices (1 ml, 100-300μg/ ml) in methanol, phosphate buffer (2.5 ml, pH 6.6) & potassium ferricyanide (2.5 ml, 1%) were added. The mixture was incubated at 50^0C for 20 min. A portion of tricholoroacetic acid was added to the mixture and then centrifuged at 4000 rpm at room temperature for 10 min. The upper layer (2.5 ml) was mixed with (2.5 ml) distilled water and (0.5 ml, 0.1%) $FeCl_3$ & absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power; ascorbic acid was used as reference compound.

DPPH radical scavenging activity

Radical scavenging activity of the juices was measured using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl). The procedure followed was according to Blosis (1958). 0.1mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml various quantities of *Citrus* juices (50-250 µg/ ml) in methanol. After 30 min absorbance was measured at 517 nm. BHA was used as reference compound. Percentage inhibition was calculated by comparing the absorbance of control and sample.

$$\% \text{ Inhibition} = (A_0-A_1)/A_0*100$$

A_0=Absorbance of control

A_1=Absorbance of juice/ reference compound

Statistical analysis

The data are expressed as the mean and standard deviation. The difference of the mean values using one-way analysis of variance (ANOVA) following by Duncan's multiple range tests (DMRT) at the level of $p \leq 0.05$, n =3.

Table 1. Various nutritional constituents present in fruit juices of different *Citrus* species.

S. No.	**Constituent** (mg/100 ml)	***Citrus maxima*** (Chakotra)	***Citrus limon*** (Lemon)	***Citrus sinensis*** (Mosambi)	***Citrus reticulata*** (Orange)
1.	Total Soluble Sugar	4.87 ± 0.09	4.37 ± 1.0	13.43 ± 0.32	15.43 ± 0.7
2.	Total RNA & Pentose Sugar	59.60 ± 6.33	131.07 ± 4.01	103.63 ± 2.33	80.17 ± 11.58
3.	Total Free Amino Acids	5.30 ± 0.07	3.60 ± 0.64	14.07 ± 0.81	15.80 ± 0.52
4.	Total Soluble Proteins	60.51 ± 3.42	180.67 ± 20.33	115.10 ± 1.58	97.17 ± 4.08
5.	Total Phenolic compounds	16.08 ± 0.65	20.90 ± 2.73	23.07 ± 1.01	21.20 ± 1.56
6.	Total Vitamin C	55.25 ± 4.71	67.97 ± 4.46	60.47 ± 3.29	60.20 ± 3.01

RESULTS AND DISCUSSION

Nutritional Analysis

The results of analysis are given in table-1 reflect that there is no specific trend observed in quantity of various constituents in the juice of different species of *Citrus*. The juice of *C. reticulata* contains the highest amount (15.43 mg/100 ml) of total soluble sugar, and frees amino acids (15.8 mg/100 ml) as compared to other species of *Citrus* whereas *C. limon* contains the lowest amount of the same compounds i.e. 4.37 mg/100 ml and 3.6 mg/100 ml, respectively. Mostly researchers have already reported the same findings (Willimott, 1928; Levi, 1972; Monselise, 1973; Einset, 1978;Bansode & Chavan, 2012).

Table-1 shows that *C. sinensis* contains the highest amount (23.07 mg/100 ml) of total phenolic compounds followed by *C. reticulata* (21.2 mg/100 ml) as compared to the juices of other species studied, whereas, *C. maxima* contains the lowest amount (14.08 mg/100 ml).

From the table-1, it was found that *C. limon* contains the highest amount (180.67 mg/100 ml) of total soluble proteins but lowest amount (60.51 mg/100 ml) was observed in *C. maxima*. Similar trend for proteins was reported by Paul & Shaha (2004). Total RNA & pentose sugar content (mg/100 ml) was observed maximum (131.07) in *C. limon*, followed by *C. sinensis* but minimum amount (59.6) was recorded in *C. maxima* (Table-1).

The results of the study presented in table-1 reflect that highest amount of Vitamin C (ascorbic acid) in C. limon (67.97 mg/100 ml) followed by C. sinensis (60.47 mg) and C. reticulata (60.2 mg) whereas minimum amount was found in C. maxima. The composition of fruit nutrients and vitamins may vary largely due to climatic conditions, topographical variation nature of soil and maturity of fruit.

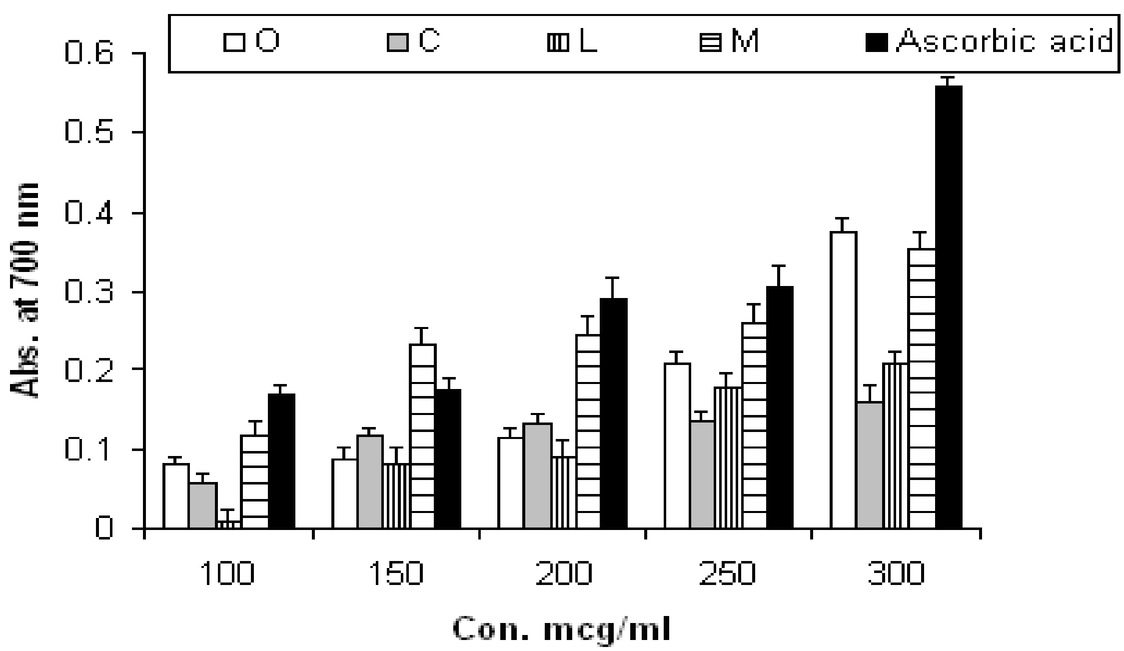
Antioxidant activity

Reducing power activity

Fig 1 shows the reductive capacity of juices compared to ascorbic acid. For the measurements of the reductive ability, it has been investigated from the Fe^{+3} - Fe^{+2} transformation in the presence of juices using the method followed by Oyaizu (1986). Earlier authors (Duh, 1998, Tanaka, Kule, Nagashina & Taguchi, 1988) have observed a direct correlation between antioxidant activity and reducing power of certain plants extracts. The reducing properties are generally associated with the presence of reductones (Duh, 1998), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing power of the tested juices suggest that it is likely to contribute significantly towards the observed antioxidant effect.

However, the antioxidant activity of antioxidants has been attributed by various mechanisms, among which some of them are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997). The reducing power of fruit juices increases with increasing amount of sample, and the maximum activity was observed for *C. reticulata* at the concentration 300 µg/ml but it is less as compared to standard. Whereas, *C. sinensis* have overall good reducing activity at all concentration range.

Fig.1. Reducing power of *Citrus* fruit juices. Results are mean ± SD of three parallel measurements.



DPPH radical scavenging activity

DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (Oyaizu, 1986). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, Dins, Cunha, & Ameida, 1997). The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. BHA was used as standards. The juices were able to reduce the stable radical DPPH to the yellow-coloured diphenylpicrylhydrazine at all concentrations. The experimental data reveal that all these juices are likely to have the effect of scavenging free radical. The involvement of free radicals, especially their increased production, appears to be a feature of most, if not all human diseases, including cardiovascular disease and cancer (Deighton, Brennan, Finn, & Davies, 2000). It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins, and aromatic amines (p-

Karimi, *et al.*, 2012 also reported the same activity in *C. aurantium* bloom.

phenylene diamine, p-aminophenol, etc.), reduce and decolourise DPPH by their hydrogen donating ability (Blois, 1958, Yokozawa *et al*., 1998; Ghasemi *et al.*2009). The results for DPPH study proves that phenolic compounds as well as ascorbic acid content of the fruit juices are probably involved in their antiradical activity

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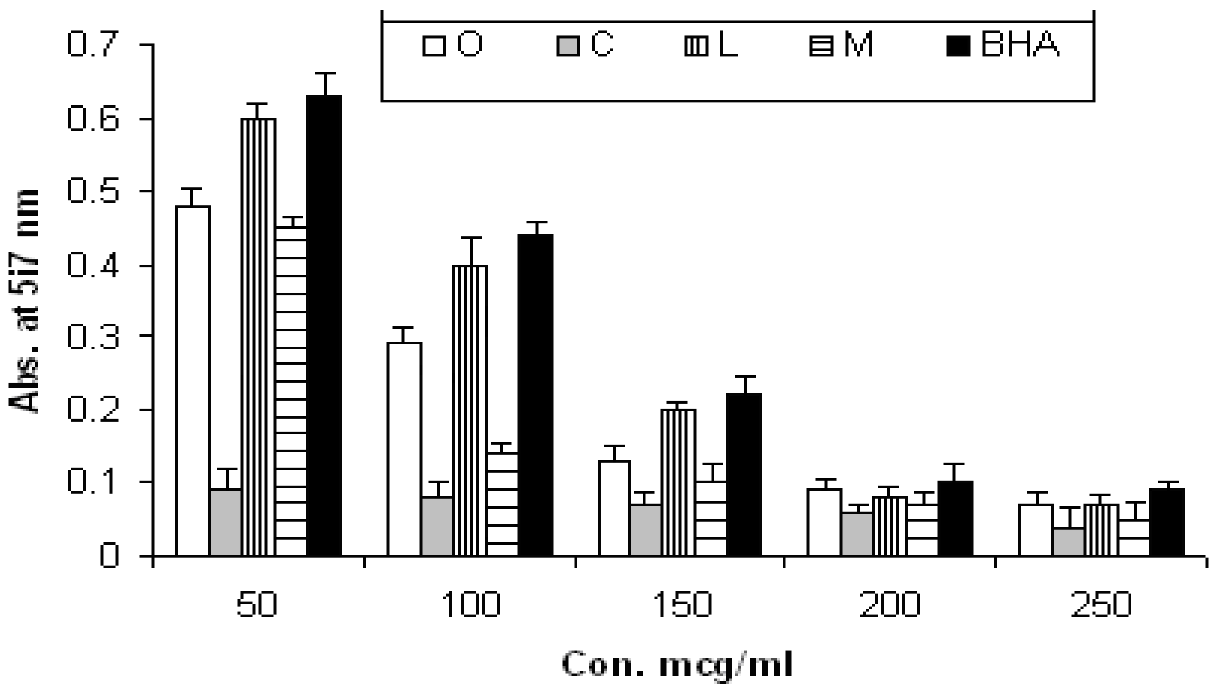
Fig.2. Scavenging activity of *Citrus* fruit juices against 1,1-diphenyl-2-picrylhydrazyl radical. Results are mean ± SD of three parallel measurements.



CONCLUSION

From the present study it can be concluded that all the *Citrus* fruits are rich source of various nutrients and can be helpful in fighting against malnutrition problem in developing countries.

C.limon is a good source of vitamin C and phenolic compounds. It is available in every season and very economical as compared to other studied fruits. Therefore, daily consumption of the juice of *C. limon* with food may reduce malnutrition and the risk of cardiovascular and cancer diseases as well.

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