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Nutritional and Antioxidant Potential of *Psidium Guajava* as a Functional Ingredient

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ABSTRACT
There are innumerable fruits which are consumed for their nutritional and medicinal potential. Bioactive constituents present in various fruits act as radical scavengers and helps in preventing non-communicable diseases such as cardiovascular diseases, diabetes mellitus, hypertension etc. The study aimed to characterize the nutritional, mineral composition and antioxidant potential of guava cultivars viz; Allahabad safeda and L-49. The nutritional composition estimation was done according to standard protocol given by AOAC, mineral contents were determined by using Atomic absorption Spectrophotometer and antioxidant content (Total phenols content and total flavonoids content) and activity (reducing power assay and hydroxyl radical scavenging activity) were performed by standard protocol.According to results obtained, Allahabad safeda cv guava had significantly high amount of fibre(2.91±0.04 g/100g), calcium(145± 0.24 mg/100g),magnesium (59.92±1.02 mg/100g) and vitamin-C(214±0.41 mg/100g) content as compared to L-49 cv guava. In addition, it also possesses excellent total phenol content (130.25±0.72 mg GAE/100g), total flavonoids content (99.60± 0.32 mg RE/100g) and antioxidant activity. Therefore, it can be used as a potential ingredient in the development of functional food products and its utilization would be a viable alternative to combat various chronic metabolic diseases.

Keywords: Psidium guajava, Nutritional composition, Minerals, Antioxidant potential, Non-communicable diseases

I. INTRODUCTION
Fruits are one of the oldest forms of food known to man and present an important part of human diet in all cultures of the world [1].India is the second largest producer of fruits after China sharing 13.28% production in world. Is production of fruits stands at 64 million tonnes, making up for around 12% of fruits production of world [2]. Nutritional value of fruits is generally high in fibre, water, minerals and vitamins. It also contains various bioactive components which are required for proper long-term cellular health and disease prevention. Regular consumption of fruits is associated with reduced risks of cancer, cardiovascular diseases, stroke, Alzheimer disease, cataracts and some of the functional declines associated with aging [3].

Psidium guajava (Guava) is a delicious fruit of the plant family Myrtaceae and commonly known as "Poor man's apple", Amrud, Peru, Piyara, Koyya, Sede, Pandu etc [4]. It was originated in tropical America, stretching from Mexico to Peru and gradually became a crop of commercial significance in several countries [5]. In India, guava has become an important fruit crop contributing to 4 per cent of total fruit production and ranks fourth in production after mango, banana and citrus with an estimated production of 4083 lakh tonnes from 251 lakh hectares [6].It is widely grown all over the tropical and subtropical areas Viz., Uttar Pradesh, Bihar, Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu, West Bengal, Assam, Orissa, Karnataka, Kerala, Rajasthan and many more states. Main varieties grown in India are Allahabad Safeda,

Lucknow-49, Chittidar, Nagpur Seedless, Bangalore, Dharwar, Akra Mridula, Arka Amulya, Harijha, Allahabad Surkha CISHG -1, CISHG - 2, CISHG – 3 etc [7].

It is a rich source of primary metabolites such as ascorbic acid, carbohydrates, proteins, minerals (calcium, phosphorus and iron) [8], vitamins like niacin, thiamine, riboflavin and provitamin A [9]. It contain broad spectrum of phytochemicals comprising of carotenoids, lectins, terpenoids, alkaloids, glycosides, steroids, phenols, flavonoids, tannins and saponins [10]. It has various pharmacological activities such as anti-septic, anti-inflammatory, anti-allergic, anti-carcinogenic, anti-diarrhoeal, anti-diabetes, anti-hypertensive, antinociceptive, antimutagenic, antispasmodic and antimicrobial [11].

II. METHODS AND MATERIALS

A. Collection of Raw Material

Two cultivars of guava viz; Allahabad safeda and L-49 were collected from Uniyara village near Bansathali Vidyapith ,Dist -Tonk, Rajastan. The samples were stored in polythene bags, properly labelled and carried to the laboratory for further processing and biochemical analyses. The samples were identified and authenticated by Horticulturist of Rajasthan Agriculture Research Institute (RARI), Jaipur.

B. Selection of Fruits and Sample Preparation

Fully matured uniform sized fruits with firm texture were selected. Extraneous materials were removed from the plant materials. The inner, fresh, tender and edible portion of each sample was retained and later cut into tiny piece and the fruits were oven dried for 3 days to remove all its moisture. The dried fruits were ground in mixer in which the ground samples were passed through 20-mesh sieve to obtain pure processed sample used for the analysis. The powder samples were stored in polyethylene bags at 4 °C with proper labelling and all chemicals and reagents used were of analytical grade.

C. Extraction For Antioxidant Content and Activity

Fruit was cut into 1 cm slices and crushed in a food processor to produce uniform slurries. The slurry was prepared fresh to preserve the extracted antioxidant compounds. In the extraction process, about 1 g of fruit slurries were weighed in universal bottles and 10 mL ethanol solvent (50%) was added. The samples were homogenized by using homogenizer at 750 rpm for 1 min and were centrifuged to get the supernatant for further analysis.

D. Determination of Nutritional Composition

Determination of moisture (Oven-Drying Method), ash (Dry Ashing Method),protein (Kjeldahl Method), fat (Soxhlet Method), fibre (Acid Alkali Method) and carbohydrate (Difference Method; Carbohydrate content (g/100g) = 100 – (Moisture Content + Ash Content + Fat Content + Fibre Content + Protein Content) and Estimation of Energy (Total energy (Kcal/100 g) = [(% available carbohydrates × 4) + (% protein × 4) + (% fat × 9)] according to the standard procedure [12], minerals such as Calcium, Magnesium and iron contents were determined by using Atomic Absorption Spectrophotometer (AAS) and Vitamin C content was analysed by the method [13].

E. Determination of Antioxidant Content

1) Total Phenols Content

Total phenols content was determined spectrophotometrically according to Folin-Ciocalteu method with slight modification [14].An amount of 0.4 mL sample or standard solution was added into 10 mL volumetric flask, containing 3.6 mL of distilled water. Folin-Ciocalteu reagent (0.4 mL) was added into the mixture. About 4 mL of 7% sodium carbonate was also added following 5 min. The solution was made up to 10 mL with distilled water, mixed thoroughly and allowed to stand at room temperature for 90 min. The absorbance was measured at 765 nm using UV-spectrophotometer against distilled water as blank. The standard curve of gallic acid was obtained using the same procedure. Total phenols content was expressed as mg of gallic acid equivalents (GAE) per 100g, which was calculated using the formula, $y = 0.0785x + 0.03$, ($R^2=0.98$) where, y is the absorbance at 765 nm and x is the amount of gallic acid equivalent (µg/mL).

2) Total Flavonoids Content

Total flavonoids content was determined by the aluminum chloride colorimetric method [15]. Five millilitres of 2% aluminum trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution at the concentration of 0.4 mg/ml. Following 10 min, the absorbance was taken against a blank that consist of the same solution but without the $AlCl_3$ at 415 nm using UV-spectrophotometer.

Similarly, a calibration curve of rutin was obtained (for concentrations ranging from 75 mg/L to 750 mg/L) and the total flavonoids content of each extract was expressed as mg of rutin equivalents (RE) per 100g, calculated using the formula, y = 0.0237x + 0.0068, (R^2 =0.9972), where, y is the absorbance at 506 nm and x is the amount of rutin equivalent (µg/mL).

3) Reducing power assay

The reducing power of was measured according to the method of [16]. One millilitre of sample was mixed with 1 mL of 20 mM sodium phosphate buffer (pH 7.0) and 1 mL of 1% potassium ferricyaninde followed by incubation in a water bath at 50 °C for 20 min. Afterwards to stop the reaction trichloroacetic acid (10%, 0.5 mL) was added. Mixture was centrifuged at 750 rpm at room temperature for 10 min and collected supernatant (2 mL) was diluted with deionized water (2 mL) and 0.1% ferric chloride (400 µL). After 10 min incubation, the absorbance of this mixture was recorded at 700 nm.

4) *Hydroxyl radical scavenging assay*

The hydroxyl radical-scavenging assay was determined using the method as described by [17].Sample (25 µL) was mixed with 25 µL of ferrous sulphate (3 mM) and 25 µL of 1,10-phenanthroline (3 mM, dissolved in 0.1 M PB (pH 7.4). Furthermore, 0.01% (v/v) hydrogen peroxide (25 µL) was added to initiate the reaction. Mixture was incubated for 1 h at 37 °C and reading was measured at 536 nm using a UV/ VIS spectrophotometer. Hydroxyl radical-scavenging capacity was calculated according to the following equation:

$$\text{Hydroxyl scavenging ability}(\%) = \frac{\text{A(Sample)} - \text{A(Blank)}}{\text{A(Control)} - \text{A(Blank)}} X\ 100$$

Where, A is absorbance, (sample) absorbance with sample, (control) solution in absence of hydrogen peroxide, (blank) solution containing all reagents except sample.

F. STATISTICAL ANALYSIS

The results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using statistical package SPSS version 16. Differences at $p<0.05$were considered to be significant. Student-t test was used to compare difference of samples.

III RESULTS AND DISCUSION

TABLE 1:
NUTRITIONAL COMPOSITION OF DIFFERENT CULTIVARS OF *PSIDIUM GUAJAVA* ON DRY WEIGHT BASIS

Parameters	*Psidium guajava*	
(g/100g)	Allahabad Safeda	L-49
Moisture	10.04±0.01	11.06±0.02
	(77.73± 1.28)*	(74.26± 5.0)*
Ash	4.28±0.03	3.62± 0.03[a]
Protein	1.49 ±0.01	1.34± 0.04[a]
Fat	0.49±0.02	0.42± 0.02
Fiber	2.91±0.04	2.04 ±0.01a
Carbohydrate	80.79±0.05	81.93±0.04[a]
Energy(Kcal/100g)	333.53±0.99	328.51±1.85[a]

The values are expressed as mean of 3 replicates ± standard deviation (SD).*Moisture content of fresh fruit. [a]superscript in each row show significant difference between valuesat $p\leq 0.05$

The nutritional composition of the Allahabad safeda and L-49 cultivars of guava are shown in Table-1. The moisture content of any food is an index of its water activity and it is used as a measure of stability for rapid deterioration of these fruits if unprocessed for long time after harvest [18]. The result indicates that the moisture content (g/100g)of the Allahabad safeda and L-49 were found to be 10.04±0.01 and 11.06±0.02 respectively but the differences were not significant at $p\leq0.05$ level. Samples with high percentage of ash content are expected to have high concentrations of various inorganic mineral elements which catalyses various metabolic processes in human body [19]. The ash content (g/100g) of the Allahabad safeda and L-49 were found to be 4.28±0.03 and 3.62± 0.03respectively which showed significant difference at $p\leq0.05$ level. The ash value of present study was comparable to that of result obtained 3% for guava [20]. Proteins are essential component of diet needed for survival of animals and humans, their basic function in nutrition is to supply adequate amounts of required amino acids in nutrition [21]. [18] found that the protein content ranged between 0.44 - 4.38% for fruits. The protein content (g/100g) found in Allahabad safeda and L-49 were1.49 ±0.01and 1.34± 0.04respectively which was within

the above stated range and showed significant difference at p≤0.05 level. Similarly [22] found that protein content for Guava variety viz Hong-kong and Ruby X supreme ranged between 0.57-1.19 %.

The low levels of fat in fruits is good indicators of nutritive quality as excess fat consumption is implicated in certain cardiovascular disorders such atherosclerosis, cancer and aging [23]. The fat content was found to be 0.49±0.02and 0.42± 0.02 for the Allahabad safeda and L-49 respectively but the differences were not significant at p≤0.05 level. The results of the present study were in agreement to those reported by [24] who revealed that the fat content to be 0.1-0.5% in guava fruit. The fibre content is an indication that it contains a portion of cellulose, hemicelluloses and lignin. However, low fibre content is also known to reduce the rate of glucose and fat absorption [25].The fibre content in Allahabad safeda and L-49 was found to be 2.91±0.04and 2.04 ±0.01 respectively but the differences were significant at p≤0.05 level. Nutrients composition of the fruits exhibited fibre in range of 0.00-3.55g/100g for fresh fruit samples of guava, banana, pawpaw, orange, apple, watermelon, bush mango and pineapple [26]. The decrease in carbohydrate content of pulp may be result of some enzyme activities on the carbohydrate as the main source of energy during the ripening process [27]. The data obtained for carbohydrate content (g/100g) in Allahabad safeda and L-49 was found to be 80.79±0.05 and 81.52±0.04respectively. The data is comparable to the carbohydrate content reported in *Aloe barbadensis* (73.07%) and *Luffa acqtangula* (66.05%) [28]. The energy content in present guava cultivars were 333.53±0.99 and334.62±1.85 Kcal/100g for Allahabad safeda and L-49.

Mineral analysis are nutritionally important because it is known that inorganic mineral element such as iron play pivotal role in preventing anemia, magnesium assist in the assimilation of phosphorus, Calcium plays an important role in impeding the development of osteoporosis and in teeth development [29]. The minerals content of the guava cultivars are represented in Figure-1. Calcium content was the predominant element among mineral analysed while iron had lowest content in both cultivars. Calcium (145.16± 0.24 mg/100g) and Magnesium (59.92±1.02 mg/100g)content were higher in Allahabad safeda and found to be insignificant whereas iron content was 4.41± 0.24 mg/100g) and found significant difference at p≤0.05 level when compared to L-49. The data obtained for Vitamin-C (mg/100g) content showed 214.42±0.41 and 212.48±0.31 for Alahabad safeda and L-49 respectively which exhibited insignificant difference at p≤0.05 level. Similarly,[30]showed that Vitamin-C content was 216±9.64mg/100g in guava pulp. Also, the data reported by [31] that white guava contain118.20±4.00, 61.70±2.00 and 1.57±1.00 mg/100g respectively for calcium, magnesium and iron content.

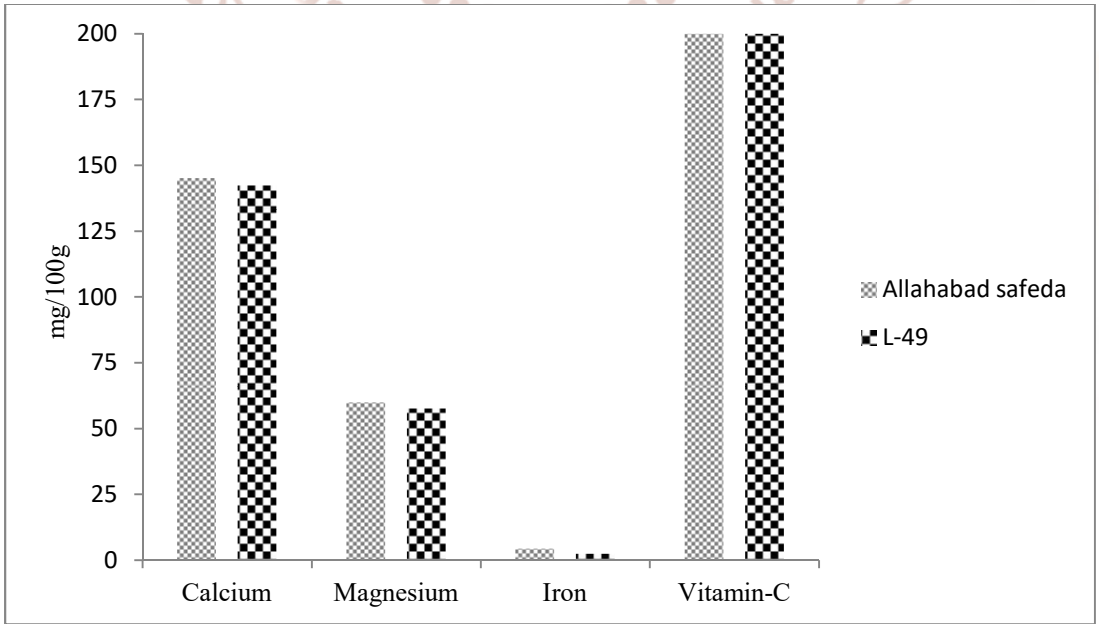

Fig.1.Minerals and vitamin-C content of different cultivars of *Psidium guajava* on dry weight basis

TABLE 2:
ANTIOXIDANT CONTENT AND ACTIVITY OF DIFFERENT CULTIVARS OF *PSIDIUM GUAJAVA*

Ethanolic extract of *Psidium guajava* cultivars	Total Phenols Content (mg GAE/100g)	Total Flavonoids Content (mg RE/100g)	Reducing Power (mg/ml)	Hydroxyl Radical Scavenging activity (mg/ml)
Allahabad safeda	130.25 ± 0.72	99.60 ± 0.32	0.62± 0.001	52.45 ± 0.02
L-49	103.55 ± 0.02*	83.30 ± 0.02*	0.38 ±0.014*	45.41 ± 0.14*

Values are Mean±SD of triplicate determination,* superscript in each column show significant difference between values at p≤ 0.05

Phenolic compounds are commonly found in both edible and inedible plants and have been reported to have multiple biological effects. Phenols are able to scavenge reactive oxygen species due to their electron donating properties [32]. The data demonstrated that Allahabad safeda (130.25 ± 0.72) had significantly higher TPC when compared to L-49 (103.55 ± 0.02) cultivar as depicted in Table2. [33] reported that Allahabad safeda extract was good source of total phenols which in turn shows the ability to retard lipid oxidation in fatty foods, thereby reducing the incidence of metabolic disorders. The study showed TPC ranged between 113.11- 143.63mg GAE/100g for different extracts of guava fruit [34]. The flavonoids are a broad class of low molecular weight, secondary plant phenolics characterized by the flavan nucleus. In plants, these compounds afford protection against ultraviolet radiation, pathogens and herbivores [35]. The data demonstrated in Table 2 that Allahabad safeda had significantly higher TFC (99.60 ± 0.32) when compared to L-49 (83.30 ± 0.02) cultivar.The results of the present study are in agreement with the report recorded by [36] that guava fruit showed 113.57 mg RE/100g on fresh weight basis.

A reducing power is an indicative of reducing agent having the availability of atoms which can donate electron and react with free radicals and then convert them into more stable metabolites and terminate the radical chain reaction [37]. The Reducing Power Assay (mg/ml) of the Allahabad safeda and L-49 were found to be 0.62± 0.00and 0.38 ±0.014 respectively which showed significant difference at p≤0.05 level. [38]Study reported that aqueous and alcoholic extracts of guava had 0.223 and 1.012 mg/ml of reducing power respectively. The hydroxyl radical is major active oxygen causing lipid peroxidation and persuades the severe damage to adjacent biomolecules such as DNA, lipid and protein as compared to other reactive oxygen species [39]**.** The hydroxyl radical scavenging activity (mg/ml) was found to be 52.45 ± 0.02 and 45.41 ± 0.14 for Allahabad safeda and L-49 respectively and showed significant difference at p≤0.05 level**.** Similar findings were reported by [40] that hydroxyl radical scavenging activity of the selected fruits (mulberries, papaya, red grapes, mango, guava and tomato) ranged from 21.88 to 52.33 mg/100 g. The results make it evident that the extracts of both cultivars were able to scavenge hydroxyl radicals.

IV. CONCLUSION

Based on the results obtained, it can be concluded that Allahabad safeda cultivar of guava had significantly high amount of fibre, minerals (calcium, magnesium) and vitamin-C content. In addition, it can also be considered as a potential source of bioactive compounds such as total phenols and flavonoids content and possess excellent antioxidant activity as compared to L-49 cultivar of guava.Thus, the above findings indicated that Allahabad safeda was best cultivar with respect to both nutritional and antioxidant quality.Therefore, It can be used as a potential nutraceutical ingredient in the development of functional food products and its utilization would be a viable alternative to combat various non-communicable diseases.

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