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Original Research Article

Antioxidant activity of *Psidium guajava* fruit (*Psidium guajava* L.)

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Abstract

Purpose: *To investigate the antioxidant effect of the methanol extract of Psidium guajava L. fruit.*
Methods: *Freshly harvested fruits of Psidium guajava were washed, and pulverized, and then 10 g of milled fruit puree was homogenized with 100 mL of methanol and centrifuged to obtain the supernatant. Catalase (CAT) activity, glutathione (GSH), and malondialdehyde (MDA) levels were determined by spectrophotometry. The findings were subjected to correlation and regression analysis.*
Results: *Levels of catalase, glutathione, and malondialdehyde were 0.074 ± 0.0021 U/L, 0.0764 ± 0.0021 mmol/g, protein and 1.0931 ± 0.1573 µmol/L, respectively. Positive correlation was detected between MDA levels and GSH, CAT and GSH, and GSH and MDA levels. Predicted values of MDA were obtained from GSH and CAT activity.*
Conclusion: *Psidium guajava L. exerts antioxidant activity which is linked to changes in MDA levels. It may therefore be considered a promising source for the development of active pharmaceutical ingredients with antioxidant properties.*

Keywords: *Psidium guajava, Antioxidant, Oxidative stress, Human health*

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INTRODUCTION

Psidium guajava L. is grown in tropical and subtropical climate conditions and has been grown in hobby gardens and partly commercially in the coastal areas of the Mediterranean region of Turkey [1]. *Psidium guajava* contains five times more ascorbic acid and different types of essential bioactive compounds compared to citrus fruits and is grown in tropical and subtropical regions of the world [2]. The main components of guajava are vitamins, tannins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids, therefore, guajava is known to have many health benefits [3].

Pharmacological studies conducted on guajava have shown its tremendous potential in the treatment of gastroenteritis, dysentery, wound healing and rheumatic ulcer treatment [2,4]. High antioxidant and anti-inflammatory potential of guajava has led to its use in the treatment of different diseases [2,4,5]. Derivation of reactive substances from oxygen and nitrogen during energy production body causes oxidative stress. Also, oxidative imbalance causes damage to reactive products and antioxidant mechanisms. Toxicity may occur as a result of excessive

accumulation of oxygen-reactive species (ROS), and damage to tissues may occur.

Reactive oxygen species (ROS) may be kept under control by antioxidant defense systems. Therefore, many phytonutrients in fruits and vegetables protect the body against ROS [6,7]. Oxidative stress is recognized to play a central role in the pathophysiology of many different diseases such as stroke, rheumatoid arthritis, and cardiovascular disorders [6,7]. Also, oxidative stress caused by oxidative damage to DNA may lead to cancer [8]. Antioxidants protect cells against oxidative stress [2,4,5]. Various enzymatic antioxidants such as catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) protect DNA from oxidative stress [9].

Accumulation of ROS in the body is modulated through antioxidant enzymes such as CAT. Non-enzymatic mechanisms such as GSH and phytochemical compounds with antioxidant activity also help prevent ROS damage [10]. One of the most frequently studied markers of lipid oxidation or lipid peroxidation is malondialdehyde (MDA) which is one of the indicators of oxidative stress [7,11]. This study therefore investigated some antioxidant effects of *Psidium guajava* L.

EXPERIMENTAL

Sample collection and handling

Psidium guajava fruits grown in the garden of a summer house in Mersin (Silifke) Province of Turkey, with a geographical location of 36° 22′ 34″ North and 33° 55′ 56″ East latitude and longitude, were used. Fruits that did not show any signs of injury and were free from insects, pests and diseases were collected. Extraction of fruit samples from *Psidium guajava* involves these steps. Firstly, the fruits were cleaned and crushed into puree. 10 g of fruit puree was homogenized with 100 mL methanol, and the mixture was centrifuged (Universal 320R, Hettich Zentrifugen, Tuttingen, Germany) for 15 minutes at 2000 rpm. After a 2-hour waiting period, the supernatant that rose to the top was separated, and samples were stored at -20 ºC for analysis.

Determination of reduced glutathione (GSH) level

Phosphate buffer (800 µL) was added to 200 µL of sample. Initial absorbance (A_1) was recorded at 412 nm with UV/visible spectra. Ellman's reagent (100 µL) was added to the same tube and the second absorbance (A_2) was obtained. Glutathione concentration (C) was calculated in mmol/g protein unit using Eq 1 [12].

$$C/1000 = (A_2 - A_1)/13{,}600 \times E_1 \times 5/2 \times ½ \quad \ldots\ldots (1)$$

Where: A_1 is the first absorbance before the addition of DTNB at 412 nm; A_2 is the second absorbance after the addition of DTNB at 412 nm; E_1 = 1; 13,600 is the molar extinction coefficient of the yellow color that formed during the interaction of GSH and DTNB (5,5-dithio-bis-(2-nitrobenzoic acid); 1000 is the conversion coefficient to mmol; C is the concentration in mmol/glutathione (mg/dL).

Determination of malondialdehyde (MDA) level

Guajava fruit (200 µL) was collected into a test tube. Thereafter, 800 µL phosphate buffer, 25 µL Butyl hydroxytoluene (BHT solution), and 500 µL of 30 % TCA (trichloroacetic acid) were added. The tubes were stirred and kept on ice for 2 h. The tubes were centrifuged at 2000 rpm for 15 min, and 1 ml from the supernatant was taken and transferred to other tubes. Thereafter, 75 µL Ethylene diamine tetra acetic acid disodium (EDTA) and 250 µL of TBA were added. The samples were mixed and kept in a hot water bath for 15 min, and later to room temperature. Absorbance was read in UV/visible spectrophotometer at 532 nm. The concentration of the samples was calculated using Eq 2 [13].

$$C = F*6.41*A \quad \ldots\ldots\ldots\ldots\ldots (2)$$

Where: C is the concentration; F is the dilution factor; A is absorbance.

Determination of catalase (CAT) activity

Hydrogen peroxide (H_2O_2) (1.4 mL of 30 mM) and 0.1 mL phosphate buffer was added to the blank tube. Thereafter, 1.4 mL of 30 mM H_2O_2 and 0.1 mL of the enzyme were added and mixed. Absorbance was measured in duplicate at 240 nm at 30-second intervals. Catalase activity was calculated in U/L using Eq 3 [14].

$$\text{Activity} = (2.3/\Delta x)*(\log A_1/\log A_2) \quad \ldots\ldots\ldots\ldots (3)$$

where Δx = 30 s; 2.3 is 1 µmol absorbance of H_2O_2 in a 1 cm light path.

Statistical analysis

Data was analyzed using Statistical Packages for Social Sciences (SPSS Inc., Chicago, USA). Measurement data were expressed in mean ± standard deviation (SD). Correlation and

regression analysis were performed to compare inter-group and intra-group means. $P < 0.05$ was considered statistically significant.

RESULTS

Antioxidant effect

Catalase level (0.0764 ± 0.0021 U/L) was significantly correlated with GSH and MDA levels ($p < 0.05$). Also, GSH level (0.0019 ± 0.0006 mmol/g protein) was significantly correlated with MDA and CAT levels ($p < 0.05$); and MDA level (1.0931±0.1573 µmol/L) was significantly correlated with GSH and CAT levels ($p < 0.05$; Table 1).

Regression model

Variables were identified by multiple linear regression analysis. The B coefficients and constant values were used to calculate the estimated model. Values of MDA were significantly predicted by GSH, and CAT values ($p < 0.05$; Table 2).

The values of MDA were predicted by GSH levels, by CAT activity values (Pearson correlation was positive) and, by total GSH levels and CAT activity. Considering the predictive power of the predictive variables included in the analysis, linear R^2 was taken into account. R^2 was obtained as 0.709 between GSH levels and MDA levels (Figure 1), and linear R^2 was obtained as 0.330 between CAT activity and MDA levels (Figure 2). Additionally, linear R^2 was obtained as 0.754 between CAT activity and GSH levels (Figure 3).

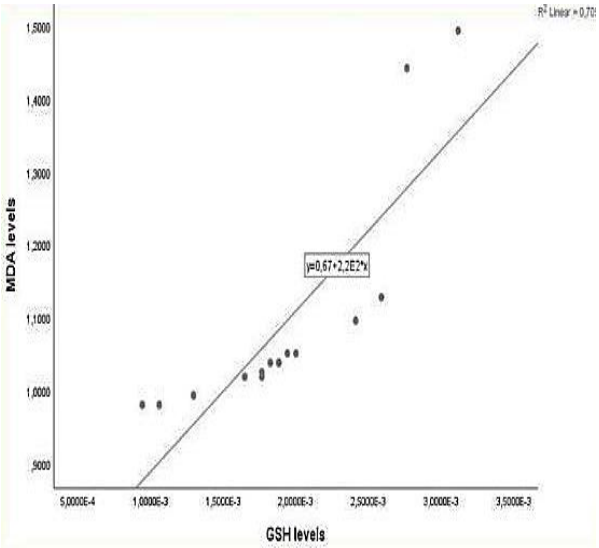

Figure 1: Regression line for MDA levels and GSH levels

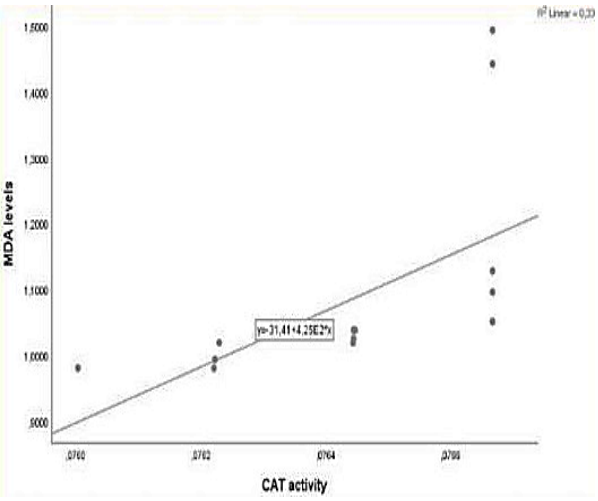

Figure 2: Regression line for MDA levels and CAT activity

Table 1: Descriptive statistics and correlation coefficients

Variable	Descriptive statistics	Correlation coefficient		
	Mean ± SD (n=15)	***GSH***	***MDA***	***CAT***
GSH (mmol/g protein)	0.0019±0.0006	1		
MDA (µmol/L)	1.0931±0.1573	0.842**	1	
CAT (U/L)	0.0764±0.0021	0.868**	0.575*	1

Note: *$P < 0.05$, **$p < 0.01$; SD: Standard deviation, GSH: Glutathione; CAT: Catalase, and MDA: Malondialdehyde

Table 2: Malondialdehyde (MDA) model-based regression analysis

Model	Variable	B	β	t	*P*-value	R	R^2	F	*P*-value
1	Constant	0.669	-	8.499	0.000	0.842	0.709	31.609	0.000
	GSH levels	220.117	0.842	5.622	0.000				
2	Constant	-31.413	-	-2.446	0.029	0.575	0.330	6.404	0.025
	CAT activity	425.143	0.575	2.531	0.025				
3	Constant	36.296	-	2.535	0.026	0.899	0.808	25.210	0.000
	GSH levels	364.169	1.393	5.461	0.000				
	CAT activity	-469.576	-0.635	-2.488	0.029				

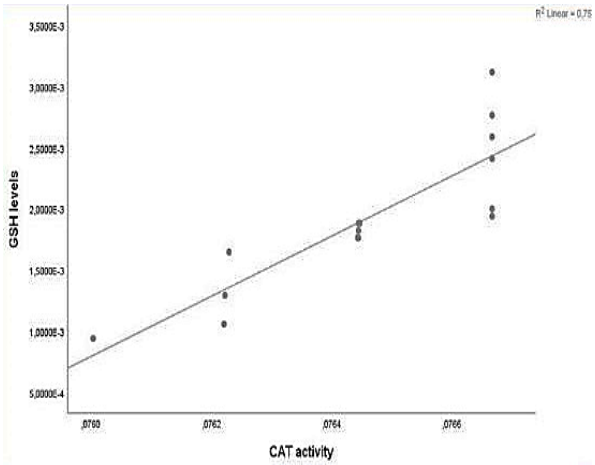

Figure 3: Regression line for GSH levels and CAT activity

DISCUSSION

Reactive oxygen species (ROS) are produced from endogenous and exogenous factors and cause the formation of different types of DNA lesions. DNA damage is devastating for cells and may lead to mutagenesis and/or cell death [8]. Antioxidants are substances that protect cells against oxidative stress. Phytochemicals such as flavonoids and polyphenols found in fruits, vegetables, and herbs are known to be natural antioxidants. Studies on antioxidants and antioxidant-rich plant extracts have emerged as an important area of study on the role of oxidative stress in health and disease [5]. Antioxidants are found naturally in plants, animals and microorganisms, and are synthesized chemically. *Psidium guajava* has a wide range of medicinal properties, and it is used in the treatment of many diseases in tropical and subtropical countries [15]. Guajava is a fruit with high nutritional value as well as high antioxidant potential. It also exhibits significant anti-hypertensive, antispasmodic, antimicrobial, hypoglycemic, anodyne and anti-inflammatory properties. As a result, it is widely used as food additive [16].

Both correlation and regression analysis were used to examine the existence of a linear relationship among the different variables [17]. The results revealed that CAT and GSH significantly predicted MDA levels. Studies have shown that guajava fruit protects the kidney through its anti-oxidative, anti-inflammatory and anti-glycation effects in rat models [16,18]. Phytochemical analysis on hydroalcoholic extract of guajava has shown that guajava has high potential antioxidant activity [4]. However, it has been reported that there was no significant difference in antioxidant enzyme activities between guajava extracts and the control and treatment groups, and MDA levels were significantly different [19]. In a study investigating the catalase activity of red guajava fruit, it was reported that guajava fruit has an effect on catalase activity [20]. Furthermore, red guajava fruit extract provided relief and healing in the alveolar tissue of rats exposed to cigarette smoke [21]. Also, aqueous extract of *Psidium guajava* provides good hepatoprotective activity [22]. Red and dark-colored fruits contain high amounts of phenolic compounds, such as anthocyanins, flavonols, and phenolic acids [23,24]. It has been documented that natural pigments of fruits provide several health advantages such as antioxidant, anti-inflammatory and anti-carcinogenic [23]. In this study, *Psidium guajava* fruits were used, and antioxidant effect was significantly linked to MDA changes.

CONCLUSION

Fruits of Psidium guajava show significant antioxidant properties which are linked to changes in MDA. Therefore, it may be considered a promising ingredient in the development of active pharmaceutical ingredients with antioxidant properties.

DECLARATIONS

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None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by them.

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REFERENCES

1. *Güler G, Gübbük H, Çelik B. An overview of Guava (Psidium guajava L.) Cultivation. Meyve Bilimi 2021; 8(2): 23-29.*
2. *Azam M, Hameed L, Qadri R, Ejaz S, Aslam A, Khan MI, Shen J, Zhang J, Nafees M, Ahmad I, et al. Postharvest ascorbic acid application maintained the physiological and antioxidant responses of Guava (Psidium guajava L.) at ambient storage. Food Sci Technol 2021; 41: 748-754.*
3. *Barbalho SM, Farinazzi-Machado FMV, Goulart RA, Brunnati ACS, Ottoboni AMMB, Nicolau CCT. Psidium guajava (Guava). Med Aromat Plants 2012; 1: 104.*
4. *Ghaderi F, Ebrahimi E, Sari AF, Koohi-Hosseinabadi O, Koohpeyma, F, Irajie C, Tanideh N, Iraji A. The effect of hydroalcoholic extract of Psidium guajava L. on experimentally induced oral mucosal wound in rat. BMC Complement Med Ther 2022; 22: 201.*
5. *Papaefthimiou M, Kontou PI, Bagos PG, Braliou GG. Antioxidant activity of leaf extracts from Stevia rebaudiana Bertoni exerts attenuating effect on diseased experimental rats: A systematic review and meta-analysis. Nutrients 2023; 15: 3325.*
6. *Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol 2011; 25: 287-299.*
7. *Amar SAA, Eryilmaz R, Demir H, Aykan S, Demir C. Determination of oxidative stress levels and some antioxidant enzyme activities in prostate cancer. Aging Male 2019; 22: 198-206.*
8. *Maynard S, Schurman SH, Harboe C, de Souza-Pinto NC, Bohr VA. Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogenesis 2009; 30(1): 2-10.*
9. *Hamam H, Demir H, Aydın M, Demir C. Determination of some antioxidant activities (superoxide dismutase, catalase, reduced glutathione) and oxidative stress level (malondialdehyde) in cirrhotic liver patients. Mid Blac Sea J Health Sci 2022; 8: 506-514.*
10. *López-Mejía A, Ortega-Pérez LG, Magaña-Rodríguez OR, Ayala-Ruiz LA, Piñón-Simental JS, Hernández DG, Rios-Chavez P. Protective effect of Callistemon citrinus on oxidative stress in rats with 1,2-dimethylhydrazine-induced colon cancer. Biomed Pharmacother 2021; 142: 112070.*
11. *Maryanto S. The effects of red guava (Psidium guajava L) fruits on lipid peroxidation in hypercholesterolemic rats. Basic Res J Med Clin Sci 2013; 2: 116-121.*
12. *Tietz F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. Anal Biochem 1969; 27(3): 502–522.*
13. *Bird RP, Draper HH. Comparative studies on different methods of malondialdehyde determination. Methods Enzymol 1984; 105: 299–305.*
14. *Aeibi H. Catalase. Meth Enzymol 1984; 105: 121-126.*
15. *Gutiérrez RMP, Mitchell S, Solis RV. Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2008; 117: 1-27.*
16. *Vasconcelos AG, Amorim ADGN, Dos Santos RC, Souza JMT, De Souza LKM, Araújo TSL, Nicolau LAD, De Lima CL, De Aquino PEA, Da Silva MC, et al. Lycopene-rich extract from red guava (Psidium guajava L.) displays an anti-inflammatory and antioxidant profile by reducing suggestive hallmarks of acute inflammatory response in mice. Food Res Int 2017; 99: 959–968.*
17. *Bewick V, Cheek L, Ball J. Statistics review 7: Correlation and regression. Crit Care 2003; 7: 451-459.*
18. *Lin CY, Yin MC. Renal protective effects of extracts from guava fruit (Psidium guajava L.) in diabetic mice. Plant Foods Hum Nutr 2012; 67: 303–308.*
19. *Molla T, Azene HA. Systemic review on antioxidant and hepatoprotective effect of Psidium guajava leaf and fruit extract. Food Sci Qual Manag 2017; 60: 28-47.*
20. *Sulastri A, Soemardji AA, Sukrasno S, Amaliya A. The Influence of maturity stage on superoxide dismutase and catalase activity in red guava fruit. Adv Health Sci Res 2019; 11: 160-163.*
21. *Meles DK, Mustofa I, Wurlina W, Susilowati S, Utama S, Suwasanti N, Putri DKSC. The restorative effect of red guava (Psidium guajava L.) fruit extract on pulmonary tissue of rats (Rattus norvegicus) exposed to cigarette smoke. Vet Med Int 2021; 1: 9931001.*
22. *Roy CK, Kamath JV, Asad M. Hepatoprotective activity of Psidium guajava Linn. leaf extract. Indian J Exp Biol 2006; 44: 305-311.*
23. *Demir H, Güler A, Demir C. Determination of some antioxidant enzyme activities in fresh and dry grapes (Vitis vinifera L.). Van Med J 2020; 27: 150-154.*
24. *Cosme F, Gonçalves B, Bacelar EA, Ines A, Jordão AM, Vilela A. Genotype, environment and management practices on red/ dark-colored fruits phenolic composition and its impact on sensory attributes and potential health benefits. In Tech; 2017. Available from: http://dx.doi.org/10.5772/66881*