



## Studies on the Chemical Constituents from the Seeds of *Zizyphus jujuba* var. *inermis*

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**Abstract** – This study analyzed the seeds of *Zizyphus jujuba* var. *inermis* commonly used as a remedy in traditional Chinese medicine, in order to determine its various biologically active compounds. Through process 3-pentadecylcatechol,  $\rho$ -menth-8-ene, and  $\gamma$ -bisabolene were isolated and identified for the first time which are urushiol, monoterpenoidal, and sesquiterpenoidal compounds, respectively. Also, found were another sesquiterpenoidal compounds, vomifoliol, and four steroidal compounds,  $\beta$ -sitosterol, stigmaterol, stigmasta-5,23-dien-3 $\beta$ -ol, and stigmast-4-en-3-one. In addition, fourteen triterpenoidal compounds were isolated and identified. These were lupeol, betulinic acid, betulinol, aliphatic acid, 3-O-cis- $\rho$ -coumaroyl-aliphatic acid, 3-O-trans- $\rho$ -coumaroyl-aliphatic acid, 2-O-cis- $\rho$ -coumaroyl-aliphatic acid, 2-O-trans- $\rho$ -coumaroyl-aliphatic acid, zizyberanic acid, ceanothic acid, oleanolic acid, maslinic acid, 3-O-cis- $\rho$ -coumaroyl-maslinic acid, and 3-O-trans- $\rho$ -coumaroyl-maslinic acid. The structures were identified by comparing of the spectroscopic experiments, NMR and MS, and then compared that reported data, respectively. Three extracts of water, methanol, and chloroform from the seeds showed a weak anti-proliferative effect, anti-microbial activity, and anti-oxidant effect, respectively.

**Keywords** – The seed of *Zizyphus jujuba* var. *inermis*, 3-pentadecylcatechol, Anti-proliferative effect, Anti-microbial activity, Anti-oxidant effect

### Introduction

The dried ripe fruit of *Zizyphus jujuba* var. *inermis* and *Zizyphus jujuba* has been used in traditional Chinese medicine as an effective herbal remedy.

Various phytochemicals are present in the *Zizyphus jujuba* var. *inermis* and *Zizyphus jujuba* ranging from alkaloids, benzoids, cyclicpeptides, flavonoids, nucleosides, saponins, sesquiterpenoids, steroids, to triterpenoids. However, a full chemical analysis of the seed from *Zizyphus jujuba* var. *inermis* had not yet been performed.

In this study, we isolated and identified the structure of twenty two compounds (**1** – **22**) and observed anti-proliferative effect, antimicrobial activity, and anti-oxidant effect from the seeds of *Z. jujuba* var. *inermis*.

### Experimental

**General experimental procedures** – The nuclear magnetic resonance (NMR) spectra data, including  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC, HMBC and NOESY experiments, were obtained on a Bruker Biospin Avance II 400 MHz

spectrometer. Fast Atom Bombardment Mass spectrometry (FAB-MS) and Liquid Chromatography (LC)-MSMS spectrometric data were acquired with a JMS-600W/ JEOL, JMS-700 (JEOL) and AQUITY TQD (Waters) mass spectrometer. Silica Gel (230 - 400 mesh, Merck) was used for column chromatography. Thin Layer Chromatography (TLC) was carried out using Merck silica Gel 60 F<sub>254</sub>. HPLC (high performance liquid chromatography) was performed using a Waters 510 pump, Sdex RI71 detector [Porasil™ Silica 1~20  $\mu\text{m}$ , 19 mm ID  $\times$  300 mm] and Jasco pump PU-1580, UV 2075 plus [ODS (octadecoxysilane, C<sub>18</sub>) 5  $\mu\text{m}$ , 10 mm ID  $\times$  250 mm (Daiso Co. Ltd.)]. HPLC solvents were from JT Baker, USA.

**Materials** – The dried red fruits of *Zizyphus jujuba* var. *inermis* were purchased from herbal market, Gyeongsang-si, Korea in October 2012. The materials were confirmed taxonomically by KGC raw materials headquarters, Korea Ginseng Corp., Daejeon, Korea and voucher specimen ZJI-2012 was deposited at the R&D headquarters, Korea Ginseng Corp., Daejeon, Korea. The seeds were separated and collected from the dried red fruits of *Z. jujuba* var. *inermis*, the sarcocarp was washed off, and then the seeds were pulverized.

**Extraction and isolation (Fig. 3)** – The powder of the seeds (2 kg) were refluxed twice with water for 4 hours which yielded the water extract (719 g). The residue was

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refluxed twice with methanol and chloroform for 4 hours which yielded the methanol extract (70.8 g) and chloroform extract (4.2 g). The chloroform extract (4.2 g) was chromatographed on a silica gel column chromatography and eluted with a gradient of chloroform → chloroform:methanol = 50:1 → 10:1 → 9:1 → 8:1 → 7:1 → 6:1 → 5:1 → 1:1 → methanol in 2 fractions (C-1, C-2). Fraction C-1 (356 mg) was subjected to reverse phase HPLC eluting with a C<sub>18</sub> ODS column (methanol-water = 95:5) to create 3-pentadecylcatechol (compound **1**, 18 mg). The methanol extract (70.8 g) was chromatographed on a silica gel column chromatography and eluted with a gradient of n-hexane → n-hexane: ethyl acetate = 50:1 → 10:1 → 9:1 → 8:1 → 7:1 → 6:1 → 5:1 → 1:1 → ethyl acetate in 5 fractions (H-1, 2, 3, 4, 5). Fraction H-1 (2.76 g) was subjected to normal phase HPLC eluting with a silica gel column (n-hexane:ethyl acetate = 50:1) to create p-menth-3-ene (**2**, 23 mg), γ-bisabolene (**3**, 15 mg), vomifoliol (**4**, 18 mg). Fraction H-2 (13.2 g) was subjected to normal phase HPLC eluting with a silica gel column (n-hexane: ethyl acetate = 5:1) to create β-sitosterol (**5**, 28 mg), stigmasterol (**6**, 35 mg), stigmasta-5,23-dien-3β-ol (**7**, 15 mg), stigmast-4-en-3-one (**8**, 10 mg), lupeol (**9**, 12 mg), betulinic acid (**10**, 890 mg), betulinaldehyde (**11**, 56 mg), alphitolic acid (**12**, 12 mg), 3-O-cis-ρ-coumaroyl-alphitolic acid (**13**, 8 mg), 3-O-trans-ρ-coumaroyl-alphitolic acid (**14**, 5 mg), 2-O-cis-ρ-coumaroyl-alphitolic acid (**15**, 17 mg), 2-O-trans-ρ-coumaroyl-alphitolic acid (**16**, 23 mg), zizyberanolic acid (**17**, 11 mg), ceanothic acid (**18**, 33 mg), oleanolic acid (**19**, 25 mg), maslinic acid (**20**, 26 mg), 3-O-cis-ρ-coumaroyl-maslinic acid (**21**, 13 mg), and 3-O-trans-ρ-coumaroyl-maslinic acid (**22**, 35 mg).

**3-Pentadecylcatechol (1)** – Yellow liquid, UV (MeOH) λ<sub>max</sub> (log ε) 280 (3.3), 220 (3.8) nm; FAB-MS m/z: 321.52 [M+H]<sup>+</sup> (C<sub>21</sub>H<sub>37</sub>O<sub>2</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>3</sup>

**ρ-Menth-8-ene (2)** – Yellow liquid, FAB-MS: m/z 139.25 [M+H]<sup>+</sup> (C<sub>10</sub>H<sub>19</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>8</sup>

**γ-Bisabolene (3)** – Yellow liquid, FAB-MS m/z: 205.36 [M+H]<sup>+</sup> (C<sub>15</sub>H<sub>25</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>9</sup>

**Vomifoliol (4)** – Yellow liquid, FAB-MS m/z: 225.30 [M+H]<sup>+</sup> (C<sub>13</sub>H<sub>21</sub>O<sub>3</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>10</sup>

**β-Sitosterol (5)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 415.72 [M+H]<sup>+</sup> (C<sub>29</sub>H<sub>51</sub>O). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR)

to published values.<sup>1,12</sup>

**Stigmasterol (6)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 413.71 [M+H]<sup>+</sup> (C<sub>29</sub>H<sub>49</sub>O). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>12</sup>

**(23E)-Stigmasta-5,23-dien-3β-ol (7)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 413.72 [M+H]<sup>+</sup> (C<sub>29</sub>H<sub>49</sub>O). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>11</sup>

**Stigmast-4-en-3-one (8)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 411.70 [M+H]<sup>+</sup> (C<sub>29</sub>H<sub>47</sub>O). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>1</sup>

**Lupeol (9)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 427.73 [M+H]<sup>+</sup> (C<sub>30</sub>H<sub>51</sub>O). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>13</sup>

**Betulinic acid (10)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 457.72 [M+H]<sup>+</sup> (C<sub>30</sub>H<sub>49</sub>O<sub>3</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>13</sup>

**Betulinaldehyde (11)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 441.72 [M+H]<sup>+</sup> (C<sub>30</sub>H<sub>49</sub>O<sub>2</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>14</sup>

**Alphitolic acid (12)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 473.70 [M+H]<sup>+</sup> (C<sub>30</sub>H<sub>49</sub>O<sub>4</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>2</sup>

**3-O-cis-ρ-Coumaroyl-alphitolic acid (13)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 619.86 [M+H]<sup>+</sup> (C<sub>39</sub>H<sub>55</sub>O<sub>6</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>2</sup>

**3-O-trans-ρ-Coumaroyl-alphitolic acid (14)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 619.86 [M+H]<sup>+</sup> (C<sub>39</sub>H<sub>55</sub>O<sub>6</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>2</sup>

**2-O-cis-ρ-Coumaroyl-alphitolic acid (15)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 619.87 [M+H]<sup>+</sup> (C<sub>39</sub>H<sub>55</sub>O<sub>6</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>2</sup>

**2-O-trans-ρ-Coumaroyl-alphitolic acid (16)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 619.86 [M+H]<sup>+</sup> (C<sub>39</sub>H<sub>55</sub>O<sub>6</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>2</sup>

**Zizyberanolic acid (17)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 471.40 [M+H]<sup>+</sup>.

This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>15</sup>

**Ceanothic acid (18)** – Amorphous powder; LC-MSMS

(ES<sup>+</sup>) m/z: 487.70 [M+H]<sup>+</sup> (C<sub>30</sub>H<sub>47</sub>O<sub>5</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>16</sup>

**Oleanolic acid (19)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 457.71 [M+H]<sup>+</sup> (C<sub>30</sub>H<sub>49</sub>O<sub>3</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>16</sup>

**Maslinic acid (20)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 473.71 [M+H]<sup>+</sup> (C<sub>30</sub>H<sub>49</sub>O<sub>4</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>17</sup>

**3-O-trans- $\rho$ -Coumaroyl-maslinic acid (21)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 619.86 [M+H]<sup>+</sup> (C<sub>39</sub>H<sub>55</sub>O<sub>6</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>17</sup>

**3-O-cis- $\rho$ -Coumaroyl-maslinic acid (22)** – Amorphous powder; LC-MSMS (ES<sup>+</sup>) m/z: 619.86 [M+H]<sup>+</sup> (C<sub>39</sub>H<sub>55</sub>O<sub>6</sub>). This compound exhibited comparable spectroscopic data (<sup>1</sup>H and <sup>13</sup>C-NMR) to published values.<sup>17</sup>

**Cell proliferation assays** – All cancer cell lines (K562, MEG-01, HL-60, KG-1, MOLT-4, L1210, P388D1, A549, HepG2, MCF-7, SK-OV-3, and SW-620) were maintained in RPMI 1640 which included 10% FBS and 1% penicillin/streptomycin. Cells were cultured at 37 in a 5% CO<sub>2</sub> incubator. Each cell line was plated in 96-well plates at 1 × 10<sup>4</sup> cells/well. Cells were incubated with serial dilutions of each reagent for 48 hours. Cell proliferation was measured using the WST-1 assay with the Premix WST-1 cell proliferation assay system. In addition, IC<sub>50</sub> values were calculated using CalcuSyn software (Biosoft, Cambridge, UK).

**Evaluation of antioxidant effect** – The antioxidant effect of the samples, based on the scavenging activity of the stable 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical, was determined by the method described by Blois, Mokbel and Hashinaga. The sample solutions (1.0 ml) in methanol at different concentrations were added to a 2 ml 0.004% (w/v) solution of DPPH in methanol, prepared daily and protected from light. The reaction mixture was incubated at room temperature in the dark for 30 min, and then the absorbance of the reactive mixture was recorded using spectrophotometer at 517 nm. Inhibition of the DPPH free radical in percent (I%) was calculated in following way: I% = [(A<sub>blank</sub> - A<sub>sample</sub>) / A<sub>blank</sub>] × 100; where A<sub>blank</sub> is the absorbance of the control reaction (containing all reagents except the test compound), and A<sub>sample</sub> is the absorbance of the test compound. Compound concentration providing 50% inhibition (IC<sub>50</sub>) was calculated from the graph plotting inhibition percentage against solution concentration. Tests were carried out in

triplicate. Six different concentrations of each sample studied have been assayed in order to check the linearity of response and to establish the antioxidant effect values in the adequate linear range. Methanol was tested against DPPH · radical and this resulted in null effect on the absorbance at 517 nm. Vitamin C, a known antioxidant, was used as positive control.

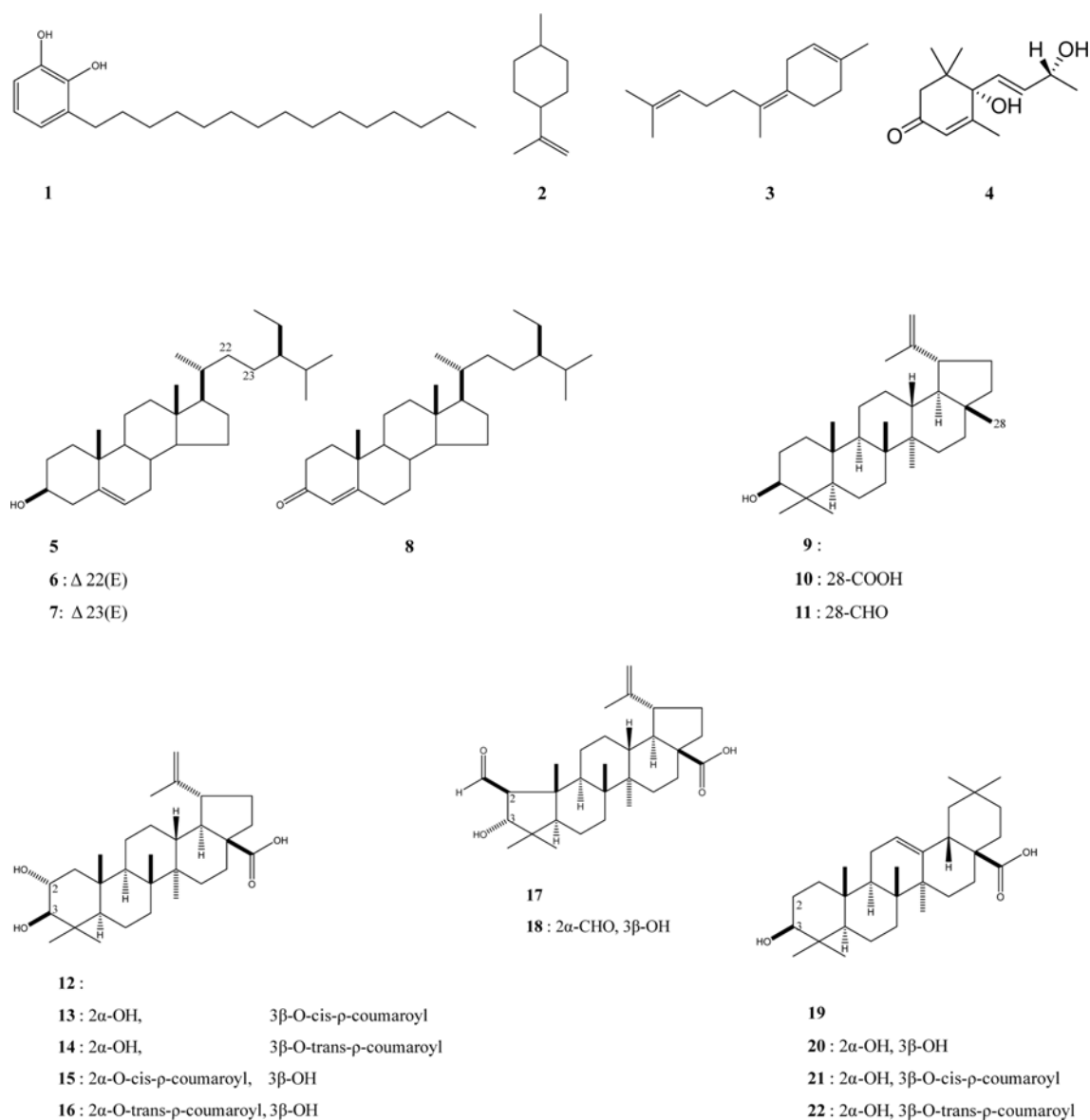
**Antimicrobial activity** – The samples showing activity against any of these were further tested against the following microorganisms: *Staphylococcus aureus* ATCC 6538<sup>TM</sup>, *Pseudomonas aeruginosa*<sup>TM</sup> *Escherichia coli* ATCC 8739<sup>TM</sup>, *Candida albicans* 10231<sup>TM</sup>, and *Aspergillus brasiliensis* 16404<sup>TM</sup>. The media used for testing the activity were Tryptic soy agar (236950, BD Difco<sup>TM</sup>, USA). The infusion was done at 30°C for 2-6 hours. The plates of microorganisms were incubated for 24 hours at 30°C. During assay, the fungal plates were incubated for 4-7 days at 30°C. The zone of inhibition of microbial growth was used as a measure of antimicrobial activity of the samples.

## Result and Discussion

One urushiol, 3-pentadecylcatechol (**1**) was isolated for the first time from the seeds of *Zizyphus jujuba* var. *inermis*. The structure was identified through comparing of the FAB-MS, <sup>1</sup>H- and <sup>13</sup>C-NMR data with related data from the cited literature (Fig. 1).<sup>3</sup> 3-Pentadecylcatechol (**1**), which was isolated from *Rhus vernicifera*,<sup>3</sup> is toxic, causing irritation, inflammation, and blistering of the skin,<sup>4</sup> and has strong antioxidant,<sup>5</sup> anticancer,<sup>6</sup> and antimicrobial activity.<sup>7</sup>

One monoterpene,  $\rho$ -menth-8-ene (**2**) and one sesquiterpene,  $\gamma$ -bisabolene (**3**) were isolated for the first time from the seeds of *Z. jujuba* var. *inermis*. These structures were identified through comparing of the FAB-MS, <sup>1</sup>H- and <sup>13</sup>C-NMR data with related data from the cited literatures (Fig. 1).<sup>8,9</sup>  $\gamma$ -Bisabolene (**3**) showed potent antiproliferative effect against human oral squamous cell carcinoma cells.<sup>21</sup>

Additionally, one sesquiterpene, vomifoliol (**4**),<sup>10</sup> and four steroids:  $\beta$ -sitosterol (**5**),<sup>1,12</sup> stigmasterol (**6**),<sup>1,12</sup> stigmasta-5,23-dien-3 $\beta$ -ol (**7**),<sup>11</sup> and stigmast-4-en-3-one (**8**),<sup>1</sup> were isolated and identified (Fig. 1). Vomifoliol (**4**) showed significant antimicrobial activity against *Neisseria gonorrhoeae*.<sup>22</sup>  $\beta$ -Sitosterol (**5**) showed antihyperlipidemic effect<sup>23</sup> and antiproliferative effect against HL-60 cell line.<sup>24</sup> Stigmasterol (**6**) showed remarkable antimicrobial activity against *Streptococcus mutans*,<sup>25</sup> *Mycobacterium smegmatis*, and *M. aurum*.<sup>28</sup>  $\beta$ -Sitosterol (**5**) and stigma-

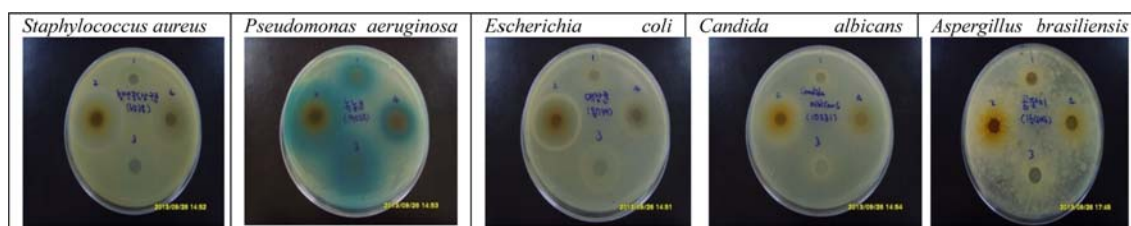


**Fig. 1.** The structures of compounds (1~22).

sterol (**6**) inhibit prostate cancer growth.<sup>26</sup>

Also, fourteen triterpenes: lupeol (**9**),<sup>13</sup> betulinic acid (**10**),<sup>13</sup> betunaldehyde (**11**),<sup>14</sup> alphitolic acid (**12**),<sup>2</sup> 3-O-cis-p-coumaroyl-alphitolic acid (**13**),<sup>2</sup> 3-O-trans-p-coumaroyl-alphitolic acid (**14**),<sup>2</sup> 2-O-cis-p-coumaroyl-alphitolic acid (**15**),<sup>2</sup> 2-O-trans-p-coumaroyl-alphitolic acid (**16**),<sup>2</sup> zizyberanic acid (**17**),<sup>15</sup> ceanothic acid (**18**),<sup>16</sup> oleanolic acid (**19**),<sup>16</sup> maslinic acid (**20**),<sup>17</sup> 3-O-cis-p-coumaroyl-maslinic acid (**21**),<sup>17</sup> and 3-O-trans-p-coumaroyl-maslinic acid (**22**),<sup>17</sup> were isolated and identified as a by spectroscopic experiments, NMR and MS, and comparison with reported data (Fig. 1). Lupeol (**9**) showed antiproliferative effect against HELA- cell line.<sup>27</sup> Lupeol

(**9**) and betulinic acid (**10**) showed appreciable antimicrobial activity against *M. smegmatis*, *M. aurum*.<sup>28</sup> Betulinic acid (**10**) showed potent anticancer activity.<sup>29</sup> Betunaldehyde (**11**) exhibited antimicrobial activity against *M. tuberculosis*.<sup>30</sup> Alphitolic acid (**12**) exhibited antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Escherichia coli*,<sup>31</sup> and antiproliferative effect against oral squamous cell carcinoma cells.<sup>32</sup> Betulinic acid (**10**), 3-O-cis-p-coumaroyl-alphitolic acid (**13**), and 3-O-trans-p-coumaroyl-alphitolic acid (**14**) showed high antiproliferative effects against K562, B-16, SK-MEL-2, PC-3, LOX-IMVI, and A-549 tumor cell.<sup>18</sup> Ceanothic acid (**18**) exhibited antimicrobial activities against



The seeds powder and extracts (1: powder, 2: water, 3: methanol, 4: chloroform)

**Fig. 2.** The antimicrobial effects of the three kinds of seeds extracts (water, methanol and chloroform) against *Staphylococcus aureus* ATCC 6538<sup>TM</sup>, *Pseudomonas aeruginosa*<sup>TM</sup>, *Escherichia coli* ATCC 8739<sup>TM</sup>, *Candida albicans* 10231<sup>TM</sup>, and *Aspergillus brasiliensis* 16404<sup>TM</sup>.

*Streptococcus mutans*, *Actinomyces viscosus*, *Porphyromonas gingivalis*, and *Prevotella intermedia*.<sup>33</sup> Maslinic acid (**20**) showed high antiproliferative effects against HT29 colon-cancer cell.<sup>34</sup>

3-Pentadecylcatechol (**1**),  $\gamma$ -bisabolene (**3**),  $\beta$ -sitosterol (**5**), stigmasterol (**6**), lupeol (**9**), betulinic acid (**10**), aliphatic acid (**12**), 3-O-cis- $\rho$ -coumaroyl-aliphatic acid (**13**), 3-O-trans- $\rho$ -coumaroyl-aliphatic acid (**14**) and maslinic acid (**20**) showed antiproliferative effects against HL-60, human oral squamous cell carcinoma, prostate cancer, HELA, K562, B-16, SK-MEL-2, PC-3, LOX-IMVI, A-549, and HT29 colon-cancer cells.<sup>7,18,21,24,26-27,29,32-34</sup> The chloroform extract of seeds contains compounds (**1**, **3**, **5**, **6**, **9**, **10**, **12**, **13**, **14** and **20**), showed antiproliferative effects with IC<sub>50</sub> values of 100.5, 107.0, 68.0, and 15.2  $\mu$ g/ml against K562, MOLT-4, L1210, and P388D1 cancer cell lines, respectively. But, the water extract was mostly inactive.

3-Pentadecylcatechol (**1**), vomifoliol (**4**), stigmasterol (**6**), lupeol (**9**), betulinic acid (**10**), betulinic aldehyde (**11**), and aliphatic acid (**12**) showed for their antimicrobial activities against *Actinomyces viscosus*, *Enterococcus faecalis*, *Escherichia coli*, *Mycobacterium smegmatis*, *M. aurum*, *Neisseria gonorrhoeae*, *Porphyromonas gingivalis*, and *Prevotella intermedia* *Staphylococcus aureus*, *Streptococcus mutans*.<sup>6,22,25,28,30-31</sup> The seeds extracts contain compounds (**1**, **4**, **6**, **9**, **10**, **11**, **12**, and **18**) showed for their antimicrobial activities with IC<sub>50</sub> values of water: methanol:chloroform = 760:210:110 mg/mL against *Staphylococcus aureus* ATCC 6538<sup>TM</sup>, *Pseudomonas aeruginosa*<sup>TM</sup>, *Escherichia coli* ATCC 8739<sup>TM</sup>, *Candida albicans* 10231<sup>TM</sup>, and *Aspergillus brasiliensis* 16404<sup>TM</sup>, respectively (Fig. 2). However, the sarcocarp of jujube did not show antimicrobial effects against *Staphylococcus aureus*.<sup>19</sup>

Pentadecylcatechol (**1**) has strong antioxidant activity<sup>20</sup> Chemical constituents such as total phenolic are some of the most important materials of antioxidant activity from jujube.<sup>20</sup> 3-Pentadecylcatechol (**1**),<sup>5</sup> 3-O-cis- $\rho$ -coumaroyl-aliphatic acid (**13**), 3-O-trans- $\rho$ -coumaroyl-aliphatic acid

(**14**), 2-O-cis- $\rho$ -coumaroyl-aliphatic acid (**15**), 2-O-trans- $\rho$ -coumaroyl-aliphatic acid (**16**), 3-O-cis- $\rho$ -coumaroyl-maslinic acid (**21**), and 3-O-trans- $\rho$ -coumaroyl-maslinic acid (**22**) are compounds containing a phenolic structure, there is a high possibility of exhibiting an antioxidative effect. The chloroform and methanol extracts of seed contain compounds (**1**, **13**, **14**, **15**, **16**, **21**, and **22**). The water, methanol, and chloroform extract of seeds were evaluated for antioxidant activity by scavenging of DPPH radical and showed IC<sub>50</sub> values of 0.65, 0.32, and 3.27 mg/ml, respectively.

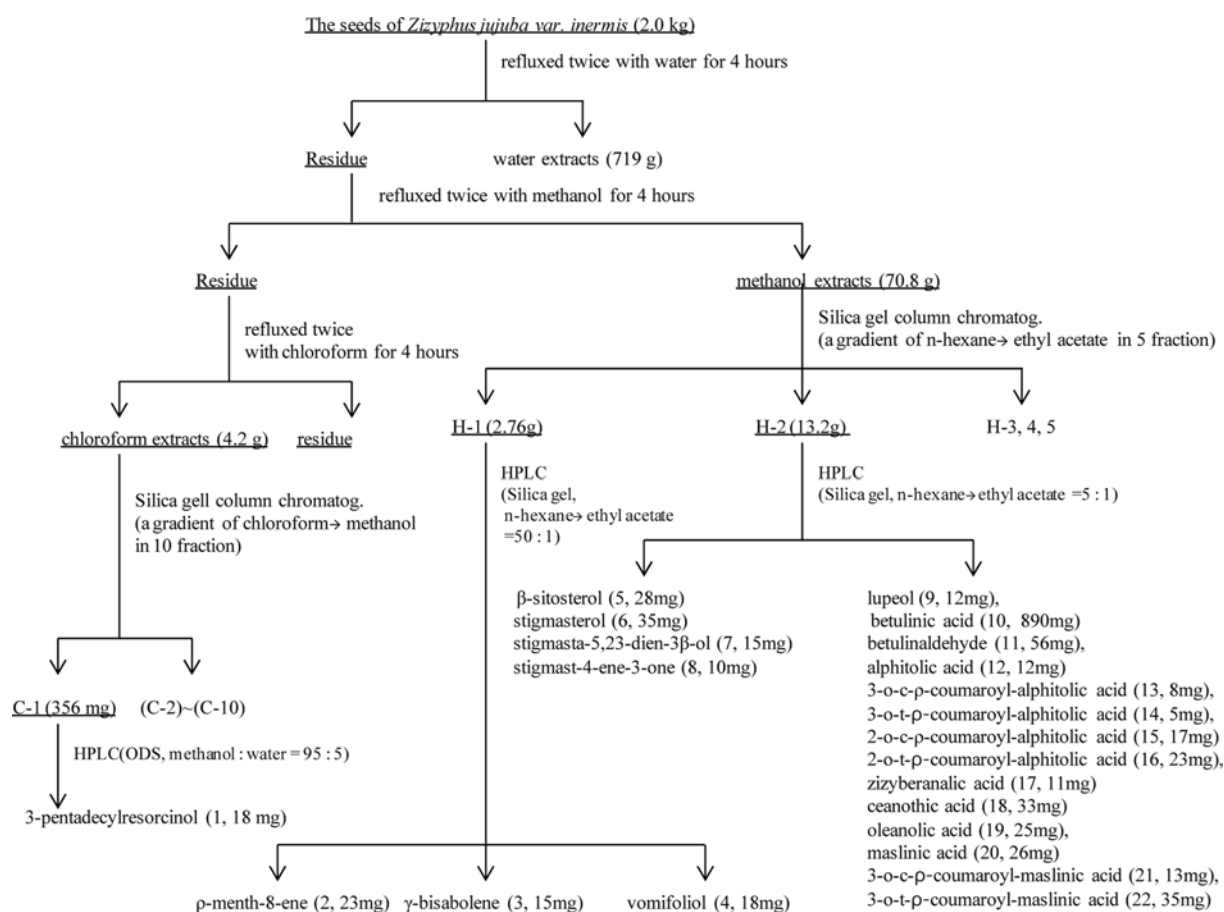
In this study, we investigated the component specificity of the jujube seed (*Zizyphus jujuba* var. *inermis*). Twenty-two compounds (**1** - **22**) were isolated from the extracts of seed. The structures were identified through comparing of the FAB-MS, <sup>1</sup>H- and <sup>13</sup>C-NMR data with related data from the cited literatures<sup>1-3,8-13,16-17</sup>: one urushiol (**1**), one monoterpene (**2**), two sesquiterpenoids (**3** and **4**), four steroids (**5** - **8**), and fourteen triterpenoids (**9** - **22**).

Compounds (**1** - **3**) were isolated and identified for the first time from *Zizyphus jujuba* var. *inermis*.

However, components such as alkaloids, cyclic peptides, flavones, nucleosides, and saponins found in fruit, cortex, root, etc. of *Zizyphus jujuba* var. *inermis* were not found in seeds.

Compounds (**1**, **3**, **5**, **6**, **9**, **10**, **12**, **13**, **14** and **20**) showed anti-proliferative effects,<sup>7,18,21,24,26-27,29,32-35</sup> compounds (**1**, **4**, **6**, **9**, **10**, **11**, **12**, and **18**) showed for their antimicrobial activities,<sup>6,22,25,28,30-31</sup> compounds (**1**, **13** - **16**, and **21** - **22**) showed antioxidant activity.<sup>3,5,20</sup> Similar to the data cited in the above literatures, each of the water, methanol, and chloroform extracts of the jujube seed showed a weak anti-proliferative effects, antimicrobial activity, and antioxidative effects, respectively.

Since the seed of jujube can cause allergies, it is instructed to roast seed in a folk medicine, heavy metals and proteins are being studied as the cause.<sup>35</sup> However, as a result of this study on the components of the jujube seed, we could estimate that urushiol (**1**) was one of the



**Fig. 3.** Extraction and isolation of compounds (1~22) from the seeds of *Zizyphus jujuba* var. *inermis*.

cause of the allergy.

Furthermore, based on the results of the efficacy test and data cited in the literatures of compounds (1 - 22), it is confirmed that the jujube seed is an excellent food material having useful effects such as antioxidant, antibacterial, anticancer, anti-inflammatory, anti-diabetic, and antihyperlipidemic effect.

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