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ORIGINAL ARTICLE

Antibacterial Activity of Triterpenoids from *Clerodendron trichotomum*

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Abstract The aim of this research was to investigate the antibacterial activity of *Clerodendron trichotomum*. Antibacterial activities of the *n*-hexane, methylene chloride (MC), ethyl acetate, and *n*-butanol fractions from *C. trichotomum* were tested against *Staphylococcus aureus*, *Escherichia coli*, and *Helicobacter pylori*. The *n*-hexane and MC fractions showed antibacterial activity against *H. pylori* at a concentration of 1.7 mg/mL and showed inhibition zones of 10 and 11 mm in disc assay, respectively. Further testing of 22-dehydroclerosterol and β-amyrin (each 3.4 mg/mL) from the MC fraction of *C. trichotomum* revealed moderate antibacterial effects against *E. coli*, *S. aureus*, and *H. pylori*. In particular, β-amyrin showed clear zones of 12 and 13 mm against *E. coli* and *H. pylori,* respectively, suggesting its potential as an antibacterial agent. The active compounds from *C. trichotomum* might provide a promising therapeutic agent against infections by *E. coli*, *S. aureus*, and *H. pylori*.

Keywords antibacterial activity · β-amyrin · *Clerodendron trichotomum* · 22-dehydroclerosterol

Introduction

Various pathogenic bacteria including *Escherichia coli* and *Staphylococcus aureus* are responsible for infectious diseases. *E. coli* is a common cause of urinary tract infections and bacteremia in humans, and is frequently resistant to aminopenicillins, such as amoxicillin and ampicillin (Allen et al., 1999; Karlowsky et al., 2002; Landgren et al., 2005). In addition, *S. aureus* is a common cause of infection in hospitalized patients (Westh et al., 2004). The outer cell membranes of Gram-negative bacteria such as *E. coli* are known to be covered with a lipopolysaccharide layer 1–3 μm in thickness, while the surfaces of Gram-positive bacteria such as *S. aureus* have a peptidoglycan layer, to which teichoic acid, teichuronic acid, and proteins are covalently bound (Sonohara et al., 1995). *Helicobacter pylori*, a Gram-negative bacterium, invokes pro-oxidant and pro-inflammatory mechanisms that may lead to chronic conditions such as gastritis, peptic ulcers, and gastric cancer.

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow et al., 2003). Plant products, either as pure compounds or as standardized extracts, provide promising opportunities for new anti-infective drugs. There is an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for use in the treatment of new and re-emerging infectious diseases (Rojas et al., 2003). Therefore, researchers are increasingly turning their attention to natural products as a source for new and better antimicrobial drugs (Benkeblia, 2004; Kang et al., 2005).

Clerodendron trichotomum Thunb., whose Japanese name is Kusagi and which belongs to the Verbenaceae family, grows wild in the fields and mountains of Korea, Japan, and China (Inchi et al., 1996; Lee, 1996). *C. trichotomum* is a deciduous shrub, growing 10–15' tall in warmer climates and regarded as an herbaceous perennial in cold northern climates. While in flower or while fruiting it is quite beautiful, but is not very appealing otherwise, as it tends to die back and appear unkempt. It tends to flower in the summer time, producing white, 1 1/2"-wide flowers in clustered cymes, 6–9" across. The fruit is a small bright blue drupe framed by a reddish leathery calyx. The dried leaf and stem of *C. trichotomum*, known as 'Chou Wu Tong', exhibits diverse

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pharmacological activities such as blood pressure reduction, sedation, soothing, and paralysis activity (Huang, 1993; Ahn, 1998). Several flavonoids (Okigawa et al., 1970; Morita et al., 1977), diterpenes (Kato et al., 1971; Kawai et al., 1998; 1999), blue pigments (Iwadare et al., 1974), sterols (Kawano et al., 1967), and phenylpropanoid glycosides (Sakurai and Kato, 1983; Kim et al., 2001) have been isolated from *C. trichotomum*. Activity studies have revealed antihypertensive and antioxidant effects (Chae et al., 2005), as well as an inhibitory effect on human immunodeficiency virus (HIV)-1 integrase activity (Kim et al., 2001) by *C. trichotomum*. In addition, Lee et al. (1998) and Jung et al. (2011) reported the antibacterial activity of extract from *C. trichotomum* and screening on antimicrobial effect of Korean herbs including *C. trichotomum*. However, the antimicrobial effect of fractions and active compound has been elucidated. Nothing is known yet about the anti-bacterial compounds of *C. trichotomum*. To the best of our knowledge, there are no reports on the isolation of anti-bacterial compounds from *C. trichotomum*. This study reports on the anti-bacterial activities of phytochemical compounds from *C. trichotomum* against *E. coli*, *S. aureus*, and *H. pylori*.

Materials and Methods

Plant materials. *C. trichotomum* Thunb. collected at Gwangneung, Korea in 2011, was provided by Korea National Arboretum, Korea. A voucher specimen was deposited at the Herbarium of the Department of Integrative Plant Science, Chung-Ang University, Korea.

Instruments and reagents. ^{1}H-NMR spectra were recorded with a Bruker AVANCE 400 NMR (Germany) spectrometer in acetone or $CDCl_3$ using tetramethyl silane (TMS) as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in Hertz (Hz). Thin layer chromatography (TLC) analysis was conducted with Kiesel gel 60 F254 (Art. 5715, Merck Co., Germany) plates (silica gel, 0.25-mm layer thickness), with compounds visualized by spraying with 10% H_2SO_4 followed by charring at 60°C. All other chemicals and reagents were of analytical grade.

Extraction, fractionation, and identification. The dried stems of *C. trichotomum* were extracted with MeOH (10 L × 3) under reflux. The combined MeOH extracts were then suspended in H_2O and subsequently partitioned with equal volumes of *n*-hexane, MC, EtOAc, and *n*-BuOH. Compounds **1** and **2** were identified from the MC fraction.

Compound **1** - ^{1}H-NMR (400 MHz, acetone): δ 5.33 (m, H-6), 5.32 (m, H-22), 5.27 (m, H-23), 3.53 (m, H-3), 1.72 (s, 27-Me), 1.08 (d, *J*=6.3 Hz, 21-Me), 0.97 (s, 19-Me), 0.91 (m, 29-Me), 0.68 (s, 18-Me).

Compound **2** - ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.19 (1H, t, *J*=3.2 Hz, H-12), 3.22 (1H, dd, *J*=4.8, 9.6 Hz, H-3), 2.02 (1H, m, H-9), 1.99 (1H, dd, *J*=4.0, 13.6 Hz, H-18), 1.92 (1H, m, H-1), 1.88 (1H, dd-like, *J*=3.8, 6.4 Hz, H-11), 1.69 (1H, m, H-15), 1.65 (1H, m, H-2), 1.58 (1H, m, H-6), 1.57 (1H, m, H-19), 1.56 (1H, m, H-16), 1.55 (1H, m, H-1), 1.54 (1H, m, H-7), 1.53 (1H, m, H-6), 1.48 (1H, m, H-21), 1.44 (1H, m, H-22), 1.36 (1H, m, H-16), 1.33 (1H, m, H-22), 1.31 (1H, m, H-21), 1.20 (1H, m, H-19), 1.18 (1H, m, H-11), 1.15 (3H, s, H-27), 1.10 (3H, s, H-29), 1.01 (3H, s, H-23), 0.99 (3H, s, H-26), 0.96 (3H, s, H-25), 0.88 (3H, s, H-30), 0.85 (3H, s, H-28), 0.80 (3H, s, H-24), 0.76 (1H, m, H-5).

Microorganisms and media preparation. *E. coli* and *S. aureus* used in this study were provided by the Korean Culture Center of Microorganisms (KCCM, Korea), and *H. pylori* was provided by the Korean Type Culture Collection (KTCC, Korea). Strains were maintained at 4°C in trypticase soy agar (TSA; Becton, Dickinson and Company, USA). Original cultures were maintained at −70°C. The TSA culture medium contained 15 g of a pancreatic casein digest, 5 g of a papaic soybean digest, 5 g NaCl, and 15 g agar in 1 L distilled water. The pH of the medium was adjusted to 7.3.

Antibacterial activity. The antibacterial activity of *C. trichotomum* was tested by the disc agar diffusion method (Davidson and Parish, 1989). TSA plates were inoculated with 0.1 mL of culture, and sterile filter paper discs (8 mm) containing the fractions (1.7 mg/mL) and compounds (3.4 mg/mL) were distributed on the surface. Inhibition zones were determined after an incubation period of 24 h at 37°C. Penicillin (1.7 mg/mL) was used as positive control.

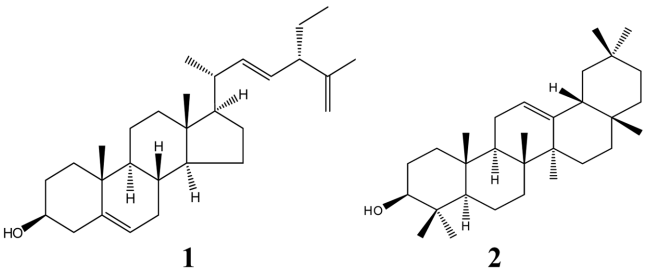
Results and Discussion

The antibacterial activities of *C. trichotomum* against *E. coli*, *S. aureus*, and *H. pylori* were evaluated and the microbial growth inhibition abilities of the fractions from *C. trichotomum* are summarized in Table 1. Previous paper showed the antibacterial activity of the methanol extract of *C. trichotomum* against *H. pylori* (Jung et al., 2011). The fractions did not demonstrate an inhibitory effect on the growth of *E. coli* and *S. aureus*. However, the *n*-hexane and MC fractions inhibited the growth of *H. pylori*, forming inhibition zones larger than 10 mm. The MC fraction exhibited the greatest antibacterial activity against *H. pylori*, forming an inhibition zone of 11 mm. Lee et al. (1998) reported the antimicrobial effect and inhibitory activities of 54 odorant mixtures from 41 Korean aromatic herbs including *C. trichotomum* against *S. aureus* SA 2 that has resistance to 10 usual antibioitics. In particular, among the test samples *C. trichotomum* exerted the

Table 1 Antibacterial activities of the fractions from *C. trichotomum*

Samples (1.7 mg/mL)	Clear zone (mm)		
	E. coli	*S. aureus*	*H. pylori*
n-Hexane fr.	-	-	10
MC fr.	-	-	11
EtOAc fr.	-	-	-
n-BuOH fr.	-	-	-
Penicillin	16	17	17

-: Not detected
Penicillin was used as a positive control.



1 2

Fig. 1 Structures of compounds **1** and **2**.

Table 2 Antibacterial activities of compounds **1** and **2** from *C. trichotomum*

Samples (3.4 mg/mL)	Clear zone (mm)		
	E. coli	*S. aureus*	*H. pylori*
1	11	11	9
2	12	10	13
Penicillin (1.7 mg/mL)	16	17	17

Penicillin was used as a positive control.

strong and does-dependent inhibition in the growth of antibiotics resistant *S. aureus* SA 2 in combination of chloromphenicol. In addition, the present result supported the antibacterial effect of *C. trichotomum* against *H. pylori.*

Compounds **1** and **2** were identified from the MC fraction and elucidated as 22-dehydroclerosterol and β-amyrin (Fig. 1), respectively, by comparison with the spectral data described in the literature (Boar and Allen, 1973; Pech et al., 2002; Cho et al., 2005; Barros et al., 2011). The antibacterial activities of 22-dehydroclerosterol (**1**) and β-amyrin (**2**) against *E. coli*, *S. aureus*, and *H. pylori* are summarized in Table 2. The inhibition zones of 22-dehydroclerosterol at a concentration of 3.4 mg/mL against *E. coli*, *S. aureus*, and *H. pylori* were found to be 11, 11, and 9 mm, respectively. In addition, β-amyrin showed clear inhibition zones of 12, 10, and 13 mm in the disc assay, respectively. In particular, β-amyrin showed the highest antibacterial activity against *H. pylori*, with an inhibition zone greater than 13 mm in the disc assay. Penicillin as a positive control exhibited inhibition zone of 16–17 mm against all tested bacteria.

22-Dehydroclerosterol was previously isolated from the aerial parts of *C. fragrans*, *C. inerme*, *C. infortunatum*, *C. scandens*, and *C. siphonanthus*, and the seeds of *C. infortunatum* (Akihisa et al., 1988; 1990). Chemical investigations have revealed the presence of β-amyrin and pharmacological studies of *Protium heptaphyllum* have revealed its anti-inflammatory, anti-pruritic, gastroprotective and hepatoprotective effects (Oliveira et al., 2004a; 2004b; 2005; Holanda Pinto et al., 2008). Also, a few other studies have shown its efficacy in suppressing acute visceral and/or orofacial nociception and bladder inflammation (Lima-Júnior et al., 2007; Holanda Pinto et al., 2008). Recently, Aragão et al. (2009) demonstrated sedative, anxiolytic, and antidepressant activities in mixtures of α- and β-amyrins, which may involve the γ-amino butyric acid (GABA) energic or the noradrenergic systems. However, a study on the bioactivity of 22-dehydroclerosterol has not been carried out yet. Thus, although 22-dehydroclerosterol and β-amyrin have demonstrated potential utility for the treatment of a variety of disorders, the antibacterial activity of terpenoids from *C. trichotomum* remained untested until now.

There is enormous potential for developing antimicrobials from plant compounds, which may not produce the toxicity associated with synthetic antimicrobials. In summary, 22-dehydroclerosterol and β-amyrin were identified from *C. trichotomum*, and their antibacterial activities were confirmed. These biologically active constituents have potential as inhibitory substances against *E. coli*, *S. aureus*, and *H. pylori*. Consequently, the active compounds from *C. trichotomum* might provide promising therapeutic agent againsts infections by *E. coli*, *S. aureus*, and *H. pylori*.

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