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# Phytochemical composition and gastroprotective effect of *Feijoa sellowiana* Berg fruits from Sicily

Maria Teresa Monforte<sup>1\*</sup>, Francesco Lanuzza<sup>2</sup>, Fabio Mondello<sup>2</sup>, Clara Naccari<sup>3</sup>, Simona Pergolizzi<sup>4</sup>, Enza Maria Galati<sup>1</sup>

<sup>1</sup>SCIFAR Department, University of Messina, Messina, Italy

<sup>2</sup>Department of Economics, Business, Environment and Quantitative Methods, University of Messina, Messina, Italy

<sup>3</sup>Fondazione "Prof. Antonio Imbesi" – SCIFAR Department, University of Messina, Messina, Italy

<sup>4</sup>S.A.S.T.A.S. Department, University of Messina, Messina, Italy

## PEER REVIEW

### Peer reviewer

Prof. Olga Tzakou, Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimioupoli Zographou, 157 71 Athens, Greece.

Tel/Fax: +2107274591

E-mail: tzakou@pharm.uoa.gr

### Comments

This is a valuable work in which authors demonstrated the gastro-protective effect exerted by *F. sellowiana* fruit. The effect was assessed on ethanol-induced ulcers in rats, through ulcer index and histopathological observations.

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## ABSTRACT

**Objective:** To assess the fruit of *Feijoa sellowiana* Berg var. *coolidge* and *gorgiona*, cultured in Sicily, for its gastroprotective effect, in association with performing phytochemical evaluation.

**Methods:** By means of HPLC, vitamin E complex and polyphenol compounds were determined. The gastroprotective effect was investigated on ethanol-induced ulcers in rats, with sucralfate as reference drug. Samples of gastric mucosae, stained by periodic acid–Shiff and haematoxylin/eosin, were observed by light microscopy.

**Results:** In pulp and peel samples of both varieties of fruit  $\alpha$ ,  $\beta$  and  $\gamma$  tocopherols were identified, while  $\delta$  was only in var. *coolidge*. In whole fruit of two varieties, catechin, eriodictyol, eriocitrin, pyrocatechol, quercetin, rutin, ellagic, gallic and syringic acid were determined. The fruit showed gastroprotective effect (ulcer index: var. *coolidge*=1.07, var. *gorgiona*=1.02). The efficacy was comparable to that of sucralfate (ulcer index 1.10). Histological examination confirmed the inhibition of ulcerogenic activity of the ethanol.

**Conclusions:** Active principles of *Feijoa sellowiana* can play an important role in human diet and show beneficial effects on various diseases, particularly those caused by oxidative processes resulting in cell damage. The amount of polyphenols and vitamin E complex confirms the nutritional value of this fruit, grown in pedoclimatic condition very different from the origin area.

### KEYWORDS

*Feijoa sellowiana* Berg var. *coolidge* and *gorgiona*, Polyphenols, Tocopherols, Gastroprotective effect, HPLC, Peel, Pulp

## 1. Introduction

At the present in Sicily the overproduction of traditional fruits (citrus fruits, apples, pears, table grapes) causes negative economical impact. A Sicilian Region Project supports the cultivation of tropical and subtropical fruit trees, such as feijoa, papaya, mango, as an alternative to local production. *Feijoa sellowiana* Berg (*F. sellowiana*) is a plant native to central and southern America that has been acclimatized in the coastal areas of Italy as Liguria and

Sicily[1].

This research is focused on fruit of *F. sellowiana* grown in the experimental cultivations, in Sicily. The aim of this study is to assess whether the pedoclimatic conditions in Sicily allow to obtain fruits with nutritional value comparable to those grown in native countries.

The fruit ripens in autumn as a spherical berry, 4–8 cm long and weighs 20–30 g. The most known varieties (*apollo*, *coolidge*, *gemini*, *mammoth*, *moore triumph*) differ in maturation time, greatness and quality of fruit. The fruit

\*Corresponding author: MT Monforte, SCIFAR Department, Vill. S.S. Annunziata, 98168 Messina, Italy.

Tel: +39 90 6766548

Fax: +39 90 6766474

E-mail: mmonforte@unime.it

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contains terpenes, tannins, steroidal saponins, pectins, polyphenols<sup>[2–4]</sup>. Some flavones and flavanones have been isolated from various parts of this plant<sup>[5,6]</sup>. Many volatile compounds, as both methyl- and ethyl-benzoate, that account for about 90% of the volatile fraction, are responsible for the fruit aroma<sup>[7–9]</sup>. The fruit contains large amounts of ascorbic acid and minerals<sup>[2]</sup>. High concentrations of iodine have also been reported<sup>[10]</sup>.

*F. sellowiana* fruit shows several biological activities: antibacterial<sup>[11]</sup>, analgesic and anti-inflammatory<sup>[12–14]</sup>, antioxidant and anticancer<sup>[12,15–18]</sup>. Our preliminary studies demonstrated a protective effect of *Feijoa* fruit methanol extract on gastric mucosa<sup>[19]</sup>. Flavonoids, vitamins, pectins, and pigments play a very important role in human diet and these substances, known as “nutraceuticals”, show beneficial effects on various diseases, particularly those caused by oxidative processes resulting in cell damage<sup>[18]</sup>.

In this study the characterization of *F. sellowiana* fruit polyphenols and its gastroprotective effect on ethanol-induced ulcer in rat, are reported. Moreover, the presence of tocopherols was tested on the basis of the significant antioxidant property of this fruit.

## 2. Materials and methods

### 2.1. Plant materials

Fruits of *F. sellowiana* Berg. var. *coolidge* and var. *gorgiona*, collected from experimental cultivations (Istituto Professionale di Stato per l’Agricoltura, Milazzo, Messina), in different times (October 2010 and December 2011). This time corresponds to maximum reopening period of these fruits, in Sicily. The fruit was immediately stored at –20 °C until utilization.

A voucher specimen was deposited in the Herbarium of the SCIFAR Department, University of Messina.

### 2.2. Water and ash determination

Water and ash determination of the fresh fruit of *F. sellowiana* was carried out according to the methods proposed by Pharmacopoea Italica. XII ed. The water content of *Feijoa* fruit was 80.62% for mesocarp and 75.73% for epicarp. The ash content was 4.3 mg/g.

### 2.3. Phytochemical analysis

Preliminary phytochemical investigations on *F. sellowiana* var. *coolidge* and var. *gorgiona* fruit were carried out. The assays for terpenes, mucilages, tannins and flavonoids were positive, whereas the test for nitrogen compounds was negative.

#### 2.3.1. Determination of tocopherol content

The content of vitamin E was determined in the pulp and peel of *Feijoa* fruits of both varieties.

#### 2.3.1.1. Sample preparation

The fruit were seedless, and the esocarp (peel) was separated from the mesocarp (pulp). From each fruit the pulp was cut in small pieces and the peel into strips. Samples were dried by lyophilization, to avoid tocopherols degradation at high temperatures.

The samples were ground with a rotating blades mill MF 10 basic (Ika Werke GmbH & Co, Staufen, Germany), using a sieve with holes of 1.5 mm diameter. Ground samples were put in hermetic vials, and stores at dark until tocopherol extraction. Aliquotes of 3 g of pulp and peel were extracted with *n*-hexane (20 mL) containing butylhydroxytoluene (BHT) (0.1%), stirring for two minutes.

BHT was added to increase the stability of the samples by reducing oxidative processes. The extraction was repeated two times with 15 mL *n*-hexane. The extracts were collected, dried under vacuum in rotavapor at 35 °C (Büchi Laboratoriums-Technik, Flawil, Switzerland), reconstituted with 2 mL *n*-hexane. After filtration with 0.22 µm teflon filters (National Scientific, Cardiff Valley Road Rockwood, TN, USA, supplied by Superchrom, Milan, Italy), the samples were submitted to HPLC analysis.

#### 2.3.1.2. Chemicals

*n*-hexane and isopropanol were both HPLC grade (J. T. Baker, Mallinckrodt Baker B.V. Exacta-Optech Labcenter S.p.A., Modena, Italy). High-quality water was obtained by a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA).

Tocopherols  $\alpha$  (99.5%),  $\beta$  (99.0%),  $\gamma$  (98.9%) e  $\delta$  (93.1%) were purchased from Supelco (Sigma-Aldrich, Milan, Italy), BHT from Fluka, (Sigma-Aldrich, Milan, Italy).

#### 2.3.1.3. Analytical method

A liquid chromatograph Perkin-Elmer LC serie 4 (Norwalk Connecticut, USA), equipped with a Rheodyne valve 8125 (loop 10 µL) (Redwood Drive Cotati, CA, USA), a Spectrofluorimetry Perkin-Elmer LC 240, a column Chromopack Chromospher 5 Si 250×0.2 mm I.D. (Varian, Superchrom s.r.l. Milan, Italy), pre-column Guard-Pak Waters Inserts Resolve™ Silica (Waters, Vimodrone Milan, Italy), was used. Chromatographic data were processed with a Chrom Card (Fisons Instruments S.p.A., Rodano, Milan, Italy). Chromatographic separation was carried out, at room temperature, in isocratic, using a sonicated mixture of *n*-hexane (99.8%) and isopropanol (0.2%), degassed for 10 min. The flow rate was 0.3 mL/min. The spectrofluorimetric detection was performed at  $\lambda_{ex}$  290 nm and  $\lambda_{em}$  330 nm.

Qualitative analysis of tocopherols  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  was carried out comparing the chromatographic behavior and the UV excitation and emission spectra of the components with pure standard solutions, under the same chromatographic conditions, registered with “stopped flow” technique. Quantitative analysis was carried out by using linear regression obtained by injecting solution of known content of tocopherol standards. The linearity response of the detector was verified in the range between 0.002 and 0.06 mg/mL for

$\alpha$ -tocopherol, 0.002 and 0.02 mg/mL for  $\beta$ -tocopherol, 0.002 and 0.04 mg/mL for  $\gamma$ -tocopherol and 0.0003 and 0.008 mg/mL for  $\delta$ -tocopherol. Samples were analyzed in triplicate.

### 2.3.2. Determination of polyphenol content

The content of polyphenols was determined in whole fruit.

#### 2.3.2.1. Sample preparation

Aliquots of 60 g of *F. sellowiana* whole fruit var. *coolidge* and var. *gorgiona* were mixed through Ultra-Turrax (Ika Werk), centrifuged at 20 000 r/min for 20 min at 15 °C (Avanti Centrifuge J 25 Bekman Coulter) and then decanted, obtaining about 20 mL of juice.

To remove mucilage, 8 mL of juice were warmed b.m. at 40°C, added with methanol (1:4) to promote mucilage precipitation, and finally centrifuged at 10 000 rpm for 15 min, at 15 °C. The supernatant was evaporated in rotary vacuum (Buchi V-800) to remove methanol. Aliquots of 0.5 mL of samples were introduced in cartridges SPE column (Varian Bond Elut C18), previously rinsed with 2.5 mL of methanol and 5 mL of H<sub>2</sub>O. Finally, 2 mL of H<sub>2</sub>O were added to sugar elimination. The purified extract was eluted with 2.5 mL of methanol and 50  $\mu$ L of H<sub>2</sub>O. The eluate was evaporated to dryness under a stream of nitrogen. The yield was of 10 mg/mL of starting juice.

#### 2.3.2.2. Chemicals

Acetonitrile and methanol, both HPLC grade, were purchased from Fluka (Sigma Aldrich, Milan), ortho-phosphoric 85% acid from Carlo Erba (Carlo Erba Reagenti SpA, Limite, Milan, Italy). High-quality water was obtained by a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA). Flavonoids and phenolic acids (ellagic acid, gallic acid, syringic acid, catechin, eriodictyol, eriocitrin, pyrocatechol, quercetin, rutin) used as standards, were purchased from Extrasynthèse, (Genay, France) and Fluka (Sigma Aldrich, Milan, Italy).

#### 2.3.2.2. Analytical method

A Spectra SYSTEM® Gradient Pumps 4000 Menus liquid chromatograph (Thermo Separation Products, FL, USA), equipped with Vacuum Membrane Degasser SCM 1000 and a Rheodyne valve 8125 (loop 20  $\mu$ L) (Redwood Drive Cotati, CA, USA) were used. Liquid chromatograph was coupled to a Photodiode Array Detector Spectra SYSTEM® UV6000 LP (Thermo Separation Products, FL, USA), working in the range 220–450 nm. Chromatographic data were processed with a ChromQuest Chromatography Workstation (ThermoQuest Italiy S.p.A., Rodano, Milan, Italy). Analyses were carried out at room temperature with a reversed phase column Hypersil Gold 5  $\mu$ m (250×4.6 mm), protected with an Hypersil Gold 5  $\mu$ m drop-in guard (Varian-Superchrom, Milan, Italy). Chromatographic separation was carried out using three eluents [A: water-phosphoric acid 0.3% (v/v), B: methanol; C: acetonitrile] in a linear gradient program shown in Table 1. The flow rate was 0.9 mL/min. The UV detection was carried out at maximum absorption

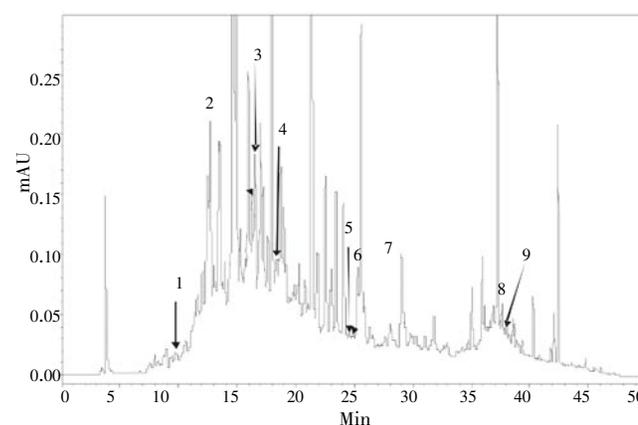
wavelength of single components. A typical chromatogram, registered at 280 nm, is in Figure 1. Qualitative analysis was carried out comparing the chromatographic behavior and the UV spectra of the components with pure standards, under the same chromatographic conditions. Peak purity was established carefully by studying the DAD data of all the peaks of interest. Quantitative analysis was carried out by using linear regression obtained by injecting solution of known content of standards. The linearity response of the detector was verified in the range from 0.002 to 0.400 mg/mL for the identified compounds. Samples were analyzed in triplicate.

**Table 1**

Mobile phase gradient programme for HPLC separation.

Eluent A %	Eluent B %	Eluent C %	Time (min)
100	0	0	0
87	0	13	10
72	9	19	30
12	18	70	40
5	15	80	55
100	0	0	56
100	0	0	60

A: Water phosphoric acid 0.3%, B: Methanol, C: Acetonitrile.



**Figure 1.** HPLC profile at 280 nm of purified extract of *F. sellowiana* Berg fruit var. *coolidge*. Chromatographic conditions: see text.

1: Gallic acid; 2: Pyrocatechol; 3: Catechin; 4: Syringic acid; 5: Ellagic acid; 6: Rutin; 7: Eriocitrin; 8: Eriodictyol; 9: Quercetin.

## 2.4. Biological activity

The *Feijoa* fruit homogenized, centrifuged and purified from mucilage and sugar was used to evaluate the gastroprotective activity.

### 2.4.1. Animals

Adult male Wistar rats, weighing 180–200 g (Harlan Italy), were used. They were kept in standardized conditions [temperature (22±2) °C; humidity (60±4) %; natural lighting], fed with a standard diet (S. Morini Mill rat GPL) and water was provided *ad libitum*. In all experiments rats were divided in groups of ten animals each. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192), as well as with the EEC regulations (O.J. of E.C.L. 358/1 12/18/1986).

### 2.4.2. Antiulcer activity

The rats were divided into six groups of ten animals each, fasted for 18 h and treated by gavage in the morning.

The first group of rats (control) received orally the ulcerogenic agent, EtOH 90% at a dose of 0.5 mL/rat.

The second and third group of rats (curative treatment) received the ulcerogenic agent orally (0.5 mL/rat). After 15 min, one group was treated with *F. sellowiana* fruit var. *coolidge* and the other received *F. sellowiana* fruit var. *gorgiona* (2 g/kg in propylene glycol 0.5 mL/100 g body weight).

The fourth and fifth groups (preventive treatment) received *F. sellowiana* fruit of varieties *coolidge* and *gorgiona* (2 g/kg in propylene glycol 0.5 mL/100 g body weight), respectively. After 1 h from oral administration of the fruit, ethanol (0.5 mL) was administered.

The sixth group of rats (reference drug) received the ulcerogenic agent orally (0.5 mL). After 15 min, the rats were administered with sucralfate (Sucral, Bioprogress), as reference drug, at the dose of 100 mg/kg suspended in propylene glycol (0.5 mL/100 g body weight).

One hour later, all the rats were sacrificed using ether anaesthesia, the stomachs were removed, opened along the great curvature and delicately washed with saline solution, so as not to remove the mucus layer from the mucosal surface.

### 2.4.3. Macroscopic observation

For the macroscopic observations, the number, lengths and severity of ulcers were noted and scored on an arbitrary 0–6 point scale [20]. The ulcer index (UI) of each stomach was the sum of its scores. The ulcer index was reported as arithmetic mean±SE.

### 2.4.4. Light microscopy observation

After the macroscopic observations, the stomachs were extended on a cork surface to avoid deformities. Small pieces of every stomach, were cut and fixed in neutralized 4% (p/v) paraformaldehyde (Immunofix®, Bio-Optica Milano) in phosphate buffer 0.06 mol/L, for 4 h at 4 °C. The samples were washed with phosphate buffer solution (0.2 mol/L) and PBS for 2 h, dehydrated in graded ethanols (30–100°) and, embedded in Bioplast® (Bio-Optica Milano). The stomach serial sections (5 µm thick), obtained by a rotary microtome (Leika 2065 Supercut), were stained with hematoxylin–eosin (HE) for general histology. Another set of sections was stained using the reaction to periodic acid–Schiff (PAS) for glycoprotein histochemistry. PAS reacts with mucopolysaccharides to produce a characteristic carmine colour.

All samples were observed and photographed with an optical microscope Axioshop, Zeiss, equipped with camera Sony® DSC–85.

### 2.5. Statistical analysis

For chromatographic analysis, the results were given as

mean±SD of three determinations.

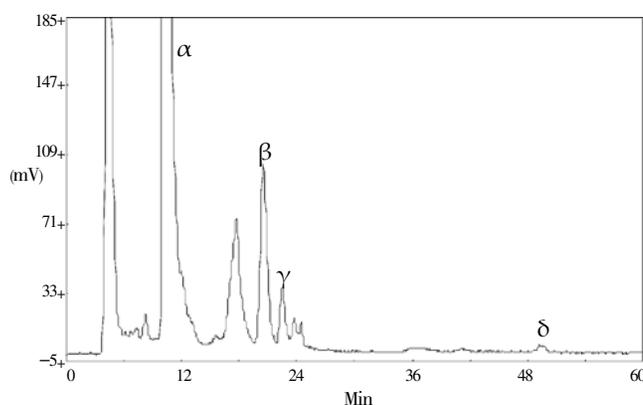
For biological assay, data were expressed as mean±SE of ten determinations. The results were statistically analysed by Student's *t*-test and *P*<0.05 versus control was taken as significant.

## 3. Results

### 3.1. Determination of tocopherol content

In pulp and peel samples of *F. sellowiana* var. *coolidge* and *gorgiona*, the components of vitamin E complex, α, β, γ and δ–tocopherols, were separated and identified through chromatographic analysis.

The total tocopherols content was similar in both fruit varieties, with higher levels in peel than in pulp (Table 2). Relating to different components of vitamin E complex, α–tocopherol was found in similar concentrations in peel samples of both varieties (var. *coolidge*: 73.71 mg/kg and var. *gorgiona*: 74.83 mg/kg), but higher in pulp of var. *coolidge* (24.20 mg/kg) than in var. *gorgiona* (19.54 mg/kg). β–tocopherol was found in higher concentration in peel than in pulp of both varieties; in all samples of pulp β–tocopherol was in greater levels than γ tocopherol. Instead, δ–tocopherol resulted lower than detection limit (Figure 2), except for var. *coolidge* (Table 2).



**Figure 2.** HPLC chromatogram of tocopherols (α, β, γ, δ) presents in pulp samples of *F. sellowiana* Berg. fruit var. *coolidge*. Chromatographic conditions: See text.

**Table 2**

Tocopherols content in pulp and peel samples of *F. sellowiana* var. *coolidge* and *gorgiona* (mean±SD).

Samples	Tocopherols (mg/kg)				Total
	α	β	γ	δ	
<i>F. sellowiana</i> Berg. var. <i>coolidge</i>	pulp 24.2±2.4	2.3±0.2	0.5±0.1	–	27.0
<i>F. sellowiana</i> Berg. var. <i>coolidge</i>	peel 73.7±12.2	11.5±1.7	2.9±0.4	0.9±0.2	89.0
<i>F. sellowiana</i> Berg. var. <i>gorgiona</i>	pulp 19.5±2.0	1.2±0.1	3.7±0.4	–	24.4
<i>F. sellowiana</i> Berg. var. <i>gorgiona</i>	peel 74.8±11.2	8.1±1.6	3.3±0.7	–	86.2

### 3.2. Determination of polyphenol content

Quercetin, ellagic acid, catechin, rutin, eriodictyol, gallic acid, pyrocatechol, syringic acid and eriocitrin were identified in both varieties of *Feijoa* fruit juices (Figure 1).

The qualitative and quantitative composition of the purified extract of *F. sellowiana* var. *coolidge*, collected at different times (Samples A and B), is presented in Table 3. Polyphenolic components not identified in juices were assayed as equivalent amounts of gallic acid. No significant differences in total polyphenol content were observed in the extract of *F. sellowiana* var. *gorgiona* respect to var. *coolidge*.

**Table 3**

Qualitative and quantitative composition of purified extract of *F. sellowiana* Berg. fruit var. *coolidge*, collected in different times (mean±SD).

Polyphenols	Sample A (mg/kg)	Sample B (mg/kg)
ellagic acid	105.2±12.9	219.9±13.9
gallic acid	12.1±2.4	17.8±2.8
syringic acid	21.5±2.7	3.1±2.4
catechin	613.1±17.0	385.3±15.6
eriodictyol	1.1±0.2	11.6±11.6
eriodictin	5.4±1.4	4.1±0.9
pyrocatechol	296.9±12.3	116.5±8.5
quercetin	12.3±1.9	3.7±0.8
rutin	4.7±1.2	15.7±15.7
Total unknown polyphenols*	4774.3±146.5	6064.3±159.5

Sample A: October 2010, Sample B: December 2011. \*: Calculated as equivalent quantity of gallic acid.

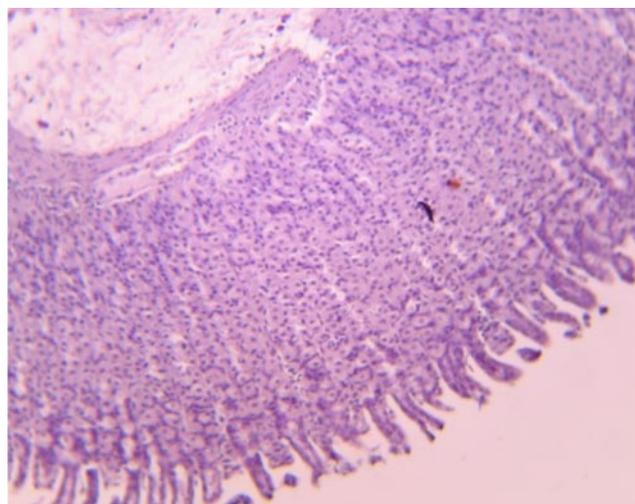
### 3.3. Antiulcer activity

#### 3.3.1. Macroscopic observations of gastric mucosa

In the control rats that received only ethanol, intense and widespread gastric hyperaemia and a thickened lesion were evident and the UI (8.36±1.50) was calculated. The pre-treatment test, with both varieties of *F. sellowiana* fruits, inhibited the mucosal injury caused by ethanol. This protective effect is evident by stomach aspect close to normality, with a significant reduction in gastric hyperaemia, number and severity of the lesions. The UI significantly decreased to 1.07±0.80 for var. *coolidge* and to 1.02±0.50 for var. *gorgiona* ( $P<0.05$ ) with respect to the control (UI 8.36±1.50), reaching the UI value of rats treated with sucralfate (UI 1.10 ± 0.90). The curative treatment with both varieties of fruit showed no activity with UI (8.13±1.10) and UI (7.96±1.20).

#### 3.3.2. Light microscopy observations

The changes of gastric mucosa were observed by light microscopy to confirm the gastroprotective effect exercised by oral administration of both varieties *F. sellowiana* fruits. The gastric mucosa of control rat showed a considerable superficial desquamation, predominantly in the glands apex. There is a marked area in the glandular region of the stomach at the level of gastric folds. Some of the superficial epithelial cells showed signs of necrosis and large portions of gastric pit took part in the erosion process. The glands kept a reticular tubular trend with a large whitening of the interglandular space and a minor hyperemia at the base of glands. In addition, it is possible to observe dilated vessels with large lumens in the submucosa. Staining PAS allowed to appreciate the lack of mucous production on glands surface and neck cells and to study the glandular turn-over (Figure 3).

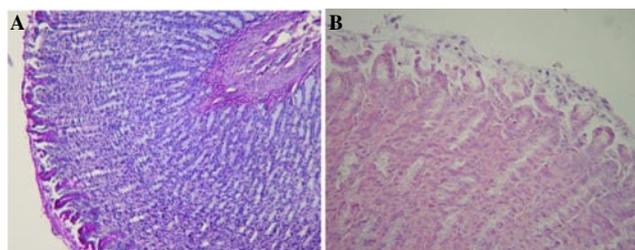


**Figure 3.** Gastric mucosa of ethanol treated rats.

The gastric mucosa shows a considerable superficial desquamation, predominantly in the glands apex. It's evident necrosis of superficial epithelial cells. Staining PAS allows to appreciate the lack of mucous production on glands surface and neck cells. (PAS magnification 20×).

In curative treatment, the glandular lumens appeared moderately dilated. The apex presented cells with reduced dimensions compared to normal cells and with rarefied cytoplasm containing a modest amount of PAS positive material. The interglandular spaces appeared dilated with larger vessels than normal.

The preventive treatment with *Feijoa* fruit significantly inhibited mucosal injury caused by ethanol. Histological examination showed glands with characteristic straight arrangement and the glandular lumens not very dilated. The glandular edges, the surface and neck cells were regularly organized, with PAS-positive substance in cytoplasm and covered with a thin layer of mucous. There was a good appearance of parietal and principal cells. Glandular fundi were normal (Figure 4). The gastric mucosa of rats treated with sucralfate appears intact and showed a layer of mucous in the glandular pits and in the neck cells.



**Figure 4.** Gastric mucosa of *F. sellowiana* var. *coolidge* and var. *gorgiona*.

A: *F. sellowiana* var. *coolidge* fruit treated rats (PAS magnification 20×), B: *F. sellowiana* var. *gorgiona* fruit treated rats (HE magnification 40×). The treatment significantly inhibits mucosal injury caused by ethanol. Histological examination shows glands with characteristic straight arrangement and regular glandular lumens. The glandular edges, the surface and neck cells are regularly organized, with PAS-positive substance in cytoplasm and a thin layer of mucous. Parietal and principal cells are normal. These results are evident both with HE and PAS staining.

## 4. Discussion

This study, within Sicilian Region Research Project “Fruttiferi tropicali e sub-tropicali”, enhances the

cultivation of tropical and subtropical fruit trees, such as feijoa, papaya, mango, in Sicily, as a new economical source and a valid alternative to the local production. Particularly, this project, through phytochemical and pharmacological studies and evaluations of pedoclimatic conditions in Sicily, aims to obtain fruit with a nutritional value comparable to those grown in native countries.

The fruit of both varieties, even if show a low lipid content (INRAN) present a significant levels of tocopherols<sup>[21]</sup>. Due to the low content in lipids and interfering substances for fluorimetric determination, we did not carried out the process of hot saponification<sup>[22–24]</sup>.

From the chromatographic analysis  $\alpha$ ,  $\beta$  and  $\gamma$  tocopherols were present at similar levels in both varieties, while  $\delta$ -tocopherol was found only in var. *coolidge* peel. The peel presented a higher content of  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  tocopherols than pulp, but it is known that vitamins are present in peel in most fruit. The tocopherol content in peel admit to take into consideration a possibility recover of vitamin E from waste matter.

*Feijoa* fruit of both varieties *coolidge* and *gorgiona*, collected in the same production site, at different times, showed by chromatographic analysis a consistent polyphenolic content, characterized by ellagic, gallic and syringic acid, catechin, eriodictyol, eriocitrin, pirocatechol, rutin and quercetin. The results obtained show that the compounds present in the fruit in higher concentration are always catechin, ellagic acid and pyrocatechol, even if the quantitative ratios have a different trend. In both varieties, polyphenol components that could not be identified were present in considerable quantities. The pedoclimatic conditions play a significant role to plant development and could influence the bioavailability of intermediate metabolites in biosynthesis of active principles. The observed variations can depend on various factors, particularly environmental conditions and harvest period. The environmental factors, as temperature, relative humidity, daylight duration, exert a direct influence on active principles contained in the fruits. The ripeness time and the collection period, instead, are correlated to biosynthesis of substances responsible of organoleptic characters and biological activities.

Relating to the biological activities, *F. sellowiana* fruit possesses a protective effect against the ulcerogenic action of ethanol, responsible for disturbances in gastric secretion, gastric mucosal damage, permeability alterations, gastric mucus depletion and free-radical production<sup>[25]</sup>. The gastric ulceration induced by oral administration of ethanol to rats involves other mechanisms in addition to the increase of oxidative stress and ROS formation. So ethanol also depletes PG concentration, due to its necrotizing action on the gastric mucosa, increased vascular permeability and decreased gastric mucosa circulation. The necrotizing action of ethanol decreases gastric mucus secretion<sup>[26]</sup>.

In ethanol treated rats (controls) histological findings showed marked mucosal damage including hyperaemia, sub-mucosal oedema and severe congestion of vessel. The pre-treatment with *F. sellowiana* fruit of both varieties inhibited the formation of gastric lesions. The severity of ulcers is

reflected on ulcer score, with values significantly lower in the pre-treated rats respect to the controls.

The gastro-protective effects of *Feijoa* could be linked to the presence in the fruit of polyphenols (ellagic, gallic and syringic acid, catechin, eriodictyol, eriocitrin, pirocatechol, rutin and quercetin), vitamin C, vitamin E and other nutraceutical substances, able to decrease oxidative stress and to increase antioxidant enzyme activity. The presence of a considerable amount of polyphenols fully justifies the already known antioxidant activity of the *Feijoa* fruits, just reported in our previous study<sup>[12]</sup>. Polyphenolic compounds can protect gastrointestinal mucosa with various mechanisms and exert a preventive action on modification of biochemical parameters and on morphological structure<sup>[27,28]</sup>. Literature data report the gastro-protective effect of quercetin by inhibition of lipid peroxidation, and consequent reduction of malonylaldehyde content, an indicator of lipid peroxidation. Sisodia *et al.*<sup>[29]</sup> showed that rutin possess significant antiulcer effect, producing in albino rats a significant reduction in volume of gastric secretion, of free and total acidity and a increase in total carbohydrate/total protein ratio. This flavonoid causes inhibitory effect on the release of gastric hydrochloric acid, protecting the gastric mucosa. It is quite likely that the antioxidant property plays a role in these effects.

Ellagic acid, a plant-derived polyphenol with high antioxidant power, is known for its anti-inflammatory, antiulcer and gastroprotective effects, but its mechanisms of action have not been fully elucidated<sup>[30]</sup>. Studies suggested that some plant drug, which contain ellagic acid and gallic acid, as *Quercus infectoria* galls, exhibited anti-ulcer activity<sup>[31]</sup>. Ellagic acid exerts its antiulcer activity by strengthening the defensive factors and attenuating the offensive factors<sup>[32]</sup>.

Also flavonoids present specific gastro-protective effects. It is known that flavones and catechins are the most powerful flavonoids with protective effects against reactive oxygen species and their biological activities are correlated to their antioxidant effects. Among flavanones present in *Feijoa*, the eriodictyol and its glycoside eriocitrin, after hepatic metabolization to eriodictyol, methylated eriodictyol, 3,4-dihydroxyhydrocinnamic acid and their conjugates, are able to reduce the lipidic peroxidation<sup>[33]</sup>, showing the highest antioxidant activity<sup>[34]</sup>. Literature data report, in addition, that flavonoids with least four hydroxyl groups, in particular of catechol-type as eriodictyol and its derivatives, can inhibit gastric  $H^+$  and  $K^+$  ATPase, with a gastroprotective effect, on acid secretion<sup>[35]</sup>. Catechins and tannins are able to protect gastric mucosa by inhibition of histidine decarboxylase and decreased synthesis of histamine<sup>[36]</sup>. Therefore, the mucus accumulation, observed in gastric mucosa of the rats treated with the fruit, could be related to a possible reaction between tannins and mucopolysaccharides<sup>[37]</sup>. Also the oral administration of vitamin E and vitamin C has been shown to attenuate ethanol- and aspirin-induced gastric damage with an effective gastroprotective action<sup>[38–40]</sup>. Particularly, the protective effect of vitamin C involves the reduction of lipid peroxidation in gastric mucosa, stimulates the expression of

antioxidant and vasodilator heme–oxygenase enzyme in the gastric epithelium and inhibits the expression of inducible oxide–synthase enzyme<sup>[41]</sup>.

As regard vitamin E, the gastro–protective effects and antiulcer properties against the damage induced by ethanol, are correlated to its antioxidant effect with reduction of lipid peroxydation and enhanced activity of endogenous antioxidant enzymes, like superoxide dismutase, catalase, and glutathione peroxidase<sup>[40,42]</sup>.

The gastro–protective effect exercised by *Feijoa* fruit depends not only on the removal of damaging factors, but probably also on an increased mucosal barrier defence. According to histological findings, in the preventive treatment, the increase of the mucus layer on the gastric mucosa of rats was confirmed by PAS stained. Actually, PAS reacts with mucopolysaccharides and produces a more rich characteristic carmine colour respect to control rats.

The results admit to sustain a greater consumption of *Feijoa* fruits in human diet, for the high content of polyphenols and tocopherols, with significant antioxidant and antiulcer activities. In addition, the amount of all components of vitamin E complex, found in edible portion of *Feijoa* fruit of both varieties, confirm the nutritional quality of this fruit, produced in pedoclimatic condition very different from origin area. Therefore, it's possible sustain the cultivation of *Feijoa* from Sicily and the consumption of its fruit.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### Comments

#### Background

Gastric disorders are very common and could be prevented by proper feeding. Tropical fruits, containing nutraceutical antioxidants, may be useful in the prevention of these diseases.

#### Research frontiers

In the present manuscript the fruits of the tropical plant *F. sellowiana* grown in Sicily were studied revealing the presence of substances with antioxidant activity and

gastroprotective effects on ethanol–induced ulcer.

#### Related reports

Ethanol may cause gastric lesions by means of free radical production, depletion of gastric mucus, permeability alterations, increase of vascular permeability. The nutraceutical substances, decreasing oxidative stress, may show gastroprotective effect.

#### Innovations and breakthroughs

The cultivation of tropical plants in Sicily can be increased, due to the favorable climate, moisture, and soil salinity. The fruits grown in Sicily may have the same nutritional value as those from the areas of origin.

#### Applications

*Feijoa* fruit, in addition to its nutrition significance, can play a role in preventing widespread diseases, such as gastritis and ulcers. Also *Feijoa* fruits or their waste is a source of substances with high added value.

#### Peer review

This is a valuable work in which authors demonstrated the gastro–protective effect exerted by *F. sellowiana* fruit. The effect was assessed on ethanol–induced ulcers in rats, through UI and histopathological observations. This effect seems to depend not only on the removal of damaging factors, but probably also on an increased mucosal barrier defence. The authors attributed the gastroprotective activity to the presence of antioxidant substances in the fruit, as polyphenols and tocopherols. *F. sellowiana* fruits of both varieties, var. *coolidge* and var. *gorgiona*, were studied and according to the reported results no significant differences were observed between them.

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