MORPHOLOGICAL AND NUTRACEUTICAL CHARACTERIZATION OF GUAVA (*Psidium guajava* L.) GENOTYPES

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
HORTICULTURE (FRUIT SCIENCE)
(Minor Subject: Biochemistry)

By

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CERTIFICATE - I

This is to certify that the thesis entitled, **"Morphological and nutraceutical characterization of guava (*Psidium guajava* L.) genotypes"** submitted for the degree of **Master of Science** in the subject of **Fruit Science** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Mr. Pankaj Kumar (L-2017-A-98-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the thesis entitled, **"Morphological and nutraceutical characterization of guava (*Psidium guajava* L.) genotypes"** submitted by **Mr. Pankaj Kumar** (Admn. No. **L-2017-A-98-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.,** in the subject of **Fruit Science** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee after an oral examination on the same.

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ABSTRACT

Nine guava genotypes were categorized under three groups *i.e.* white fleshed, pink fleshed and red skinned which were evaluated for their morpho-physiological and nutraceutical parameters. White fleshed genotypes had higher fruit diameter and fruit weight than other genotypes, with maximum values observed in Punjab Safeda (8cm and 244g respectively). Core diameter was minimum in Punjab Pink (4.07cm) and maximum in Punjab Apple Guava (5.53cm), whereas Punjab Safeda and Lalima had maximum pulp thickness (1.63cm). Lalit had highest seed number per fruit (507) and seed weight per fruit (5.78g), while 100 seed weight was maximum in Lalima (1.53g). Total soluble solids ranged from 7.77 percent in Lalima to 11.27 percent in AC 1-4. The TSS: acidity ratio as well as juice pH were higher in Allahabad Safeda (21.3 and 5.35 respectively) than other genotypes. Regarding nutraceutical parameters, white fleshed genotype Punjab Safeda had maximum ascorbic acid (178.99 mg/100g pulp) and total phenols content (170.30mg/100g fw), while pink fleshed genotypes had minimum ascorbic acid and total phenols content. Carotenoids were maximum in Punjab Safeda (0.45mg/100g fw) and minimum in Lalima (0.32 mg/100g fw). While, flavonoids and anthocyanins were maximum in the red skinned genotypes. Flavonoids content was maximum in Punjab Apple Guava (115.48 mg/100g fw) and the anthocyanins content was maximum in AC 1-4 (65.77 mg/100g fw) as compared to other genotypes. Regarding enzymatic activities, Polyphenol oxidase activity was found to be non-significant in all the genotypes. Peroxidase activity was maximum in Lalit and minimum in Punjab Kiran. Phenylalanine ammonia lyase enzyme activity was found maximum in white fleshed genotypes, with maximum value observed in Punjab Safeda. Total antioxidant activity, measured by DPPH assay was maximum in red skinned genotypes, followed by white fleshed and pink fleshed genotypes. Punjab Appla Guava had highest total antioxidant activity (71.11%). The antioxidant activity had significant correlation with ascorbic acid (r=0.92) and anthocyanins content (r=0.802). Red skinned genotype namely Punjab Apple Guava had highest nutraceuticlal potential and can be promoted for commercial cultivation among the fruit growers.

Key words: Guava, phenol, flavonoids, nutraceutical, antioxidant activity

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Signature of Major Advisor **Signature of the Student**

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ਸਾਰ ਅੰਸ਼

ਮੌਜੂਦਾ ਅਧਿਐਨ ਦੌਰਾਨ ਅਮਰੂਦ ਦੀਆਂ ਨੌ ਕਿਸਮਾਂ ਦਾ ਦਿਸਣਯੋਗ ਅਤੇ ਪੌਸ਼ਟਿਕ-ਚਕਿਤਸਕ ਮਾਪਦੰਡਾਂ ਲਈ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ। ਇਹਨਾਂ ਕਿਸਮਾਂ ਨੂੰ ਤਿੰਨ ਸ਼੍ਰੇਣੀਆਂ-ਚਿੱਟਾ ਗੁੱਦਾ, ਗੁਲਾਬੀ ਗੁੱਦਾ ਅਤੇ ਲਾਲ ਛਿੱਲ ਵਾਲੀਆਂ ਕਿਸਮਾਂ ਵਿੱਚ ਵੰਡਿਆ ਗਿਆ। ਚਿੱਟੇ ਗੁੱਦੇ ਵਾਲੇ ਅਮਰੂਦਾਂ ਦਾ ਫ਼ਲ ਵਿਆਸ ਅਤੇ ਭਾਰ ਬਾਕੀ ਕਿਸਮਾਂ ਦੇ ਮੁਕਾਬਲੇ ਵੱਧ ਦਰਜ ਕੀਤਾ ਗਿਆ ਅਤੇ ਪੰਜਾਬ ਸਫੈਦਾ ਵਿੱਚ ਇਹ ਮਾਪਦੰਡ ਸਭ ਤੋਂ ਵੱਧ (ਕ੍ਰਮਵਾਰ 8 ਸੈ.ਮੀ. ਅਤੇ 244 ਗ੍ਰਾਮ) ਪਾਏ ਗਏ। ਅੰਦਰੂਨੀ ਕੋਰ ਦਾ ਵਿਆਸ ਸਭ ਤੋਂ ਘੱਟ ਪੰਜਾਬ ਪਿੰਕ (4.01 ਸੈ.ਮੀ.) ਅਤੇ ਸਭ ਤੋਂ ਵੱਧ ਪੰਜਾਬ ਐਪਲ ਗੁਆਵਾ (5.53 ਸੈ.ਮੀ.) ਵਿੱਚ ਪਾਇਆ ਗਿਆ। ਗੁੱਦੇ ਦੀ ਚੌੜਾਈ ਪੰਜਾਬ ਸਫੈਦਾ ਅਤੇ ਲਾਲੀਮਾ ਵਿੱਚ ਦੂਜੀ ਕਿਸਮਾਂ ਦੇ ਮੁਕਾਬਲੇ ਵੱਧ ਦਰਜ ਕੀਤੀ ਗਈ (1.63 ਸੈ.ਮੀ.)। ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਠੋਸ ਦੀ ਮਾਤਰਾ 7.77% (ਲਾਲੀਮਾ) ਤੋਂ 11.27% (ਏ ਸੀ 1-4) ਤੱਕ ਪਾਈ ਗਈ। ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਠੋਸ : ਖਟਾਸ ਅਨੁਪਾਤ ਅਤੇ ਜੂਸ ਪੀ.ਐੱਚ. ਸਭ ਤੋਂ ਵੱਧ ਅਲਾਹਾਬਾਦ ਸਫੈਦਾ (ਕ੍ਰਮਵਾਰ 21.3 ਅਤੇ 5.35) ਵਿੱਚ ਪਾਏ ਗਏ। ਪ੍ਰਤੀ ਫ਼ਲ ਬੀਜਾਂ ਦੀ ਗਿਣਤੀ ਅਤੇ ਭਾਰ ਲਲਿਤ ਵਿੱਚ ਵੱਧ ਪਾਏ ਗਏ (ਕ੍ਰਮਵਾਰ 507 ਅਤੇ 5.78 ਗ੍ਰਾਮ), ਜਦਕਿ 100 ਬੀਜਾਂ ਦਾ ਭਾਰ ਸਭ ਤੋਂ ਵੱਧ ਲਾਲੀਮਾ (1.53 ਗ੍ਰਾਮ) ਵਿੱਚ ਦਰਜ ਕੀਤਾ ਗਿਆ। ਗੁਲਾਬੀ ਗੁੱਦੇ ਵਾਲੀਆਂ ਕਿਸਮਾਂ ਵਿੱਚ ਵਿਟਾਮਿਨ ਸੀ ਅਤੇ ਕੁੱਲ ਫੀਨੋਲਿਕ ਮਾਤਰਾ ਬਾਕੀ ਕਿਸਮਾਂ ਦੇ ਮੁਕਾਬਲੇ ਘੱਟ ਸੀ। ਵਿਟਾਮਿਨ ਸੀ ਅਤੇ ਕੁੱਲ ਫੀਨੋਲਿਕ ਮਾਤਰਾ ਸਭ ਤੋਂ ਵੱਧ ਪੰਜਾਬ ਸਫੈਦਾ ਵਿੱਚ (178.99 ਮਿ. ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ ਅਤੇ 170.30 ਮਿ. ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ ਕ੍ਰਮਵਾਰ) ਅਤੇ ਸਭ ਤੋਂ ਘੱਟ ਪੰਜਾਬ ਪਿੰਕ (120.51 ਮਿ. ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ ਅਤੇ 116.40 ਮਿ. ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ ਕ੍ਰਮਵਾਰ) ਵਿੱਚ ਦਰਜ ਕੀਤੀ ਗਈ। ਕੈਰੋਟੀਨਾਇਡਜ ਸਭ ਤੋਂ ਵੱਧ ਪੰਜਾਬ ਸਫੈਦਾ ਅਤੇ ਸਭ ਤੋਂ ਘੱਟ ਲਾਲੀਮਾ ਵਿੱਚ ਪਾਏ ਗਏ। ਫਲੇਵੋਨੋਇਡਜ ਅਤੇ ਐਂਥੋਸਾਇਨਿਨ ਦੀ ਮਾਤਰਾ ਲਾਲ ਛਿੱਲ ਵਾਲੇ ਅਮਰੂਦਾਂ ਵਿੱਚ ਵੱਧ ਪਾਈ ਗਈ। ਫਲੇਵੋਨੋਇਡਜ ਦੀ ਮਾਤਰਾ ਪੰਜਾਬ ਐਪਲ ਗੁਆਵਾ (115.40 ਮਿ. ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ) ਅਤੇ ਐਂਥੋਸਾਇਨਿਨ ਦੀ ਮਾਤਰਾ ਏ ਸੀ 1-4 (65.77 ਮਿ. ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ) ਵਿੱਚ ਬਾਕੀ ਕਿਸਮਾਂ ਦੇ ਮੁਕਾਬਲੇ ਵੱਧ ਦਰਜ ਕੀਤੀ ਗਈ। ਸਭ ਕਿਸਮਾਂ ਵਿੱਚ ਪੀ ਪੀ ਓ ਐਨਜਾਇਮ ਦੀ ਕਿਰਿਆ ਨਾ ਦੇ ਬਰਾਬਰ ਸੀ। ਪੀ ਓ ਡੀ ਐਨਜ਼ਾਇਮ ਦੀ ਕਿਰਿਆ ਸਭ ਤੋਂ ਵੱਧ ਲਲਿਤ ਅਤੇ ਸਭ ਤੋਂ ਘੱਟ ਪੰਜਾਬ ਕਿਰਨ ਵਿੱਚ ਪਾਈ ਗਈ। ਪੀ ਏ ਐਲ ਐਨਜਾਇਮ, ਜੋ ਕਿ ਫੀਨੋਲਿਕ ਤੱਤ ਬਣਾਉਣ ਲਈ ਜ਼ਿੰਮੇਵਾਰ ਹੁੰਦਾ ਹੈ, ਦੀ ਕਿਰਿਆ ਸਭ ਤੋਂ ਵੱਧ ਚਿੱਟੇ ਗੁੱਦੇ ਵਾਲੇ ਅਮਰੂਦਾਂ ਵਿੱਚ ਪਾਈ ਗਈ। ਕੁੱਲ ਐਂਟੀਆਕਸੀਡੈਂਟ ਕਿਰਿਆ ਇਸ ਤਰਤੀਬ ਵਿੱਚ ਪਾਈ ਗਈ : ਲਾਲ ਛਿੱਲ ਵਾਲੇ ਅਮਰੂਦ > ਚਿੱਟੇ ਗੁੱਦੇ ਵਾਲੇ ਅਮਰੂਦ > ਗੁਲਾਬੀ ਗੁੱਦੇ ਵਾਲੇ ਅਮਰੂਦ । ਪੰਜਾਬ ਐਪਲ ਗੁਆਵਾ ਵਿੱਚ ਕੁੱਲ ਐਂਟੀਆਕਸੀਡੈਂਟ ਕਿਰਿਆ ਸਭ ਤੋਂ ਵੱਧ (71.11%) ਸੀ। ਕੁੱਲ ਐਂਟੀਆਕਸੀਡੈਂਟ ਕਿਰਿਆ ਦੇ ਵਿਟਾਮਿਨ ਸੀ (r=0.92) ਅਤੇ ਐਂਥੋਸਾਇਨਿਨ (r=0.802) ਨਾਲ ਅਰਥਪੂਰਨ ਸਬੰਧ ਪਾਏ ਗਏ। ਰਾਜ ਵਿੱਚ ਅਮਰੂਦਾਂ ਦੀ ਕਾਸ਼ਤ ਨੂੰ ਹੋਰ ਵਧਾਉਣ ਲਈ ਵਧੇਰੇ ਪੌਸ਼ਟਿਕ ਚਕਿਤਸਕ ਸਮਰੱਥਾ ਵਾਲੀਆਂ ਲਾਲ ਛਿੱਲ ਵਾਲੀਆਂ ਕਿਸਮਾਂ ਜਿਵੇਂ ਕਿ ਪੰਜਾਬ ਐਪਲ ਗੁਆਵਾ ਦੀ ਕਾਸ਼ਤ ਨੂੰ ਹੱਲਾਸ਼ੇਰੀ ਦੇਣ ਦੀ ਲੋੜ ਹੈ।

ਮੁੱਖ ਸ਼ਬਦ: ਅਮਰੂਦ, ਫੀਨੋਲਿਕ ਮਾਤਰਾ, ਫਲੇਵੋਨੋਇਡਜ, ਪੌਸ਼ਟਿਕ-ਚਕਿਤਸਕ, ਐਂਟੀਆਕਸੀਡੈਂਟ ਕਿਰਿਆ

ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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CHAPTER – I

INTRODUCTION

Guava (*Psidium guajava* L.) belongs to the family Myrtaceae. It is well distributed throughout the South America, Asia, Europe and Africa (Rios *et al* 1977). Genus *Psidium* contains nearly 150 species, out of which only a few produce edible fruits, with common guava being the commonly cultivated (Morton 1987). The guava is native to tropical America (from Peru to Mexico). In the early seventeenth century, it was introduced into India by Portuguese. It has spread to most of the tropical and subtropical world and has become naturalized in several countries (Menzel 1985).

The fruits of guava have unique flavour and taste, along with high levels of ascorbic acid (vitamin C), which is approximately 260 mg/100g of fruit pulp. The fruits are also a good source of pectin and fibre. Due to its easy availability and high nutrition value, it is known as 'apple of the tropics'. It is hardiest among tropical fruits and can be grown well in different climatic conditions, even upto 1500m above mean sea level. The fruits of guava have a number of uses-and can be eaten raw or can be processed into a number of products like jam, jellies, juices etc. (Singh *et al* 2016). Guava has wider adaptability in diverse soils and agro-climatic regions, along with prolific bearing, low cost of cultivation and highly remunerative value, so it is gaining more popularity among the fruit growers in different countries (Das *et al* 1995).

In India, guava is the fourth most important fruit crop after mango, citrus and banana with 265 thousand hectares of area with an annual production of 4054 thousand MT. Guava is cultivated in both tropical and sub-tropical areas of India. Uttar Pradesh is the leading state in guava production followed by Bihar, Madhya Pradesh, Chhattisgarh, West Bengal, Odisha, Maharashtra, Haryana, Gujarat, Tamil Nadu, Jharkhand and Punjab (Anonymous 2018). In Punjab, Guava is the 2nd most important fruit crop after citrus. It is cultivated on 9142 hectares of area with a total of 206.10 thousand MT of production (Anonymous 2019).

The nutraceutical industry is one of the emerging industries for future businesses in the modern world. The term nutraceutical, coined by Stephen DeFelice in 1989, consists of two words, "nutrient" means a nourishing food component and "pharmaceutical" means a medicinal drug (Radhika *et al* 2011). So, nutraceutical is a food or part of nourishment which provides medical or health benefits, along with providing basic nutrition (DeFelice 1995). Nutraceuticals are different from pharmaceuticals in way that pharmaceuticals are the drugs used mainly to treat diseases, whereas nutraceuticals are those which help to prevent diseases. Pharmaceuticals have patent protection as they are the result of expensive testing to meet the specifications of respective governments (Rajasekaran *et al* 2008). Nutraceuticals are seen as

an alternative to pharmaceuticals, because the later may have some side-effects sometimes. These days, consumers prefer foods, which apart from providing nutrition, plays a role in prevention of diseases and improving the physical and mental health. Nutraceuticals have received great attention these days owing to their potential nutritional, safety and therapeutic effects (Dutta *et al* 2017).

Fruits are potential sources of nutraceuticals due to their inherent composition of beneficial elements. The daily intake of fruits, as well as vegetables in sufficient quantity promotes the health and prevents the risk of several chronic diseases. Guava is rich in high-profile nutrients and due to its health-promoting qualities, it is labelled as "super-fruits". The fruits are rich source of vitamin C and minerals like iron (1%), calcium (0.01%) and phosphorus (0.04%) (Nanjundaswamy *et al* 1964), and contains some other phytonutrients and antioxidants like polyphenols and carotenoids. The main phytochemicals present in guava are triterpenes, flavonoids, tannins, ellagic acid, pentacyclic triterpenoid, guajanoic acid, quercetin, lectins, oleanolic acid, carotenoids, leucocyanidin, saponins, amritoside, ursolic acid, beta-sitosterol, uvaol and other compounds. It has anti-diarrhoeal, lipid lowering, hypoglycemic (due to presence of fibre), hepatoprotective, antioxidant and antibacterial activities. In ancient times, people of India used decoction of leaves and bark of guava for curing vomiting, diarrhea, sore throats and inflammated intestinal problems. The leaves have sedative effects and the people used to chew the guava leaves to get relief from mouth sores and bleeding gums. Guava fruits have hypoglycemic effect due to fair amount of fibre present. They are also fat and cholesterol free (Kamath *et al* 2008). A study of 9 week was performed to check the effect of consuming guava 400g/day on lipid profile (total cholesterol, triglycerides, low-density lipoprotein [LDL] and high-density lipoprotein [HDL] cholesterol) and total antioxidant status. The guava consumption significantly reduced oxidative stress and altered lipid profile by increasing good quality (HDL) cholesterol, hence reducing the risk of diseases caused by high blood cholesterol and free radical activities (Rahmat *et al* 2004). So guava fruit has high nutraceutical potential.

Despite of such unique benefits of guava, very little work has been done at national level to exploit its nutraceutical potential. So, there is a need to exploit the health benefits of major guava varieties grown in tropical and sub-tropical areas of India. The coloured fleshed varieties show deviations in antioxidant activity than the colourless fleshed varieties, probably due to the presence of different pigments in them (Flores *et al* 2015). So, the nutraceutical potential of different varieties with different flesh colour needs to be compared to get the variety with better antioxidant potential and nutraceutical traits.

Thus, keeping in view the nutraceutical potential and scope of guava for mass cultivation in the state, the studies on morpho-physiological and nutraceutical characteristics

in guava genotypes was undertaken with the following core objective:

- To study the physiological and nutraceutical traits of cultivated guava and their association analysis with yield.

CHAPTER – II

REVIEW OF LITERATURE

Guava, being a heterozygous crop, has a wide variation in morphological characters. To form an effective genetic plant improvement programme with desirable traits, information on varietal or genetic differences is important. Though, the information on morpho-physiological parameters in potential guava genotypes is documented to certain extent, however, a very little information on nutraceutical properties of guava is available in the guava genotypes having potential under North Indian conditions. Accordingly, in this chapter, an attempt has been made to review the literature with respect to the present investigation "Morphological and nutraceutical characterization of guava (*Psidium guajava* L.) genotypes". The available literature on various guava genotypes and their morpho-physiological and nutraceutical profiling is reviewed as follows:

Morpho-physiological characterization

Ghosh *et al* (2013) studied the performance of 21 guava cultivars viz., Lucknow-49, S8, Apple colour, Allahabad Safeda, Harijha, F1, S1, Behat Coconut, Seedless, Arka Mridula, Red Fleshed, Arka Amulya, Patialo, Supreme, Chittidar, Kairala Seedlings, Khaja, Almond Iskbala, Banarasi, Baruipur and Florida Seedlings in West Bengal in laterite and red soil under irrigated conditions. This study of four consecutive years concluded that Banarasi cultivar was having highest average yield (73.7 kg plant^{-1} year^{-1}) followed by Allahabad Safeda (71.6 kg plant^{-1} year^{-1}) and Apple colour (69.5 kg plant^{-1} year^{-1}). Almond Iskbala cultivar recorded maximum fruit weight (177g), followed by Red Fleshed (161g) and Apple Colour (151g). The fruits of rainy season crop were having more weight than winter season crop in most of the cultivars. Kundu *et al* (1995), Das *et al* (1995) and Singh (2004) also reported the lower values of fruit weight (76.9-116.3g) in various guava cultivars in different agro-climatic conditions. The fruits of Florida Seedling were having highest seed content (36%), followed by Allahabad Safeda (20%). The Seedless cultivar recorded lowest seed content (1.1%). Seed texture was soft in Chittidar cultivar only, while all other cultivars were having hard seed texture.

Singh *et al* (2016) evaluated seven varieties of guava in arid conditions of Punjab with respect to their growth, yield and quality traits. Plant height was maximum in Red Fleshed and L-49 while, stem diameter and plant spread were maximum in Allahabad Safeda and Sarbati. The highest yield was recorded in Allahabad Safeda (10.20 kg tree^{-1}) followed by L-49 (8.60 kg tree^{-1}). Flesh colour was red in Red Fleshed, pink in Lalit and white in other varieties. The fruit weight ranged from 87.2g (Lalit) to 152g (Sarbati). Sarbati recorded maximum fruit size and number of seeds/100 g fruit (194). Shweta recorded minimum

number of seeds/100g fruit (128). Pulp thickness was maximum in Allahabad Safeda (15.70 mm). The highest TSS was recorded in MPUAT Sel. 1 (20.4^0B), whereas acidity was maximum in Lalit (0.69 %). Acidity was minimum in Red Fleshed (0.49%). Red Fleshed had maximum total sugar content (10.16%) while, L-49 recorded maximum pectin content (1.2%).

Khehra and Bal (2006) studied the variability among eighteen distinct genotypes of guava (*P. guajava*). They recorded high range of variability in various characters like fruit traits, maturity period and yield efficiency among these genotypes. The average fruit weight ranged from 100.5 to 238.3 g, seed content from 1.8 to 5.5 g and seed number from 112 to 466 per fruit in different genotypes. The variability range for TSS was from 9.8 to 12.3 per cent. Ascorbic acid content ranged from 107.52 to 278.04 mg in 100 g of pulp. The maturity period varied from first fortnight of November to first fortnight of December.

Sharma *et al* (2010) conducted an experiment to estimate the genetic relationship between 20 genotypes of *P. guajava* and two species, *P. friedrichsthalianum* Ndz. and *P. catleianum* Sabine, by means of a morphological characterization. 16 morphological characters were studied and all the traits showed variability to considerable extent except inflorescence type, which did not show significant variation. Maximum fruit length was recorded in 'Lucknow-49' (7.62 cm) that was at par with 'Tehsildar' (7.29 cm). The minimum fruit length was recorded in genotype 'Strawberry guava' (2.63 cm) which was at par with 'Chinese guava' (3.14 cm). The highest fruit diameter was recorded in 'Lucknow-49' (7.07 cm), followed by 'Barafkhana' (6.63 cm) and 'Dharwar' (6.47 cm). The minimum fruit width was observed in 'Strawberry guava' (2.47 cm), which was at par with 'Chinese guava' (2.54 cm). The maximum fruit weight was recorded in 'Barafkhana' (213.80 g), which was at par with 'Lucknow49' (211.26 g). The minimum fruit weight was observed in 'Strawberry guava' (11. 93 g) which was at par with the 'Chinese guava' (12.92 g). TSS ranged from 9.4 to 13.5 ^{0}Brix. Maximum TSS was observed in 'Hybrid Red Supreme'. Acidity ranged from 0.37% to 0.96%. Maximum acidity was observed in 'Chinese Guava'. Ascorbic acid ranged from 51.90 to 189.73 mg/100 g of fruit pulp. Maximum amount of ascorbic acid was observed in 'Gutaniwala'.

Shukla *et al* (2012) evaluated the diverse germplasm of guava assembled at Horticulture Farm, Maharana Pratap University of Agriculture and Technology, Udaipur with respect to morphometric characteristics, quality parameters and yield attributes for further utilization in improvement programmes. The 47 guava genotypes were evaluated and they showed significantly range of variability with respect to yield attributes, plant growth and yield and quality parameters. The different fruit characters and their range of values were- fruit weight from 65g to 281 g, fruit size (L x B) from 4.40 cm x 8.61 cm to 3.60 cm x 8.24 cm, fruit yield from 25 kg/tree to 68 kg/tree, seed weight from 1.21 g/fruit to 3.26 g/fruit,

number of seeds/fruit from 125 to 450, TSS from 11% to 18.20%, reducing sugars content from 3.96% to 4.75%, non reducing sugars content from 3.45% to 4.68%, total sugars content from 7.41% to 9.43%, acidity from 0.5% to 1.01%, ascorbic acid from129 mg/100 g pulp to 268mg/100 g pulp and organoleptic score from 7 to 9.

Singh *et al* (2013) conducted an experiment at Institute of Agricultural Sciences, BHU, Varanasi during the year 2011-12. The experiment comprised of five cultivars of guava viz., Allahabad Safeda, Lalit, Sweta, Hisar Surkha and L-49. Maximum fruit length was recorded in Hisar Surkha (6 cm), followed by L-49 (5.60 cm) and Lalit (5.57 cm). Fruit diameter was maximum in Lalit (6.79 cm). The minimum length (5.22 cm) and diameter (4.92 cm) of fruit were recorded in Allahabad Safeda. These variations in fruit length and diameter might be due to genotypic variations, in association with various physiological phenomena viz. photosynthetic efficiency, translocation of photosynthates from source to sink and photo-respiration that occurs in the plant body. Lalit had maximum fruit weight (144.60 g) and Allahabad Safeda had minimum fruit weight (123.63 g). The maximum seed weight was observed in Allahabad Safeda (3.70 g) followed by L-49 (3.24 g), Sweta (3.18 g) and Hisar Surkha (2.97 g), while Lalit recorded minimum seed weight (2.83 g). Allahabad Safeda, L-49 and Sweta were having bold sized seed, whereas Lalit and Hisar Surkha were having medium sized seed. The seed texture was hard in Allahabad Safeda and L-49, whereas it was soft in Hisar Surkha, Lalit and Sweta. The maximum ascorbic acid content and acidity were recorded in Sweta, while minimum values for the same parameters were recorded in Lalit. TSS was maximum in Hisar surkha and minimum in Allahabad Safeda.

Verma and Singh (2015) studied the physico-chemical attributes of seven important cultivars of guava in winter season of the year 2011-12 at Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. The fruit shape did not vary significantly but red blush on the skin was cultivar specific. Fruit weight and volume increased with increase in fruit size, while specific gravity remained unaffected. The maximum fruit length, width, volume and weight were observed in L-49 and Gorakh Bilash Pasand, followed by Sweta. The highest keeping quality was recorded in L-49 (7 days) with maximum weight (244.52g) and minimum fruit quality was recorded in Sweta (4 days) with fruit weight of 236.16g. The maximum acidity was observed in L-49 which was comparable to Allahabad Safeda and Sweta. The ascorbic acid was recorded maximum in L-49, followed by Allahabad Safeda and Sweta. The maximum pH and TSS were observed in Lalit and Sangam. Correlation between pH and acidity and dry matter content was significantly positive. The soft texture of fruit was considered important for better pulp quality, while taste and flavour were essential for palatability. The soft texture and appealing taste and flavour were recorded in the fruits of L-49 and Allahabad Safeda.

Mehmood *et al* (2014) collected the one hundred and thirty two accessions from 12

regions in Pakistan to study the genetic diversity. A total of 33 traits, comprised of 18 qualitative and 15 quantitative traits, were studied for assessment of the genetic variability and structure of guava germplasm. Traits like fruit acidity, seed weight, fruit diameter, fruit skin colour, outer flesh thickness, number of seeds, non-reducing sugars, fruit sweetness, longitudinal ridges, longitudinal grooves, leaf twisting, fruit shape at the stalk and flesh colour were found highly variable. Many of these traits had significant economic importance and could be used as breeding objectives to enhance fruit quality and fruit yield. There was strong positive correlation among the 15 quantitative traits related to fruit quality and fruit yield. The traits included fruit weight and diameter, fruit length and diameter, fruit weight and diameter of the fruit cavity, length and width of the leaf blade, number of seeds and seed weight, and seed weight and fruit weight. Among the rest 18 qualitative traits studied, some negative correlations were found.

Methela *et al* (2019) carried out an investigation on twelve guava germplasm, to study morphological characters viz. leaf qualitative and fruit qualitative and quantitative characters. The colour, venation, shape, surface smoothness and tips of different guava germplasm showed a significant variation among them. Fruit length was maximum in Chiangmai long (9.37 cm) and minimum in Sawadi (4.43 cm). Fruit diameter was maximum in BAU-5 (8.54 cm) and minimum in Brazil (4.27 cm). The dry weight per 100 g fruits was maximum in Thai oval (18.14 g) and minimum in BAU-6 (11.09 g). Number of seeds per fruit was maximum in Chiangmai round (352.80) and minimum in Kanchan Nagar (196.40). Weight of seeds per fruit was maximum in Chiangmai round (4.39 g) and minimum in Kanchan Nagar (1.94 g). Weight of seeds per 100 g fruit was maximum in Brazil (4.22 g) and minimum in BAU-1 (1.34 g). Weight of 1000 seeds was maximum in BAU-5 (13.07 g) and minimum in Sayedi (7.93 g). A significant variation was observed among the germplasm using analysis of variance for different characters.

Singh *et al* (2014) evaluated seven important cultivars of guava on the basis of morphological-chemical attributes of fruit in winter season crop. The guava fruits were collected from Central Institute for Subtropical Horticulture, Lucknow. The experiment was conducted in completely randomized block design and was replicated thrice, taking two plants as a unit. The maximum fruit length, width, volume and weight were observed in L-49. The specific gravity was highest in Lalit, followed by L-49. The pectin content was highest in Shweta, followed by L-49. Maximum pH was observed in Lalit (6.15), followed by Allahabad Safeda (6.08) and L-49 (6.05). The TSS was maximum in L-49. The TSS: acid ratio was highest in L-49, followed by Allahabad Safeda and Lalit. Significant positive correlation was found between pH and acidity and dry matter content.

Pandey *et al* (2007) conducted an experiment for evaluation of eleven guava hybrids/selections for various morphological and physico-chemical attributes for their

suitability under north Indian conditions. Tree height, canopy spread and stem circumference were maximum in Hybrid-21, while same characters were minimum in Arka Mridula, Hisar Safeda and CISH-G-1 respectively. Maximum fruit weight was observed in Shweta (CISH-G-4) (275 g) and minimum in Hisar Safeda (130 g). Maximum fruit yield was observed in Lalit (24.27 kg per tree) and minimum in Hybrid-21 (4.27 kg per tree). Fruit length was maximum in Hybrid-21 (8.40 cm) and minimum in Lalit (5.83 cm). Fruit breadth was maximum in Shweta (7.43 cm) and CISH-G-31 (7.43 cm) and minimum in Lalit (6.37 cm). Hisar Surkha recorded maximum TSS (15.67^0B), reducing sugar (6.11%) and total sugar contents (9.32 %) and minimum acidity (0.157%). TSS was minimum in Hybrid-21 (11.27^0B). Acidity was maximum in Sangam (0.35%). Total sugars were minimum in Hisar Safeda (6.67%). Reducing sugars content was minimum in CISH-G-1 (4.41%). Ascorbic acid content was maximum in cultivar Shweta (CISH-G-4) (243.75 mg/100 g pulp) and minimum in Pant Prabhat (104.17 mg/100 g pulp). Based on this evaluation, it was observed that cultivars Hisar Surkha, Shweta (CISH-G-4), Lalit and Sangam performed better as compared to other tested cultivars.

Patel *et al* (2011) carried out variability studies in guava genotypes in mid-hills of Meghalaya for growth, yield and quality attributes. Eleven guava genotypes viz., RCGH-1, RCGH-4, RCGH-7, RCG-1, RCG-2, RCG-3, RCG-11, L-49, Sangam, Allahabad Safeda and Lalit showed a lot of variation with respect to plant growth, yield and quality traits. The range of values for different parameters were: Plant height (m) from 2.04 to 3.07, stem diameter (cm) from 4.17 to 6.65, canopy spread (m^2) from 2.35 to 7.66, shoot length (cm) from 61.75 to 96.83, shoot diameter (mm) from 9.80 to 11.57, no. of leaves/shoot from 36.83 to 45.50, yield (kg/tree) from 5.40 to 14.18, fruit weight (g) from 92.48 to 184.50, fruit length (cm) from 5.16 to 7.08, fruit diameter (cm) from 5.25 to 7.08, no. of seeds/100 g fruit weight from 53.29 to 361.44, test weight 100 seeds (g) from 0.96 to 2.07, TSS (%) from 9.35 to 11.88, acidity (%) from 0.45 to 0.65, ascorbic acid (mg/100 g pulp) from 149.13 to 246, total sugar (%) from 6.04 to 8.39, pectin (%) from 0.82 to 1.38, phenol (mg GAE/100 g FW) from 250.50 to 377.45 and dietary fibre (%) from 2.38 to 4.70. RCGH-1 was found superior in stem diameter (6.65 cm), plant height (3.07m), fruit yield (14.18 kg/tree) and highest ascorbic acid (246 mg/100 g) content. The hybrid RCGH-4 was having maximum fruit weight (184.50 g) and fruit diameter (7.08 cm) whereas, the cultivar RCG-11 recorded least seed number (53.29 Nos/100 g fruit weight) with highest TSS (11.88%). The RCGH-7 was found to be superior in quality attributes like highest total sugars content (8.39%) and pectin content (1.38 %) and lowest acidity (0.45%). The genotypes viz., RCGH-1, RCGH-7 and RCG-11 were observed to be superior to other cultivars for growth, yield and quality attributes.

Kumar *et al* (2017) studied the physico-chemical and morphological characters of 15 guava genotypes viz. MPUAT Sel-1, MPUAT Sel-2, RCGH-1, RCGH-7, RCGH-11, CISH-

G-35, KG-1, Arka Kiran, Allahabad Safeda, Kayamganji, Barafkhana, Hisar Surkha, Hisar Safeda, Sangareddy and Bariyapur. The differences were significant among different genotypes. MPUAT Sel-1 recorded maximum fruit weight (210 g), fruit diameter (68.87 mm), fruit size (4865.93 mm^2), fruit volume (225 ml) and total sugar percentage (7.44%). 'Kayamganzi' genotype recorded maximum reducing sugars content (4.55%) and fruit length (78.06 mm). Allahabad Safeda exhibited maximum length: diameter ratio (1.06). RCGH-1 recorded maximum plant height (4.85 m), stem girth (7.35 in), plant canopy spread (5.47 m^2), non-reducing sugars (4.30%), TSS (12.40^0B), sugar: acid ratio (49.43) and minimum titraTable acidity (0.15%). RCGH-11 recorded maximum specific activity (1.24). Sangareddy recorded maximum ascorbic acid content (149.41 mg/100 g).

Deshmukh *et al* (2013) carried out a comparative study on three guava hybrids viz. RCGH 1, RCGH 4 and RCGH 7 and three commercial cultivars viz. L-49, Allahabad Safeda and Lalit with respect to growth, yield and quality traits for two years (2011 and 2012) at ICAR Research Complex for NEH Region, Umiam, Meghalaya. The significantly maximum fruit yield was recorded in RCGH 1 (39.05 kg/plant), followed by Allahabad Safeda (31.37 kg/plant), however minimum in L 49 (21.26 kg/plant) which was significantly less than RCGH 7 (24.26 kg/plant). The fruit weight was recorded highest in RCGH 4 (183.52 g). Fruit length and fruit diameter were maximum in RCGH 4 (6.54 cm and 6.99 cm respectively) and minimum in Allahabad Safeda (5.98 cm and 6.15 cm respectively). While less number of seeds/100 g fruit weight was observed in RCGH 7 (111.18). In fruit quality, hybrid RCGH 1 exhibited maximum ascorbic acid (231.86 mg/ 100g), TSS (10.83ºB) and total sugar (8.07%) with lowest acidity (0.50 %), followed by RCGH 7 (205.26 mg/100g, 10.39ºB, 8.05% and 0.51%), respectively, and found superior over L-49 and Allahabad Safeda under mid-hills situations. TSS: acid ratio was maximum in RCGH 1 (21.52) and minimum in Lalit (14.43). RCGH 1 and RCGH 7 recorded maximum pectin content (1.33% and 1.31% respectively) and Allahabad Safeda and L-49 recorded minimum pectin content (0.92% and 0.97% respectively).

Mehta *et al* (2016) conducted an experiment at Horticulture Research Centre of H.N.B. Garhwal University, Srinagar Garhwal (Uttarakhand), India to compare the physico-chemical characters of three guava cultivars viz., Lucknow-49, Allahabad Safeda and Pant Prabhat under sub-tropical valley conditions. Lucknow-49 recorded maximum fruit weight (158.08gm), fruit length (6.10cm), fruit diameter (6.45cm) and fruit volume (160.87ml). Number of seeds/100gm fruit weight was minimum in Pant Prabhat (133.45) and maximum in Allahabad Safeda (146.51). Pant Prabhat recorded minimum fruit weight (108.18 g), fruit length (4.94 cm), fruit diameter (5.43cm) and fruit volume (109.14ml). Allahabad Safeda recorded maximum fruit specific gravity (0.99ml) and acidity (0.60%). Pant Prabhat recorded maximum total soluble solids (11.82^0Brix), TSS: acid ratio (44.29), pectin content (0.98%),

total sugar content (7.38%), ascorbic acid content (230.44mg/100gm) and minimum acidity (0.27%). Thus the cultivars Pant Prabhat and Lucknow-49 were found superior with respect to chemical and physical characteristics respectively in this investigation.

Ulemale *et al* (2018) carried out evaluation of nine guava genotypes viz., GRS_1, GRS_2, GRS_3, GRS_4, GRS_5, GRS_6, GRS_7, GRS_8 and L-49 for yield and bio-chemical parameters. Fruit yield was maximum in red fleshed genotype GRS_8 (52.9 kg/tree) and minimum in white fleshed genotype GWS_8 (17.27 kg/tree). White fleshed genotype GWS_6 contained maximum total sugar (8.4%) and non-reducing sugar content (3.28%). Minimum total sugar and non-reducing sugar content was present in GWS_8 (6.42%) and GRS_1 (1.44%) respectively. Red fleshed genotype GRS_4 contained maximum reducing sugar (5.41%) and lowest acidity (0.38%) content. GWS_6 contained minimum reducing sugar (3.77%) and highest acidity (0.38%) content. White fleshed genotype GWS_6 recorded maximum ascorbic acid content (297.93 mg/00 g) and highest TSS (12.45 ^{0}Brix). Red fleshed genotype GRS_3 recorded minimum ascorbic acid content (176.79 mg/100 g) and lowest TSS (9.34 ^{0}Brix).

Pandey *et al* (2017) studied the various morphological characters of 20 genotypes of the *Psidium* sp. The various characters observed were petiole length, leaf surface area, length and width of leaf blade, upper and lower leaf surface colour, vein number, leaf apex and base shape, pubescence, leaf texture, leaf shape, leaves colour during winters and leaf lamina thickness. *Psidium guineense* was having maximum leaf surface area (106.12cm^2). Lalit was having maximum leaf blade length (14.89cm) and maximum leaf blade width (6.09cm). Petiole length was maximum for Sardar variety (1.08cm), while number of veins was maximum for Riverside variety (23.4). Maximum diversity was found in *Psidium chinensis*.

Alam *et al* (2017) studied the morphological characters of 8 guava varieties grown in Pakistan. Different characters studied were leaf petiole colour, leaf shape from base of leaves, branching habit, pubescence on apical leaves, leaf shape, branch colour, colour of apical leaves, mature leaf colour, leaf twisting, colour of leaf vein and leaf shape from tip of leaves. They found significant variations among varieties, which may help in better selection of plants for breeding programs.

Mahmoud and Peter (2014) carried out physical screening on hundred guava genotypes. They studied physical fruit characters and found significant variations among different genotypes. Thirteen genotypes (Genotype no. 100, 99, 50, 4, 30, 94, 88, 5, 57, 33, 88, 3, 8 and 67) were having better fruit physical performance. Genotype no. 99 showed highest fruit weight (300.5 g). These values were more than those recorded by Jana *et al* (2010), who obtained maximum fruit weight 139.8g for cv. Eskwala. Tandon *et al* (1983) recorded maximum fruit weight in var. Sardar Guava (162 g), followed by var. Apple Colour (135 g) and var. Chittidar (126 g). Mahmoud and Peter (2014) recorded maximum fruit diameter in genotype no. 99 (7.36 cm). They recorded least number of seeds in genotype no

99 (0) and 192 100 (0), followed by 94 (58), 3 (69) and 57 (103). Variation in seed number might be due to the fact that some varieties were diploid 2n=2x=22 and some were triploid 2n=3x=33. This was in accordance with Raman *et al* (1971) who reported that triploidy in guava is the cause of seedlessness. Lowest seed weight was recorded in genotype no. 99 (0 g) and 100 (0 g), followed by 57 (0.70 g), 94 (1.43 g) and 33 (1.53 g). Pulp thickness was highest in genotype no 99 (3.2 cm)

Morphological characterization of 4 guava varieties (L-49, Allahabad Safeda, Lalit and Shweta) grown in hot-arid Rajasthan zone was done by Singh *et al* (2017). Number of new leaves/bunch (4.66) and increase in plant height (25.93%) were maximum for Shweta. L-49 was having maximum total chlorophyll content as well as chlorophyll a and chlorophyll b contents. The leaf area (80.91cm^2), relative water content (60.19%) and specific leaf area (36.61cm^2/g) were more for Sweta than other varieties. Lalit variety was observed with highest net photosynthetic rate (10.84µmol $CO_2/m^2/s$), water use efficiency and carboxylation efficiency. The performance of Shweta variety was found to be better than other varieties under hot-arid conditions of Rajasthan.

Singh *et al* (2015) studied the tree, reproductive, vegetative, seed and fruit characters of 35 varieties of guava grown under Punjab conditions. They divided the varieties into 6 clusters for inter-cluster and intra-cluster studies. The different vegetative, reproductive, fruit and seed characters under study were young twig diameter, leaf length, leaf length to width ratio, flower size, inter node length, leaf chlorophyll index, leaf width, petiole length, number of petals, fruit weight, fruit diameter, pedicel length, outer flesh thickness, titraTable acidity, fruit length, fruit length/width ratio, calyx cavity diameter, TSS, vitamin C, seed core diameter, seed number/ fruit, seed weight/ fruit, seed weight/100g fruit and 100 seed weight.

Singh *et al* (2016) studied the morphological characters of some promising varieties of guava grown under north-Indian sub-tropical environment. Various leaf and flower characters studied were leaf shape, leaf apex, colour of midrib on lower side, leaf base, relief on upper surface of leaf, undulation of margin, petiole length, leaf blade width, leaf blade length, leaf length: width ratio, leaf chlorophyll index, number of fully developed petals and flower size. Variety Pear Shaped was having maximum chlorophyll index. Leaf length was maximum in Punjab Pink (151.27mm) while leaf blade width was maximum in L-49 (65.54mm). Young shoots with red colour were found in Lalit, Pear Shaped, Allahabad Safeda, Shweta and Punjab Pink. For winter season crop, the mean flower size was maximum in L-49 (47.01mm).

Various stem, fruit, leaf and root descriptors used by Kidaha *et al* (2015) were pubescence on apical leaf, colour of leaf vein, orientation of petiole, leaf retention, leaf shape, branch colour, petiole colour, leaf colour, root hair, colour of root pulp, root shape, colour of root cortex, colour of fruit endocarp and colour of fruit. They characterize the guava landraces

from coastal and western landraces. The landraces didn't show any differences based on root descriptors but they showed variations based on leaf, fruit and branching of stem. Different leaf shapes were trapezoidal, ovate, oblong and elliptical, and different branching habits were axial, irregular or erect.

Shiva *et al* (2017) characterized 20 genotypes of *Psidium guajava* and two species *viz., P. pumilum* and *P. friedrichsthalianum* based on fruit and leaf parameters. 24 parameters were studied. Fruit weight was maximum in Thai guava (228.26g) and minimum in *P. pumilum* (13.0g). Leaf length was maximum in Sour Type (15.41cm); leaf width was maximum in Pant Prabhat (7.62cm); petiole length was maximum for Sasni Collection (1.16cm); leaf area was maximum for Red type (60.92cm^2); leaf length and width ratio was maximum for *P. pumilum* (3.16). Other physio-chemical parameters studied were core diameter, TSS, antioxidant, fruit weight, seed weight/fruit, vitamin-C, number of seeds/fruit and titrable acidity. Lalit was having maximum core diameter (4.46cm) and TSS (15.43%); Hissar Surkha was having maximum number of seeds/fruit (439.33) and seed weight/fruit (4.12); Red type was having maximum titrable acidity (1.45%); L-49 was having maximum vitamin-C (187.7mg/g) and Hafsi Red was having maximum antioxidant activity (26.93mmol/g TROLEX).

Nutraceutical characterization

Lin and Yin (2012) worked on the extracts from guava fruit and studied the renal protective effects of extract in diabetic mice. The study led to the conclusion that guava fruit has anti-glycative, anti-oxidative and anti-inflammatory effects and could be a potential source to protect kidney from harmful effects of diabetes. The intake of extracts at 2% concentration showed good results and was effective to increase the plasma insulin levels in diabetic mice ($p< 0.05$) and decreased blood urea nitrogen and glucose levels. The activity of reactive oxygen species was also decreased. Treatments of GAE and GEE at 2 % decreased renal Nε-(carboxymethyl) lysine, pentosidine and fructose levels ($p<0.05$), interleukin (IL)-6, tumor necrosis factor-α and IL-1β levels in kidney ($p< 0.05$), and suppressed renal activity of aldose reductase ($p<0.05$).

Thuaytong and Anprung (2011) studied the bioactive compounds and prebiotic activity in Thailand-grown red and white guava fruit. The varieties under study were Pansithong for white guava and Samsi for red guava. The respective average values for white and red guava were: reducing sugar (mg glucose/g FW)= 43.90 and 47.87, total acidity (%)= 0.79 and 0.62, total dietary fiber (g/100g FW)= 3.28 and 4.99, soluble dietary fibre (g/100g FW)= 1.14 and 1.27, insoluble dietary fibre (g/100 g FW)= 2.14 and 3.72. The antioxidant activity was determined by 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging and ferric reducing antioxidant power (FRAP) assays. The values for DPPH assay were 10.28 µg fresh weight (fw)/µg DPPH for white guava and 7.82 µg fresh weight (fw)/µg DPPH for red

guava. The values for FRAP assay were 78.56 µM Trolox equivalent (TE)/g fw for white guava and 111.06 µM TE/g fw for red guava. Ascorbic acid contents were 130mg/100g fw for white guava and 112 mg/100 g fw for red guava. The amount of total phenolics contents were 145.52 and 163.36 mg gallic acid equivalents (GAE)/100 g fw for white and red guava respectively. Similarly, the amount of total flavonoids contents were 19.06 and 35.85 mg catechin equivalents (CE)/100g fw for white and red guava respectively. Solid-phase microextraction (SPME)/gas chromatography (GC)/mass spectrometry (MS) method was used to analyse the volatile compounds in guava. The major volatile compounds identified were cinnamyl alcohol, β-caryophyllene, α-bisabolene, ethyl benzoate and (E)-3-hexenyl acetate.

In a study conducted by Bashir and Abu-Goukh (2003) to analyze the compositional changes during ripening of white and pink fleshed guava, they found that the peel showed more values of total phenolic compounds, ascorbic acid and total protein than the pulp. Also, the white-fleshed guava had higher values of TSS, reducing sugars, phenolic compounds, total sugars, titraTable acidity and ascorbic acid than pink fleshed fruits.

Biegelmeyer *et al* (2011) carried out comparative analysis of the antioxidant activity and chemical composition of red (*P. cattleianum*) and yellow (*P. cattleianum* var. *lucidum*) strawberry guava fruits. The cultivars taken were Irapua for red (*P. cattleianum*) and Ya-Cy for yellow (*P. cattleianum* var. *lucidum*) strawberry guava. The phenolic compounds were determined by HPLC-DAD method and total flavonoid content was analyzed by Folin-Ciocalteu assay. The antioxidant activity was determined by total reactive antioxidant potential (TRAP) method. The red strawberry guava possessed a higher amount of polyphenolic compounds (501.33 mg/100 g) than yellow strawberry guava (292.03 mg/100 g). Similarly, the total flavonoids were higher in red strawberry guava (100.20 mg/100 g) than yellow strawberry guava (35.12 mg/100 g), with hyperoside being major flavonoid identified for both cultivars. Strawberry guava also contained an anthocyanin, named as cynidin, in addition to flavonoids. The volatile oils in fruits and fatty acids in seeds were evaluated by GC-EM. β-caryophyllene was found to be the main component of fruit oils. Linoleic acid was the major fatty acid present.

McCook-Russell *et al* (2012) conducted an experiment for nutraceutical and nutritional comparison of Jamaican *P. cattleianum* (strawberry guava) and *P. guajava* (common guava) fruits. They compared the total phenolics, proximate contents, anti-inflammatory, antimicrobial and antioxidant activities of *P. cattleianum* and *P. guajava.* Strawberry guava was superior to common guava in total phenolics, vitamin C content, antioxidant and antimicrobial activities. TEAC value of strawberry guava was three times to that of common guava (11.3 compared to 3.8 µmol Trolox/g fresh weight). Strawberry guava contained more than twice as much polyphenols as common guava (4439 compared to 1952

µg GAE/g FW). Both red and yellow strawberry guava fruits from Mauritia showed phenolic contents exceeding 5000 µg GAE/g fresh weight. These values are much higher as compared to those reported by Luximon-Ramma *et al* (2003) in pink and white Mauritian common guavas having phenolic content of 1264 + 60 and 2473 + 45 µg GAE/g fresh weight respectively. Ascorbic acid contents were 2091 µg/g FW for *P. cattleianum* and 1200 µg/g FW for *P. guajava.* Strawberry guava also contained higher fibre content (24.9%) as compared to common guava. The hexane and ethyl acetate extracts of *P. cattleianum* fruits showed cyclooxygenase-2 enzyme inhibitory activities of 18.3% and 26.5%, respectively (250 µg/mL), expressing anti-inflammatory activity.

Dolkar *et al* (2017) studied the biochemical parameters in seven cultivars of guava at three development stages like green mature stage (GMS), half ripe stage (HRS) and full ripe stage (FRS). They found out that with the advancement of fruit maturity at different stages, ascorbic acid, the total soluble solids (TSS) and sugar (total sugar, non-reducing and reducing sugar) contents increased significantly, while pectin and acidity decreased during the fruit ripening. Out of all the cultivars studied, ascorbic acid (265.09 mg/100g FW) and total sugar (8.50%) were highest in L-49, followed by Allahabad Safeda (245.24 mg/100g FW and 8.20% respectively) at full ripe stage. L-49 showed maximum pectin content (0.77%) and minimum acidity (0.26%) followed by Allahabad Safeda (0.73% and 0.39% respectively). Pectin methyl esterase (PME) activity increased progressively in all the cultivars up to half ripe stage and subsequently decreased at full ripe stage. PME activity was maximum in L-49 (56.25 units/g FW) at half ripe stage whereas, it was found to be decreased at full ripe stage (52.25 units/g FW), followed by Allahabad Safeda and Lalit. It was found out that among all the commercial cultivars of guava grown under sub-tropical conditions, L-49 was superior followed by Allahabad Safeda and Lalit.

Guo *et al* (2003) carried out an experiment to determine the antioxidant activities of pulp, peel and seed fractions of 28 fruits consumed commonly in China. Antioxidant activity was measured using the ferric reducing antioxidant power (FRAP) assay. They found out that among all fruit pulps, hawthorn pulp had the maximum FRAP value (13.42 mmol/100g wet weight), followed by date (6.98 mmol/100g wet weight), guava (6.07 mmol/100g wet weight), kiwifruit (4.38 mmol/100g wet weight), purple mulberry (4.11 mmol/100g wet weight), strawberry (3.29 mmol/100g wet weight), white pomegranate (3.10 mmol/100g wet weight), lukan tangerine (2.29 mmol/100g wet weight) and honey tangerine (2.19 mmol/100g wet weight) pulps. Hence guava stood 3rd among the 28 fruits pulps in antioxidant potential.

Luximon-Ramma *et al* (2003) carried out an experiment to analyze the antioxidant potential, phenolics, proanthocyanidins, flavonoids and vitamin C contents of 17 commonly consumed Mauritian exotic fruits. Antioxidant potential was measured by two independent methods. The antioxidant activity measured by Trolox equivalent antioxidant capacity

(TEAC) ranged from 1 to 47 µmol g^{-1} fresh weight and that measured by ferric reducing antioxidant power (FRAP) ranged from 11 to 360 µmol g^{-1} fresh weight. Total phenolics ranged from 118 to 5638 µg g^{-1} fresh weight, proanthocyanidins ranged from 7 to 2561 µg g^{-1} fresh weight, flavonoids ranged from 21 to 712 µg g^{-1} fresh weight and vitamin C content ranged from 8 to 1426 µg g^{-1} fresh weight. The correlations between antioxidant activity and proanthocyanidins and total phenolics were strong. The contribution of flavonoids to the antioxidant potential seemed to be less, whereas correlations between ascorbate content and antioxidant activity were very poor. Red and yellow *P. cattleianum* Sabine 'Chinese guava' recorded highest antioxidant potential, followed by sweet and acid *Averrhoa carambola* L 'starfruit', *Syzygium cumini* L Skeels 'jamblon' and white *P. guajava* L 'guava'. The total phenolics contents were 1264 µg gallic acid g^{-1} fresh weight in pink fleshed guava and 2473 µg gallic acid g^{-1} fresh weight in white fleshed guava. The flavonoids contents were 110 µg quercetin g^{-1} fresh weight in pink fleshed guava and 209 µg quercetin g^{-1} fresh weight in white fleshed guava. The proanthocyanidins and vitamin C contents were 109 µg cyaniding chloride g^{-1} fresh weight and 722 µg ascorbic acid g^{-1} fresh weight respectively in pink fleshed guava and 263 µg cyaniding chloride g^{-1} fresh weight and 1426 µg ascorbic acid g^{-1} fresh weight respectively in white fleshed guava. The antioxidant activities measured by TEAC and FRAP assay were 7 µmol trolox g^{-1} fresh weight and 8 µmol Fe(II) g^{-1} fresh weight respectively in pink fleshed guava and 17 µmol trolox g^{-1} fresh weight and 14 µmol Fe(II) g^{-1} fresh weight respectively in white fleshed guava.

Kumar *et al* (2016) studied the biochemical attributes like antioxidant activity (DPPH), total phenolics, total flavonoids, ascorbic acid content and H_2O_2 scavenging activity in guava fruits cv. Allahabad Safeda at ICAR-CSSRI, Karnal, Haryana. The fruits produced in winter season exhibited higher flavonoids content, total phenolics content, antioxidant activity, ascorbic acid and H_2O_2 scavenging activity as compared to summer season fruits. Immature fruits recorded higher antioxidant activity, total phenol content and flavanoids content (49.35%, 24.84 mg TA/g and 26.98 mg/100g respectively) during both seasons than mature (40.02%, 23.95 mg TA/g and 24.22 mg/100g respectively), ripe (31.76%, 23.62 mg TA/g and 20.54 mg/100g respectively) and overripe fruits (25.7%, 23.17 mg TA/g and 17.04 mg/100g respectively). H_2O_2 scavenging activity increased as the maturity advances in both the seasons and was maximum in over ripe fruits (501.48 µmol/g). Ascorbic acid content was maximum in ripe fruits (205.03 mg/100 g FW) that decreased considerably in over ripe fruits (137.25 mg/100 g FW). Hence the season of crop and maturity stages differ significantly in relation to the various biochemical parameters.

Melo *et al* (2006) carried out an experiment to analyze the concentration of some bioactive phytochemicals in 15 vegeTables and 15 fruits consumed commonly in Recife-PE, Brazil. They determined polyphenols, ascorbic acid and total carotenoid contents in these

fruits and vegeTables. In guava, the total phenolics content was 186.68 mg catechin 100 g^{-1} fresh weight and total flavonols content was 5.45 mg quercetin 100 g^{-1} fresh weight. Total proanthocyanidins content was 182.97 mg catechin 100 g^{-1} fresh weight, ascorbic acid content was 89.29 mg 100 g^{-1} fresh weight and total carotenoids content was 42.98 µg g^{-1} fresh weight.

Moreno *et al* (2014) studied the antioxidant potential and main phytochemicals present in fresh fruits as well as flour obtained by lyophilization of ripe fruits of common guava. Total phenolics were 50.35 mg GAE/100 g FW in fruits and 970.34 mg GAE/100 g in flour, non-flavonoids phenolics were 0.56 mg GAE/100 g FW in fruits and 13.22 mg GAE/100 g in flour, flavonoid phenolics were 49.79 mg GAE/100 g FW in fruits and 957.12 mg GAE/100 g in flour, condensed tannins were 8.51 mg procyanidin B2/100g FW in fruits and 208.02 mg procyanidin B2/100g in flour, anthocyanins were 2.3 mg C3GE/100g FW in fruits and 16 mg C3GE/100g in flour, ascorbic acid content was 19.73 mg/100g FW in fruits and 143.49 mg/100g in flour, and carotenoids were 821.49 mg E β-C/100 g FW in fruits and 2300 mg E β-C/100 g in flour. The antioxidant activity of fruits and flour samples were also analyzed using ABTS assay, and all the extracts obtained from flour exhibited more activity than fresh fruits.

Nora *et al* (2014) studied the bioactive compounds profile in red guava (*P. cattleyanum* Sabine) and guabiju (*Myrcianthes pungens* (O. Berg) D. Legrand), both fruits native to southern Brazil. They evaluated the physicochemical composition, functional compounds and antioxidant activity of both fruits. Dietary fibre was more in red guava. Guabiju exhibited higher anthocyanin content, higher carotenoid content and higher antioxidant activity. β-carotene was the predominant carotenoid in guabiju fruit, representing 40.4% of the total carotenoids, and malvidin-3-glycoside was the predominant anthocyanin, representing 60% of the total anthocyanins. In the red guava, β-cryptoxanthin represented 44.8% of the total carotenoids and cyanidin 3-glycoside represented 51.7% of the total anthocyanin content.

Vissotto *et al* (2013) determined the contents of total phenolic compounds, ascorbic acid and total flavonoids of 18 frozen fruit pulps and observed their scavenging capacities against peroxyl radical (ROO*), hydroxyl radical (*OH) and hydrogen peroxide (H_2O_2). Whole guava fruit was taken for the experiment. Total phenolic compounds content recorded from frozen guava pulp was 88 mg GAE/100 g, total flavonoids content was 25 mg CE/100 g of frozen pulp and ascorbic acid content was 29 mg/100g of frozen pulp. The scavenging capacity of guava against peroxyl radical was 1575 µmol trolox/100 g of frozen pulp, against hydrogen peroxide was 1454 µg/mL (IC_{50}) and against hydroxyl radical was 65 µg/mL (IC_{50}). There was strong correlation between total phenolics and capacity to scavenge ROO*. Flavonoids showed high capacity to scavenge *OH, but less capacity to scavenge H_2O_2.

Saroja (2015) conducted a study to analyze the total polyphenolic content (TPC) and total flavonoid content (TFC) in two varieties of papaya and guava fruits i.e. yellow and red fleshed varieties of papaya and white and red fleshed varieties of guava from Agasteeswaram taluk in Kanyakumari district. The concentrations of total polyphenols and total flavonoids were three times higher in guava fruits than papaya fruits. The TFC and TPC are proportional to the antioxidant activity, hence the antioxidant activity due to TPC was in order: white guava (3.355µg/ml) > red guava (3.255µg/ml) > red papaya (1.32µg/ml) > yellow papaya (1.143µg/ml). The antioxidant activity due to TFC was in order: red guava (1.991µg/ml) > white guava (1.663 µg/ml) > yellow papaya (0.795 µg/ml) > red papaya (0.584 µg/ml).

Vasco *et al* (2008) analyzed 17 fruits from Ecuador to determine their ascorbic acid content, total soluble phenolic compounds content and antioxidant capacity, using DPPH, FRAP and ABTS assay. Guava fruits contained ascorbic acid in the range of 74 to 84 mg/100 g FW and total soluble phenolic compound content 462±128 mg GAE/100 g FW. The antioxidant activity measured by DPPH assay was 30±7 µmol trolox/g FW. Andean blackberry, capuli cherry peel and banana passion fruit contained maximum total phenolic contents and hence were having highest antioxidant activities.

Wilberg and Rodriguez-Amaya (1995) quantifiedd major carotenoids in 52 samples of guava, papaya, mango and some of their processed products by HPLC by both standard addition and external standardization calibration techniques. β-carotene concentrations in 7 different samples of pink-fleshed guava obtained by external standardization were 5.36, 4.11, 5.84, 4.42, 3.19, 3.02 and 3.08 µg/g FW and those obtained by standard addition were 5.62, 4.16, 5.61, 4.51, 3.18, 3.03 and 3.11 µg/g FW respectively. Lycopene concentrations in same guava samples obtained by external standardization were 48.2, 44.8, 58.6, 52.6, 60.6, 52.8 and 54.2 µg/g FW and those obtained by standard addition were 49.8, 44.8, 58.1, 51.3, 61, 52 and 55.1 µg/g FW respectively. Hence these two calibration techniques were not significantly different. These concentrations were found to be coherent with those obtained previously with open column chromatography.

Ahmed *et al* (2013) studied the antioxidant activities of guava and mango fruits. One local variety of guava and 4 varieties of mango (Apple Mango, Keitt Mango, Vandyke Mango and Local Mango) were tested for their antioxidant potential to reduce potassium hexacyanoferrate (Fe^{3+}) to ferrous (Fe^{2+}) form. FRAP assay was used to measure antioxidant potential. The FRAP value obtained from guava fruits was 2.39 mg AA/g sample while those obtained from Keitt, Vandyke, Apple Mango and Local Mangos were 1.95, 1.85, 1.76 and 1.66 mg AA/g sample respectively. In percent reducing power as compared to ascorbic acid, highest reducing power was recorded in guava fruit (96.98%). Percent reducing powers of Keitt Mango, Vandyke Mango, Apple Mango and Local Mango varieties were 82.44%, 78.96%, 76.18% and 71.34% respectively.

Correa *et al* (2011) reported the contents of free ascorbic acid, flavonoids, lycopene, β-carotene, phenolic compounds and total antioxidant activity in a collection of guava and araca (*Psidium* spp.) accessions from different Brazilian regions. Ascorbic acid content varied from 44.66 to 409.77 mg/100g, and maximum concentration was observed in G03MA, G47PE and G38PE accessions. Total flavonoids were expressed in rutin concentration and ranged from 10.67 to 46.82 mg/100g in guava accessions, with maximum concentration found in G21MA (dark orange pulp), G24MA (dark pink) and G55SE (pale orange) accessions. Lycopene content varied from 4.04 mg/100g in the accession G73RO (dark pink coloured pulp) to 0.04 mg/100g in G96AM accession (white coloured pulp). The β-carotene content varied from 0.13 to 2.54 mg/100g in guava accessions. Total phenols were expressed in equivalent of GA (GAE) and ranged from 158 to 447 mg GAE/100g in guava accessions, and these contents were maximum in G03MA, G10MA and G01MA having dark orange, dark pink and pale orange pulp respectively. The antioxidant activity varied from 280 to 812 mg/100g expressed as AAE in guava accessions. The antioxidant activity varied from 23.87 to 70.42 µmoles/g in guava when expressed as µmoles of reduced DPPH/g of sample. They found high positive correlation between β-carotene and flavonoid content and between phenolic content and antioxidant activity.

Joseph and Priya (2011) reviewed the pharmacological and nutritional value of guava. Toxicity studies on leaf and fruit extracts in mice showed no side-effects. The compounds isolated from various parts of plant like quercetin, galactose-specific lecithins, guaijaverin and flavonoids have anti-diarrheal, anti-bacterial, anti-spasmodic activities, which greatly add to the medicinal value of guava. Various pharmacological studies on guava have proved its antihypertensive, anti-mutagenic, anti-microbial, anti-diarrheal, hypoglycemic, hepatoprotective and anti-microbial potential.

Yan *et al* (2006) compared guava, banana, orange, dragon fruit, sugar apple, star fruit and water apple for ascorbic acid content, total phenols content and antioxidant potential. The antioxidant activity was assessed by DPPH and FRAP assay. They found out that guava was having highest antioxidant potential among given fruits, containing rich amount of phenol compounds and ascorbic acid. The total phenols contents were 138 mg GAE/100g in seeded guava and 179 mg GAE/100g in seedless guava. Ascorbic acid contents were 144 mg/100g in seeded guava and 132 mg/100g in seedless guava. Antioxidant potential measured by DPPH assay was 218 mg AA/100g in seeded guava and 176 mg AA/100g in seedless guava while that measured by FRAP assay was 2.09 mg GAE/g in seeded guava and 1.65 mg GAE/g in seedless guava. IC_{50} value measured by DPPH assay was 1.71 mg/mL in seeded guava and 2.11 mg/mL in seedless guava.

Alothman *et al* (2009) observed phenol content and antioxidant potential of three fruits-Thai seedless guava, honey pineapple and banana. Ferric reducing assay was used to

evaluate antioxidant potential of fruits. The phenolic content in Thai seedless guava was 123-191 GAE/100g (GAE=gallic acid equivalents), and in banana was 24.4-72.2 GAE/100g, and that in honey pineapple was 34.7-54.7 GAE/100g. So, the Thai seedless guava was having highest phenolic content, which is positively related with antioxidant activity.

Flores *et al* (2015) studied the antioxidant potential of seven varieties of guava with different pulp colours. There were four pink-pulp varieties- Homestead, Sardina 2, Barbie Pink, Sardina 1; two white-pulp varieties- Sayla and Yen-2; and one red-pulp variety-Thai Maroon. DPPH and ABTS assays were performed to estimate the antioxidant potential of guava fruits. In the DPPH assay, the red pulp fruits showed maximum activity, followed by pink fleshed fruits. The activity of white pulp fruits was lower than coloured pulp fruits. The ABTS assay showed the same results as those of DPPH assay for first 20 minutes, but after that the Barbie Pink (pink-pulp variety) showed lesser activity than white-pulp guavas. So, these studies lead to the conclusion that composition of phytoconstituents and antioxidant potential of guava fruits vary with pulp-colour and varieties.

Escrig *et al* (2001) studied the dietary fibre and antioxidant content of guava fruit. Experiments were performed on peel and pulp fractions which showed high proportion of polyphenols (2.62–7.79%) and dietary fibre (48.55–49.42%).

Mercadante *et al* (1999) isolated the various carotenoid compounds from guava fruit and explained their structures. The experiments were performed on Brazilian red guavas and sixteen carotenoid compounds were extracted from the fruit flesh. The various compounds discovered were (all-E)-, (9Z)-, (13Z)-, and (15Z)-β-carotene, (all-E,3R)-rubixanthin, (all-E)-, (9Z)-, (13Z)-, and (15Z)-lycopene, (all-E,3R,3‘R,6‘R)-lutein, phytofluene, (all-E,3R)-β-cryptoxanthin, (all-E)-γ-carotene, (all-E,3S,5R,6R,3‘S,5‘R,8‘R)-, (all-E,3S,5R,6R,3‘S,5‘R,8‘S)-neochrome and (all-E,3S,5R,8S)-cryptoflavin. Out of all carotenoid compounds, the lycopene was obtained in major amount. Garbanzo *et al* (2017) also worked on the carotenoid profile of the guava fruits. They also found 15-cis-lycopene, all-trans-β-carotene, all-trans-lycopene in the fruit pulp and all-trans-lycopene were present in dominant amount (63% to 92% of the total carotenoids).

The guava fruit contains both hydrophilic and lipophilic antioxidant compounds. Thaipong *et al* (2005) studied the lipophilic antioxidant activity (AOAL) and hydrophilic antioxidant activity (AOAH) of guava fruit. They found that major antioxidant activity of guava comes from hydrophilic part. The hydrophilic antioxidant activity is attributed to vitamin C and phenolics, and lipophilic antioxidant activity is attributed to carotenoids. The experiment was performed on one white pulp clone (Allahabad Safeda) and three pink pulp clones (Fan Retief, Ruby Supreme and an advanced selection). Both AOAH and AOAL levels were high for white pulp clone [AOAH 33.3 microM Trolox equivalents (TE)/g fresh weight (FW) and AOAL (0.25 microM TE/g FW)] than pink pulp clone [AOAH 15.5 to 30.4

microM TE/g FW and AOAL 0.12 to 0.13 microM TE/g FW]. A positive correlation was obtained for AOAH with vitamin C ($r = 0.92$, $p < 0.01$) and with total phenolic ($r = 0.97$, $p < 0.01$), but there was negative correlation with beta-carotene ($r = -0.73$, $p = 0.03$).

The flavonoid profile and antioxidant activity of pink guava were assessed by Musa *et al* (2015). They worked on 2 varieties of pink guava named Semenyih and Sungkai. The experiments were performed differently on skin fraction, flesh and whole fruits. Ascorbic acid and 6 flavonoid compounds (quercetin, kaempferol, apigenin, luteolin, isorhamentin and myricetin) were assessed. Vitamin C, antioxidant activity and phenolic content were more in Semenyih as compared to Sungkai variety. Skin fraction was rich in antioxidant potential. Out of flavonoid compounds, kaempferol was the major flavonoid compound and minor compound was quercetin in both varieties. The total phenolic contents were 671, 227.9 and 193.1 mg GAE/100 g fresh weight for Sungkai skin, fruit and flesh respectively, while for Semenyih skin, fruit and flesh, the levels were 841, 383 and 344.7 mg GAE/100 g fresh weight respectively. Similarly, the amount of myricetin were 80.38, 51.60 and 93.75 mg/kg for Sungkai fruit, skin and flesh respectively, while for Semenyih fruit, skin and flesh, the levels were 83.05, 73.75 and 84.00 mg/kg respectively. Apigenin was absent in both varieties. So, they concluded that fruit skin is the richest source of the antioxidants with more amount of ascorbic acid and flavonoid compounds than fruit flesh.

Ademiluyi *et al* (2016) studied the antioxidant and antihypertensive properties of phenolic compounds extracted from four guava varieties (Giant white, Small white, Stripped and Pink). The dominant phenolic compounds found were eugenol, catechin, rosmarinic acid, caffeic acid and carvacrol. Seshadri *et al* extracted the polyphenols from the leaves of guava. The dominant polyphenolic compunds found were quercetin, guaijaverin, leucocyanidin, 4-gentiobioside of ellagic acid and a little amount of ellagic acid too.

Santos *et al* (2017) found the concentrations of different phenolic compounds in guava fruits at two stages- ripe and green. The main phnolic compounds were rutin (5.09-4.02), chlorogenic acid (1.83-10.75), catequin (1.09-13.09), gallic acid (2.43-7.28) and ellagic acid (5.72-30.60). Total flavonoid content was 35.26-75.19 mg QE/100g and total polyphenol content was 55-516 mg GAE/100g. The antioxidant potential in DPPH assay was 5.22-5.62 TEAC/100g DW and in ABTS assay was 17.63-18.74 TEAC/100g DW. The TPC and TLC were higher for green samples than ripe samples and the phenolics which were responsible for this variability are-catequin, rutin and: syringic, transcinamic, elagic, cafeic and p-coumaric acids.

CHAPTER – III

MATERIALS AND METHODS

3.1 Experimental Site

The present study entitled **"Morphological and nutraceutical characterization of guava (*Psidium guajava* L.) genotypes"** was carried out in Department of Fruit Science, Punjab Agricultural University, Ludhiana and at Regional Fruit Research Station, PAU, Bahadurgarh, Patiala during the year 2018-19.

3.2 Experimental Plant Material

The present research was carried out on nine genotypes of guava, being maintained at Regional Fruit Research Station, PAU, Bahadurgarh, Patiala. The plants of these varieties were being maintained as per according to the Punjab Agricultural University's recommended package of practices for guava orchard cultivation during the study. The detail of these evaluated nine genotypes of guava for morphological and nutraceutical characterization is as follows:

Table 3.1: Detail of guava genotypes investigated during the research.

Sr. No.	Variety Name	Category
1	Punjab Safeda	White fleshed
2	Shweta	White fleshed
3	Allahabad Safeda	White fleshed
4	Punjab Kiran	Pink fleshed
5	Punjab Pink	Pink fleshed
6	Lalit	Pink fleshed
7	Punjab Apple Guava (AC 6-2)*	Red skinned
8	Lalima (CISH G-5)	Red skinned
9	AC 1-4	Red skinned

* Approved as Punjab Apple Guava in 271st Research Evaluation Committee Meeting, PAU, Ludhiana held on 03-01-2019.

3.3 Experiment details

The present research problem entitled "Morphological and nutraceutical characterization of guava (*Psidium guajava* L.) genotypes" was performed in two experiments. In the first experiment, three white fleshed varieties namely Punjab Safeda, Shweta and Allahabad Safeda; three pink fleshed varieties namely Punjab Kiran, Punjab Pink and Lalit; and three red skinned varieties namely Lalima (CISH-G-5), Punjab Apple Guava (AC 6-2) and AC 1-4 (total nine guava varieties) grown at PAU Regional Fruit Research Station, Bahadurgarh, Patiala were selected for study. The standard uniform practices were

followed during the period of investigation. Fruits were harvested at maturity and various morpho-physiological traits were recorded.

Experiment - I

1. **Name of Experiment:** To study the morpho-physiological traits of cultivated varieties of guava.
2. **Location:** Number of locations- 02
   a) Fruit Research Farm and PG Laboratory, Department of Fruit Science, Punjab Agricultural University, Ludhiana.
   b) Regional Fruit Research Station, PAU, Bahadurgarh, Patiala.
3. **Crop** **:** Guava (*Psidium guajava* L.)
4. **Design** **:** Randomized Block Design
5. **Number of genotypes** (Treatments) **:** 9
6. **Number of replications** **:** 3
7. **Number of plants per replication** **:** 2
8. **Total no of experimental plants** **:** 54

Experiment II

In the second experiment, the fruits of genotypes taken in experiment no.1 were evaluated for their nutraceutical potential and antioxidant activity in Biochemistry Lab, Department of Biochemistry, Punjab Agricultural University, Ludhiana.

1. **Name of Experiment:** To estimate the nutraceuticals and antioxidant activity in different genotypes of guava.
2. **Location:** Biochemistry Lab, Department of Biochemistry, Punjab Agricultural University, Ludhiana.
3. **Crop** **:** Guava (*Psidium guajava* L.)
4. **Design** **:** Randomized Block Design
5. **Number of genotypes** (Treatments) **:** 9
6. **Number of replications** **:** 3
7. **Number of plants per replication** **:** 2
8. **Total no of experimental plants** **:** 54

3.4 Observations recorded

The observations on morpho-physiological and nutraceutical traits were recorded in the study. Fruiting traits were observed at the time of harvest. Other morpho-physiological and nutraceutical traits were observed as per the respective standard procedures. The various parameters along with the methodology followed for their assessment are discussed below:

3.4.1 Fruit traits

3.4.1.1 Fruit weight (g)

Fruits from each treatment were harvested at the maturity stage and their weight was measured on physical balance. Average weight was calculated under each treatment.

3.4.1.2 Fruit length (cm)

Fruits from each treatment were harvested at the maturity stage and their length was measured with vernier caliper. Average length was calculated under each treatment and expressed in cm.

3.4.1.3 Fruit diameter (cm)

Fruits from each treatment were harvested at the maturity stage and their diameter was measured with vernier caliper. Average diameter was calculated under each treatment and expressed in cm.

3.4.1.4 Fruits per tree (no)

The total number of fruits per tree were counted during second week of October (winter season crop) for each treatment and recorded separately. Average fruit number per tree was calculated under each treatment.

3.4.1.5 Yield (kg/tree)

The total number of fruits per tree was counted during second week of October (winter season crop) for each treatment. Yield was estimated from average fruit weight and expressed in kg/tree.

3.4.1.6 Fruit colour

Fruits from each treatment were selected randomly and their colour was observed with Colour Difference Meter (Model: Mini Scan XE Plus, and Made: Hunter Lab, USA). The recorded data for fruit colour was expressed as L, a and b values.

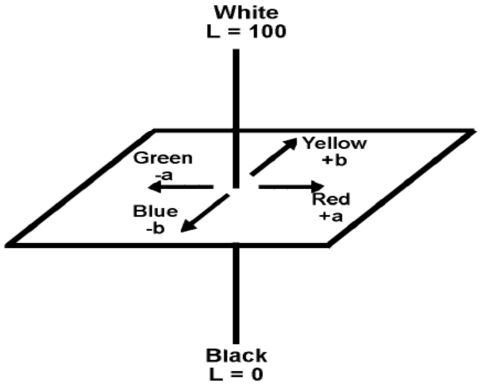


Fig. 1: Chromaticity Diagram

L= lightness; a &b chromaticity coordinates; +a= red to purple;

-a= green to blue; +b= yellow; -b=blue

The higher 'L' value indicates the lightness (>50) and lower value represents the darkness.

3.4.1.7 Fruit firmness (kg/cm^2)

Fruits from each treatment, harvested at maturity stage, were taken and their firmness was measured with Penetrometer (Model FT-327, QA Supplies, Norfolk, VA, USA). The pressure needed to drive a probe (made of stainless steel with diameter 8mm) into the fruit flesh was recorded in terms of pound-force (lbf).

3.4.1.8 Core diameter (cm)

Fruits from each treatment were harvested at the maturity stage and fruits were cut into two equal halves with the help of knife. Vernier caliper was used to measure core diameter. Average core diameter was calculated under each treatment and expressed in cm.

3.4.1.9 Pulp thickness (cm)

Fruits from each treatment were harvested at the maturity stage and fruits were cut into two equal halves with the help of knife. Vernier caliper was used to calculate pulp thickness. Average pulp thickness was calculated under each treatment and expressed in cm.

3.4.2 Seed traits

3.4.2.1 Number of seeds per fruit (no)

Randomly selected fully matured fruits were taken for each treatment. From the fruit pulp, the seeds were extracted and were counted. Average seed number per fruit was calculated for each treatment.

3.4.2.2 Seeds per 100g pulp (no)

Randomly selected fully matured fruits were taken for each treatment. After weighing the fruits, seeds were extracted from them. Seeds were counted and then divided by pulp weight and multiplied by 100 to get seed number per 100g fruit. Average value was calculated for each treatment.

3.4.2.3 Seed weight per fruit (g)

Randomly selected fully matured fruits were taken for each treatment. From the fruit pulp, the seeds were extracted and their weight was calculated with electronic balance. Average seed weight for each treatment was calculated and was expressed in grams.

3.4.2.4 Seed hardness (kg/cm^2)

Randomly selected fully matured fruits were taken for each treatment. Seeds were extracted from the fruit pulp. Seed hardness was measured using a pressure analyzer. Average value for seed hardness was calculated for each treatment.

3.4.3 Biochemical Traits

3.4.3.1 Total soluble solids (^{0}Brix)

From randomly selected fruits, the juice was extracted and stained through muslin cloth for each replication. Total soluble solids (TSS) were calculated with Erma hand refractometer at the room temperature, in terms of degree Brix (%). From extracted juice of each sample, one drop was taken and placed on dry refractometer prism and the readings were

recorded. The readings obtained were corrected with temperature correction chart (AOAC 1990). After each observation, the refractometer prism was cleaned with double-distilled water.

3.4.3.2 Titratable acidity (%)

The titratable acidity was measured with the help of titration method as reported by Ranganna (1986). Two ml fruit juice was taken and diluted with distilled water to 20 ml. It was titrated against 0.1 N NaOH solution. The phenolphthalein was used as indicator in this titration process. The endpoint of this titration was marked by colour change from colourless to light pink. The titratable acidity (%) was figured out and expressed in terms of citric acid percentage using the following formula:

$$\text{Titratable acidity (\%)} = \frac{0.0064 \times \text{Vol. of NaOH used (ml)}}{\text{Volume of juice taken (ml)}} \times 100$$

3.4.3.3 TSS: acid ratio

The TSS: acid ratio was determined by dividing the average TSS with corresponding average titratable acidity.

3.4.3.4 Juice pH

The pH of fruit juice was determined using microprocessor-based pocket pH meter (Eutech instrument Pvt. Ltd. Singapore). Before using the pH meter, it was standardized in the standard solution (pH 4). The juice pH was calculated by dipping the pH meter into the juice for a few seconds, allowing the reading to stabilize and then reading was noted. Before every measurement, the bulb of the pH meter was cleansed with double-distilled water so as to remove the residual effect.

3.4.4 Nutraceutical Traits

3.4.4.1 Ascorbic Acid

The ascorbic acid content of fruits was determined by 2, 6-dichlorophenol indophenols dye (DCPIP) visual titration method.

Reagents

1. **Metaphosphoric acid-acetic acid extraction solution:** 7.5 g of glacial metaphosphoric acid (HPO_3) sticks or pellets were dissolved in 20 ml glacial acetic acid and 100 ml distilled water. Dilute this solution to 250 ml with distilled water and filter it.
2. **Standard Indopheols solution:** 50 mg 2, 6-dichlorophenol indophenols dye and 42 mg $NaHCO_3$ were taken and dissolved in distilled water with final volume made to 200 ml.
3. **Ascorbic acid standard solution:** 100mg of ascorbic acid was taken in 100 ml volumetric flask. It was diluted upto the mark with reagent 1.

Standardization of indophenols solution: Two ml of standard solution of ascorbic acid was

taken in each of two flasks. In both flasks, 5 ml reagent No. 1 was taken and titrated with standard indophenols solution. Titration was continued until rose pink colour persisted for 5 seconds. Two blanks were also titrated by taking 2 ml of distilled water in two flasks and 5 ml of reagent 1. Average blanks were subtracted from standard titration and Dye factor was determined by following formula:

$$\text{Dye factor} = \frac{\text{Ascorbic acid conc. per ml}}{\text{Volume of dye used}}$$

Estimation:

The two ml juice was taken and 5 ml reagent No. 1 was added. It was titrated against reagent No. 2. When the endpoint appeared, the amount of the reagent No. 2 (dye) used was noted. The vitamin C content was calculated using the following formula:

$$\text{Vitamin C} = \frac{\text{Dye factor} \times \text{vol. of dye used (ml)}}{\text{Vol. of sample taken (ml)}} \times 100$$

3.4.4.2 Carotenoids

Total carotenoids content was calculated as per Kirk and Allen (1965) method by using 80 per cent acetone. One hundred milligrams of fresh guava tissue was homogenized in pestle and mortar thoroughly with 2.0 ml acetone (80%) and then transferred to the centrifuge tubes. The tubes were centrifuged at 3000 × g for 10 minutes, and the supernatant was collected into the other test tubes. The remaining pellet was again extracted with 2.0 ml acetone, again centrifuged for 10 minutes and two supernatants collected were pooled. The final volume of supernatant was made with acetone to 10 ml. The absorbance was noted at three different wavelengths: 665, 645 and 480 nm. These values were put into the following equation to determine the total carotenoids content:

$$\text{Total carotenoids} = {}^{A}480 + (0.114\ {}^{A}665) - (0.638\ {}^{A}645) \times V/ (1000 \times W)$$

Where ${}^{A}480$ = Absorbance at 480 nm

${}^{A}665$ = Absorbance at 665 nm

${}^{A}645$ = Absorbance at 645 nm

V = Total volume of the extract (ml), W = Weight of the sample (g)

The value of total carotenoids content was expressed in units mg/g fresh weight (FW).

3.4.4.3 Total phenols

Total phenols content was measured by the assay given by Swain and Hills (1959). This assay is based on the principle that tissue is extracted with the 80% methanolic solution and the intensity of the colour produced is measured colourimetrically**.**

Reagents

1. 80% methanol

2. 1 N Folin & Ciocalteau's phenol reagent

3. Sodium carbonate solution (saturated)

Estimation

Five hundred milligrams of fresh tissue was taken in test tubes which were fitted with water condensers. 5 ml of 80% methanol was added to each tube. The tubes were kept in a boiling water bath and refluxed for one hour. Refluxed material was filtered off and final volume was made to 10 ml with methanol (80%). The methanolic extract was utilized for the quantification of total phenols.

Procedure

From the methanolic extract, a fixed volume of 0.5 ml was taken and evaporated to dryness. The residue left after evaporation was dissolved in 6.5 ml of distilled water. 0.5 ml Folin & Ciocalteau's phenol reagent was added to this. After keeping it for 5 minutes, 1 ml sodium carbonate solution (saturated) was added. It was mixed thoroughly and was kept for one hour at room temperature. Blue colour was developed, indicating the presence of phenols and absorbance was noted at wavelength 760 nm using spectrophotometer against blank. The total phenols content was calculated by preparing standard curve of gallic acid of different concentrations ranging from 10 to 60 µg and content was expressed as mg/g FW.

3.4.4.4 Flavonoids

Total flavonoids content was determined by method suggested by Balababa *et al* (1974).

Reagents

1. 80% methanol

2. 0.1 M Methanolic solution of aluminium chloride.

Extraction

Five hundred milligrams of guava tissue was taken and refluxed with 10 ml of hot methanol (80%) for one hour. The refluxed material was filtered and final volume was made to 10 ml with hot methanol. This extract was used for the determination of total flavonoids.

Procedures

The three ml of extract was taken and evaporated to dryness. Five ml methanolic solution of aluminium chloride (0.1 M) was added to residue left. The yellow colour was developed which was read at wavelength 420 nm against the blank. The total flavonoids content was figured out from the standard curve prepared by using rutin of different concentrations from 40 to 200 µg and content was expressed as mg/g FW.

3.4.4.5 Anthocyanins

The anthocyanins content was measured by method suggested by Ranganna (1997).

Reagents

1. 0.1% HCl in methanol

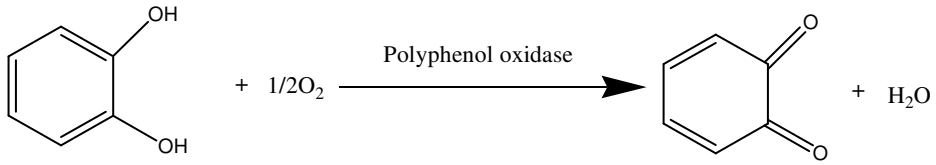
Procedure

One hundred milligrams of guava tissue was homogenized in 2 ml solution of 0.1% solution of HCl in methanol at low speed. The extract obtained was kept at room temperature for 20 hours. The absorbance of extracts was measured at 530 and 657 nm against blank (0.1% HCl in methanol). The anthocyanin content was expressed as mg $100g^{-1}$ FW.

3.4.5 Enzymes and Antioxidant Assays

3.4.5.1 Polyphenol oxidase (PPO)

The assay used for estimation of polyphenol oxidase activity was suggested by Zauberman *et al* (1991). Polyphenol oxidase (PPO) catalyzes the oxidation reaction of phenolic compounds to quinone compounds.



O-quinone

Reagents

1. 0.05 M Phosphate buffer (pH 6.5)
2. 0.1 M Catechol

Extraction

One hundred milligrams of fresh tissue of guava was homogenized in pestle mortar using 2.0 ml of 0.05 M sodium phosphate buffer (ice-cold) containing 1% polyvinylpyrolidone. Homogenized material was then centrifuged at 10000 rpm for 20 minutes at 4°C and supernatant obtained was used for assay of polyphenol oxidase enzyme.

Enzyme assay

About 1 ml of phosphate buffer (0.05 M) was taken in a spectrophotometric cuvette and 0.5 ml of enzyme extract was added into it. The reaction was started by adding 0.5 ml catechol. The absorbance was read at 410 nm for 3 minutes at the interval of 30 seconds against the buffer. The activity of polyphenol oxidase was expressed as change in O.D. min^{-1} g^{-1} fresh weight.

3.4.5.2 Peroxidase (POD)

The peroxidase activity was estimated by the assay suggested by Shannon *et al* (1965). Peroxidase catalyses the breakdown reaction of hydrogen peroxide at the expense of electron acceptors like quinines, ascorbate and cytochrome C. They utilize electron donor for oxidation reaction of hydrogen peroxide.

$$\text{Guaiacol} + H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + \text{Tetrahydroguaiacol}$$

Peroxidase

$$ROOR` + 2\ e^- + 2\ H^+ \Longrightarrow ROH + R`OH$$

Reagents

1. 0.05 M Sodium phosphate buffer (pH 6.5)
2. 0.1% ortho-dianisidine solution in methanol
3. 6% H_2O_2 in 0.1 M sodium phosphate buffer (pH 6.5)

Extraction

One hundred milligrams of fresh tissue of guava was homogenized in a pestle mortar using 2.0 ml of 0.05 M sodium phosphate buffer (ice-cold) containing 1% polyvinylpyrolidone. Homogenized material was then centrifuged at 10000 rpm for 20 minutes at 4°C and supernatant obtained was used for the assay of peroxidase enzyme.

Enzyme assay

Approximately 3.5 ml sodium phosphate buffer (pH 6.5) was taken in test tube and 0.1 ml of enzyme extract was added to it, followed by the addition of 0.1 ml of ortho-dianisidine solution. The temperature of the mixture was brought to 30°C in a constant temperature water bath. The contents were then transferred to a spectrophotometric cuvette. The reaction was initiated by the addition of 0.1 ml of hydrogen peroxide directly in the cuvette and mixing it well. Absorbance was noted at 430 nm for 3 minutes at an interval of 30 seconds against the reagent blank. The activity of the peroxidase enzyme was expressed as change in O.D. min^{-1} g^{-1} FW.

3.4.5.3 Phenylalanine ammonia lyase (PAL)

The Phenylalanine ammonia lyase enzyme activity was determined by the method proposed by Lister *et al* (1996).

Phenylalanine ammonia lyase (PAL) enzyme catalyses the following reaction:

$$\text{L-Phenylalanine} \xrightarrow{PAL} \text{trans-Cinnamate} + NH_3$$

Reagents

1. 0.05 M Sodium borate buffer
2. 0.1% Polyvinylpyrrolidone
3. 5 mM Mercaptoethanol
4. 20 mM L-phenylalanine
5. 6 M Trichloroacetic acid

Extraction

The enzyme extract was prepared by homogenizing one hundred milligrams of fresh guava tissue in a pestle mortar using 0.05 M sodium borate buffer (pH 8.8) containing 1.0 per cent of polyvinylpyrrolidone and 5 mM 2-Mercaptoethanol. The homogenized material was

then centrifuged at 12000 rpm for 15 minutes at 4ºC in the cooling centrifuge. The extract was filtered through muslin cloth. The supernatant obtained was used for the assay of phenylalanine ammonia lyase.

Enzyme assay

0.1 ml of enzyme extract was taken, and 1.9 ml of 0.05 M sodium phosphate buffer (with pH 8.8) and 1 ml of L-phenylalanine were added to it. This mixture was then kept in a constant water bath at 37^0 C for 1 hour. The control was also prepared by replacing the enzyme extract with sodium borate buffer. The reaction was terminated by the addition of 0.2 ml of 6 M trichloroacetic acid. This mixture was transferred to the spectrophotometric cuvette and the reading was set to zero at 290 nm. The readings were taken for different samples for the absorbance at 290 nm. The enzymatic activity was calculated from the standard curve prepared from different concentrations of cinnamic acid ranging from 5 to 35 µg. The enzymatic activity was expressed as µg t-cinnamic acid formed min^{-1} g^{-1} FW of tissue.

3.4.5.4 Total antioxidant activity (DPPH assay)

The total antioxidant activity was estimated by the DPPH radical scavenging capacity assay suggested by Liyana and Shahidi (2005).

Reagents

1. Methanol
2. DPPH reagent

Procedure

One hundred milligrams of fresh tissue of guava was homogenized in 2.0 ml of methanol absolute. After doing centrifugation at 10,000 × g for 10 minutes, 1.0 ml of extract was collected, and 4.0 ml of DPPH reagent was added to it. The tubes were placed in dark at room temperature for half an hour. The blank was prepared by using 3.94 ml of DPPH and 60 µl methanol. The absorbance of sample was recorded at 517 nm. The absorbance of the sample was recorded as A and of blank as A_0. The DPPH radical scavenging activity was measured by using following formula:

$$\text{DPPH scavenging activity } (\%) = (1-A/A_0)*100$$

Where A and A_0 were the absorbances at 517 nm for the sample solution and control, respectively.

Statistical analysis:

The experiment was performed in Randomised Block Design as per the procedure suggested by Singh *et al* (1998), taking three replications for each treatment. The data were analyzed for a variance by using computer software SAS (Statistical analysis system). The treatment means are subjected to mean separation by least significant difference at 5% level of probability.

CHAPTER – IV

RESULTS AND DISCUSSION

The data related to the present investigtion entitled "Morphological and nutraceutical characterization of guava (*Psidium guajava* L.) genotypes" is presented and discussed in this chapter. The observations on morpho-physiological and nutraceutical characteristics of guava genotypes were recorded during year 2018-19. The results obtained for parameters under study are presented and discussed under suitable headings and sub-headings after tabulation and statistical analysis as follow:

4.1 Fruit parameters

4.1.1 Fruit weight (g)

The data regarding fruit weight of different guava genotypes is shown in Table 1. Maximum fruit weight was recorded in cv. Punjab Safeda (244.0 g), followed by Shweta (235.33 g) and these values were found significantly higher as compared to other genotypes. Punjab Pink had minimum fruit weight (163.67 g), followed by Punjab Kiran (177.67 g), Lalit (181.67 g) and Punjab Apple Guava (183.67 g) and these varieities were at par with each other. White fleshed varieties recorded more fruit weight in comparison to other varieties. Khehra and Bal (2006) found the average fruit weight in guava ranged from 100.5 to 238.3 g. Sharma *et al* (2010) found fruit weight ranged from 11.93 to 213.80 g. Shukla *et al* (2012) found fruit weight ranging from 65 to 281 g and Pandey *et al* (2007) found this range between 130 to 275 g.

Table 1: Comparative performance of guava genotypes for fruit weight, fruit length and fruit diameter.

Sr. No.	Variety	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)
1	Punjab Safeda	244.0	7.22	8.0
2	Shweta	235.33	7.22	7.76
3	Allahabad Safeda	207.33	7.68	7.18
4	Punjab Kiran	177.67	7.49	6.85
5	Punjab Pink	163.67	8.19	6.54
6	Lalit	181.67	6.70	6.96
7	Punjab Apple Guava	183.67	6.39	7.24
8	Lalima	197.67	7.46	6.55
9	AC 1-4	200.67	6.75	7.41
	CD(p≤0.05)	23.49	0.29	0.37

4.1.2 Fruit length (cm)

The data regarding fruit legth of various genotypes is presented in Table 1. The fruit

length values ranged from 6.39 to 8.19 cm. Maximum fruit length was recorded in cv. Punjab Pink, which was significantly higher as compared to other genotypes, followed by Allahabad Safeda (7.68cm), Punjab Kiran (7.49cm) and Lalima (7.46cm). The minimum fruit length was observed in Punjab Apple Guava (6.39 cm), which was significantly lower than rest of the varieties, followed by Lalit (6.70cm) and AC1-4 (6.75cm). Methela *et al* (2019) also reported that fruit length varied from 4.43 to 9.37 cm, while Pandey *et al* (2007) found this range from 5.83 to 8.4 cm.

4.1.3 Fruit diameter (cm)

The data for fruit diameter of guava genotypes is shown in Table 1. The fruit diameter ranged from 6.54 to 8.00 cm and Punjab Safeda recorded maximum fruit diameter, followed by Shweta (7.76 cm) and these values were significantly higher than other varieties. Minimum fruit diameter was recorded in Punjab Pink which was at par with Lalima (6.55 cm) and Punjab Kiran (6.85 cm) and these values were significantly lower than rest of the varieties. On an average, white fleshed varieties were recorded with more fruit diameter than other varieties, with pink fleshed varieties having minimum values for fruit diameter. Methela *et al* (2019) worked on fruit diameter of various guava varieties and found this range between 4.27 to 8.54 cm. Pandey *et al* (2007) found fruit diameter in the range from 5.83 to 7.43 cm with Shweta having maximum fruit diameter.

4.1.4 Fruits per tree (no)

The data pertaining to fruits per tree is presented in Table 2. Punjab Pink had maximum fruit number per tree (408.33), which was significantly higher than all other genotypes. Minimum fruit number per tree was recorded in Lalit (300.67), Punjab Safeda (302.33), Lalima (303.67), Punjab Apple Guava (314.00), Allahabad Safeda (321.00) and AC 1-4 (321.67) and these varieties were at par with each other and significantly lower than Shweta (330.33) and Punjab Kiran (336.67).

Table 2: Comparative performance of guava genotypes for fruits and yield per tree.

Sr. No.	Variety	Fruits per tree (no)	Fruit yield (kg/tree)
1	Punjab Safeda	302.33	73.77
2	Shweta	330.33	77.74
3	Allahabad Safeda	321.00	66.55
4	Punjab Kiran	336.67	59.81
5	Punjab Pink	408.33	66.83
6	Lalit	300.67	54.62
7	Punjab Apple Guava	314.00	57.67
8	Lalima	303.67	60.02
9	AC 1-4	321.67	64.55
	CD($p \leq 0.05$)	29.93	6.01

4.1.5 Fruit yield (kg/tree)

Data recorded for yield per tree for different varieties is presented in Table 2. Shweta had maximum yield (77.74 kg/tree), followed by Punjab Safeda (73.77 kg/tree) and these values were significantly higher than all other varieties. Minimum fruit yield was recorded in Lalit (54.62 kg/tree), which was however, at par with Punjab Apple Guava (57.67 kg/tree), Punjab Kiran (59.81 kg/tree) and Lalima (60.02 kg/tree) and these values were significantly lower than other varieties. Ghosh *et al* (2013) found somewhat similar results in Allahabad Safeda (71.6 kg/tree) and Apple colour (69.5 kg/tree). Shukla *et al* (2012) found the fruit yield varying from 25 to 68 kg/tree.

4.1.6 Fruit colour

The fruit skin colour of different varieties representing white flesh, pink flesh and red coloured skin was measured and the values recorded as 'L', 'a' and 'b' are shown in Table 3.

Table 3: Comparative performance of guava genotypes for fruit colour.

Sr. No.	Variety	Fruit colour (colour flex lab)		
		'L' value	**'a' value**	**'b' value**
1	Punjab Safeda	74.62	4.79	37.12
2	Shweta	66.36	-2.31	37.45
3	Allahabad Safeda	61.28	-7.83	33.06
4	Punjab Kiran	63.50	6.26	40.77
5	Punjab Pink	64.73	6.96	40.17
6	Lalit	60.25	3.30	37.66
7	Punjab Apple Guava	41.99	32.86	18.80
8	Lalima	54.00	28.75	27.36
9	AC 1-4	44.12	25.73	18.16
	CD($p \leq 0.05$)	10.99	8.49	8.84

Punjab Safeda recorded highest 'L' value (74.62), followed by Shweta (66.36) and Punjab Pink (64.73) and these values were significantly higher than other varieties. Red skinned varieties had lowest 'L' value and minimum 'L' value was recorded in Punjab Apple Guava (41.99) and AC 1-4 (44.12). The negative 'a' value indicates the greenness, while the more positive value denotes the redness. The minimum 'a' value was recorded in Allahabad Safeda (-7.83) and Shweta (-2.31) which was significantly lower than other varieties. Maximum 'a' value i.e. more redness of skin (Plate 1) was recorded in Punjab Apple Guava (32.86), followed by Lalima (28.75) and AC 1-4 (25.73) and these were significantly higher than other varieties. The higher 'b' value indicates the yellowness of the fruit. The white fleshed and pink fleshed varieties (Plate 2) were at par with each other, with maximum value

observed in Punjab Kiran (40.77), followed by Punjab Pink (40.17), Lalit (37.66), Shweta (37.45), Punjab Safeda (37.12) and Allahabad Safeda (33.06). Red skinned varieties had significantly lower 'b' values than other varieties, with minimum value recorded in AC 1-4 (18.16) and Punjab Apple Guava (18.80).

4.1.7 Fruit firmness (kg/cm^2)

Fruit firmness measurement is a good technique to monitor the fruit softening and predict the bruising damage during harvest and post harvest handling of fruits. The values for fruit firmness of different varieties have been shown in Table 4. There were no significant differences for this parameter among all varieties. Punjab Kiran (7.50kg/cm^2) recorded higher fruit firmness as compared to other varieties, followed by AC 1-4 (6.87kg/cm^2), Shweta (6.83kg/cm^2) and Lalit (6.80kg/cm^2). Punjab Pink (5.50kg/cm^2) and Punjab Apple Guava (5.53kg/cm^2) recorded lower fruit firmness than other varieties.

Table 4: Comparative performance of guava genotypes for fruit firmness, core diameter and pulp thickness.

Sr. No.	Variety	Firmness (kg/cm^2)	Core diameter (cm)	Pulp thickness (cm)
1	Punjab Safeda	6.37	4.60	1.63
2	Shweta	6.83	4.97	1.57
3	Allahabad Safeda	6.40	4.87	1.53
4	Punjab Kiran	7.50	4.87	1.27
5	Punjab Pink	5.50	4.07	1.17
6	Lalit	6.80	4.63	1.17
7	Punjab Apple Guava	5.53	5.53	1.27
8	Lalima	6.33	4.60	1.63
9	AC 1-4	6.87	4.73	1.13
	CD(p≤0.05)	NS	0.22	0.19

4.1.8 Core diameter (cm)

The core diameter for different guava varieties is shown in Table 4. The core diameter varied from 4.07 cm to 5.53 cm. Punjab Pink had minimum core diameter, which was significantly lower than other varietie, followed by Lalima (4.60 cm), Punjab Safeda (4.60 cm) and Lalit (4.63 cm). Punjab Apple Guava recorded significantly higher core diameter than other varieties, followed by Shweta (4.97 cm), Allahabad Safeda (4.87 cm) and Punjab Kiran (4.87 cm). Shiva *et al* (2017) found similar results for Lalit variety and recorded core diameter 4.46 cm.

Punjab Apple Guava

Lalima

AC 1-4

Plate 1: Red skinned varieties of guava.

Punjab Kiran

Lalit

Punjab Pink

Plate 2: Pink fleshed varieties of guava.

Punjab Safeda

Shweta

Allahabad Safeda

Plate 3: Pulp thickness in White fleshed guava varieties.

Punjab Kiran

Punjab Pink

Lalit

Plate 4: Pulp thickness in Pink fleshed guava varieties.

Punjab Apple Guava

Lalima

AC 1-4

Plate 5: Pulp thickness in Red skinned guava varieties.

4.1.9 Pulp thickness (cm)

The data pertaining to pulp thickness was recorded in white fleshed (Plate 3), pink fleshed (Plate 4) and red skinned (Plate 5) guava varieties and is shown in (Table 4). Punjab Safeda (1.63 cm) and Lalima (1.63 cm) recorded maximum pulp thickness, which was at par with Shweta (1.57 cm) and Allahabad Safeda (1.53 cm). All other varieties were significantly lower than these varieties, with lowest values recorded in AC 1-4 (1.13 cm), Lalit (1.17 cm), Punjab Pink (1.17 cm), Punjab Kiran (1.27 cm) and Punjab Apple Guava (1.27 cm) and these varieties were at par with each other. Singh *et al* (2016) found the similar results and recorded the pulp thickness range from 1.06 cm to 1.57 cm in different varieties of guava with Allahabad Safeda having 1.57 cm of pulp thickness.

4.2 Seed characteristics

4.2.1 Seed number per fruit (no)

The seed number per fruit for different varieties has been discussed in Table 5. A lot of variation was observed and values for seed number per fruit ranged from 242.00 to 696.33. Maximum seed number per fruit was observed in Lalit (507.00) which was at par with Punjab Pink (482.67). Minimum seed number per fruit was observed in Lalima (242.00) and Punjab Safeda (271.33) and these values were significantly lower than other varieties. Khehra and Bal found the values for seed number per fruit from 112 to 466 among 18 distinct genotypes of guava. Shukla *et al* (2012) found this range from 125 to 450 seed number per fruit.

Table 5: Comparative performance of guava genotypes for seed characteristics.

Sr. No.	Variety	Seed number per fruit (no)	Number of seeds/100g pulp (no)	Seed weight per fruit (g)	Seed hardness (kg/cm^2)
1	Punjab Safeda	271.33	107.68	3.81	9.33
2	Shweta	396.33	168.27	3.41	6.07
3	Allahabad Safeda	439.00	222.95	2.84	8.03
4	Punjab Kiran	433.00	236.54	3.38	7.83
5	Punjab Pink	482.67	299.05	5.30	8.27
6	Lalit	507.00	279.86	5.78	8.80
7	Punjab Apple Guava	382.33	202.67	4.33	9.77
8	Lalima	242.00	125.01	3.70	9.03
9	AC 1-4	308.67	152.50	3.76	8.87
	CD($p \leq 0.05$)	30.50	17.67	0.34	0.91

4.2.2 Seeds per 100g pulp (no)

The data for number of seeds/100 g pulp of white fleshed (Plate 6), pink fleshed (Plate 7) and red skinned varieties (Plate 8) is discussed in Table 5. A lot of variation was observed for this trait and the values ranged from 107.68 to 299.05. The seed number/100 g pulp was highest in Punjab Pink (299.05). Minimum seed number/100 g pulp was observed in Punjab Safeda (107.68) and it was at par with Lalima (125.01). Allahabad Safeda (222.95) and Punjab Kiran (236.54) were at par with each other. Patel *et al* (2011) found this range

4.2.3 Seed weight per fruit (g)

The data pertaining to seed weight per fruit has been presented in Table 5. Lalit was recorded with maximum seed weight per fruit (5.78 g) and it was significantly higher than all other varieties. Other varieties with higher seed weight per fruit were Punjab Pink (5.30 g) and Punjab Apple Guava (4.33 g). Minimum seed weight per fruit was recorded in Allahabad Safeda (2.84 g) which was significantly lower than rest of the varieties. Three varieties i.e. Punjab Safeda (3.81 g), AC 1-4 (3.76 g) and Lalima (3.70 g) contained no significant differences among them and were at par with each other. Khehra and Bal (2006) found the seed content from 1.8 to 5.5 g per fruit.

4.2.4 Seed hardness (kg/cm^2)

The data given in Table 5 reveals the seed hardness in different guava varieties, which ranged from 6.07 to 9.77 kg/cm^2. The seed hardness was minimum in Shweta (6.07 kg/cm^2) and all other varieties were significantly higher than Shweta in seed hardness index. Maximum seed hardness was recorded in Punjab Apple Guava (9.77 kg/cm^2) which was at par with Punjab Safeda (9.33 kg/cm^2), Lalima (9.03 kg/cm^2) and AC 1-4(8.87 kg/cm^2). Punjab Kiran (7.83 kg/cm^2), Allahabad Safeda (8.03 kg/cm^2) and Punjab Pink (8.27 kg/cm^2) were at par with each other.

4.3 Biochemical parameters

4.3.1 Total soluble solids (^{0}Brix)

Total soluble solids include the carbohydrates, organic acids, fats, proteins and minerals of the fruit. The data pertaining to TSS of different varieties is presented in Table 6. The TSS among all genotypes ranged from 7.77 to 11.27^{0}Brix. AC 1-4 was recorded with significantly higher TSS value (11.27^{0}Brix) than rest of the genotypes. Minimum TSS values were recorded in Lalima (7.77^{0}Brix), Punjab Kiran (7.83^{0}Brix) and Allahabad Safeda (7.93^{0}Brix) and these varieties were statistically at par with each other. Three varieties Punjab Pink (8.40^{0}Brix), Punjab Apple Guava (8.53^{0}Brix) and Shweta (8.77^{0}Brix) were at par with each other. Punjab Safeda (9.27^{0}Brix) variety was at par with Lalit (9.43^{0}Brix) and Shweta (8.77^{0}Brix). Khehra and Bal (2006) found the variability range for TSS from 9.8 to 12.3%, while Sharma *et al* (2010) found this range from 9.4 to 13.5%. Patel *et al* (2011) found this range lying between 9.35 to 11.88%. Musa *et al* (2015) worked on two pink fleshed guava

Allahabad Safeda

Punjab Safeda

Shweta

Plate 6: Seeds of white fleshed guava varieties.

Punjab Pink

Punjab Kiran

Lalit

Plate 7: Seeds of pink fleshed guava varieties.

AC 1-4

Punjab Apple Guava

Lalima

Plate 8: Seeds of red skinned guava varieties.

varieties and found TSS values of 7.5 and 8.57%.

Table 6: Comparative performance of guava genotypes for total soluble solids, acidity and TSS: acid ratio.

Sr. No.	Variety	TSS (^{0}Brix)	Acidity (%)	TSS: acid ratio
1	Punjab Safeda	9.27	0.49	18.90
2	Shweta	8.77	0.53	16.45
3	Allahabad Safeda	7.93	0.37	21.30
4	Punjab Kiran	7.83	0.41	19.34
5	Punjab Pink	8.40	0.59	14.33
6	Lalit	9.43	0.51	18.42
7	Punjab Apple Guava	8.53	0.48	17.78
8	Lalima	7.77	0.50	15.51
9	AC 1-4	11.27	0.61	18.53
	CD(p≤0.05)	0.47	0.03	1.23

4.3.2 Titratable acidity (%)

The titratable acidity content of different guava genotypes is presented in Table 6. Maximum titratable acidity content was shown by AC 1-4 (0.61%), which was at par with Punjab Pink (0.59%) and significantly higher than rest of the genotypes. Minimum titratable acidity content was observed in Allahabad Safeda (0.37%) which was significantly lower than all other genotypes. Singh *et al* (2016) found titratable acidity ranging from 0.49 to 0.69% and Sharma *et al* (2010) found this range varied from 0.37 to 0.96%. Shukla *et al* (2012) found acidity ranging from 0.5 to 1.01%, while Patel *et al* (2011) found this range from 0.45 to 0.65%. Dolkar *et al* (2017) found that Allahabad Safeda contained 0.39% acidity and Lalit contained 0.46% acidity. Musa *et al* (2015) worked on two pink fleshed guava varieties and found acidity values of 0.46 and 0.50%.

4.3.3 TSS: acid ratio

The evaluation of TSS: acid ratio exhibited a significant difference among the evaluated guava genotypes, which is presented in Table 6 and values ranged from 14.33 to 21.30. Allahabad Safeda was recorded with significantly higher value of TSS: acid ratio as compared to rest of the genotypes, followed by Punjab Kiran (19.34), Punjab Safeda (18.90), AC 1-4 (18.53) and Lalit (18.42). Punjab Pink contained minimum TSS: acid ratio (14.33) which was at par with Lalima (15.51) and significantly lower than other genotypes. Musa *et al* (2015) worked on two pink fleshed guava varieties and found TSS: acid values of 16.30

and 17.1.

4.3.4 Juice pH

The juice pH for different genotypes has been discussed in Table 7. Allahabad Safeda recorded maximum juice pH (5.35) which was significantly higher than rest of the genotypes. It was followed by Punjab Apple Guava (5.25) and AC 1-4 (5.23) which were at par with each other. Varieties Punjab Pink (5.10), Lalima (5.11), Lalit (5.12) and Shweta (5.15) were at par with each other. Minimum values was recorded in Punjab Safeda (4.96) which was at par with Punjab Kiran (4.99) and significantly lower than rest of the genotypes. Singh *et al* (2014) recorded pH value of 6.15 in Lalit and 6.08 in Allahabad Safeda.

Table 7: Comparative performance of guava genotypes for juice pH, ascorbic acid and carotenoids.

Sr. No.	Variety	Juice pH	Ascorbic acid (mg/100g pulp)	Carotenoids (mg/100g fw)
1	Punjab Safeda	4.96	178.99	0.45
2	Shweta	5.15	169.28	0.36
3	Allahabad Safeda	5.35	163.41	0.41
4	Punjab Kiran	4.99	143.91	0.33
5	Punjab Pink	5.10	120.51	0.39
6	Lalit	5.12	143.48	0.37
7	Punjab Apple Guava	5.25	173.70	0.36
8	Lalima	5.11	167.46	0.32
9	AC 1-4	5.23	175.43	0.36
	CD($p \leq 0.05$)	0.06	3.81	0.02

4.4 Nutraceutical Traits

4.4.1 Ascorbic Acid (mg/100g pulp)

The data pertaining to ascorbic acid content of different genotypes of guava has been presented in Table 7. Punjab Safeda recorded maximum ascorbic acid content (178.99 mg/100g pulp) which was at par with AC 1-4 (175.43mg/100g pulp). Minimum ascorbic acid content was observed in Punjab Pink (120.51mg/100g pulp) which was significantly lower than other genotypes. It was followed by Lalit (143.48 mg/100g pulp) and Punjab Kiran (143.91 mg/100g pulp). The data reveals that pink fleshed varieties contained lower ascorbic acid content as compared to white fleshed and red skinned varieties. Khehra and Bal (2006) found the ascorbic acid content ranged from 107.52 to 278.04 mg/100g pulp in different guava varieties. Sharma *et al* (2010) found this range from 51.90 to 189.73 mg/100g of fruit pulp. Shukla *et al* (2012) found the ascorbic acid content ranging from 129 to 268 mg/100g

pulp. Ulemale (2018) found white fleshed varieties containing more ascorbic acid content than pink fleshed varieties. Thuaytong and Anprung (2011) also found the similar results. Bashir and Abu-Goukh (2003) found more ascorbic acid content in white fleshed guava than pink fleshed guava. Dolkar *et al* (2017) found that Allahabad Safeda contained ascorbic acid 169.07 mg/100g fw and Lalit at half ripe stage contained ascorbic acid 139.99 mg/100g fw. Kumar *et al* (2016) found that Allahabad Safeda contained ascorbic acid content of 158.36 mg/100g fw.

4.4.2 Carotenoids (mg/100g fw)

The total carotenoids content in different guava genotypes has been discussed in Table 7. Punjab Safeda contained maximum amount of total carotenoids (0.45mg/100g fw) which was significantly higher than rest of the genotypes. Lalima recorded significantly minimum amount of total carotenoids (0.32 mg/100g fw). Four varieties Lalit (0.37 mg/100g fw), Punjab Apple Guava (0.36 mg/100g fw), AC 1-4 (0.36 mg/100g fw) and Shweta (0.36 mg/100g fw) were at par with each other. Wilberg and Rodriguez-Amaya (1995) found that total carotenoids content in guava ranged from 0.30 to 0.58mg β-carotene/100g fw, while Correa *et al* (2011) found this content ranged from 0.13 to 2.54mg/100g in white and coloured fleshed guava accessions. Jain *et al* (2001) found that white fleshed guava contained this value upto 0.52mg.

4.4.3 Total phenols (mg/100g fw)

Total phenolics content in different guava varieties is presented in Table 8. Punjab Safeda recorded maximum total phenols content (170.30 mg/100g fw) which was significantly higher than rest of the genotypes. Punjab Pink recorded minimum total phenols content (116.40 mg/100g fw) which was significantly lower than other genotypes. From the data, it was observed that pink fleshed varieties contained lower total phenols content than white fleshed varieties. Saroja (2015) found the same results that white fleshed guava contained more polyphenolic content than red fleshed guava. Bashir and Abu-Goukh (2003) and Yeshiwas and Mekonnen (2018) found similar results that white fleshed guava contains more phenolic compounds than pink fleshed guava. Thaipong *et al* (2005) found that Allahabad Safeda contained more phenolic content than pink pulp clones. Russell *et al* (2012) found that common guava contains polyphenols content 195.2 mg/100g fw. Luximon-Ramma *et al* (2003) reported that pink and white fleshed Mauritian guava had phenolic content of 126.4 and 247.3 mg/100g fw respectively. Melo *et al* (2006) found that total phenolics content was 186.68mg/100g fw. Yan *et al* (2006) found this range from 138 to 179mg/100g fw in guava and Alothman *et al* (2009) found this range from 123 to 191mg/100g fw.

4.4.4 Flavonoids (mg/100g fw)

The results of total flavonoids content in different guava varieties is shown in Table 8. The total flavonoids content ranged from 43.89 mg/100g fw to 115.48 mg/100g fw. The

maximum value was observed in Punjab Apple Guava (115.48 mg/100g fw) which was at par with AC 1-4 (114.92 mg/100g fw). Lalima contained 89.37 mg/100g fw total flavonoids content. Red skinned varieties contained maximum values of total flavonoids content, followed by pink fleshed varieties and white fleshed varieties. Saroja (2015) found the same results that red fleshed guava contained more flavonoids content than white fleshed guava. Biegelmeyer *et al* (2011) found that red guava possessed total flavonoids content 100.20 mg/100g fw and yellow guava possessed 35.12 mg/100g fw. Kumar *et al* (2016) found that Allahabad Safeda contained flavonoids content 28.02 mg/100g fw. Moreno *et al* (2014) found that flavonoid content in guava fruits was 49.79 mg/100g fw. Rodrigues *et al* (2013) worked on frozen guava pulp and found that total flavonoids content was 25 mg/100g of frozen pulp. Correa *et al* (2011) worked on a collection of white fleshed and coloured fleshed guava varieties and found that total flavonoids content ranged from 10.67 to 46.82mg/100g fw. Santos *et al* (2017) found that total flavonoid content was 35.26 to 75.19mg/100g fw for different guava varieties.

Table 8: Comparative performance of guava genotypes for total phenols, flavonoids and anthocyanins.

Sr. No.	Variety	Total phenols (mg/100g fw)	Flavonoids (mg/100g fw)	Anthocyanins (mg/100g fw)
1	Punjab Safeda	**170.30**	48.17	21.58
2	Shweta	149.03	56.27	14.93
3	Allahabad Safeda	150.12	43.89	17.86
4	Punjab Kiran	129.63	61.51	11.58
5	Punjab Pink	116.40	61.75	12.32
6	Lalit	135.89	69.21	11.47
7	Punjab Apple Guava	158.30	**115.48**	57.17
8	Lalima	145.33	89.37	53.24
9	AC 1-4	124.94	114.92	**65.77**
	CD(p≤0.05)	5.01	4.73	1.75

4.4.5 Anthocyanins (mg/100g fw)

The data pertaining to the anthocyanins content of the guava varieties has been discussed in the Table 8. Red skinned varieties contained higher anthocyanins content than white fleshed and pink fleshed varieties. The AC 1-4 recorded significantly higher anthocyanins content (65.77 mg/100g fw) than rest of the varieties, followed by Punjab Apple Colour (57.17 mg/100g fw) and Lalima (53.24 mg/100g fw). Pink fleshed varieties contained minimum anthocyanins content. Lalit recorded minimum anthocyanins content (11.47 mg/100g fw) which was at par with Punjab Kiran (11.58 mg/100g fw) and Punjab Pink (12.32

mg/100g fw).

4.5 Enzymes and Antioxidant Assays

4.5.1 Polyphenol oxidase (PPO)

Enzymatic browning is an important reaction occurring in fruits and vegetables having negative effects on colour, flavour, taste and nutrition value. This browning occurs due to oxidation of phenolic compounds and this reaction is carried out by polyphenol oxidase (PPO) enzyme (Holderbaum *et al* 2010). The polyphenol oxidase enzymatic activity was measured for different guava varieties and presented in Table 9. There was a very little change in OD during the assessment of enzymatic activity and enzymatic activity was not so significant. Mowlah and Itoo (1982) found the same results that there was very low activity of polyphenoloxidase enyme in mature fruits of white and pink fleshed guava and enzymatic activity was noticeable at full ripening stages. AC 1-4 recorded significantly higher enzymatic activity (5.78 ΔA/min/g fw) than rest of the genotypes. Punjab Kiran recorded minimum enzymatic activity (2.89 ΔA/min/g fw) which was at par with Lalima (3.11 ΔA/min/g fw) and Punjab Pink (3.44 ΔA/min/g fw).

Table 9: Comparative performance of guava genotypes for enzymatic activities.

Sr. No.	Variety	PPO (ΔA/min/g fw)	POD (ΔA/min/g fw)	PAL (Units/min/g FW)
1	Punjab Safeda	4.44	2.09	0.27
2	Shweta	4.56	3.87	0.21
3	Allahabad Safeda	3.56	1.57	0.17
4	Punjab Kiran	2.89	1.00	0.17
5	Punjab Pink	3.44	1.56	0.14
6	Lalit	4.11	3.89	0.22
7	Punjab Apple Guava	4.44	1.58	0.20
8	Lalima	3.11	1.18	0.16
9	AC 1-4	5.78	3.76	0.15
	CD(p≤0.05)	0.57	0.34	0.02

4.5.2 Peroxidase (POD)

The peroxidase enzyme plays role in ethylene biosynthesis, membrane integrity, hormone balance, respiration control, in ripening and senescence and changes associated with these processes (Haard 1973). Peroxidase enzyme is associated with enzymatic browning of fresh fruits and off-flavour generation in frozen or canned horticultural products (Vamos-Vigyazo and Haard 1981). The data recorded in Table 9 showed a considerable range (1.00 to 3.89 ΔA/min/g FW) of peroxidase enzyme activity among the different guava genotypes with

significant differences. Maximum enzymatic activity was found in Lalit which was at par with Shweta (3.87 ΔA/min/g fw) and AC 1-4 (3.76 ΔA/min/g fw). Minimum enzymatic activity was observed in Punjab Kiran which was at par with Lalima (1.18 ΔA/min/g fw). Three varieties Punjab Apple Guava (1.58 ΔA/min/g fw), Allahabad Safeda (1.57 ΔA/min/g fw) and Punjab Pink (1.56 ΔA/min/g fw) were at par with each other.

4.5.3 Phenylalanine ammonia lyase (PAL)

The data on phenylalanine ammonia lyase enzyme activity from guava genotypes were recorded in and presented in Table 9. PAL is the first enzyme in the phenylpropanoid pathway and is key-point in the biosynthesis of phenolic compounds (Kim and Hwang 2014). PAL catalyzes the first step and results in formation of trans-cinnamate and trans-4-hydroxycinnamate which leads to formation of flavanol, caffeic acid derivatives, anthocyanin and chlorogenic acid, hence PAL enzyme controls the phenolic compounds formation (Jones 1984, Tomas-Barberan *et al* 1997, Pina and Errea 2008). The different genotypes had PAL activity between 0.14 to 0.27 U/min/g fw. The enzymatic activity was significantly higher in Punjab Safeda than all other genotypes. The minimum activity was observed in Punjab Pink.

4.5.4 Total antioxidant activity (DPPH assay)

Different guava genotypes were analysed and found that they differed significantly with respect to their antioxidant potential, which ranged from 40.00 to 71.11% (Table 10). Among the evaluated genotypes, Punjab Apple Guava had highest total antioxidant activity (71.11%) which was at par with Lalima (68.15%) and AC 1-4 (66.67%). The red skinned varieties had highest total antioxidant potential, followed by white fleshed varieties and pink fleshed varieties. Punjab Pink had lowest total antioxidant activity which was significantly lower than rest of the genotypes. Kumar *et al* (2016) found that Allahabad Safeda possessed total antioxidant activity of 51.44% in winter season. Correa *et al* (2011) found that DPPH

Table 10: Comparative performance of guava genotypes for total antioxidant activity.

Sr. No.	Variety	Total antioxidant activity (%age)
1	Punjab Safeda	62.89
2	Shweta	60.74
3	Allahabad Safeda	56.30
4	Punjab Kiran	44.81
5	Punjab Pink	40.00
6	Lalit	45.93
7	Punjab Apple Guava	**71.11**
8	Lalima	68.15
9	AC 1-4	66.67

	CD(p≤0.05)	4.55

activity (µmoles of reduced DPPH/g of sample) for different coloured fleshed varieties of guava ranged from 23.87 to 70.42. Verma *et al* (2018) found that DPPH activity of guava ranged from 12.24 to 74.18%. Yeshiwas and Mekonnen (2018) found white flesh guava containing more antioxidant activity than pink flesh guava.

4.5.5 Nutraceutical potential

Among the different guava genotypes, red skinned varieties possessed maximum antioxidant activity, followed by white fleshed and pink fleshed varieties. Punjab Apple Guava possessed maximum flavonoids content and total antioxidant activity. AC 1-4 possessed maximum content of anthocyanins. Out of white fleshed varieties, Punjab Safeda had highest antioxidant activity, possibly as it contained higher amount of total phenols and ascorbic acid content than all other genotypes. Although, the total carotenoids content was low in all the varieties investigated, however, the maximum carotenoid content was recorded in Punjab Safeda. Phenylalanine ammonia lyase (PAL) enzyme catalyses the reactions involved in biosynthesis of phenolic compounds, which ultimately enhances the phenolic content. PAL activity was highest in Punjab Safeda.

Table 11: Nutraceutical potential of guava genotypes.

S.No.	Variety	Nutraceutical parameters with highest content
1.	Punjab Apple Guava	Flavonoids, Total antioxidant activity
2.	AC 1-4	Anthocyanins
3.	Punjab Safeda	Total phenols Ascorbic acid Total carotenoids PAL activity

4.6 Correlation Studies

4.6.1 Correlation analysis of morpho-physiological parameters and yield of guava genotypes

Correlation matrix of morpho-physiological parameters of different guava genotypes has been presented in Table 12. Yield was positively correlated with fruit weight ($r= 0.756$, $p<0.05$), but no significant correlation was found between yield with rest of the morpho-physiological characters. Significant positive correlations were found between fruit number per tree and fruit length ($r= 0.62$, $p<0.05$), fruit diameter and fruit weight ($r= 0.857$, $p<0.01$), pulp thickness and fruit weight ($r= 0.752$, $p<0.05$), fruit colour 'L' value and fruit colour 'b' value ($r= 0.900$, $p<0.01$), seed number per fruit and seed weight per fruit ($r= 0.748$, $p<0.05$),

seed no per fruit and seed number/100g pulp (r= 0.924, p<0.01), and seed weight per fruit and seed/100g pulp (r= 0.701, p<0.05). Significant negative correlations were found between core diameter and fruit length (r= -0.693, p<0.05), fruit colour 'L' value and fruit colour 'a' value (r= -0.794, p<0.05), fruit colour 'a' value and fruit colour 'b' value (r= -0.801, p<0.01), acidity and TSS: acid ratio (r= -0.672, p<0.05), and seed number per fruit and seed hardness (r= -0.725, p<0.05),

4.6.2 Correlation analysis of nutraceutical parameters and yield of guava genotypes

Correlation matrix of nutraceutical parameters and yield of different guava genotypes has been presented in Table 13 and Fig 2 to Fig 8. No significant correlations were found between yield data with nutraceutical characters. Total antioxidant activity recorded strong correlation with ascorbic acid (r= 0.92, p<0.01) and anthocyanins content (r= 0.802, p<0.01) and also positive correlation with total phenols content (r= 0.61) and flavonoids content (r= 0.56). Significantly, direct positive correlations of total phenols were recorded with vitamin C (r= 0.725, p<0.05) and phenylalanine ammonia lyase activity (r= 0.772, p<0.05). The total flavonoids also had a considerable positive correlation with anthocyanins content (r= 0.896, p<0.01). Polyphenol oxidase and peroxidase enzymes, both responsible for enzymatic browning, were strongly correlated with each other (r= 0.742, p<0.05). There was no significant correlation found between total antioxidant activity and total carotenoids content, but the r value came out to be negative (-0.069). Thaipong *et al* (2005) found the same that a positive correlation was obtained for antioxidant activity with vitamin C (r = 0.92, p < 0.01) and with total phenolic (r = 0.97, p < 0.01), but negative correlation with beta-carotene (r = -0.73, p = 0.03). Ahmad and Abdullah (2014) found that there was significant correlation between total phenolics content and DPPH (r = 0.901, p<0.05, r2= 0.812). Verma *et al* (2018) found that there were positive significant linear correlations present between antioxidant activity (DPPH assay) and contents of phenolics (R= 0.92) and flavonoids (R= 0.89) in guava. Pathare *et al* (2017) found positive correlations between DPPH antioxidant activity with phenols (r= 0.42) and also with vitamin C (r= 0.29). Luximon-Ramma *et al* (2003) found strong correlation between antioxidant activity (FRAP assay) and total phenolics (r= 0.95). Flavonoids were also positively correlated with antioxidant activity (r= 0.69).

Table 12: Correlation matrix of morpho-physiological parameters of guava genotypes

	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Firmness (kg/cm^2)	Fruit no per tree	Fruit colour (L)	Fruit colour (a)	Fruit colour (b)	TSS (^{0}Brix)	pH	Acidity (%)	TSS: acid ratio	Seed test weight (g)	Seed no per fruit	Seed wt per fruit (g)	Seed hardness (kg/cm^2)	Seed/100 g pulp	Core diameter (cm)	Pulp thickness (cm)
Fruit diameter (cm)	-0.41																		
Fruit weight (g)	-0.13	**0.857****																	
Firmness (kg/cm^2)	-0.11	0.17	0.22																
Fruit no per tree	**0.682***	-0.41	-0.49	-0.35															
Fruit colour(L)	0.58	0.24	0.41	0.20	0.19														
Fruit colour (a)	-0.48	-0.23	-0.28	-0.31	-0.19	**-0.794***													
Fruit colour (b)	0.62	-0.09	0.03	0.26	0.36	**0.900****	**-0.801****												
TSS (^{0}Brix)	-0.52	0.45	0.20	0.17	-0.17	-0.30	0.21	-0.42											
pH	-0.22	-0.02	-0.09	-0.28	-0.08	-0.61	0.10	-0.58	0.11										
Acidity (%)	-0.12	0.02	-0.06	-0.24	0.33	-0.21	0.36	-0.24	0.66	-0.08									
TSS: acid ratio	-0.27	0.39	0.25	0.45	-0.51	0.02	-0.34	-0.06	0.11	0.27	**-0.672***								
Seed test weight (g)	-0.23	-0.03	0.11	-0.30	-0.27	-0.16	0.64	-0.34	0.28	-0.36	0.52	-0.47							
Seed no per fruit	0.02	0.06	-0.05	0.19	0.25	0.28	-0.60	0.50	-0.07	0.11	0.04	-0.09	-0.60						
Seed wt per fruit (g)	-0.21	-0.01	-0.12	-0.06	0.14	0.15	-0.19	0.31	0.20	-0.13	0.48	-0.45	0.06	**0.748***					
Seed hardness (kg/cm^2)	-0.39	-0.14	-0.25	-0.43	-0.31	-0.43	0.64	-0.52	0.19	0.00	0.05	0.09	0.63	**-0.725***	-0.27				
Seed/100g pulp	0.18	-0.25	-0.40	0.03	0.50	0.19	-0.51	0.51	-0.17	0.11	0.06	-0.19	-0.61	**0.924****	**0.701***	-0.59			
Core diameter (cm)	**-0.69***	0.36	0.17	0.06	-0.52	-0.50	0.29	-0.52	-0.05	0.41	-0.40	0.42	-0.26	0.04	-0.17	0.07	-0.11		
Pulp thickness (cm)	0.23	0.37	**0.752***	0.02	-0.40	0.44	-0.22	0.15	-0.43	-0.12	-0.39	0.07	0.14	-0.20	-0.31	-0.19	-0.43	0.08	
Fruit yield (kg/ tree)	0.35	0.65	**0.756***	0.01	0.20	0.57	-0.47	0.29	0.09	-0.13	0.17	-0.09	-0.13	0.17	-0.02	-0.56	-0.04	-0.17	0.54

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

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Table 13: Correlation matrix of nutraceutical parameters and yield of guava genotypes.

Parameters	Total phenols (mg/100g fw)	lavonoids (mg/100g fw)	Anthocyanins (mg/100g fw)	Ascorbic acid (mg/100g fw)	Carotenoids (mg/100g fw)	POD activity (ΔA/min/g fw)	PPO activity (ΔA/min/g fw)	PAL activity (Units/min/g fw)	Total antioxidant activity (%)
Flavonoids (mg/100g fw)	-0.155								
Anthocyanins (mg/100g fw)	0.086	**0.896****							
Ascorbic acid (mg/100g fw)	**0.725***	0.313	0.591						
Carotenoids (mg/100g fw)	0.429	-0.512	-0.337	0.122					
POD activity (ΔA/min/g fw)	-0.096	0.114	0.005	0.192	0.064				
PPO activity (ΔA/min/g fw)	0.115	0.464	0.479	0.554	0.234	**0.742***			
PAL activity (Units/min/g fw)	**0.772***	-0.314	-0.258	0.425	0.536	0.270	0.235		
Total antioxidant activity (%)	0.610	0.560	**0.802****	**0.920****	-0.069	0.069	0.496	0.201	
Fruit yield (kg/tree)	.384	-.495	-.268	.278	.503	.219	.267	.286	.132

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

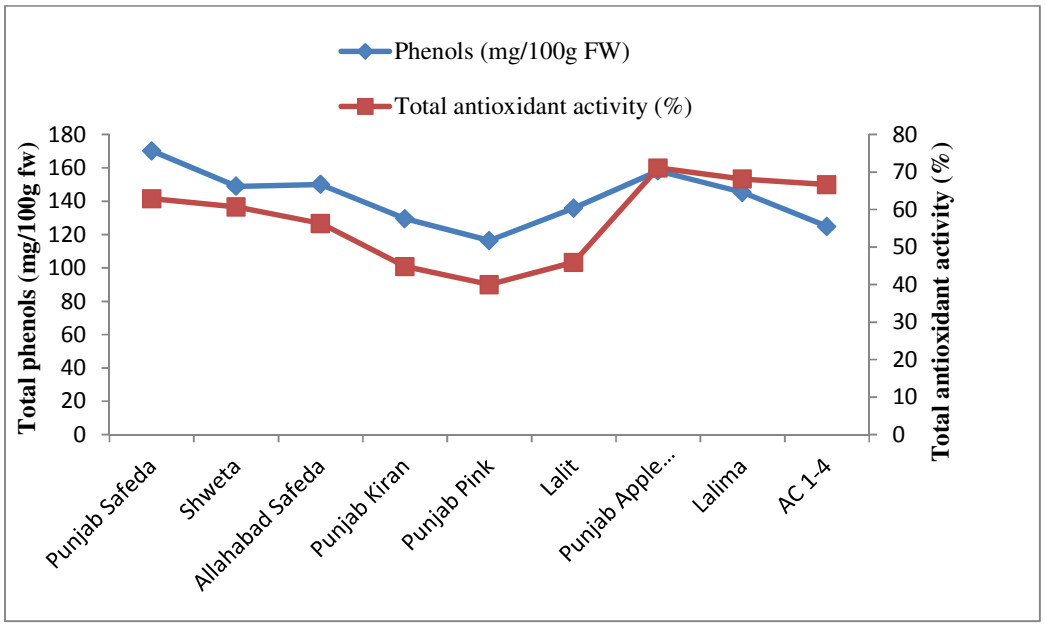

Fig 2: Correlation between total phenols and total antioxidant activity

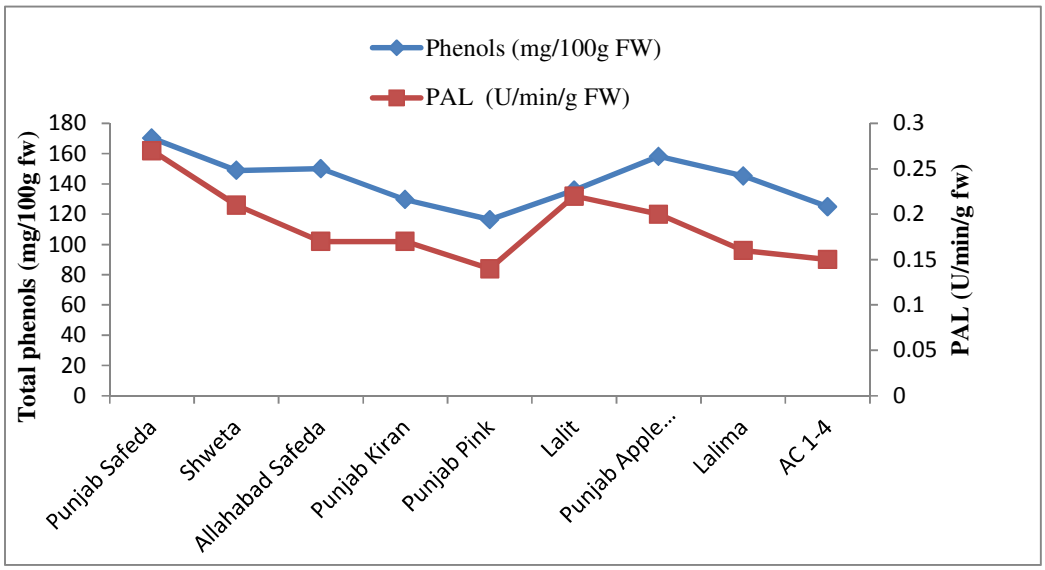

Fig 3: Correlation between total phenols and phenylalanine ammonia lyase activity

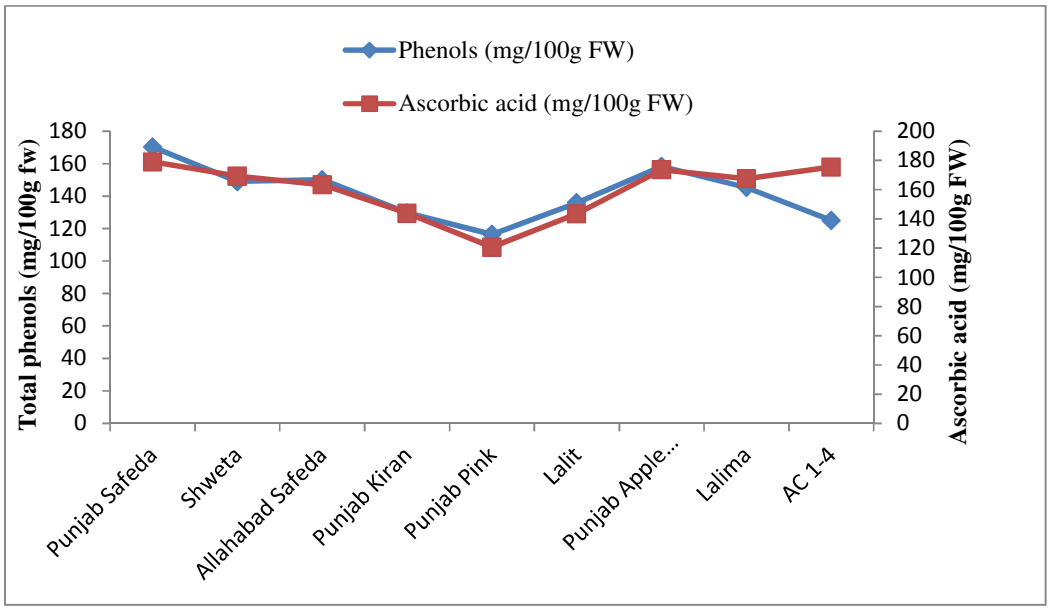

Fig 4: Correlation between total phenols and ascorbic acid content

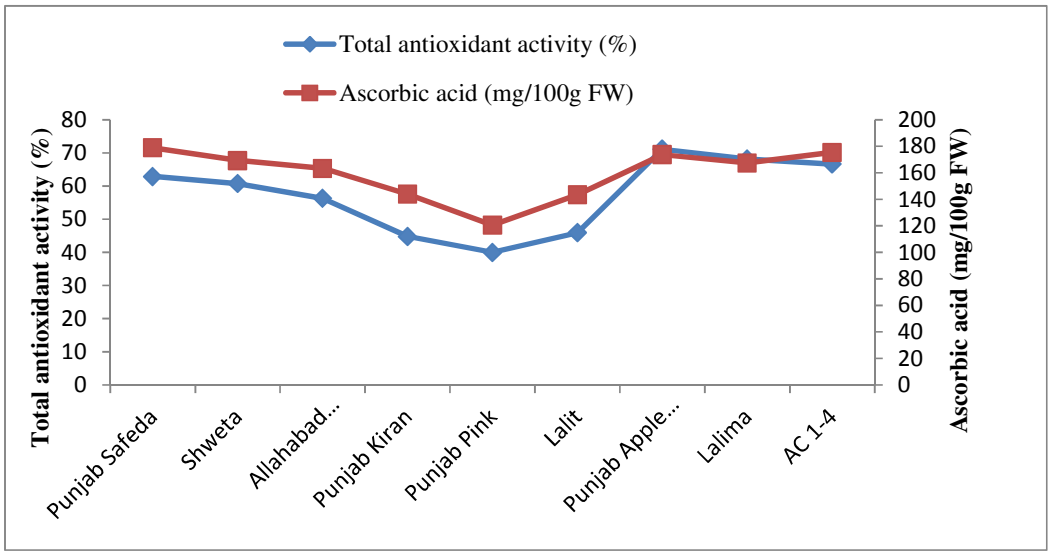

Fig 5: Correlation between total antioxidant activity and ascorbic acid content

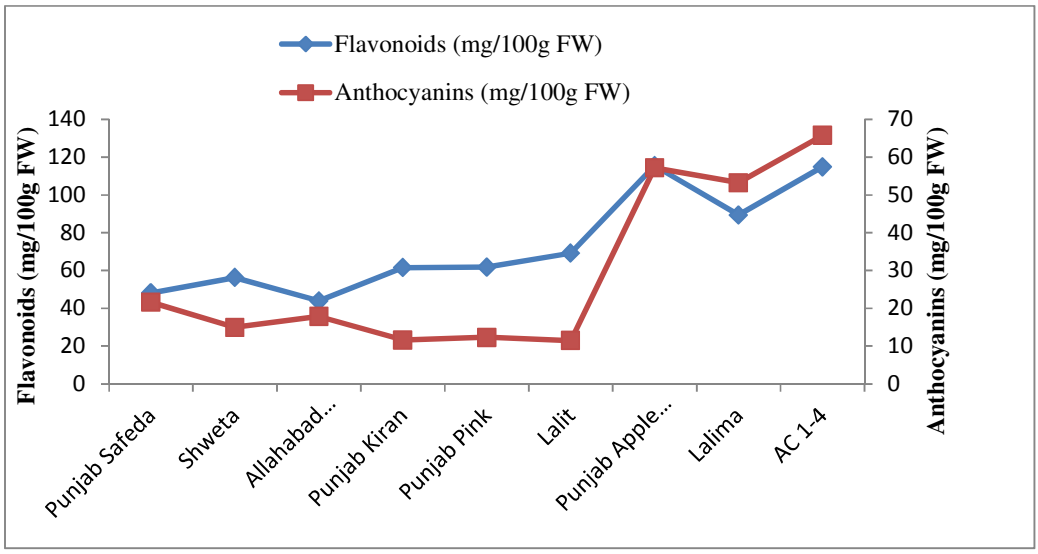

Fig 6: Correlation between total flavonoids and anthocyanins content

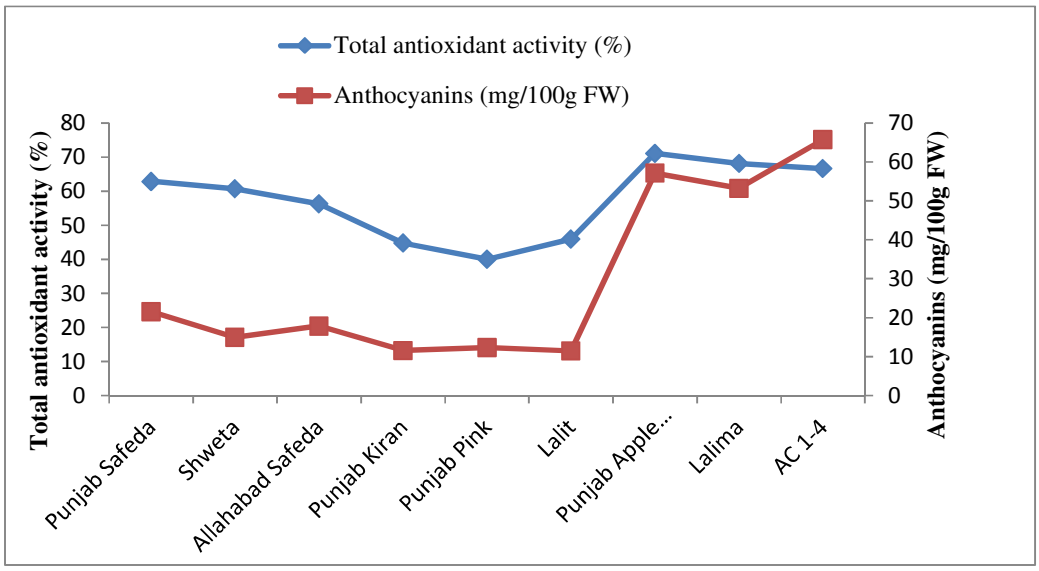

Fig 7: Correlation between total antioxidant activity and anthocyanins content

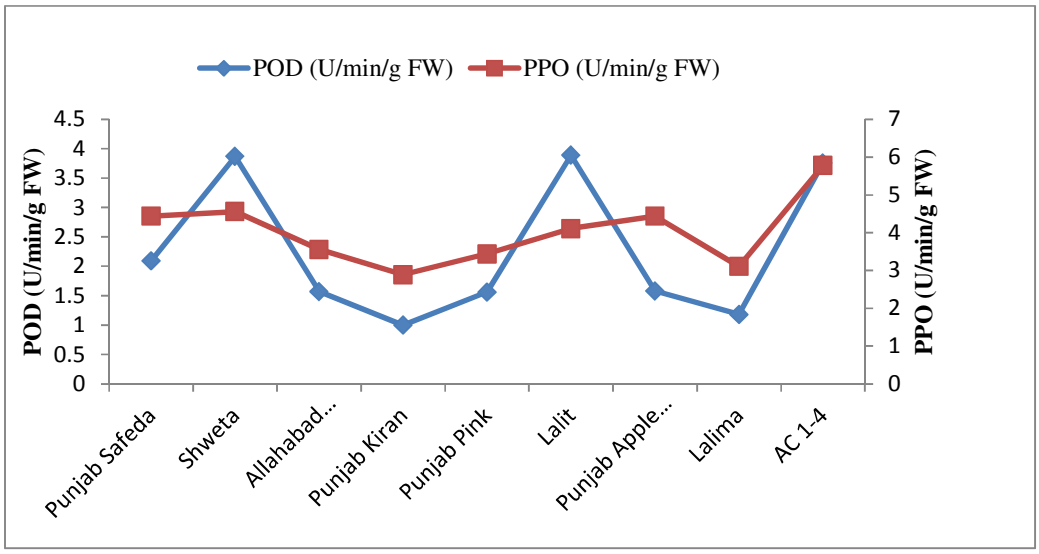

Fig 8: Correlation between peroxidase activity and polyphenol oxidase activity

CHAPTER – V

SUMMARY

The present investigation entitled "Morphological and nutraceutical characterization of guava (*Psidium guajava* L.) genotypes" was carried out at Punjab Agricultural University, Ludhiana and at Regional Fruit Research Station, Bahadurgarh, Patiala. The laboratory related work was carried out at PG Laboratory, Department of Fruit Science and Biochemistry Lab, Department of Biochemisty, Punjab Agricultural University, Ludhiana during the year 2018-19. During the present studies, nine varieties of guava were evaluated for morphological and nutraceutical potential. The salient findings of the study are summarized as below:

5.1 Fruit traits

The white fleshed varieties had greater fruit diameter and fruit weight as compared to pink fleshed varieties. The fruit diameter was maximum in Punjab Safeda (8.00 cm), followed by Shweta (7.76 cm), while it as minimum in Punjab Pink (6.54 cm) closely followed by red skinned variety Lalima (6.55 cm). Fruit length was maximum in Punjab Pink (8.19 cm) and minimum in Punjab Apple Guava (6.39 cm). Fruit weight was maximum in Punjab Safeda (244.00 g), followed by Shweta (235.33 g) and minimum in Punjab Pink (163.67 g). The fruit number per tree was highest in Punjab Pink (408.33) and lowest in Lalit (300.67) followed by Punjab Safeda (302.33) and Lalima (303.67). Shweta (77.74 kg/tree) and Punjab Safeda (73.77 kg/tree) had high yield, while Lalit (54.62 kg/tree) and Punjab Apple Guava (57.67 kg/tree) recorded low yield, as compared to other varieties. Fruit colour was measured as 'L', 'a' and 'b' values. 'L' value was highest in Punjab Safeda (74.62) and lowest in Punjab Apple Guava (41.99). 'a' value was highest in Punjab Apple Guava (32.86) and lowest in Allahabad Safeda (-7.83). 'b' value was maximum in Punjab Kiran (40.77) and Punjab Pink (40.17) and minimum in AC 1-4 (18.16) and Punjab Apple Guava (18.80). There were no significant differences in fruit firmness among all varieties. Punjab Kiran recorded higher fruit firmness (7.50 kg/cm^2), while Punjab Pink (5.50 kg/cm^2) recorded lower fruit firmness than other varieties. Core diameter ranged from 4.07 cm to 5.53 cm, with Punjab Pink having minimum core diameter and Punjab Apple Guava the maximum core diameter. Pulp thickness was maximum in Punjab Safeda (1.63 cm) and Lalima (1.63 cm) while, it was minimum in Lalit (1.17 cm) and Punjab Pink (1.17 cm).

5.2 Seed traits

Lalit variety had higher seed number per fruit (507.00), while Lalima (242.00) followed by Punjab Safeda (271.33) had lower seed number per fruit as compared to other varieties. Seed hardness was minimum in Shweta (6.07 kg/cm^2). The seed hardness in Punjab Apple Guava (9.77 kg/cm^2) was at par with Punjab Safeda (9.33 kg/cm^2) and Lalima

(9.03 kg/cm^2). Number of seeds/100g pulp ranged from 107.68 to 299.05, with maximum values recorded in Punjab Pink and minimum in Punjab Safeda (107.68) followed by Lalima (125.01). Seed weight per fruit was maximum in Lalit (5.78 g) and minimum in Allahabad Safeda (2.84 g).

5.3 Biochemical traits

TSS content ranged from 7.77 to 11.27%. AC 1-4 recorded highest TSS. Lowest TSS values were recorded in Lalima (7.77%) closely followed by Punjab Kiran (7.83%) and Allahabad Safeda (7.93%). Titratable acidity was highest in AC 1-4 (0.61%) followed by Punjab Pink (0.59%) and lowest in Allahabad Safeda (0.37%). TSS: acid ratio varied from 14.33 to 21.30. Allahabad Safeda (21.30) depicted highest TSS: acid ratio while, Punjab Pink (14.33) and Lalima (15.51) had lowest TSS: acid ratio. Juice pH was maximum in Allahabad Safeda (5.35) and minimum in Punjab Safeda (4.96) followed by Punjab Kiran (4.99).

5.4 Nutraceutical traits

Pink fleshed varieties depicted lower ascorbic acid content as compared to white fleshed and red skinned varieties. Punjab Safeda recorded maximum ascorbic acid content (178.99 mg/100g pulp) which was at par with AC 1-4 (175.43 mg/100g pulp). Minimum ascorbic acid content was observed in Punjab Pink (120.51 mg/100g pulp). Carotenoids were maximum in Punjab Safeda (0.45 mg/100g fw) and minimum in Lalima (0.32 mg/100g fw). Pink fleshed varieties recorded lower total phenols content than other varieties. Minimum content was recorded in Punjab Pink (116.40 mg/100g fw) and maximum in Punjab Safeda (170.30 mg/100g fw). In case of flavonoids and anthocyanins, red skinned varieties depicted maximum content, followed by pink fleshed and white fleshed varieties. Flavonoids content was maximum in Punjab Apple Guava (115.48 mg/100g fw) followed by AC 1-4 (114.92 mg/100g fw) and was minimum in Allahabad Safeda (43.89 mg/100g fw). The anthocyanins content was maximum in AC 1-4 (65.77 mg/100g fw) and minimum in Lalit (11.47 mg/100g fw) followed by Punjab Kiran (11.58 mg/100g fw) and Punjab Pink (12.32 mg/100g fw).

5.5 Enzymes and antioxidant assays

Polyphenol oxidase (PPO) and peroxidase (POD) enzymes are associated with enzymatic browning of fresh fruits and negative effects on colour, flavour, taste and nutrition value. There was a very little change in OD during the assessment of PPO activity. The AC 1-4 had higher PPO activity (5.78 ΔA/min/g fw) than other varieties, while Punjab Kiran recorded minimum PPO activity (2.89 ΔA/min/g fw). POD activity was highest in Lalit (3.89 ΔA/min/g fw) followed by Shweta (3.87 ΔA/min/g fw) and was lowest in Punjab Kiran (1.00 ΔA/min/g fw). PAL is the first enzyme in the phenylpropanoid pathway and is key-point in the biosynthesis of phenolic compounds (Kim and Hwang 2014). The PAL activity was significantly higher in Punjab Safeda (0.27 U/min/g fw) and lower in Punjab Pink (0.14 U/min/g fw) than other varieties. Total antioxidant activity was measured by DPPH assay and

it was observed that the red skinned varieties had highest total antioxidant potential, followed by white fleshed varieties and pink fleshed varieties. Punjab Apple Guava had highest total antioxidant activity (71.11%) which was at par with Lalima (68.15%) and AC 1-4 (66.67%). Punjab Pink had lowest total antioxidant activity (40.00%).

5.6 Correlation studies

The fruit yield was positively correlated with fruit weight (r=0.756) and didn't correlate significantly with any of the nutraceutical parameters. Total antioxidant activity had strong correlation with ascorbic acid (r=0.92) and anthocyanins content (r=0.802) and also positive correlation with total phenols content (r=0.61) and flavonoids content (r=0.56). Significantly, direct positive correlations of total phenols were recorded with ascorbic acid (r=0.725) and phenylalanine ammonia lyase activity (r=0.772). The flavonoids also had a considerable positive correlation with anthocyanins content (r=0.896). Polyphenol oxidase and peroxidase enzymes, both responsible for enzymatic browning, were strongly correlated among each other (r=0.742).

Conclusion:

Among the nine guava genotypes evaluated for morphological and nutraceutical traits, the genotype Punjab Apple Guava was superior with respect to antioxidant properties and had maximum flavonoids content among all genotypes and higher phenolic content than other red skinned genotypes. Among the white fleshed genotypes, Punjab Safeda had highest total antioxidant activity. Punjab Safeda also had maximum total phenols content, PAL activity, total carotenoids and ascorbic acid among all the guava genotypes. Hence, significant differences for morpho-physiological and nutraceutical characters were recorded during the evaluation of nine guava genotypes.

The potential guava genotypes like Punjab Apple Guava and Punjab Safeda can be promoted for commercial cultivation in the state. Nutraceutical traits in these varieties need futher clinical investigations and validation, particularly for their use in prevention of diseases.

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