

BIOLOGICAL ACTIVITY OF FEJJOA PEEL EXTRACTS

Hideki NAKASHIMA

Abstract

Fractions of Feijoa peel extracts were studied for anti-tumor activity, 50% cell cytotoxicity (CC_{50}), anti-human immunodeficiency virus activity and anti-bacterial activity. Two active fractions [A3] of acetone extract and [M2] of MeOH extract had potent inhibitory activity against Gram-positive and Gram-negative bacteria as well as fungi tested. These results indicate the therapeutic value of Feijoa peel extracts as potential anti-tumor and anti-microbial organism agents.

Key words: anti-bacterial, anti-HIV, anti-tumor, Feijoa extracts

Introduction

Feijoa sellowiana Berg (Myrtaceae) is mainly cultured in tropical and subtropical countries such as southern Brazil, Uruguay, Paraguay and northern Argentina. Feijoa has a pleasant flavor,



Fig. 1. Pictures of Feijoa. A; Feijoa leaves, B; Feijoa fruit in the tree, C; Feijoa flower, D; Feijoa fruit.

it is also eaten as stew, jam, jelly or juice. Feijoa contains many medicinally bioactive compounds (Figure 1).

The predominant aroma of Feijoa fruits is ascribed to methylbenzoate and ethylbenzoate (HARDY & MICHAEL 1970; HERRMANN 1994). Other volatile components have also been identified (PINO 1997; BINDER & FLATH 1989; Di CESARE et al. 1998; STARODUBTSEVA & KHAREBAVA 1978; STARODUBTSEVA & KHAREBAVA 1986). Feijoa is known to contain high amounts of vitamin P (P)-active polyphenols, such as catechin, leucoanthocyanins, flavonols, proanthocyanidins, and naphthoquinones (BABA-ZADE 1972; KRIVENTSOV & KARAKHANOVA 1972b; GERSHTEIN & GABISONIYA 1972; VANIDZE 1991). Leaves of Feijoa also contain catechins, such as (+)-catechin, (-)-epicatechin, (+)-gallocatechin and (-)-epigallocatechin (VANIDZE et al. 1991). Further, tannins in Feijoa fruits and leaves have been identified (FOO & PORTER 1981). The flower part of Feijoa contains anthocyanin-3-glucoside of polyphenols (LOWRY 1976). Moreover, Feijoa fruits are rich in vitamin C (KRIVENTSOV & KARAKHANOVA 1972a; MGALOBILISHVILI & LOLOSIDI 1971; BABA-ZADE 1972; GERSHTEIN & GABISONIYA 1972; SALVO et al. 1987). Vitamine C in concentrated Feijoa juice can be up to three times higher than that in fresh juice (Di CESARE et al. 1998). Provitamin A, such as α -carotene, β -carotene and β -cryptoxanthin, has also been determined (HOMNAVA et al. 1990). Both water-soluble and water-insoluble fibers are found in Feijoa (SALVO et al. 1987; LINTAS & CAPPELLONI 1992). Amino acids such as tryptophan, lysine, methionine and asparagine with nutritional value are also found in Feijoa extracts (TSIKORIDZE et al. 1986; SALVO et al. 1987).

Feijoa peel contains more than twice as much vitamin C and catechin's vitamin P (P)-active polyphenols, such as leucoanthocyanins, flavonols and naphthoquinones (KRIVENTSOV & KARAKHANOVA 1972a). Although the chemical constituents of Feijoa fruit have been reported, pharmaceutical studies of constituents of the peel have barely been carried out. Feijoa fruit peel is generally wasted. However, the peel is rich in biologically and nutritiously interesting compounds. With this consideration in mind, we have screened for new biologically active agents various fruits and vegetables that are consumed by many people, including Feijoa. The purpose of this study is to investigate anti-tumor activity, 50% cell cytotoxicity (CC₅₀), anti-human immunodeficiency virus (HIV) activity and anti-bacterial activity of fractions of Feijoa peel extracts.

Materials and Methods

Preparation of Feijoa peel extracts.

Feijoa peel (330 g) was cut into small pieces and successively extracted with hexane, acetone, MeOH and 70% MeOH at room temperature. The solvent was concentrated *in vacuo*, and the hexane extract [H 0](0.44 g), acetone extract [A0](5.9 g), MeOH extract [M 0](16.7 g) and 70% MeOH extract [70M0](6.1 g) were obtained, respectively. First, the aliquot of hexane extract [H0](0.4 g) was subjected to silica gel column chromatography, which was then eluted with a hexane-acetone gradient. The hexane fraction [H 1](32 mg), hexane-acetone (9:1) fraction [H 2](157 mg), hexane-acetone (4:1) fraction [H 3](47 mg) and [H 4](45 mg) were eluted stepwise. Second, the acetone extract [A 0](5.5 g) was subjected to silica gel column chromatography, which was then eluted with a benzene-EtOAc gradient. The benzene fraction [A1](92 mg), benzene-AcOEt (10:1) fraction [A2] (103 mg), [A3](145 mg), benzene-AcOEt (1:1) fraction [A4](170 mg), AcOEt fraction [A 5](582 mg), AcOEt-EtOH (5:1) fraction [A6](836 mg) and [A7](854 mg) were eluted stepwise. Third, the MeOH extract [E 0](16.7 g) was subjected to

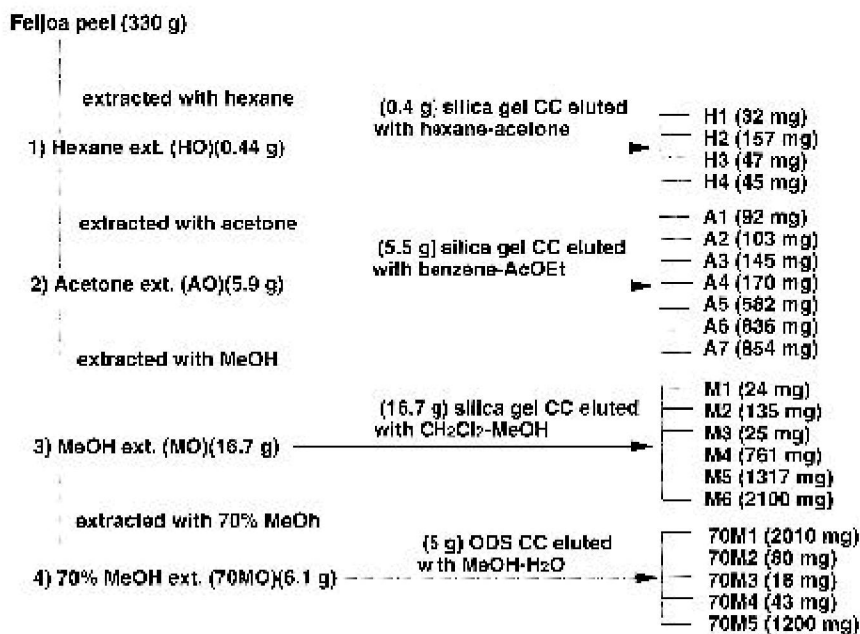


Fig. 2. Fractional separation of Feijoa peel extracts. CC: column chromatography.

silica gel column chromatography, which was then eluted with a CH₂Cl₂-MeOH gradient. The CH₂Cl₂ fraction [M 1](24 mg), CH₂Cl₂-MeOH (50:1) fraction [M 2](135 mg), CH₂Cl₂-MeOH (9:1) fraction [M 3](25 mg), [M 4](761 mg), CH₂Cl₂-MeOH (4:1) fraction [M 5](1317 mg), and [M 6](2100 mg) were eluted stepwise. Finally, the 70% MeOH extract [70M0](5 g) was subjected ODS column chromatography, which was then eluted with a H₂O-MeOH gradient. The H₂O-MeOH (2:1) fraction [70 M 1] (2010 mg), [70M2](80 mg), H₂O-MeOH (1 : 1) fraction [70 M 3](18 mg), [70 M4] (43 mg), and MeOH fraction [70 M 5](1200 mg) were eluted stepwise (Figure 2).

Assay for anti-tumor and cytotoxic activity.

Human oral squamous cell carcinoma cells (HSC-2), human oral salivary tumor cells (HSG) and human oral gingival fibroblasts (HGF) (5-7 population doubling levels) were cultured in Dulbecco's modified Eagle's minimum essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). These cells were incubated for 24 h with the indicated concentrations of test compounds, and the viable cell number was then determined by 3-(4,4-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. In brief, cells were washed with phosphate buffered saline (PBS), and incubated for 4 h with a fresh culture medium containing 0.2 mg/ml MTT. After removing the medium, cells were lysed with 100 μ l DMSO and the absorbance at 540 nm of the cell lysate was measured with Labsystems Multiskan^R (Biochromatic) with Star/DOT Matrix printer JL-10. The CC₅₀ was determined from the dose-response curve.

Anti-HIV assay

The suppression of HIV-induced cytopathic effects by Feijoa extracts was studied. Human T cell leukemia virus 1 (HTLV-1)-bearing CD4 positive human T cell lines, MT-4 cells, were infected with HIV-1_{MB} at a multiplicity of infection (m.o.i.) of 0.01. HIV-infected or mock-infected MT-4 cells (1.5×10^5 /ml, 200 μ l/well) were placed into 96-well microtiter plates in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) and incubated in the presence of varying concentrations of test compounds. After incubation for 5 days at 37°C in a CO₂ incubator, cell viability was quantified by a colorimetric assay (at 540 and 690 nm), monitoring the ability of viable cells to reduce MTT to a blue formazan product (NAKASHIMA et al. 1992b). All data represent the mean values of triplicate measurements.

Anti-bacterial assay.

Escherichia coli LE 140 F⁺lac, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Candida albicans*, and *Candida glabrata* were isolated from clinical specimens obtained from the Department of Clinical Microbiology, Medical University of Szeged, Hungary. *Helicobacter pylori* (ATCC43504) was purchased from the American Type Culture Collection (Rockville, MD).

The anti-bacterial experiments were made by adding 10 μ l of original solutions as a droplet on a minimal medium supplemented with 1% trypton and 0.5% yeast extract (MTE) broth and blood agar plates incubated with 10^5 cells of the tested strains. The plates were incubated at 37°C for 24 h, then the inhibitory zones were measured. As a control 10 μ l of DMSO was examined on each strain. It was found that *C. albicans*, *C. glabrata* and *P. aeruginosa* were sensitive to DMSO. The growth of *E. coli* and *S. epidermidis* was not suppressed by DMSO. Ampicillin disc was used as positive control in the studies of antibacterial effects.

The micro-dilution broth method was used to determine the minimum inhibitory concentration (MIC) against *H. pylori* growth. Mueller-Hilton broth containing 5% FBS was used as the medium, and was cultured in a jar conditioned with Campylo Pack (Dia Iatron) for 48 h. Briefly, *H. pylori* strains were inoculated on a Brucella agar plate containing 10% horse serum, and cultured at 37°C for 48 h. The collected bacterial colonies were diluted to a 10^7 colony forming unit (UFC)/ml with 0.9% saline. The fractions were dissolved in DMSO, and then diluted with Mueller-Hilton broth. To the solution of the fractions, a suspension solution of the bacteria was added to make 10^6 CFU/100ml/well. The mixture was incubated at 37°C for 48 h. The MIC values of tested fractions were determined by observation of colony formation.

Results and Discussion

Anti-tumor and cytotoxic activity.

It is widely accepted that, in tumor cells, agents act as apoptosis-inducers and an important reason for nonresponsiveness is the insufficiency of these drugs to trigger apoptosis (HICKMAN 1992). The cytotoxicity of all fractions was evaluated in two human oral tumor cell lines (HSG-2, HSG) and one human gingival fibroblasts (HGF), using the microculture tetrazolium (MTT) assay. Crude hexane [H0], acetone [A0], MeOH [M0] and 70% MeOH [70M0] extracts showed weak cytotoxic activity against these cells. However, after separation by column chromatography (Figure 2), cytotoxic activities became detectable in several fractions (Table 1). Most fractions exhibited only low cytotoxicity (IC₅₀ values >100 μ g/ml). However, fraction [A3] displayed significant potency and was relatively cytotoxic to two tumor cell lines (HSC-2 and HSG) and the

Table 1. Activity of anti-cancer, anti-HIV and anti-bacteria of Feijoa peel extracts

| Fraction | Cytotoxic activity (CC ₅₀ µg/ml) | | | Anti-HIV activity | | | Anti-bacterial activity (MIC ₅₀ , µg/ml) | | | | | | |
|-----------------|---|------|------|-------------------|-----------------------------|-----------------------------|---|--|----------------|-----------------------------------|-----------------------------|-----------------------------|--------------------------------|
| | HSC-2 | HSG | HGF | SI (HGF/HSC-2) | CC ₅₀ (µg/ml) | EC ₅₀ (µg/ml) | SI (CC ₅₀ /EC ₅₀) | <i>Staph. lococcus epidermidis</i> | <i>E. coli</i> | <i>Pseudomonas aeruginosa</i> | <i>Candida albicans</i> | <i>Candida glabrata</i> | <i>Helicobacter pylori</i> |
| H0 | 381 | 386 | 395 | 1.0 | 100 | >200 | <1 | - | - | - | - | - | ND ^b |
| H1 | >500 | >500 | >500 | ><1 | >200 | >200 | ><1 | - | - | - | - | - | >100 |
| H2 | 313 | 357 | 370 | 1.2 | 162 | >200 | <1 | + | + | - | + | - | >100 |
| H3 | >500 | >500 | >500 | ><1 | >200 | >200 | ><1 | - | + | - | - | - | >100 |
| H4 | 269 | 356 | 409 | 1.5 | 117 | >200 | <1 | - | + | - | - | - | >100 |
| A0 | 444 | 462 | 452 | 1.0 | 119 | >200 | <1 | - | - | - | - | - | ND ^b |
| A1 | 160 | 302 | 305 | 1.0 | 121 | >200 | <1 | - | +- | - | ++ | - | >100 |
| A2 | 132 | 123 | 165 | 1.3 | 113 | >200 | <1 | - | ++ | + | ++ | - | >100 |
| A3 | 81 | 79 | 80 | 1.0 | 22 | >40 | <1 | ++ | ++ | + | ++ | + | >100 |
| A4 | 274 | 318 | 382 | 1.4 | 46 | >200 | <1 | -a | - | - | - | - | >100 |
| A5 | 335 | 383 | 403 | 1.2 | 127 | >200 | <1 | - | ++ | - | - | - | >100 |
| A6 | >500 | >500 | >500 | ><1 | >200 | >200 | ><1 | - | - | - | - | - | >100 |
| A7 | >500 | >500 | >500 | ><1 | >200 | >200 | ><1 | - | - | - | - | - | >100 |
| M0 | >500 | >500 | >500 | ><1 | >200 | >200 | ><1 | - | - | - | - | - | ND ^b |
| M1 | 378 | 421 | 441 | 1.2 | >200 | >200 | ><1 | -a | - | + | + | - | >100 |
| M2 | 119 | 101 | 152 | 1.3 | 120 | >200 | <1 | + | ++ | + | ++ | - | >100 |
| M3 | 338 | 268 | 277 | 0.8 | 78 | >200 | <1 | -a | +- | + | + | + | >100 |
| M4 | >500 | 458 | 235 | <0.5 | >200 | >200 | ><1 | - | - | - | - | - | >100 |
| M5 | >500 | >500 | >500 | ><1 | >200 | >200 | ><1 | - | - | - | - | - | >100 |
| M6 | 458 | >500 | >500 | >1.1 | >200 | >200 | ><1 | - | - | - | - | - | >100 |
| 70M0 | 380 | >500 | >500 | >1.3 | >200 | >200 | ><1 | - | + | - | - | - | ND ^b |
| 70M1 | 396 | >500 | >500 | >1.3 | >200 | >200 | ><1 | - | - | - | - | - | >100 |
| 70M2 | 394 | >500 | >500 | >1.3 | 99 | 15 | 7 | -a | + | - | - | - | >100 |
| 70M3 | 250 | >500 | >500 | >2 | 83 | >200 | <1 | -a | + | - | - | - | >100 |
| 70M4 | 208 | 280 | 419 | 2.0 | 102 | >200 | <1 | -a | + | - | - | - | >100 |
| 70M5 | 488 | >500 | >500 | >1.0 | >200 | >200 | ><1 | - | - | - | - | - | >100 |
| Curdlan sulfate | | | | | >1000 | 1.16 | >861 | | | | | | |
| Dextran sulfate | | | | | >1000 | 13.00 | >71 | | | | | | |
| AZT (µM) | | | | | 268 | 0.34 | 787 | | | | | | |
| ddC (µM) | | | | | 2395 | 6.66 | 360 | | | | | | |

++ very potent; + potent, +- slightly potent; - no effect. ^ainhibited haemolysis. ^bNot detected.

Metronidazole, Clarithromycin, and Erythromycin were used as reference anti-bacterial compounds for *H. pylori*, and their MIC₅₀ values were 74 µg/ml, 1.9 µg/ml, and 1.8 µg/ml, respectively.

healthy cell line (HGF). When water solubility was increased, the relative cytotoxic activity against normal cells (SI=HGF/HSC-2) generally declined (Table 1).

Anti-HIV activity.

Table 1 demonstrated that most fractions did not significantly inhibit HIV-induced cyopathic effects on MT-4 cells (selectivity index (SI)=1-7), compared to higher anti-HIV activities of curdian sulfate (CRDS), dextran sulfate (DS), AZT, and ddC (SI=71-861). However, fraction [70M2] showed some anti-HIV activity. The present study demonstrates that fraction [70M2] with relatively high water-solubility has the highest anti-HIV activity (Table 1). This is consistent with our previous finding that anti-HIV activity increases with water-solubility of the compounds: in the order of lignin (NAKASHIMA et al. 1992a) > hydrolyzable tannins > condensed tannins (NAKASHIMA et al. 1992b) > flavonoids (SAKAGAMI et al., unpublished data), and higher molecular weight lignins and tannins (NAKASHIMA et al. 1992b) show higher anti-HIV activity

in comparison with their lower molecular compounds.

Anti-bacterial activity.

Chemotherapy with antibiotics sometimes induces serious side effects, such as diarrhea, nausea, abnormal taste, dyspepsia, abdominal pain/discomfort, headache, and angioedema. Therefore, there is a strong demand for compositions having all of the beneficial anti-bacterial properties with reduced side effects. Antibacterial agents of fruit or vegetable origin may be superior as compared with many antibiotics. All fractions were tested against a panel of microorganisms, including the Gram-positive bacteria, *Staphylococcus epidermidis*, and Gram-negative bacteria, *Escherichia coli*, and *Pseudomonas aeruginosa*, and the fungi, *Candida albicans* and *Candida glabrata* (Table 1). The highest level of activity consistently arose in the acetone and MeOH extract fractions. Two fractions [A3] and [M2] were found to exhibit potent anti-bacterial activity against both Gram-positive and Gram-negative bacteria as well as *Candida*.

Anti-Helicobacter pylori activity.

The current medical consensus suggests that *H. pylori* is the primary causative organism for acute gastritis (BLASER 1992). Since the drug will directly come in contact with the lining of the stomach, edible plants may be a good source of oral antiulcer agents. However, all Feijoa extract fractions did not exhibit potent anti-*H. pylori* activity ($MIC_{50} > 100 \mu\text{g/ml}$), in contrast with three effective positive controls: metronidazole, clarithromycin and erythromycin (Table 1).

In conclusion, it is important to note that fractions [A3] and [M2] showed promising anti-bacterial activity. Although the activities were not strong, some fractions of Feijoa peel extracts showed anti-tumor and anti-HIV activity. The results of this study apparently indicate therapeutic value for Feijoa peel extracts. Further work is required to identify and isolate the compounds responsible for the observed biological activity.

Acknowledgements

The author is grateful for the collaboration of Dr. N. Motohashi (Meiji Pharmaceutical Univ.), Dr. M. Kawase and Dr. Y. Shirataki (Faculty of Pharmaceutical Sciences, Josei Univ.), and Dr. H. Sakagami (Meikai Univ. School of Dentistry).

References

- BABA-ZADE, F. I. 1972. Accumulation of vitamin C in fruits of the feijoa. In: Tr Vses Semin Biol Aktiv (Lech) Veshchestvam Plodov Yagod, 4th. (ed. FRANCHUK, E.P.), 183-186. Vses Nauch-Issled Inst Sadovod, Michurinsk, USSR.
- BINDER, R. G. & FLATH, R. A. 1989. Volatile components of pineapple guava. J Agric Food Chem. 37: 734-736.
- BLASER, M. J. 1992. *Helicobacter pylori*: its role in disease. J. Clin. Infect. Dis. 15: 381-393.
- Di CESARE, L. F., NANI, R., CITRO, D. & PROIETTI, M. 1998. Concentration of aroma compounds and soluble substances in a feijoa juice by cold finger freezing-out. Ind Bevande 27: 126-130 (Chem. Abst. 128: 320839a).
- FOO, L. Y. & PORTER, L. J. 1981. The structure of tannins of some edible fruits. J Sci Food Agric. 32: 711-716.
- GERSHTEIN, L. A. & GABISONIYA, L. D. 1972. Biologically active substances of the fruit of the Feijoa. In: Tr Vses Semin Biol Aktiv (Lech) Veshchestvam Plodov Yagod, 4th. (ed.

- FRANCHUK, E.P.), 187-189. Vses Nauch-Issled Inst Sadovod, Michurinsk, USSR.
- HARDY, P. J. & MICHAEL, B. J. 1970. Volatile components of Feijoa fruits. *Phytochemistry* 9: 1355-1357.
- HERRMANN, K. 1994. The aromatic substances of exotic fruits (part 2): Passion fruit, guava, feijoa. *Fluss Obst*. 61: 398-401.
- HICKMAN J. A. 1992. Apoptosis induced by anticancer drugs. *Cancer Metastasis* 11: 121-139.
- HOMNAVA, A., ROGERS, W. & EITENMILLER, R. R. 1990. Provitamin A activity of specialty fruit marketed in the United States. *J Food Compos Anal*. 3: 119-133.
- KRIVENTSOV, V. I. & KARAKHANOVA, S. V. 1972a. Biologically active substances of several subtropical fruit crops of the Crimea. In: *Tr Vses Semin Biol Aktiv (Lech) Veshchestvam Plodov Yagod*, 4th. (ed. FRANCHUK, E.P.), 82-86. Vses Nauch-Issled Inst Sadovod, Michurinsk, USSR.
- KRIVENTSOV, V.I. & KARAKHANOVA, S. V. 1972b. Changes in biochemically active substances in fruits of subtropical crops mediated by calcium iodide. In: *Tr Vses Semin Biol Aktiv (Lech) Veshchestvam Plodov Yagod*, 4th. (ed. FRANCHUK, E.P.), 267-270. Vses Nauch-Issled Inst Sadovod, Michurinsk, USSR.
- LINTAS, C. & CAPPELLONI, M. 1992. Dietary fiber content of Italian fruit and nuts. *J Food Compos Anal*. 5: 146-151.
- LOWRY, J. 1976. Anthocyanins of the *Melastomataceae*, *Myrtaceae* and some allied families. *Phytochemistry* 15: 513-516.
- MGALOBlishvili, T. S. & LOLOSIDI, S. I. 1971. Biochemical characteristics of the fruits from different forms of feijoa. *Subtrop Kul't* 6: 122-124.
- NAKASHIMA, H., MURAKAMI, T., YAMAMOTO, N., NAOE, T., KAWAZOE, Y., KONNO, K. & SAKAGAMI, H. 1992a. Lignified Materials as Medicinal Resources. V. Anti-HIV (human immunodeficiency virus) activity of some synthetic lignins. *Chem. Pharm. Bull.* 40: 2102-2105.
- NAKASHIMA, H., MURAKAMI, T., YAMAMOTO, N., SAKAGAMI, H., TANUMA, S., HATANO, T., YOSHIDA, T. & OKUDA, T. 1992b. Inhibition of human immunodeficiency viral replication by tannins and related compounds. *Antiviral Res.* 18: 91-103.
- PINO, J.A. 1997. Volatile constituents of tropical fruits. III. Feijoa, sapodilla and cashew apple. *Alimentaria (Madrid)* 286: 41-45 (*Chem. Abst.* 127: 3304474w).
- SALVO, F., TOSCANO, M. A. & DUGO, G. 1987. Chemical composition of *Feijoa sellowiana* fruit. *Riv Soc Ital Sci Aliment.* 16: 471-474.
- STARODUBTSEVA, V. P. & KHAREBAVA, L. G. 1978. Volatile complex of *Feijoa sellowiana*. Part 3. *Subtrop Kul't*. 5: 70-72.
- STARODUBTSEVA, V P. & KHAREBAVA, L. G. 1986. Volatile components of feijoa. Part 4. Composition of the essential oil. *Subtrop Kul't* 5: 118-123.
- TSIKORIDZE, N. G., TKESHELASHVILI, C. F. & KHURTSILAVA, D. I. 1986. Study of the free amino acids of Feijoa fruit. *Subtrop Kul't*. 2: 137-139.
- VANIDZE, M. R. 1991. Quantitative changes in foliar catechins, proanthocyanidins, and flavonols. *Subtrop Kul't*. 3: 80-81.
- VANIDZE, M. R., SHALASHVILI, A. G., CHKHIKVISHVILI, I. D. & KVESITADZE, E. G. 1991. Catechins of feijoa leaves. *Subtrop Kul't*. 6: 87-90.