Kagoshima University Research Center for the Pacific Islands, Occasional Papers No.34, 169-175, 2001
Part 2, Section 2, Report 5. The Progress Report of the 1999 Survey of the Research Project
"Social Homeostasis of Small Islands in an Island-zone"

BIOLOGICAL ACTIVITY OF FEIJOA PEEL EXTRACTS

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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;

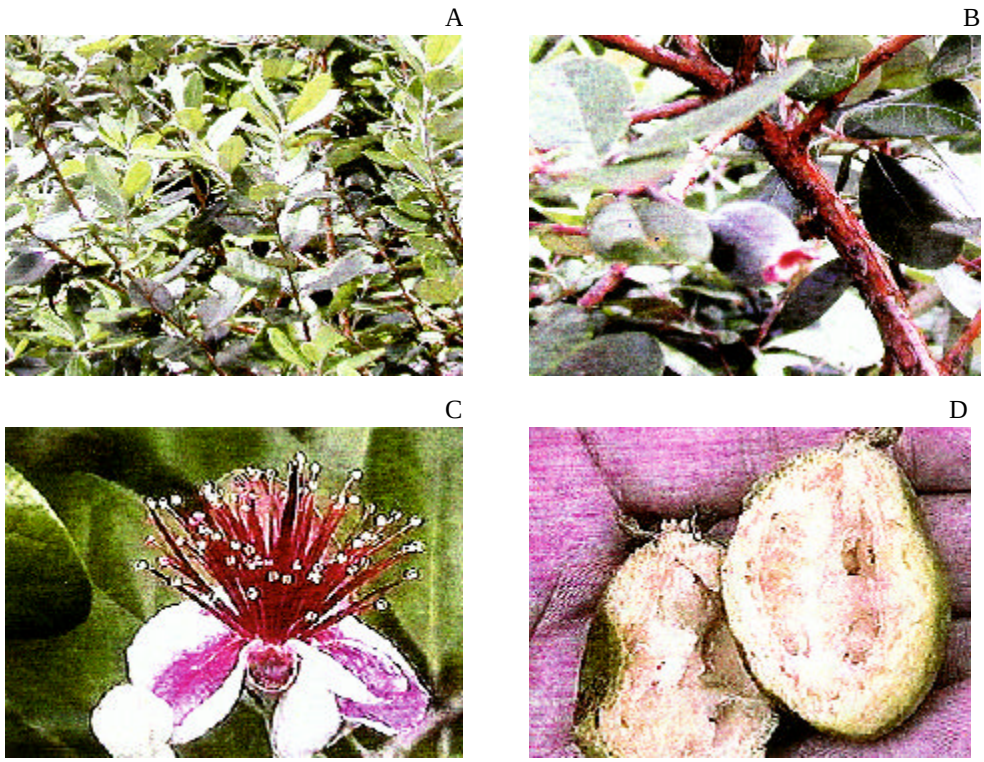

Fig. 1. Pictures of Feijoa. A; Feijoa leaves, B; Feijpa fruit in the tree, C; Feijor flower, D; Feijoa fruit.

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; MGALOBLISHVILI & 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 (CC_{50}), 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-aceton (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

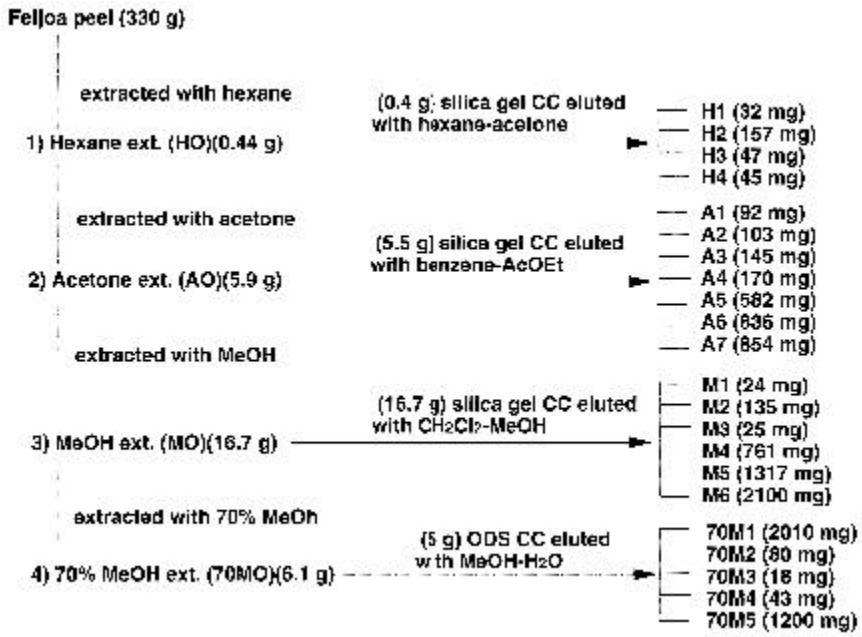

Fig. 2. Fractional separation of Feijoa peel extracts. CC: column chromatography.

silica gel column chromatography, which was then eluted with a CH_2Cl_2-MeOH gradient. The CH_2Cl_2 fraction [M 1](24 mg), CH_2Cl_2-MeOH (50:1) fraction [M 2](135 mg), CH_2C1_2-MeOH (9:1) fraction [M 3](25 mg), [M 4](761 mg), CH_2C1_2-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 H_2O-MeOH gradient. The H_2O-MeOH (2:1) fraction [70 M 1] (2010 mg), [70M2](80 mg), H_2O-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-tetrazonium 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 µl 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 CC_{50} 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_{IIIB} at a multiplicity of infection (m.o.i.) of 0.01. HIV-infected or mock-infected MT-4 cells (1.5 x 10^5/ml, 200 µl/well) were placed into 96-well microtiter plates in RPMI 1640 medium supplemented with 10% heat-inactivted fetal calf serum (FCS) and incubated in the presence of varying concentrations of test compounds. After incubation for 5 days at 37ºC in a CO_2 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 epidermidts*, *Candida albioans*, 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 10µl 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 37ºC for 24 h, then the inhibitory zones were measured. As a control 10µl 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 37ºC 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 37ºC 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 (IC_{50} values >100 µg/ml). However, fraction [A3] displayed significant potency and was relatively cytotoxic to two tumor cell lines (HSC-2 and HSG) and the

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

Fraction	Cytitoxic 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})	*Staph.lococcus epidermidis*	*E.coli*	*Pseudomonas aeruginosa*	*Candia albicans*	*Candida glabrata*	*Helicobacter pylori*
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. [a]inhibited haemolysis. [b]Not detected.
Metronidazole, Clarithromycin, and Eruthromycin were used as reference anti-bacterial compounds for *H.pylori*, and their MIC_{50} 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 curdlan 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-HlV 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-Helicobaoter pylori aotivity.

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 μg/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).

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