Nutraceuticals & Food
Vol. 7, p.215~218 (2002)

Nutraceuticals and Food

Antibacterial Activity of Oleanolic Acid from *Physalis angulata* against Oral Pathogens

– Research Note –

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Abstract

A methanol extract of *Physalis angulata* exhibited *in vitro* antibacterial activity against oral pathogens such including *Streptococcus mutans* and *Porphyromonas gingivalis*. The methanol extract of *Physalis angulata* was further fractionated with ethyl acetate, *n*-butanol and water, in which the ethyl acetate fraction exclusively showed antibacterial activity. An active antibacterial compound from the ethyl acetate fraction was purified to a single compound using silica gel column chromatography and identified as oleanolic acid by ^{13}C-NMR, ^{1}H-NMR and EI-MS. MIC of oleanolic acid against *S. mutans* and *P. gingivalis* were determined to be 50 and 25 μg/mL, respectively. The Antibacterial activity of oleanolic acid from *Physalis angulata* suggested that it has potential as an anticarcinogenic and antiperiodontic ingredients in various foods and oral care products.

Key words: *Physalis angulata*, oleanolic acid, antibacterial activity, *Streptococcus mutans*, *Porphyromonas gingivalis*

INTRODUCTION

Dental plaque plays an important role in the development of caries and periodontal disease, which result in both tooth dysfunction and loss (1). Extensive effort has been made to search out effective antiplaque agents from a variety of chemical and biological compounds for incorporation into dental products, for the mitigation of plaque-mediated diseases (2,3). To date, only chlorhexidine and listerine (a combination of essential oils) have gained the approval of the American Dental Association Council on Dental Therapeutics (4). However, various adverse effects such as teeth staining and increased calculus formation are commonly observed with the currently used chemicals (5). These drawbacks justify further research and development of alternative agents that are safe for the host while retaining antimicrobial specificity and efficacy (6).

Recently, many studies have been conducted to search for antibacterial agents from edible plants, food spices and beverages (7). The spice extracts, cinnamon bark oil, papuamace extract, and clove bud oil were reported to inhibit the growth of many oral bacteria (8). Green tea extract, which is customarily drunk after every meal in Japan, is known to contain several polyphenols that inhibit the growth of *S. mutans* (9). Sanguinarine is an alkaloid extract from the rhizome of the plant *Sanguinaria canadensis* that reportedly possess broad antiseptic action spectrum against a wide variety of oral bacteria (10).

Physalis angulata (Solanaceae) has long held a place in natural medicine in the tropical countries. It is employed for chronic rheumatism, skin diseases and dermatitis, as a sedative and diuretic, for fever and vomiting, and for many types of kidney, liver and gallbladder problems (11). Phytochemical studies on *Physalis angulata* revealed that it contained flavonoids, alkaloids and many different types of plant steroids, some of which were previously unidentified (12,13).

In this study, the active ethyl acetate fraction, obtained from a menthanol (MeOH) extract of *Physalis angulata*, was subjected to silica gel column chromatography and oleanolic acid was isolated as an active compound against oral pathogens. There have been a few previous reports on the antibacterial action of oleanolic acid (14). However, antibacterial activity against oral pathogens have not been reported previously. This study isolated and identified oleanolic acid from *Physalis angulata* and assess its antibacterial activity against oral pathogens.

MATERIALS AND METHODS

Plant materials

Dried *Physalis angulata* L. (Solanaceae) was purchased at a local market in Yokjakarta, Indonesia in Feb. of 1998. A voucher specimen is deposited at 4°C in the Bioproducts Research Center, Yonsei University in Seoul, Korea. All other chemicals used were of reagent grade.

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Bacterial strain and culture conditions

Streptococcus mutans ATCC 25175, *Streptococcus sobrinus* ATCC 27351, *Streptococcus sanguis* ATCC 35105 and *Porphyromonas gingivalis* ATCC 53978 were used for screening and measuring the antibacterial activity of *Physalis angulata* extract. The strains were maintained in BHI agar (Difco) and *P. gingivalis* ATCC 53978 was maintained in anaerobic conditions. Prior to testing, each strains were inoculated into 5 mL of BHI broth (Difco) and incubated at 37°C for 10~14 h.

Antibacterial assay

A test compound was dissolved in DMSO, which was then added to the first tube containing 1 mL of BHI broth and a two-fold serial dilution was performed (15,16). 0.1 mL of bacterial suspension (about 2×10^5 cells/mL) was added to each tube and incubated at 37°C for 24 h. Minimum inhibitory concentration (MIC) was determined by judging visually the bacterial growth in the series of test tubes and minimum bactericidal concentration (MBC) by the concentration required to suppress the survivability of *S. mutans* completely (17).

Isolation of an antibacterial compound

The dried rhizomes (100 g) of *Physalis angulata* was ground and extracted twice with 75% MeOH (400 mL, v/v) for 24 h at room temperature and the extract was concentrated, frozen and lyophilized (6.8 g). The MeOH extract was further fractionated successively with ethyl acetate, *n*-butanol and water. Each fraction was evaporated and dried under reduced pressure (ethyl acetate fraction 1.3 g, butanol fraction 2.9 g, water fraction 1.6 g).

As shown in Fig. 1, the ethyl acetate fraction was further separated using silica gel column chromatography (Merck Kieselgel 60, 70~230 mesh). The sample was mixed with silica gel and loaded on the column packed with silica gel. The sample was eluted with a solution of *n*-hexane, ethyl acetate and methanol (3:5:3, v/v), divided into four fractions (Fr. I~Fr. IV) on a silica TLC plate (Merck, Silica gel 60 F_{254}), and each fraction assayed for antibacterial activity. The active fraction (Fr. I) was further separated in the following manner: Fr. I yielded compound Fr. I-B after passing through a silica gel column eluted with *n*-hexane and ethyl acetate (10:3), Fr. I-B yielded compound Fr. I-B2 when eluted with *n*-hexane and ethyl acetate (11:3) and Fr. I-B2 yielded compound Fr. I-B2-c when eluted with *n*-hexane and ethyl acetate (11:2).

Instrumentation

^{1}H-NMR, and ^{13}C-NMR spectra were measured with a JNM-LA400 ($CDCl_3$, 400 MHz, JEOL, Japan). Mass spectra (EI-MS) were measured with a VG Platform II (FISONS, UK).

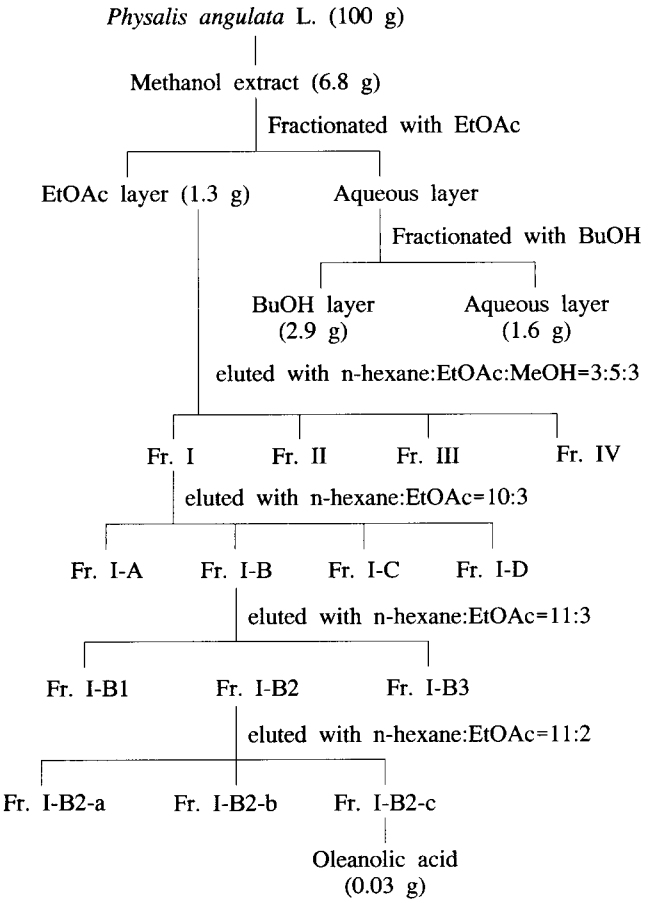

Fig. 1. Isolation of active antibacterial compound from *Physalis angulata*.

RESULTS AND DISCUSSION

Since the antibacterial activity against *S. mutans* was observed in the ethyl acetate fraction, further separation was performed for the ethyl acetate fraction using silica gel column chromatography. The sample was eluted with a solution of *n*-hexane, ethyl acetate and methanol (3:5:3, v/v), divided into four fractions (Fr. I~Fr. IV). Fr. I provided considerable inhibitory activity against *S. mutans*. Fr. I-B fractionated from Fr. I was found to contain three or more compounds with similarities in solubility and polarity besides the active antibacterial component materials. Fr. I-B yielded compound Fr. I-B2 when eluted with *n*-hexane and ethyl acetate (11:3) using a different organic solvent base to differentiate between the compounds and Fr. I-B2 yield the purified compound Fr. I-B2-c eluted with *n*-hexane and ethyl acetate (11:2).

Compound Fr. I-B2-c : colorless powder; IR ($CDCl_3$, ν, max) 3420 (OH), 2975, 2930 (CH), 1680 (COOH), 1445 (CH), 1040, 1020 (C-O) cm^{-1}; EI-MS (*m/z*) 456, 438, 248; ^{13}C-NMR ($CDCl_3$, 400 MHz) : 16.02 (C-25), 16.24 (C-24), 17.87 (C-6), 27.70 (C-11), 25.15 (C-16), 24.30 (C-27), 27.94 (C-2), 28.83 (C-23), 30.40 (C-21), 33.20

(C-7), 37.86 (C-10), 39.19 (C-4), 39.49 (C-8), 40.07 (C-20), 41.78 (C-11), 42.37 (C-14), 47.28 (C-9), 48.41 (C-17), 56.02 (C-5), 76.76 (C-3), 123.39 (C-12), 144.34 (C-13), 178.10 (C-28)

EI-MS of compound Fr. I-B2-c indicated that it has an α-amyrin or a β-amyrin skeleton with one carboxyl group at rings D and E. The ^{1}H-NMR spectrum of compound Fr. I -B2-c exhibited seven angular methyl signals at δ 0.80 (3H), 0.97 (6H), 1.03 (3H), 1.05 (3H), 1.08 (6H), a carbomethoxyl signal at 3.66 (3H), an olefinic proton at 5.33 (1H, brs) and a multiplet centered at 2.42 (2H) assignable to methylene protons neighboring to ketone. The ketone group can only be located at C-3 because of biosynthetic considerations. Careful comparison of several spectral data of compound Fr. I-B2-c including ^{1}H-NMR (Fig. 2) and ^{13}C-NMR (Fig. 3) with those of literature (18) concluded the chemical structure to be oleanolic acid (Fig. 4).

As shown in Table 1, oleanolic acid isolated and identified from the methanol extract of *Physalis angulata* was evaluated for growth inhibitory activity against the oral pathogens: *Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguis* and *Porphyromonas gingivalis*. MIC and MBC of the oleanolic acid against *S. mutans* were determined to be 50 μg/mL and 100 μg/mL, respectively.

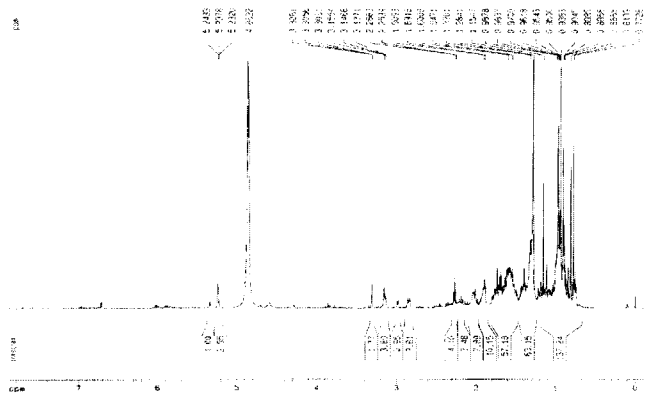

Fig. 2. ^{1}H-NMR spectrum of oleanolic acid.

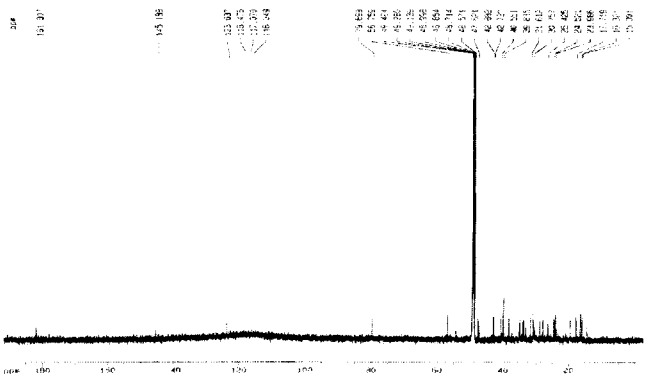

Fig. 3. ^{13}C-NMR spectrum of oleanolic acid.

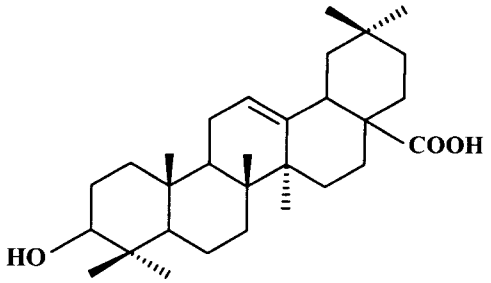

Fig. 4. The chemical structure of oleanolic acid.

Table 1. MIC and MBC of oleanolic acid against oral pathogens

Oral pathogens	MIC (μg/mL)	MBC (μg/mL)
Streptococcus mutans ATCC 25175	50	100
Streptococcus sobrinus ATCC 27351	100	100
Streptococcus sanguis ATCC 35105	100	200
Porphyromonas gingivalis ATCC 53978	25	50

Oleanolic acid also possesses potent antimicrobial activity against other cariogenic oral bacteria, *S. sobrinus* and *S. sanguis*, with MIC values of 100 μg/mL and periodontal pathogen *P. gingivalis* with a MIC value of 25 μg/mL. Antibiotics such as chlorhexidine, penicillin, ampicillin, tetracyline, erythromycin and vancomycin have been reported to effectively prevent dental caries *in vivo* and *in vitro*, but they also result in dearrangements of the oral and intestinal flora and cause undesirable side effects (5).

It was reported that tea polyphenols, consisting of catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, etc, can could effectively inhibit the growth of *S. mutans*, *S. sobrinus* and *P. gingivalis*. Among them, gallocatechin and epigallocatechin were more active than catechin and epicatechin, with reported MIC values of 250 ~ 1000 μg/ mL (9). Hattori et al. (19) studied the antibacterial properties of lignan components and reported that isoeugenol (MIC 200 μg/ mL) exhibited the most pronounced inhibitory effect on the growth of *S. mutans*.

These results suggested that oleanolic acid, with MIC values of 25 and 50 μg/mL of MIC against *P. gingivalis* and *S. mutans*, could be employed as a natural antibacterial agent for preventing dental caries and periodontitis brought about by the growth of *S. mutans* and *P. gingivalis*.

ACKNOWLEDGMENTS

This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the Bioproducts Research Center at Yonsei University.

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(Received January 5, 2002; Accepted April 27, 2002)