Korean Society for Biotechnology and Bioengineering Journal 29(3): 179-182 (2014)
http://dx.doi.org/10.7841/ksbbj.2014.29.3.179 ISSN 1225-7117 / eISSN 2288-8268

연구논문

Antimicrobial Activity of Essential Oil of *Pinus koraiensis* Seed Against Pathogens Related to Acne

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접수: 2014년 4월 15일 / 게재승인: 2014년 5월 2일
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Abstract: The purpose of the present research was to evaluate the antimicrobial activity of the essential oil extracted from *Pinus koraiensis* seed against pathogens related to acne. The essential oil was extracted by steam distillation method. The chemical compositions of essential oil were analyzed by GC-MS. Alpha-pinene (29.87%), D-limonene (19.26%), beta-pinene (11.19%), beta-myrcene (3.84%), n-hexadecanoi acid (3.2%), beta-caryphyllene (2.72%), and cyclohexene (2.17%) were main components. This essential oil had antimicrobial activities against *Malasseizia furfur, Propionibacterium acnes*, and *Staphylococcus epidermidis*.

Keywords: *Pinus koraiensis* seed, Essential oil, Antimicrobial activity

1. INTRODUCTION

Pinus koraiensis, commonly called Korean nut pine, is an evergreen tree species across Korea, Japan, and the north-eastern part of China. It only grows in locations higher than 1,000 m above sea level and can reach 1.0 m in diameter and 20~30 m in height. The seed of *P. koraiensis* have been used as food supplement and the plant has been used in oriental medicine for thousands of years. It has also been reported *Pinus* bark extract, including that from *P. koraiensis,* exhibits antitumor, antioxidant, antiaging, and antimutation activities based on removing superfluous free radicals and enhancing immunity [1,2]. Essential oil, as odorous and volatile of the secondary metabolism of plants that are normally formed in special cells or groups of cell, have a wide application in folk medicine. Food flavoring and preservation, and fragrance industries, however, their applicability has recently expanded because of their antioxidant, antiaging, antimutation, and sedative effects [3,4]. Antimicrobial properties of essential oils have also been known for many countries and various essential oils have already been studied for antimicrobial properties against bacteria and fungi [5,6]. The antimicrobial actives of essential oils prepared from the needles of three coniferous trees, *P. densiflora, P. koraiensis,* and *C. obtus* were investigated [7], whereas essential oils from *P. densiflora* and *C. obtuse* showed some degree of antibacterial activity. Kim et al. investigated the antihyperlipidemic activities of the essential oil from the leaves of *P. koraiensis* SIEB that has been used as a folk remedy for heart disease. [8]. Previously, author investigated proximate composition and amino acid of seed and fatty acid and physicochemical properties of the oil to study chemical composition of *P. koraiensis* seed. In addition, DPPH radical scavenging activity and reducing power, nitrite scavenging activity, and the inhibitory activities of elastase and collagenase using various extracts of *P. koraiensis* seed were investigated [9]. Nonetheless, there was no information on antimicrobial activity of essential oil of *P. koraiensis* seed against pathogens related to acne such as *Malasseizia furfur, Propionibacterium acnes*, and *Staphylococcus epidermidis* so far.

In this study, to investigate antimicrobial activity of the essential oil extracted from *P. koraiensis* seed, the essential oil was extracted by steam distillation method and antimicrobial activity against pathogens related to acne was evaluated.

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2. MATERIAL AND METHODS

2.1. Sample preparation

P. koraiensis seed in October 2011 were collected at Hamra mountain at Jeonbuk, South Korea.

2.2. Essential oil extraction

The collected *P. koraiensis* seed with cone were cut into 1~2 cm pieces and the essential oil was extracted by steam distillation method for 7 hr (100 g of sample in 300 mL of distilled water). The volatile compounds containing the water-soluble fraction were allowed to settle for 1 hr. The essential oil layer was separated and stored in sealed glass vials at 4°C prior to analysis.

2.3. Identification of chemical compositions of the essential oil

GC-MS analysis method was carried out to determine the composition of the essential oil. The essential oil was analyzed on a HP-5MS capillary column (30 m × 0.35 mm × 0.2 m), and GC-2010 (Shimadzu, Japan) coupled with a GC-MS-QP2010 plus (Shimadzu, Japan). Oven temperature was increased 50~100°C at 3°C/min, 100~200°C at 2°C/min and then 200~280°C at 5°C/min, with helium as a carrier gas at 1.2 L/min and the injector temperature was 200°C. The compounds were identified by retention indices, peak matching library searches, and from the database of National Institute of Standards and Technology .

2.4. Antimicrobial Activity

To investigate antimicrobial activity of essential oil of *P. koraiensis* seed aganist pathogens related to acne, *Propionibacterium acnes* KCTC2358, *Staphylococcus epidermidis* ATCC1228, *Malasseizia furfur* KCCM 12679 were used. The each medium was shown in Table 1. *P. acnes* were cultivated at anaerobic jar CO_2 incubator at 37°C for 72 hr. *M. furfur* was cultivated at 37°C for 24 hr. *S. epidermidis* was cultivated at 37°C for 24 hr. An essential oil was added to the flasks (20, 40, 60 μL/100 mL concentration). The control was cultured without essential oil.

Table 1. Medium used in this experiment

Strain	Medium
P. acnes	Reinforced clostridial medium and heart infusion medium containing 0.1% Tween 80
M. furfur	YM medium containing 1% olive oil
S. epidermidis	Tryptic soy medium

Table 2. Chemical compositions of essential oil of *P. koraiensis* seed

Volatile compounds	Concentration (Peak area, %)
alpha-Thujene	0.22
Tricyclene	0.14
Santene	0.11
alpha-Pinene	29.87
Camphene	1.23
alpha-Phellandrene	0.42
beta-Pinene	11.19
beta-Myrcene	3.84
beta-Caryphyllene	2.72
3-Carene	1.85
Cyclohexene	2.17
p-Cymmene	0.58
D-Limonene	19.26
alpha-Terpinolene	1.87
Isolongifolene	1.27
n-Hexadecanoi acid	3.2
gamma-Muurolene	0.48
gamma-Cadinene	1.2
Terpinen-4-ol	0.23
p-Cymen-8-ol	0.17
Bornyl acetate	2.56

3. RESULT AND DISCUSSION

The chemical composition of essential oil of *P. koraiensis* seed was analyzed by GC-MS and 23 components were identified among 31 compounds. The results are shown in Table 2. The main components of essential oil of *P. koraiensis* seed were alpha-pinene (29.87%), D-limonene (19.26%), beta-pinene (11.19%), beta-myrcene (3.84%), n-hexadecanoi acid (3.2%), beta-caryphyllene (2.72%), and cyclohexene (2.17%). Part of our result is similar with those of Krauze-Baranowska et al., who reported that essential oil from pine needles contained α-pinene, β-pinene, limonene, camphene, 3-carene, myrcene, and so on [10]. Lee et al. [1] reported the chemical composition and antimicrobial activity of essential oil from cones of *P. koraiensis* without seeds. They identified 87 components comprising about 96.8% of the total oil. The main oil components were limonene (29.70%), alpha-pinene (23.89%), beta-pinene (12.02%), 3-carene (4.95%), beta-pinene (4.53%), isolongfolene (3.35%), bornylacetate (2.02%), caryophyllene (1.71%), and comphene (1.54%). Yang et al. [11] reported the analysis of essential oils of pine cones of *P. koraienssis* steb.Et Zucc and *P. sylvestris* L. from China. They identified 35 and 31 components. Alpha-pinene (35.2%), D-limonene (18.4%), beta-pinene (8.7%), beta-caryophylllene (3.5%) and myrcene (3.0%) were the main components of *P. koraienssis*. In the case of *P. sylvestris,* aromadendrene (20.2%), alpha-pinene (18.5%), alpha-longipinene (10.5%) and alpha-terpineol (5.5%) were the main components. Liu and Xu [12] reported characterization of essential oil in pine nut shells from commodity waste in China by steam distillation and GC-MS. They determined 48 volatile chemical compositions. Alpha-pinene, beta-pinene, 3-carene, 1-methy-4-(1-methylethenyl)-(S)-cyclohexene, and n-hexadecanoic acid are predominant volatile compositions in pine nut shells. Kohsuke et al. [13] reported compositions of the essential oils from the leaves of nine *Pinus* species and the cones of three of *Pinus*. The main components of *P. densiflora* cone oil were D-germacrene (20.6%), beta-caryophyllene (8.9%), delta-cadinene (8.4%) and longifolene (8.0%). The major constituents of *P. rigida* cone oil were beta-phellandrene (15.0%), pinocarveol (7.6%), alpha-terpineol (7.4%), alpha-pinene (6.5%), myrtenol (6.1%) and beta-pinene (6.0%). Those of *P. taeda* cone oil were alpha-pinene (51.8%), verbenone (4.8%), p-mentha-1,5-dien-8-ol (4.8%), pinocarveol (4.5%), beta-pinene (3.8%) and borneol (3.8%) (only *P. taeda* lists major compounds over 3.5%). The present work shows quantitative and qualitative differences from previous research. These results indicate that they were affected by the influence of the age of the plant, the harvesting period, the climate and the geographic circumstances on the components of the essential oil [12,13].

Essential oils are natural complex compounds characterized by a strong odour with volatility, formed by aromatic plants as secondary metabolites [14]. They are obtained from flowers, buds, seeds, leaves, bark, fruits and roots as aromatic oils [15]. They are highly volatile in the air and their fragrances differ from every species or plant materials. In nature, essential oils play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and herbivores by reducing their appetite for such plants [16]. Many researches are being carried out to inhibit the food and plant pathogens (*Salmonella enteritidis, Escherichia coli* and *Botrytis cinerea* etc.) by using antimicrobial activities of essential oils [17,18]. The essential oils from conifers are also applied to the cosmetics and medicines and especially good antimicrobial properties of essential oils from *Cryptomeria japonica* and *Chamaecyparis obtusa* have been reported [19]. Therefore, this study examined the antimicrobial activity of essential oils obtained from *P. koraiensis* seed against pathogens related to acne such as *P. acnes* KCTC2358, *S. epidermidis* ATCC1228, *M. furfur* KCCM 12679. Fig. 1 shows the effect of essential oil of *P. koraiensis* seed on growth of *P. acnes* in Brain Heart infusion broth containing 0.1% Tween 80 during incubation for 72 hr at 37°C.The growth of *P. acnes* was decreased with the increase of culture and essential oil concentration of *P. koraiensis* seed. Especially, when 20 μL of essential oil of *P. koraiensis* was used, the growth of *P. acnes* at 36 hr was repressed a little. However, at after 72 hr of culture, it was repressed by 10.0×10^4 CFU/mL. At 60 μL of essential oil, the growth of *P. acnes* was repressed to 8.4×10^4 CFU/mL after 72 hr of culture, which was about 23.6% of decrease compared to control (without essential oil addition). Fig. 2 is effect of essential oil of *P. koraiensis* seed on growth of *S. epidermidis* in trypic soy broth during incubation for 24 hr at 37°C. The growth of *S. epidermidis* was decreased with the increase of culture and essential oil concentration of *P. koraiensis* seed. However, at 6 hr of culture, they were similar, irrespective of essential oil concentrations except 60 μL of essential oil. When 20 and 40 μL of essential oil of *P koraiensis* seed were used, they were 10.5 and 10.1×10^5 CFU/

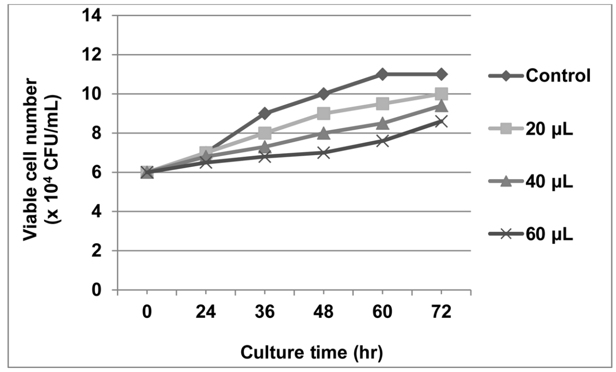

Fig. 1. Effect of essential oil of *P. koraiensis* seed on growth of *P. acnes.*

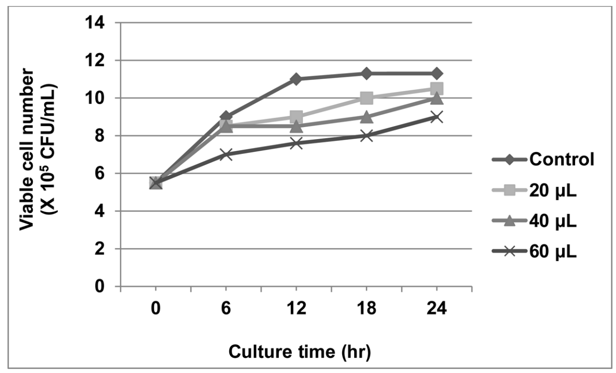

Fig. 2. Effect of essential oil of *P. koraiensis* seed on growth of *S. epidermidis*.

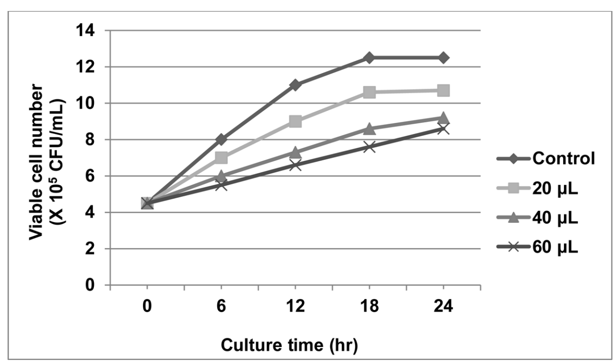

Fig. 3. Effect of essential oil of *P. koraiensis* seed on growth of *M. furfur.*

mL, respectively after 24 hr of culture. When 60 μL of essential oil of *P. koraiensis* seed was used, it was strongly affected. Especially, at 24 hr of culture, the viable cell number was 8.9×10^5 CFU/mL, which was about 20.0% of decrease compared to control (without essential oil addition). Fig. 3 is effect of essential oil of *P. koraiensis* seed on growth of *M. furfur* in YM broth containing 1% olive oil during incubation for 24 hr at 37°C. The growth of *M. furfur* was decreased with the increase of culture and essential oil concentration of *P. koraiensis* seed. When 20 μL of essential oil was used, the growth of *M. furfur* was increased with the increase of culture by 18 hr and the viable cell number was 10.7×10^5 CFU/mL after 24 hr of culture. When 40 and 60 μL of essential oil were used, the growth of *M. furfur* was strongly affected. Especially, at 24 hr of culture using 60 μL of essential oil, the viable cell number was decreased from 8.3×10^5 CFU/mL which was about 31.2% of decrease compared to control (without essential oil addition). These results indicate that the essential oil extracted from *P. koraiensis* seed, which have mild antimicrobial properties, can inhibit the growth of other pathogens related to acne.

4. CONCLUSION

The author identified 23 chemical compositions in essential oil from *P. koraiensis* seed by GC-MS. The essential oil had inhibitory activity against pathogens related to acne. In particular, the essential oil showed stronger inhibitory activity against *M. furfur.* Based on the results, the essential oil of *P. koraiensis* seed can be used as natural cosmetic or antimicrobial substances. We are investigating a relationship between the chemical structures of the main ingredients of essential oil and their antimicrobial activities.

Acknowledgements

This study Wonkwang Health Science University 2014 Conference has been done by the application fee.

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