Phytopharmacology

Inforesights Publishing

Antibacterial constituents from *Uncaria tomentosa*

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Received: 7 January 2011, **Revised**: 26 January 2011, **Accepted**: 27 January 2011

Abstract

traditionally used for treatment of several diseases including its theoretic use in microbial We have isolated three flavonoids (Artochamin C, 5'-Hydroxycudraflavone A and Dihydrocudraflavone B) based on bioactivity guided isolation. All compounds showed significant antibacterial activities against *Escherchia coli, Staphylococcus aureus*, *Klebsiella pneumoniae* and *Bacillus subtilis.* Artochamin C (**1**) was the most active among all compounds with MIC values ranging from 4.1 µg/mL to 6.7 µg/mL. Other compounds also exhibited considerable antibacterial activities. Neomycin was used as a positive control. Preliminary results of isolated compounds have justified ethnopharmacological use of *Uncaria tomentosa* in microbial infections.

Keywords: *Uncaria tomentosa*; Artochamin C; 5'-Hydroxycudraflavone A; Dihydrocudraflavone B; antibacterial; flavonoids

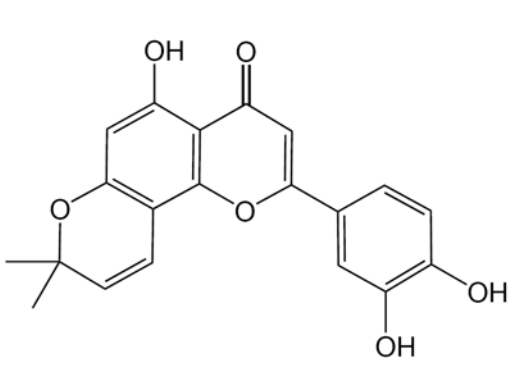
Introduction

Newly emerging resistance to microbial infections is a hard challenge for global pharmaceutical industry. New and effective therapeutic agents are necessary to strive for better health of mankind. Antimicrobial agents from natural products are one the major sources to discover potential antimicrobial agents. *Uncaria tomentosa* DC (Rubiaceae), is generally called as Cats claw, is a medicinal plant found natively in Peruvian Amazon. Traditional uses by Ashaninka Indians includes its use to treat disorders like arthritis, infections, heart disease, cancer, and other inflammatory diseases (Heitzman et al., 2005; Cheng et al., 2007). Woody vines are typically prepared in a ground tea-like preparation and served as a hot water concoction (Pilarski et al., 2007). Phytochemical studies have been

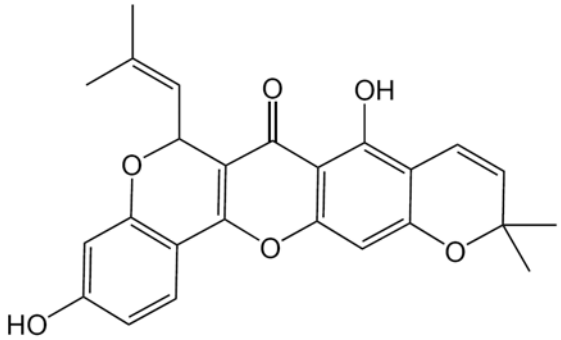
employed to isolate bioactive constituents having significant antioxidant, anti-viral and anti-mutagenic properties (Goncalves et al., 2005; Reis et al., 2008). However no considerable work has been done so far on antibacterial activities of bioactive constituents from *Uncaria tomentosa*. Therefore, a study was designed to explore potentially active compounds isolated from *Uncaria tomentosa* against bacterial pathogens.

Extraction & Bioactivity guided Isolation

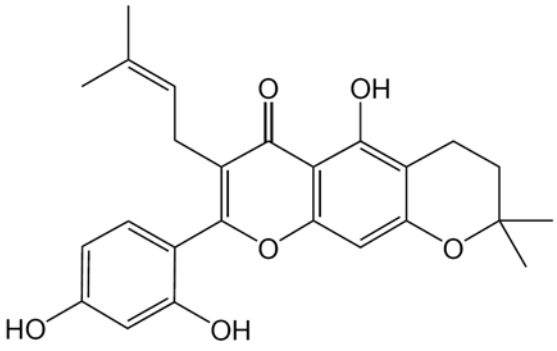
Arial parts of *Uncaria tomentosa* were collected and subjected to shade drying. Air-dried and powdered stem bark (1.6 kg) was extracted with methanol for 48 hours. The extract was filtered through Whatman no. 1 filter paper, and the combined solvent evaporated under reduced pressure at 40°C. Vacuum liquid chromatography (VLC) (Silica gel 550 g) of the methanol extract (92 g), using hexane: ethyl acetate (1: 0–0 : 1) and subsequently ethyl acetate: methanol (1 : 0–0 : 1) step gradients, yielded 26 fractions. These were pooled by TLC profile into 13 major subfractions (Sfr.1–Sfr.13) and afterward submitted for antibacterial assay. Subfractions 4 (7.9 g) and 6 (9.2 g) both eluted with hexane: ethyl acetate (9:1) were found to be the most active. Further purification of Sfrs. 4 and 6 combined, by gravity column chromatography ((silica gel), 140 g) using the same solvent step gradients, produced 122 fractions, which were later combined into 10 subfractions (Sfr.6A–Sfr.6J) after TLC analysis. After antibacterial testing of the ten subfractions, Sfr.6G (943 mg) was found to be the most active.



Artochamin C (**1**)



5'-Hydroxycudraflavone A (**2**)



Dihydrocudraflavone B (**3**)

Figure1. Chemical structures of isolated compounds.

Table 1. Minimum inhibitory concentrations (MIC) of isolated compounds against selected bacterial strains

Test sample	MIC (µg/mL)			
	E.c	***S.a.***	***K.p.***	***B.s.***
Artochamin C (**1**)	4.1	4.6	6.7	5.9
5'-Hydroxycudraflavone A (**2**)	9.8	10.4	16.9	12.2
Dihydrocudraflavone B (**3**)	5.3	5.1	8.3	7.7
Neomycin (standard)	1.6	0.78	1.6	0.78

E.c.: *Escherchia coli, S.a.*: *Staphylococcus aureus, K.p.*: *Klebsiella pneumoniae, B. s. Bacillus subtilis*

Subsequent purification of Sfr 6G by repeated gravity column chromatography combined with preparative TLC (silica gel F254, 0.25 cm thickness), using hexane:ethyl acetate (3 : 2) as the solvent system, afforded three pure compounds. Chemical structures of isolated compounds were identified by comparing their mass spectrometry and NMR data with literature. Compound **1, 2** and **3** were identified as Artochamin C (Wang et al., 2004), 5'-Hydroxycudraflavone A (Syah et al., 2004) and Dihydrocudraflavone B (Groweiss et al., 2000). These compounds were again tested for antibacterial activity, and the results are summarized in Table 1.

Antibacterial activity.

Microtitre bioassay (Eloff, 1998) was used to determine the antibacterial activity. Samples were made up to 5 mg/mL with 25% ethanol. Samples (100 µL) were two-fold serially diluted with distilled water in 96-well microplates to give concentrations of 1.25–0.0098 mg/ mL. Overnight Mueller-Hinton (MH) broth cultures (grown at 37°C in a water bath with continuous shaking) of the test organisms were diluted 100-fold with MH broth, and 100 µL of the resulting bacterial culture was added to each well. Neomycin (100 µg/mL) was used as a positive control for each bacterium, with solvent and bacteria-free wells being included as negative controls. Microplates were covered and incubated overnight at 37°C. To indicate bacterial growth, 40 µL of 0.2 mg/mL *p*-indonitritetrazolium violet (INT) was added to each well and incubated at 37°C for 30 min. Clear wells with INT after incubation indicated inhibition of bacterial growth. The MIC values were recorded as the lowest concentration of extract and/or compound that completely inhibited bacterial growth. *Staphylococcus aureus* (ATCC 12600) was used throughout the isolation process, and isolated compounds were then tested against two Gram-positive bacteria – *Bacillus subtilis* (ATCC 6051) and *Staphylococc-us aureus* (ATCC 12600) and two Gram-negative bacteria – *Klebsiella pneumoniae* (ATCC 13883) and *Escherichia coli* (ATCC 11775).

Results and Discussion

Antibacterial activities of the isolated compounds were investigated against selected bacterial strains. This data supported the fact behind folk medicinal usage of *Uncaria tomentosa*in various diseases including microbial infections. In the present study, Artochamin C (**1**) showed best activity against *S. aureus*, *B. subtilis*, *E. coli* and *K.*

pneumoniae with MIC values ranging from 4.1 µg/mL to 6.7 µg/mL (Table 1). It exhibited the best antibacterial activity against both Gram-positive and Gram-negative bacteria. *E. coli* was found to be the most susceptible bacterial strain. 5'-Hydroxycudraflavone A (**2**) was found be least active among all compounds having MIC values ranging from 9.8 µg/mL to 16.9 µg/mL. Similarly Dihydrocudraflavone B (**3**) was the second most active compound with MIC values ranging from 5.1 µg/mL to 8.3 µg/mL. This data revealed considerable scope of the tested compounds to be further modified as potential lead compound to open a new arena I antibacterial drug discovery. However extensive work is demanding task to achieve scientifically possible target for new drug discovery based on natural products and ethnopharmacology.

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