

COMPARISON OF ANTIOXIDANT ACTIVITY OF *ACTINIDIA DELICIOSA* AND *PSIDIUM GUAJAVA* USING FERRIC REDUCING ANTIOXIDANT POWER ASSAY METHOD

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## ABSTRACT

**Objectives:** Antioxidant activity was studied in naturally dried seed extract and hot air oven dried extract of *Actinidia deliciosa* and *Psidium guajava* using ferric reducing antioxidant power (FRAP) assay method.

**Methods:** The dried powdered seed of *A. deliciosa* and *P. guajava* 10 g was dissolved in 100 ml of ethanol in four different conical flasks S1 (for naturally dried seeds of *A. deliciosa*), S2 (for hot air oven-dried seeds of *A. deliciosa*), S3 (for naturally dried seeds of *P. guajava*), and S4 (for hot air oven-dried seeds of *P. guajava*). The extract was carried out in shaker at 120 rpm for 72 h at room temperature by mild shaking. The extract was taken out and centrifuged at 4000 rpm for 10 min, the supernatant was taken out. The supernatant was placed in a water bath at 95°C for the solvent to evaporate and stored at room temperature.

**Results:** According to the FRAP results, *P. guajava* which was naturally dried and extracted has shown the highest antioxidant activity (sample 3) then followed by the samples S4, S1, and S2. The least activity is observed in the sample (S2).

**Conclusion:** By comparing the antioxidant activity between the *A. deliciosa* and *P. guajava* with the help of FRAP assay results, *P. guajava* has the highest amount of vitamin C (responsible for antioxidant activity) when compared to that of the *A. deliciosa*.

**Keywords:** *Actinidia deliciosa*, *Psidium guajava*, Ferric reducing antioxidant power, Antioxidant assay.

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## INTRODUCTION

*Actinidia deliciosa* (Kiwi fruit) is said to be a dietary powerhouse because of the presence of proteins, fats, carbohydrates, minerals, vitamins, and natural fiber [1]. Kiwi is also rich in Vitamin C which is responsible for its antioxidant activity. Due to the excellent folate content, it is beneficial for pregnant women. *A. deliciosa* also loaded with minerals consisting of potassium which assists to maintain blood stress and increase strength. In addition, kiwifruit includes a soluble enzyme known as *Actinidia* which facilitates inside the digestion of proteins and relieves the bloated feeling. Kiwifruits are the simplest fruit containing the actinidin enzyme, making them particular and an absolute have to have for your daily fruit basket [2]. These fruits are responsible for controlling blood sugar because it is characterized by a low glycemic index. Kiwifruit may support immune function and reduce the incidence and severity of cold- or flu-like illness in at-risk groups such as older adults and children. However, kiwifruit is allergenic, and although symptoms in most susceptible individuals are mild, severe reactions have been reported. While many research gaps remain, kiwifruit with their multiple health benefits has the potential to become part of our daily prescription for health [3]. Antioxidant activity study provides original data on the compositional profile and antioxidant capacity of kiwifruit grown in five orchards of the largest Italian production area (Lazio region). Data on macronutrients (moisture, protein, lipid, and carbohydrate), starch, total dietary fiber, oxalic acid, organic acids, minerals, trace elements, and bioactive molecules, including ascorbic acid, total polyphenols, carotenoids (lutein and  $\beta$ -carotene), and tocols ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol, and  $\gamma$ -tocotrienol) content are reported. Kiwifruit was rich in ascorbic acid (mean value 60 mg/100 g). Lutein was the most abundant carotenoid (mean value 0.2 mg/100 g) followed by  $\beta$ -carotene. Among tocopherols,  $\alpha$ -tocopherol was the most abundant (mean value 0.9 mg/100 g) followed by  $\gamma$ -tocotrienol (mean value 0.12 mg/100 g). The antioxidant activity (evaluated by means of ferric reducing antioxidant

power [FRAP], ABTS, oxygen radical absorbance capacity assays) was significantly higher ( $P < 0.05$ ) in Borgo Podgora orchard than in any other one, the fruit of this orchard showed also the highest bioactive molecules content. Antioxidant activity positively correlated with hydrophilic molecules content mostly with ascorbic acid ( $r=0.97$ ), suggesting Vitamin C as the major contributor to the total antioxidant activity in kiwifruit [4].

*Psidium guajava* (guava) is called as super fruits because of the presence of folic acid, flavonoids, manganese, copper, Vitamin A, Vitamin C, and potassium. Lycopene is present in guava fruit which fights against free radical and stops the dangers of heart disease and several types of cancer. *P. guajava* is recognized to combat off lung and oral cancers too [5]. Consuming guava helps in tightening of pores in the skin and also helps to remove wrinkles. The fruit also consists of natural fiber which removes constipation and hemorrhoids. All parts of the plant have been used for different purposes: Hepatoprotection, antioxidant, anti-inflammatory, antispasmodic, anti-cancer, antimicrobial, anti-hyperglycemic, analgesic, endothelial progenitor cells, anti-stomach-ache, and anti-diarrhea. *P. guajava* has many effects on health and that it should be researched more extensively in clinical trials. Furthermore, leaves, seeds, and peel are treated as wastes by the food processing industry and are discarded, so their use may reduce the disposal of these parts of *P. guajava* as pollutants [6]. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti-inflammatory, and antinociceptive activities, supporting its traditional uses and wide range of clinical applications for the treatment of infantile rotaviral enteritis, diarrhea, and diabetes [7]. *Pedilanthus tithymaloides* showed significant free radical scavenging activity using FRAP assay [8].

**MATERIALS AND METHODS**

*A. deliciosa* and *P. guajava* were collected from the Koyambedu market. The plant was authenticated by Prof. P. Jayaraman, Director, Institute of Herbal botany, Plant Anatomy and Research Centre, Chennai-45.

**Fruit extract preparation**

Extraction is a process used for separating bioactive constituents from solutions using specific solvents by adopting standard procedures [9].

Fruits were rinsed with distilled water and extracted the seeds manually with the help of a spatula. The flesh around the seeds was removed with the help of muslin cloth. Both the fruits were dried naturally and by hot air oven. Ground the seeds into a fine powder. Ten gram of seed powder was mixed with 100 ml of ethanol and it was placed in the shaker for 72 h in 120 rpm after taking the solvent out centrifuge in 4000 rpm for 10 min.

**Antioxidant assay**

*FRAP assay*

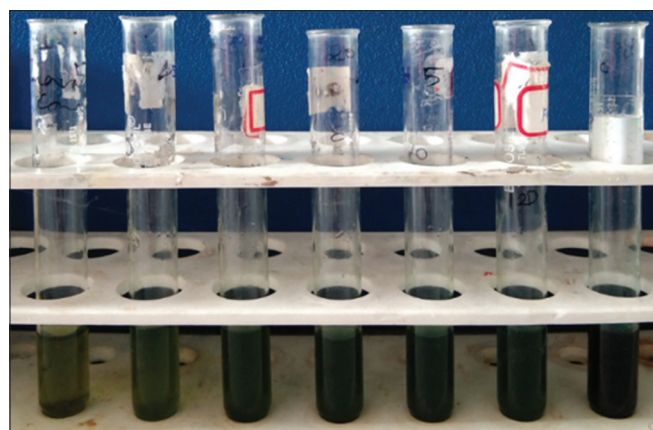
The FRAP assay measures the antioxidant potential in samples through the reduction of ferric iron (Fe<sup>3+</sup>) to ferrous iron (Fe<sup>2+</sup>) by antioxidants present in the samples. Following the reduction of the ferric iron, a blue color develops that is read calorimetrically at 594 nm.

**Table 1: Ferric reducing antioxidant power assay of naturally dried guava extract**

Concentration (µg/ml)	OD%	Sample OD
20	71.16	0.482
40	72.03	0.497
60	73.67	0.528
80	75.65	0.571
100	77.25	0.611
120	78.21	0.638

**Table 2: Ferric reducing antioxidant power assay of naturally dried kiwi extract**

Concentration (µg/mL)	OD%	Sample OD
20	66.35	0.434
40	81.77	0.801
60	82.92	0.855
80	83.72	0.897
100	84.36	0.934
120	85.65	1.018



**Fig. 1: Ferric reducing antioxidant power assay of naturally dried guava extract**

**FRAP assay**

The reducing power of the test sample was determined by Fe<sup>3+</sup> reduction method with slight modification. One milliliter of three test samples of different concentrations (20–120 µg/ml) was mixed with 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of potassium ferricyanide [K<sub>3</sub>Fe (CN)<sub>6</sub>] (1 % w/v). The mixtures were then incubated at 50°C in a water bath for 30 min. 0.5 mL of trichloroacetic acid (10% w/v) was added to each mixture. Then, 0.1 ml of freshly prepared FeCl<sub>3</sub> (0.1% w/v) solution was added and the absorbance was measured at 700 nm in UV-Vis spectrophotometer (Oyaizu, M. 1986) [10]. The percentage of reduction was calculated as:

$$\frac{(\text{Sample OD} - \text{Control OD})}{\text{Sample OD}} \times 100.$$

Antioxidant activity is found to be linearly proportional with phenolic contents [11].

**Sample preparation**

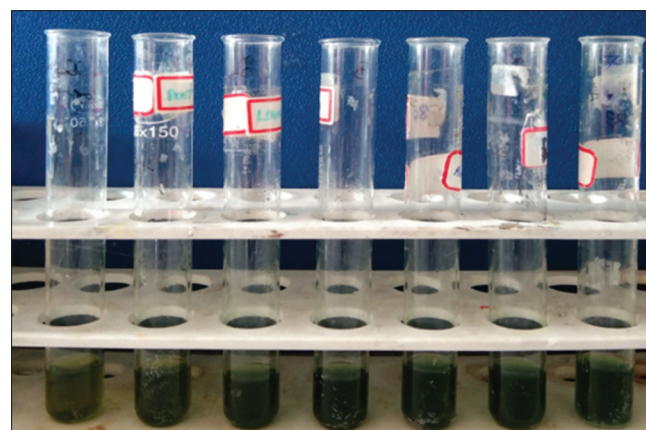
The dried powdered seed of *A. deliciosa* and *P. guajava* 10 g was dissolved in 100 ml of ethanol in four different conical flasks S1 (for naturally dried seeds of *A. deliciosa*), S2 (for hot air oven-dried seeds of *A. deliciosa*), S3 (for naturally dried seeds of *P. guajava*), and S4 (for hot air oven-dried seeds of *P. guajava*). The extract was carried out in shaker at 120 rpm for 72 h at room temperature by mild shaking. The extract

**Table 3: Ferric reducing antioxidant power assay of hot air oven-dried guava extract**

Concentration (µg/ml)	OD%	Sample OD
20	69.38	0.454
40	71.22	0.483
60	72.69	0.509
80	74.4	0.543
100	76.91	0.602
120	77.9	0.6229

**Table 4: Ferric reducing antioxidant power assay of hot air oven-dried kiwi extract**

Concentration (µg/ml)	OD%	Sample OD
20	62.5	0.389
40	81.61	0.794
60	82.26	0.823
80	83.31	0.875
100	84.11	0.919
120	85.2	0.987

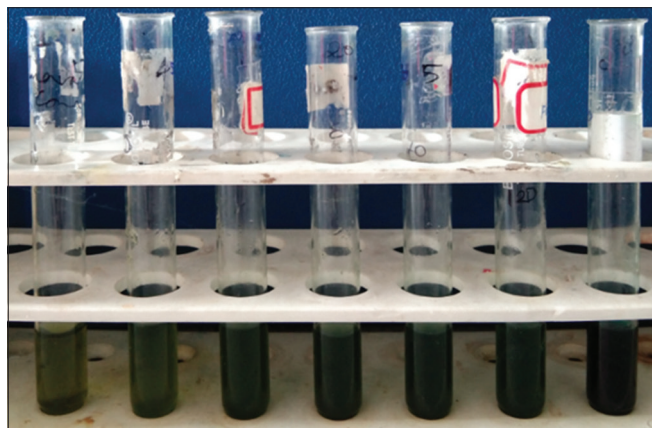


**Fig. 2: Ferric reducing antioxidant power assay of hot air oven-dried kiwi extract**

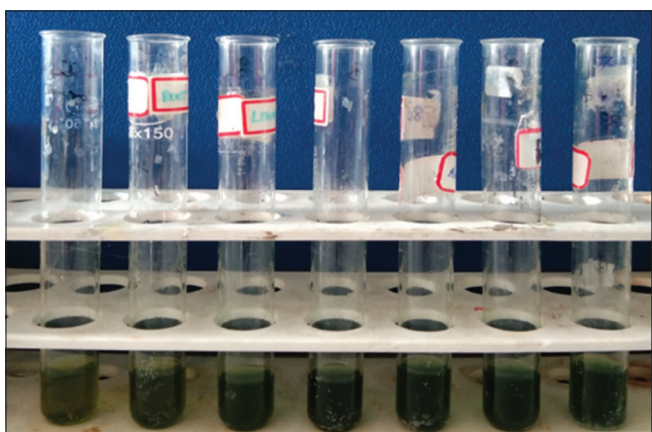
was taken out and centrifuged at 4000 rpm for 10 min, the supernatant was taken out. The supernatant was placed in a water bath at 95°C for the solvent to evaporate and stored at room temperature [12].

**RESULTS**

Ferric reducing antioxidant power assay results of naturally dried guava extract.



**Fig. 3: Ferric reducing antioxidant power assay results of naturally dried guava extracts**



**Fig. 4: Ferric reducing antioxidant power assay of hot air oven-dried kiwi extract**

**RESULTS AND DISCUSSION**

Table 1: Concentration, sample OD, and OD percentage of naturally dried guava extract.

$$\text{Sample OD} - \text{control OD} / \text{sample OD} \text{ multiplied by } 100 = \text{OD}\%$$

Control OD of naturally dried guava extract = 0.139.

Table 2: Concentration, sample OD, and OD percentage of naturally dried kiwi extract.

$$\text{Sample OD} - \text{control OD} / \text{sample OD} \text{ multiplied by } 100 = \text{OD}\%$$

Control OD of naturally dried kiwi extract = 0.146.

Table 3: Concentration, sample OD, and OD percentage of hot air oven-dried guava extract.

$$\text{Sample OD} - \text{control OD} / \text{sample OD} \text{ multiplied by } 100 = \text{OD}\%$$

Control OD of hot air oven-dried guava extract = 0.139.

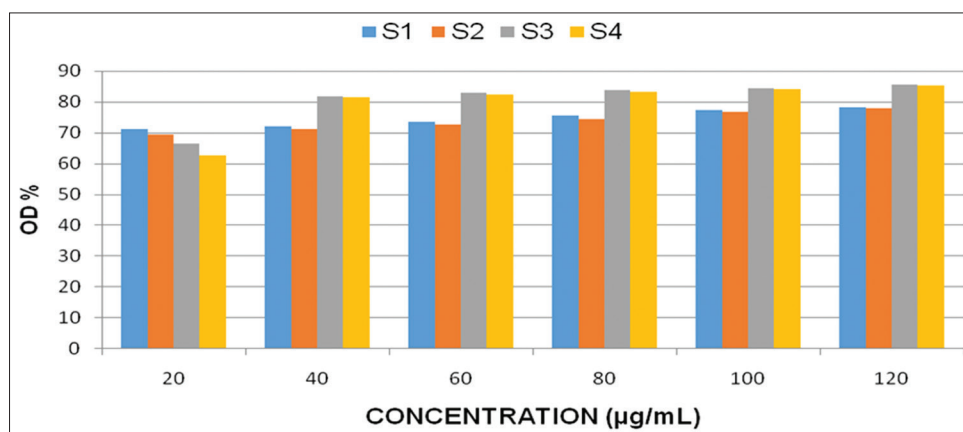
Table 4: Concentration, sample OD, and OD percentage of hot air oven-dried kiwi extract.

$$\text{Sample OD} - \text{control OD} / \text{sample OD} \text{ multiplied by } 100 = \text{OD}\%$$

Control OD of hot air oven-dried kiwi extract = 0.146.

Fig. 1 represents FRAP results of different concentrations (control, 20, 40, 60, 80, 100, and 120 [all in microgram per ml]) of naturally dried guava extract. Fig. 2 represents FRAP results of different concentrations (control, 20, 40, 60, 80, 100, and 120 [all in microgram per ml]) of naturally dried kiwi extract. Fig. 3 represents FRAP results of different concentrations (control, 20, 40, 60, 80, 100, and 120 [all in microgram per ml]) of hot air oven-dried guava extract. Fig. 4 represents FRAP results of different concentrations (control, 20, 40, 60, 80, 100, and 120 [all in microgram per ml]) of hot air oven-dried kiwi extract. Intensity of blue colour determine the presence of antioxidants. Of these, the naturally dried ethanolic *P. guajava* seed extracts sample 3 shows the highest antioxidant activity (Fig. 5). Sample 4 showed the lowest antioxidant activity according to the FRAP assay results.

FRAP assay was performed to determine the antioxidant activity of each sample. According to the FRAP results, *P. guajava* which was naturally dried and extracted has shown the highest antioxidant activity (sample 3) then followed by the samples S4, S1, and S2. The least activity is observed in the sample (S2).



**Fig. 5: Graphical representation of overall ferric reducing antioxidant power**



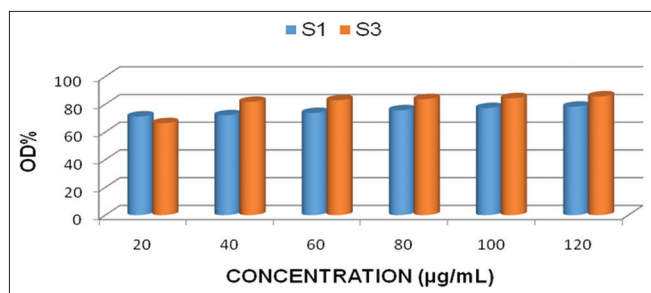


Fig. 6: Hot air oven drying ferric reducing antioxidant power results

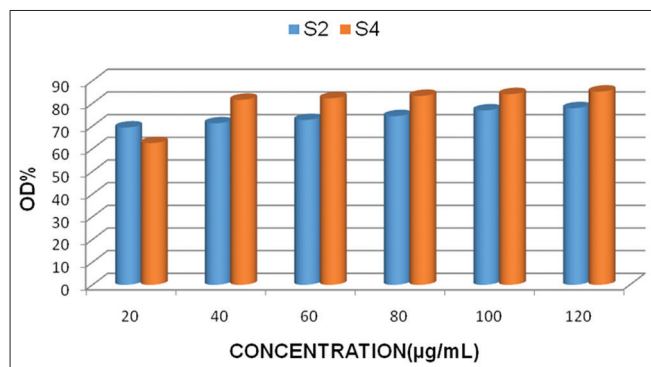


Fig. 7: Natural drying ferric reducing antioxidant power results

While comparing the natural drying and hot air oven drying, the hot air oven drying has a reducing effect on the antioxidant activity (Fig. 6), hence natural drying method is preferable than hot air oven drying. With the help of FRAP assay, we conclude that the natural drying is best than the hot air oven drying. Only at the concentration of 20 µg/ml, the hot air oven-dried seed extracts (Fig. 6) (sample S2 and S4) have shown high antioxidant activity than the other two samples (Fig. 7) (sample S1 and S3) which were naturally dried and extracted.

**CONCLUSION**

While comparing the antioxidant activity between the *A. deliciosa* and *P. guajava* with the help of FRAP assay results, *P. guajava* has the highest amount of Vitamin C (responsible for antioxidant activity) and antioxidant capacity when compared to that of the *A. deliciosa*. Further work should be carried out to isolate and purify the bioactive compound responsible for antioxidant activity.

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**AUTHORS' CONTRIBUTIONS**

The authors declare that both authors contributed equally to this research article.

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