Antibacterial Activity of Acanthoic acid Isolated from *Acanthopanax koreanum* against Oral and Skin Microfloras

Jin Kyung Kim*

Vestibulocochlear Research Center & Department of Microbiology, Wonkwang University School of Medicine, Iksan

The (-)-pimara-9 (11), 15-dien-19-oic acid, acanthoic acid was extracted from the roots of *Acanthopanax koreanum* using bioassay-guided isolation of a MeOH extract. Acanthoic acid was assayed against *Streptococcus mutans* and *Staphylococcus epidermidis* causing dental caries and opportunistic pathogen. The minimum inhibitory concentration (MIC) of acanthoic acid against *Streptococcus mutans* and *Staphylococcus epidermidis* was 2 and 4 ㎍/mL, respectively, which was much lower than those of other natural antimicrobial agents such as 8 ㎍/mL of tanshinone IIA. Acanthoic acid also significantly inhibited the growth of other cariogenic bacteria such as *Streptococcus sobrinus* and *Streptococcus sanguis*, and *Streptococcus gordonii* in the MIC range of 4~32 ㎍/mL. Our findings suggest that acanthoic acid could be employed as a potential antibacterial agent for preventing dental caries and skin infections.

Key words : *Acanthopanax koreanum*, Acanthoic acid, Antibacterial Activity

Introduction

Dental plaque plays an important role in the development of dental caries, which results in both tooth dysfunction and loss. Formation of dental caries is caused by the colonization and accumulation of oral microorganism[1]. Dental caries are the most common chronic diseases in the dental fields[2,3]. Streptococci especially *Streptococcus mutans* (*S. mutans*) have been implicated as a primary causative organism of dental caries[4]. *Staphylococcus epidermidis* (*S. epidermidis*) is recognized as normal skin and mucosal microfloras of humans, and opportunistic pathogen in numerous cases of bacteremia, the colonization of prosthetic devices, surgical wounds, urinary tract, cerebrospinal fluid, peritoneal dialysis-related, ophthalmologic and intravascular catherter-related infections[5].

S. mutanscan synthesize firmly-bound glucan from sucrose by the cooperative action of glucosyltransferase (GTF) [6]. Many attempts have been made to eliminate *S. mutans* from the oral flora. Previous reports have shown that the antibiotics such as chlorhexidine, penicillin, tetracycline and erythromycin are effective in inhibiting *S. mutans* for the prevention of dental caries[7], but they are not used in dental clinics due to the development of antibiotic resistance for long-term use [8]. These problems necessitate further search for natural antimicrobial agents that are safe for humans and specific for oral pathogens.

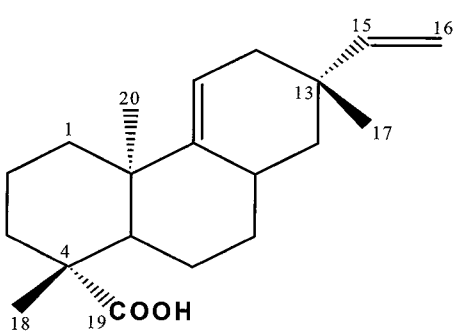

Fig. 1. The chemical structure of acanthoic acid was isolated from the roots of *Acanthopanax koreanum*.

Acanthopanax koreanum Nakai (*Araliaceae*) is a native plant that grows on Jeju Island, located in the south of Korea. Even the roots and stem barks of *A. koreanum* have been widely used in traditional Korea medicine to treat rheumatism, diabetes and hepatitis[9]. Several studies have revealed that acanthoic acid inhibited the production of the pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, in activated human monocytes, macrophases, and mononuclear cells[10,11] and that acanthoic acid was able to inhibit IL-8 production via MAPKs and NF-kB in TNF-α stimulated human intestinal epithelial cell line[12]. Recently, diterpenoids with inhibitory activity against NFAT transcription factor from *A.*

* To whom correspondence should be addressed at : Jin Kyung Kim Vestibulocochlear Research Center & Department of Microbiology, School of Medicine, Wonkwang University, Iksan 570-749, Korea

· E-mail : kommando@hanmail.net, · Tel : 063-852-0220

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koreanum[13]. However, little is known about the antimicrobial effects of *A. koreanum* on oral bacteria. In the course of our screening project of the antibacterial activities of some natural products, it was recently found that $CHCl_3$-soluble extract of stem of *A. koreanum* had antibacterial activity against *S. mutans*. In this study, an active compound (acanthoic acid, Fig 1) against *S. mutans* and *S. epidermidis* was isolated from A. koreanum and its effectiveness was investigated in comparison with other well-know commercial antibacterial agents.

Materials and Methods

1. General

Melting point was determined using an Electrothermal 9100 and are uncorrected. Optical rotation was measured on a Bellinghamand Stanley ADP 2000 polarimeter. NMR spectrum was recorded in $CDCl_3$ on a Bruker AVANCE 300 and 500 MHz spectrometers. ESI mass spectra was obtained on a Macro Mass Quatro LC with electro spray ionization method. Column chromatography was performed using silicagel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck). TLC was performed using Kieselgel 60 F254 (Merck) pre-coated plates and spots were visualized by spraying with vanillin-sulfuric acid spray and followed by heating.

2. Plant material

A. koreanum was collected in the Medicinal Herbs Shop, Jeonbuk, Korea, in November 2004. A voucher specimen (WH 32) has been deposited at the Department of Microbiology, Wonkwang University School of Medicine

3. Extraction and isolation of test material

The extraction and isolation of active principles from the roots of A. koreanum were conducted according to previous report[12]. In brief, the root of A. koreanum was extracted with MeOH and partitioned with $CHCl_3$, EtOAc and n-BuOH. The active $CHCl_3$-soluble extract from *A. koreanum* yielded compound as the active principles after chromatography in silica gel. The structure of the compound was determined by its physico-chemical and spectral data, which are in agreement with those reported in literature[14]. Copies of the original spectra are obtainable from the corresponding author.

4. Microbial strains

The microorganism used for bioassay-guided fractionation of antimicrobial compound was *S. mutans* ATCC 25715, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 21059, *Streptococcus mutans* ATCC 25175, *Streptococcus sanguinis* ATCC 10556, *Streptococcus sobrinus* ATCC 27607 and *Streptococcus ratti* KCTC 3294 was determined by the broth dilution method. The culture was grown in tryptic soy broth (TSB) (Mikrobiologie; Merck, Germany) at 37°C in the presence of 5 % CO_2.

5. Determination of minimum inhibitory concentration (MIC)

Acanthoic acid and other antibacterial agents, dissolved in 1% dimethyl sulfoxide (DMSO), were added to the first tube containing 1 mL TSB and serially diluted by the two-fold method, resulting in a range 1-1000 $\mu g/mL$[15]. A bacterial suspension (0.1 mL) containing 2×10^5 colony forming units (CFU) was added to each tube and incubated for 24 h at 37°C. MIC values were taken as the lowest concentration of test samples that resulted in a complete inhibition of visible growth in the series of test tubes. The control included an inoculated growth medium without the test compounds, and some commonly available antibacterial agents used for caries control were employed as positive controls. All experiments were performed in triplicate.

Results and Discussion

1. Isolation and identification of an active antibacterial compound

To isolate and identify the active compound, the chloroform fraction of the stems of A. koreanum exhibiting strong antibacterial activity against *S. mutans* and *S. epidermidis* was further separated using a silica gel column chromatography, and the purification procedures of the active compound were monitored by MIC and MBC in comparison with some commercially available antibacterial agents. Compound, prepared as described in methodology, had the molecular weigh 302 from the negative ESIMS spectrum (m/z, 301, [M-H+]). In conjugation with the analysis of the 1H-NMR (500 MHz, $CDCl_3$), 13C-NMR (125 MHz, $CDCl_3$), DEPT, COSY, HMQC and HMBC spectrum, its molecular formula of a compound was deduced to be one of the compound in the diterpenic acid series isolated from the stem of certain species of *Acanthopanax* plants. Comprehensive analysis of the NMR data literature[14] indicated that the chemical structure in question was consistent with (-)-pimara-9 (11), 15-dien-19-oic acid, acanthoic acid isolated from the stem of A. koreanum.

2. Antibacterial activity of acanthoic acid

Antibacterial activity of acanthoic acid was investigated in terms of MIC in comparison with some commercially available agents being used for the caries control. As shown in Table 1, the MIC of acanthoic acid for S. mutans was 2.0 μg /mL. Its MIC is much lower than those of other natural

antibacterial agents, such as tanshinon IIA (8 μg/mL) thymol and eucalyptol (500 μg/mL). It is also interesting to note in Table 1 that acanthoic acid (2 μg/mL) has a significantly lower MIC than tanshinon IIA (8 μg/mL), which is a natural compound isolated from *Salvia miltiorrhiza* Bunge. This indicates that acanthoic acid has much stronger antibacterial activity than other natural commercial agents. The activity was almost comparable with ampicillin and gentamicin. However, antibiotics are very limited in extended applications in oral care as they may product detrimental side effects such as discolouration of teeth, reduction of the immune defence, disruption of normal ecology of plaque, diarrhea and vomiting [16].

Table 1. Comparison of MIC between acanthoic acid and other antibacterial compounds against *S. mutans*

Compounds	MIC (μg/mL)
Acanthoic acid	2
Gentamicin	8
Thymol	500
Eucalyptol	500
Tanshinon IIA	8

As demonstrated in Table 2, acanthoic acid also exhibited preferential antimicrobial spectrum against other oral and skin bacteria. The MIC values was 4 μg/mL for *S. epidermidis*, 16 μg /mL of *S. pyogenes*, 4 μg/mL of *S. sanguinis*, 4 μg/mL of *S. sobrinus*, 16 μg/mL of *S. ratti*, 16 μg/mL of *S. anginosus* and 32 μg/mL of *S. gordonii*.

Table 2. MIC of acanthoic acid against oral microoragnisms

Microoragnisms	MIC (μg/mL)
Streptococcus mutans ATCC 25175	2
Streptococcus sobrinus ATCC 27607	8
Streptococcus ratti KCTC 3294	16
Streptococcus sanguinis ATCC 10556	8
Streptococcus anginosus ATCC 31412	16
Streptococcus gordonii ATCC 10558	32
Streptococcus pyogenes ATCC 21059	16
Streptococcus epidermidis ATCC 12228	4

In this study, acanthoic acid exhibited specific activity against the oral and skin bacteria *S. mutans*, *S. epidermidis*. In particular, acanthoic acid showed high activity suggest that acanthoic acid could be employed as a natural anticariogenic agent for preventing dental caries and skin infection caused by the growth of *Streptococcus* spp and *S. epidermidis*. Further studies are necessary for elucidating the antibacterial mechanism of acanthoic acid at the molecular level.

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