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Comparative assessment of Antioxidants Vitamin C, Total Phenolics and Flavonoids of selected commercial and underutilized fruits

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ABSTRACT: Background: Fruits possess several antioxidant activities like Vitamin C, Phenols and Flavenoids. It improves our health and increases the total metabolic activities. **Aim:** The present study was aimed to explore the comparative analysis of antioxidant activity in between the commercial fruits and underutilized fruits in varying their phase of maturity. **Methods:** The *Psidium guajava* L., *Anana comosus* Mill and *Mangifera indica* L., were used as commercial fruits. The *Averrhoa bilimbi* L, *Ixora coccinea* L and *Flacourtia jangomas* (Lour) Raeus, were used as underutilized fruits. The fruits were extracted by analytical methods and measured for the quantity of antioxidants using estimation method. **Result:** The antioxidant vitamin C content was highest in ripened stages of *Ixora coccinea L.* The phenol content was highest in ripened stage of *Flacourtia jangomas*. The flavanoid content was highest in ripened stage of *Mangifera indica* L. **Conclusion:** The underutilized fruits are rich in antioxidants compared to commercial fruits. Hence we included the underutilized fruits in our diet.

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INTRODUCTIONS:

Antioxidants are naturally occurring or synthetic chemicals in foods that help to counter detrimental effects of reactive oxygen species (ROS) and free radicals which causes degenerative human diseases such as cancer, heart diseases and cerebrovascular diseases [1]. Recently, natural foods and derived antioxidants such as vitamins and phenol phytochemicals have received growing attention. This is because Vitamin C is one of the most popular and least toxic antioxidant components of foods and has been most widely used as a dietary supplement to prevent oxidative stress mediated

diseases. Studies have revealed that increased consumption of grains, fruits and vegetables is associated with reduced risk of diseases. This may be attributed to the presence of natural antioxidants such as vitamin C, tocopherols, carotenoids, polyphenolics and flavonoids which prevent free radical damage [2]. The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots, etc. Antioxidants have attracted more and more attention as potential agents for preventing and treating oxidative stress related diseases. The human body is equipped with an antioxidant defense system that deactivates these highly reactive free radicals. Dietary antioxidants may play an important role in protecting the cell against damage caused by free radicals by acting as radical scavengers, reducing agents, forming complexes with pro-oxidant metals, and quenchers of singlet oxygen formation. The antioxidants comprise both synthetic and natural antioxidants. The synthetic antioxidants have been widely used in the food industry to prolong the shelf life, but some synthetic antioxidants, such as butylated hydroxytoluene and butylated hydroxyanisole, have been found to be harmful due to their potential toxicity and carcinogenicity [3,4].

Fig 1. Fruits of *Magnifera indica* plant.

Fig 2. Fruits of *Psidium guajava* plant.

The natural antioxidants are expected to be an alternative to synthetic ones because of their potential health benefits, vitamin C, it is the most important vitamin in fruits and vegetables. More than 90 % of the vitamin C in human diets is supplied by fruits and vegetables. Vitamin C is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels. It functions in collagen formation, absorption of inorganic iron, reduction of plasma Cholesterol level, inhibition of nitrosoamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals. As an antioxidant, it reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer [5,6].

The present study was focused on following objectives as to estimate vitamin C content in three underutilized, three commercial fruits at different stage of maturity, to find out the total phenol content and to evaluate variation in phenol concentration during maturation in underutilized and commercial fruits, also quantify the total flavanoids content in commercial and underutilized fruits and comparative analysis of antioxidant properties of the fruits based on results obtained.

MATERIALS AND METHODS:

The oxalic acid, ethanol and folin-ciocalteau reagent were procured from S.D. Fine Chemical, Mumbai. The aluminium chloride and tartarate were purchased from Himedia, New Delhi. All other chemicals were procure from an authorized dealer and were of analytical grade.

Selection of fruits for the study:

Different stages of fruits (Young mature and ripen) of selected commercial and underutilized fruits were collected analyzed. The underutilized fruits are collected locally while commercial fruits were collected from the market. The commercial fruits used for this study are *Psidium guajava* L., *Ananas comosus* Mill and *Mangifera indica* L. The underutilized fruits used for this study are Averrhoa bilimbi L., *Ixora coccinea* L. and *Flacourtia jangomas* (Lour.) Raeusch.

Estimation of antioxidants [7-9]:

Estimation of vitamin C:

About 5 ml of the working standard solution was Pipette out into 100 ml conical flask. It was added with 10 ml of 4 % oxalic acid. The mixture was titrated against the dye (V1 ml). End point was the appearance of pink color, which persists for a few minutes.

Fig 3. Fruits of *Anana cosmosus* plant.

Fig 5. Fruits of *Ixora coccinea bilimbi* plant.

Fig 4. Fruits of *Avverhoa bilimbi* plant.

Fig 6. Fruits of Flacourtia *jangomas* plant.

A 0.5 g of fruit sample was extracted in 4 % oxalic acid. The volume was adjusted up to 100 ml and centrifuged. A 5 ml of this supernatant was pipette out, added 10 ml of 4 % oxalic acid and it was titrated against the dye (V2 ml).

$Q=\{[(w/V1)\times(V2/v)]\times V^{*}l/W\}\times 100$ ….(1)

Where, Q is the amount of ascorbic acid (mg/100g of sample), W is the weight of the sample. V1 and V2 are volume of dye in ml, w is the weight of fruit sample in g (0.5 g), V* is the total final volume of solution (100 ml) and v is the total volume of oxalic acid in ml (15 ml).

Estimation of total Phenol:

About 0.5 to 1.0 g of the fruit sample was weighed. The fruit was grinded with 10 times volume of 80 % ethanol in a mortar and pestle. The homogenate mixture was centrifuged at 10000 rpm for 20 min. The supernatant was extracted with the 5 times the volume of 80 % ethanol. Then it was centrifuged and cooled. The supernatant was evaporated to the dryness. The residue was dissolved in known volume of distilled water (5 ml). The different aliquot of solution (0.2 to 2 ml) was pipetted out into test tube. The volume was made up to the 3 ml with water in each test tube. The folin-ciocalteau reagent (0.5 ml) was added to above mixture. After 3 min, 2 ml of 20 % sodium carbonate solution was added to each test tube. It was mixed thoroughly and the tube was placed in boiling water for exactly 1 min and then cooled. The absorbance was measured at λ_{max} of 650 nm against the blank. A standard curve was prepared by using different concentration of catechol.

Estimation of total Flavanoids:

The aluminium chloride method was used for the determination of the total flavonoid content of the sample extracts. Aliquots of extract solutions were taken and made up the volume 3 ml with methanol. Then 0.1 ml aluminium chloride, 0.1 ml Na-K tartarate and 2.8 ml of distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at λ_{max} of 415 nm was recorded after 30 min incubation. A standard calibration curve was plotted at λ_{max} of 415 nm using known concentration of flavonoid. The concentration of test samples was calculated from the

calibration plot and it was expressed as mg quercetin equivalent/g of sample.

RESULTS AND DISCUSSION:

Vitamin C:

Vitamin C content was least in *Averrhoa bilimbi* and highest in *Ixora coccinea*. Young stage of *A. bilimbi* showed only negligible amount of vitamin C (51.28 mg/100g of sample). This was a considerably increase when the fruit ripened (256.41 mg/100g of sample). In *Ixora coccinea*, the vitamin C content in mature fruit was as high as (307 mg/100g of sample). In *Flacourtia jangomas*, the three stages did not show much difference in vitamin C content; it ranged between 102.56 mg/100g of sample in young fruit to 179.48 mg/100g of sample in mature fruit as represented in Table 1 and Fig 7.

Table 1. Analysis of vitamin C in underutilized fruits.

Fruits	Stage	V_1 (ml)	V2 (ml)	Vit C (mg/W)
AB	Young	2.6	0.2	51.28
	Mature	2.6	0.3	76.92
	Ripen	2.6	1	256.41
IC	Young	2.6	0.5	128.20
	Mature	2.6	0.9	230.76
	Ripen	2.6	1.2	307.69
FJ	Young	2.6	0.4	102.56
	Mature	2.6	0.6	153.84
	Ripen	2.6	0.7	179.48

AB - *A. bilimbi*, IC - *I. coccinea* and FJ - *F. jangomas*. W is 100 g of sample).

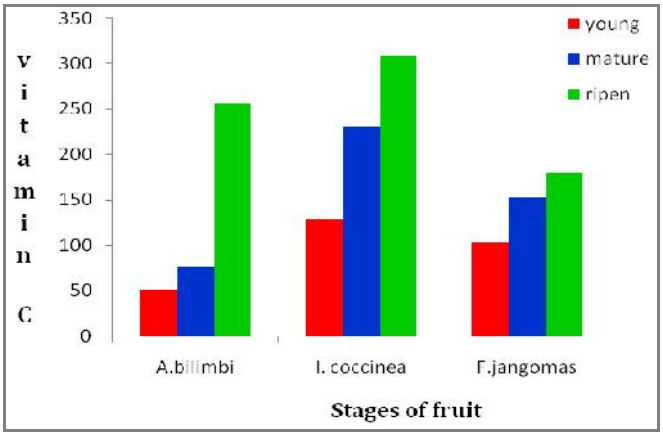

Fig 7. Analysis of vitamin C in underutilized fruits.

Among Psidium, Anana and Mangifera, vitamin C content in Psidium was least. The ripened stage contained 128.20 mg/100g of sample. As the fruit ripened, the vitamin C concentration increased and reached to a concentration of 230.76 mg/100g of fruit. *Mangifera indica* showed the lowest concentration of vitamin C in young stage (51.28 mg/100g of fruit) as given in Table 2 and Fig 8.

Table 2. Analysis of vitamin C in commercial fruits.

Fruits	Stage	V_1 (ml)	V2 (ml)	Vit C (mg/W)
PG	Young	2.6	0.3	76.92
	Mature	2.6	0.4	102.56
	Ripen	2.6	0.5	128.20
AC	Young	2.6	0.5	128.20
	Mature	2.6	0.6	153.84
	Ripen	2.6	0.9	230.76
MI	Young	2.6	0.2	51.28
	Mature	2.6	0.5	128.20
	Ripen	2.6	0.6	153.84

PG - *P. guajava*, AC - *A. cosmos* and MI - *M. indica*. W is 100 g of sample).

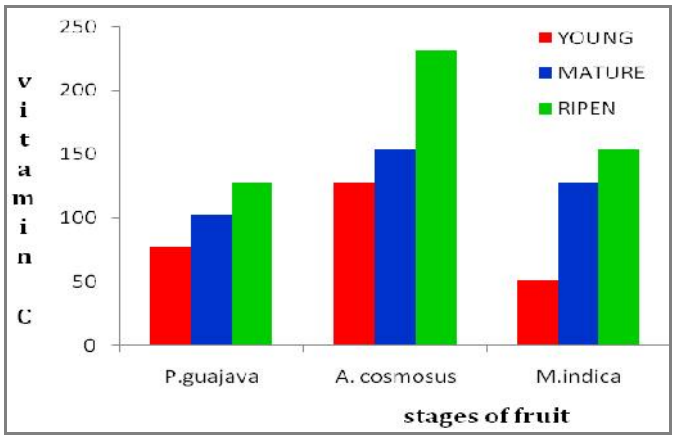

Fig 8. Analysis of vitamin C in commercial fruits.

Total Phenolics content:

In the case of underutilized fruits, total phenolics are generally higher in mature and ripen stages. *Ixora coccinea* mature stage exhibited higher amount of phenol (465 µg/50mg fresh weight), as the fruit ripen phenol content decreases (275 µg/50 mg fresh weight). Highest amount of phenol present in ripen stage of *Flacourtia jangomas* (Table 3 and Fig 9).

In *Psidium guajava*, as the fruit ripened, the phenol concentration was decreased. The phenol content was highest in young stage (470 µg/50mg fresh weight). As fruit reached maturity the amount reduced to 310 µg/50mg fresh weight) and there was a small increase in concentration as the fruit ripened reaching 315 µg/50mg fresh weight).

Highest phenol content was observed in mature stages of *Mangifera*, (505 µg/50mg fresh weight) and Anana ripened fruit (435 µg/50 mg fresh weights) as demonstrated in Table 4 and Fig 10.

Table 3. Analysis of total phenolic content in underutilized fruits.

Fruits	Stage	Total Phenol (µg/50 mg fresh weight)
A. bilimbi	Young	265
	Mature	290
	Ripen	325
I. coccinea	Young	280
	Mature	465
	Ripen	275
F. jangomas	Young	280
	Mature	475
	Ripen	545

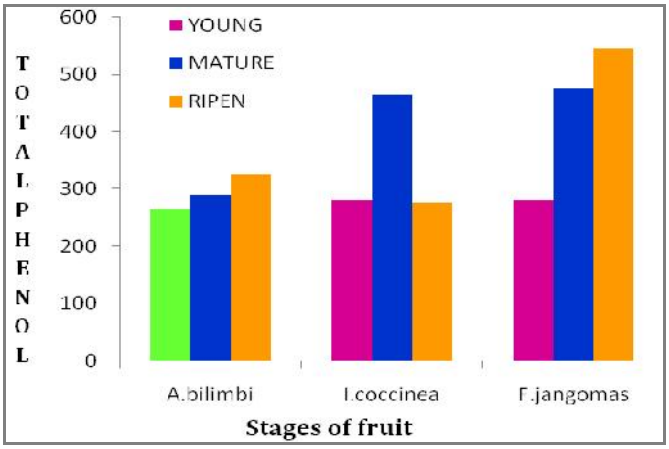

Fig 9. Analysis of total phenol in underutilized fruits.

Fruits	Stage	Total Phenol (µg/ 50mg fresh weight)
P. guajava	Young	470
	Mature	310
	Ripen	315
A. cosmosus	Young	350
	Mature	400
	Ripen	435
M. indica	Young	450
	Mature	505
	Ripen	465

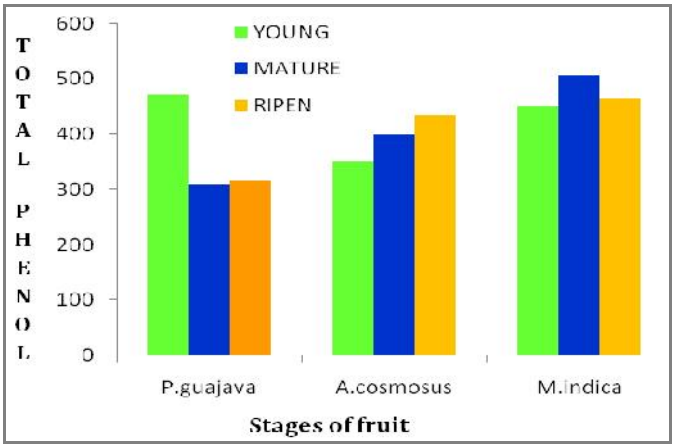

Fig 10. Analysis of total phenol in commercial fruits.

Flavonoids content:

Flavonoid is an antioxidant in which is also a secondary metabolic product found in fruits. In the case of underutilized fruits, highest concentration of flavonoid was present in ripen stage of *Ixora coccinea* (15 µg/50mg of fresh weight). Lowest amount of flavonoid was present in ripen stage of *Flacourtia jangomas* (4 µg/50mg fresh weight). As general trend, much variation was not observed in flavanoid content at different stages of maturity. Data are given in Table 5 and Fig 11.

Table 5. Analysis of total flavonoid in underutilized fruits.

Fruits	Stage	Total Flavonoid (µg/ 50mg fresh weight)
A. bilimbi	Young	13
	Mature	13.5
	Ripen	12.5
I. coccinea	Young	11
	Mature	12
	Ripen	15
F. jangomas	Young	10
	Mature	9
	Ripen	4

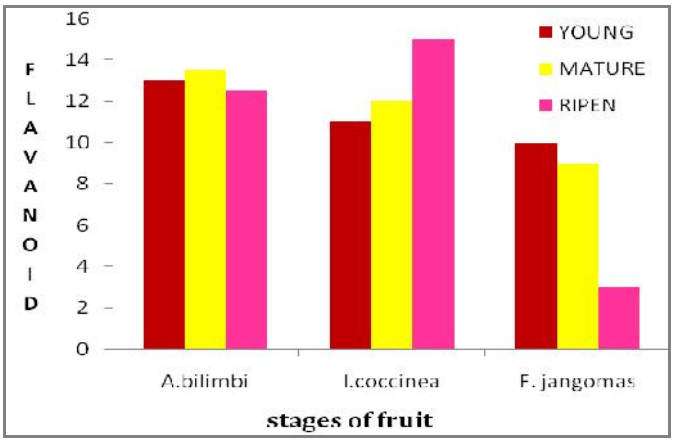

Fig 11. Analysis of total flavonoid in underutilized fruits.

Table 6. Analysis of total flavonoid in commercial fruits.

Fruits	Stage	Total Flavonoid (µg/50 mg fresh weight)
P. guajava	Young	14
	Mature	7
	Ripen	5
A. cosmosus	Young	20
	Mature	11.5
	Ripen	4
M. indica	Young	6
	Mature	24.5
	Ripen	19

In *Psidium* young stage, the fruit had highest flavonoid content of 14 µg/50mg fresh weight and 20 µg/50mg fresh weight respectively. As the fruits ripened, the flavonoid content decreased drastically. In Mangifera the mature fruit had highest flavonoid content of 24.5 µg/50mg fresh weight, as given in Table 6 and Fig 12.

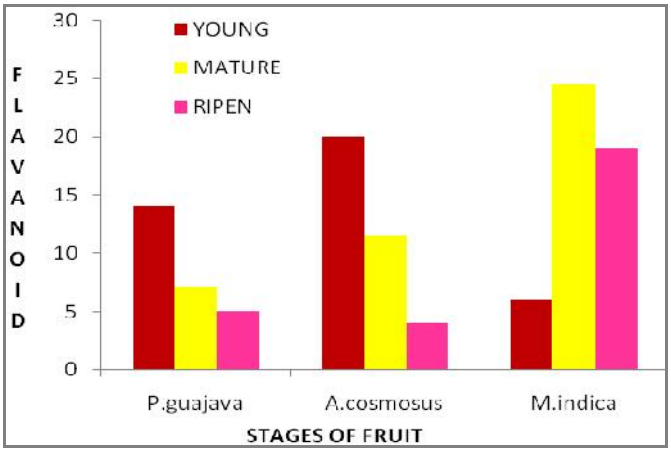

Fig 12. Analysis of total flavonoid in commercial fruits.

CONCLUSION:

The present investigation showed that, underutilized fruits are richer in antioxidants compared to commercial fruits. The present study also suggests the use of such underutilized fruits in our diet is may balance dietary equilibrium. Because they are the rich sources of nutrients and also antioxidants. While commercial fruits also nutritional but when they are comparing with underutilized fruits, underutilized fruits are more nutritional. Hence we conserve our natural vegetation cover for healthy life as well as our future generations.

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