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Antimicrobial activity of Korean camellia mistletoe (*Korthalsella japonica* (Thunb.) Engl.) extracts

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ABSTRACT

Mistletoes are evergreen and semi parasitic plant growing on a variety of trees and shrubs. They have been used in the treatment and management of many diseases for many years, both traditional and complementary medicine such as diarrhea, cough, diabetes, hypertension and cancer. The purpose of this study was to determine the antimicrobial activity of Korean camellia mistletoe (*Korthalsella japonica* (Thunb.) Engl.) depending on the harvest time (August and November 2015) and solvent (100% methanol and 70% ethanol). Antimicrobial activity of methanol and ethanol extracts was subject to screening against two gram positive (*S. epidermidis* and *B. subtilis subsps.*) and negative bacillus (*K. pneumonia* and *E. coli*) by using disc diffusion method and measured by the size of clear zone (diameter, mm). The results showed that methanol extract was slight effective in 15 mg/disc concentration on all strains regardless of harvest time, while ethanol extract has no certain antimicrobial effect on both gram positive and negative bacillus. The results obtained in this study suggested that the extracts have low potential for use in the treatment of diseases caused by these test organisms and futher studies are necessary to evaluate the anti-bacterial efficiency of Korean camellia mistletoe against different bacterial strains.

INTRODUCTION

Plants and their secondary metabolites possess antimicrobial, antifungal or antiviral activities. The various plant products, that are regularly used for their therapeutic potential, and plant or plant products that form the part of the food or as dietary components, have been receiving considerable attention (Cowan, 2009). However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection, pharmaceutical and food processing (Balandrin *et al.*, 1985; Cowan, 2009). Furthermore, nowadays most of the antibiotics and food preservatives are obtained by various synthetic processes (Reiner, 1984). Mistletoe is general term for woody shoot parasites in several plant families that also known as medical plant.

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The typical farmer or gardener sees mistletoes as notorious and devastating parasites which pose serious losses toeconomically-valuable fruit trees and medicinal plants whether growing in wild forests, gardens or orchards. Very often, host trees that have lots of mistletoes suffer from them as the triumph of mistletoes lead to poor growth and productivity and eventual death of such host plants. However in the other side, mistletoe is known as one of medical plants used in traditional/alternative medicine in Korea and other countries such as in treatment for cough, diabetes, hypertension, cancer, diuretic, smallpox, ulcer, skin infection and after child-birth (Ishizu *et al*., 2002; Kim *et al*., 2016; Osabede *et al.*, 2004). Since mistletoe is a semi-parasitic plant, it is suggests that their bioactivities could also depend on their host plant. *Camellia mistletoe*, *Korthalsella japonica* (Thunb.) Engl., is one of the Korean mistletoes species which is distributed in Jeju island, Korea. Although there are a large number of studies about bioactivity of different mistletoe species, the antibacterial activity of *Camellia mistletoe* was not found. In the present study, extracts of camellia mistletoe obtained with different harvest time and solvents were investigated for *in vitro* antibacterial activity.

MATERIALS AND METHOD

Plant material and extraction

Korean camellia mistletoe (*Korthalsella japonica* (Thunb.) Engl.) was collected in August and November of 2015, respectively, on Jeju Island, Korea. Korean camellia mistletoe was thoroughly rinse with tap and distilled water and was air-dried at room temperature. The air-dried mistletoe was grounded into powder using a blender. Dried powder (20 g) of camellia mistletoe was extracted with 100 mL of 100% methanol or 70% ethanol at 25ºC for 72 h with constant shaking. The extract was then purified by using a Sep-Pak C_{18} cartridge and a 0.45 µm membrane filter (Waters, Milford, MA, US), concentrated using a rotary evaporator (Buchi Rotavapor R-200, New Castle, DE, US), freeze dried and finally stored at -20ºC until further use. Dried extracts were reconstituted in dimethyl sulfoxide (DMSO, Amresco, Solon, Ohio, USA). DMSO acts as a solvent without changing any property of the methanol and ethanol extracts.

Growth and maintenance of test microorganisms for antimicrobial studies

Four strains were chosen for investigation of which one was reference bacteria: Gram positive (*S. epidermidis* and *B. subtilis subsps.*) and Gram-negative (*K. pneumonia* and *E. coli*) organisms were stored at -70°C and fresh subcultures were used for each experiment. *S. epidermidis*, *B. subtilis subsps.*, *K. pneumonia* and *E. coli*were grown under aerobic conditions in 1 L nutrient broth (1% polypeptone, 0.2% yeast extract, and 0.1% $MgSO_4$, pH 7.0) at 37°C.

Preparation of Inoculum

The test microorganisms were pre-cultured in nutrient broth overnight in a rotatory shaker at 37 °C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water. The cell density was monitored turbidimetrically at 600 nm. The fungal inoculum (*S. Epidermidis*, *B. subtilis subsps*, *K. pneumonia* and *E. coli*) was prepared from the culture grown on agar-medium containing 1.5% agarose and the conidia were scraped using sterile spatula.

Evaluation of antimicrobial activity

The test microorganisms were seeded into respective medium by spread plate method 250µL with the overnight cultures of bacteria growth in nutrient broth. The *in vitro* antibacterial activity of the methanol and ethanol extracts of Korean camellia mistletoe was carried out by disc agar diffusion method (Elgayyar *et al*., 2001) with modification. Briefly, first a loop of each standard strain culture media was cultured on the plates, and then paper discs (from Advantec filter with 8 mm diameter) placed on agar-medium, plates were saturated with 10-40 µL of the test compound and control allowed to dry and was introduced on the upper layer of the seeded agar plate with 15 mg/disc concentrations, were prepared in distilled water and was treated with methanol and ethanol extracts of Korean camellia mistletoe and placed in culture medium by sterile loop. Then it was fixed on the media with a light little pressure. After 12-18 h, the diameter of free zone was measured exactly by using a ruler in millimeters. The effects were compared with that of the standard antibiotic ampicillin (10 µg/disc) and vehicle (DMSO) alone served as control. All experiments were performed with 3 replicates.

Statistical analysis

All the assays were carried out in triplicates. The experimental results were expressed as mean ± SD. The data were analysis using one way analysis of variance (ANOVA) using SPSS for Windows 12.0 (SPSS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSION

The antibacterial activity of Korean camellia mistletoe extracts was assayed *in vitro* by agar disc diffusion against 4 bacterial species. Table 1 summarizes the microbial growth inhibition of both methanol and ethanol extracts of the Korean camellia mistletoe. The methanol extracts showed slight activity only against both gram positive and negative bacterial species, while the ethanol extracts on all the test organisms did not show any clear zone around the disc indicating no antimicrobial activity (Figures 1-4). On the contrary, 10 µg of ampicillin (positive control) showed wide zones of inhibition on all the test organisms which is incomparable to the 15 mg/disc concentration of the plant extracts. DMSO negative control shows no zone of inhibition (Figures 1-4).

Table 1: Antimicrobial effects of methanol and ethanol extracts of 15 mg/disc Korean camellia mistletoe (*Korthalsella japonica* (Thunb.)Engl.) harvested in August and November of 2015, on *S. epidermidis*, *B. subtilis subsps.*,*K. pneumonia* and *E. Coli*.

Microorganism	Degree of inhibition[a]				
	August		November		
	Methanol extract	Ethanol extract	Methanol extract	Ethanol extract	Ampicillin[b]
S. epidermidis	+	-	+	-	++
B. subtilis subsps.	+	-	+	-	++
K. pneumonia	+	-	+	-	++
E. coli	+	-	+	-	++

[a]Rating scale: - = No inhibition; + = Trace; ++=Inhibition.
[b]Positive control.

Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent (Marjorie, 1999). Methanol extracts obtained in this study might have higher solubility for more of active antimicrobial phytoconstituents compared with ethanol extracts, consequently displaying the relative antimicrobial activity (Figures 1-4). It is known that the content of bioactive compounds in plant materials varies greatly with, for example processing, genotype, harvest time, growing environment and conditions (Karami *et al*., 2013).

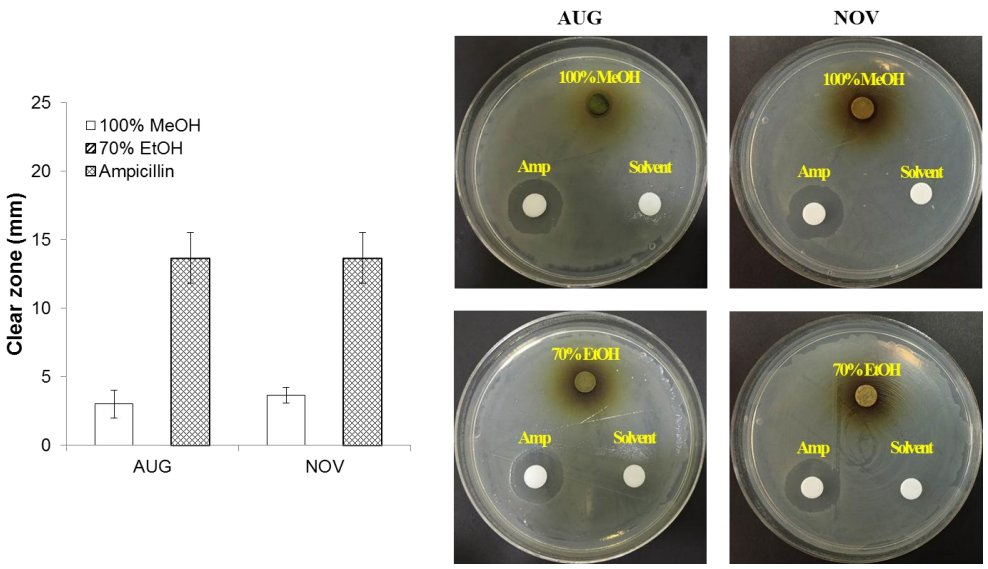

Fig. 1: Average diameter (mm) of microbial free zone area of methanol and ethanol extracts of Korean camellia mistletoe (*Korthalsella japonica* (Thunb.) Engl.) harvested in August and November of 2015, on *S. epidermidis.* Each value is expressed as mean ± S.D. (n = 3).

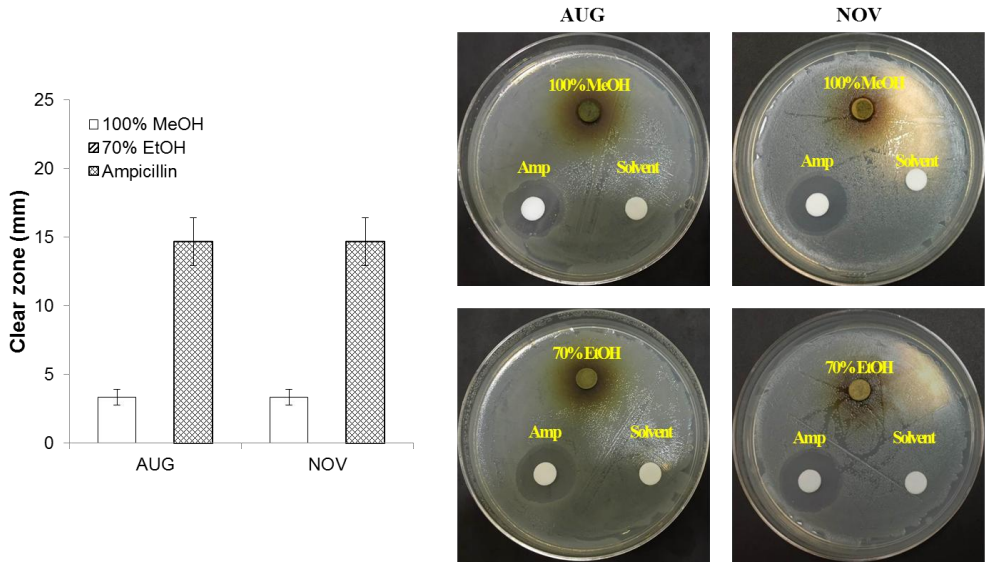

Fig. 2: Average diameter (mm) of microbial free zone area of methanol and ethanol extracts of Korean camellia mistletoe (*Korthalsella japonica* (Thunb.) Engl.) harvested in August and November of 2015, on *B. subtilis subsps.* Each value is expressed as mean ± S.D. (n = 3).

In the present study, there were no significant differences between antimicrobial activity of the Korean camellia mistletoe harvested at August and November (Figures 1-4). These results may suggest that the concentrations of the active constituent in the extracts are too low for any appreciable antibacterial activity. It is also possible that the plant showed low or no inhibitory potential on test microorganisms only. Plants remain the most common source of antimicrobial agents (Bibitha *et al*., 2002; Maghrani *et al*., 2005). Many aromatic plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast (Hulin *et al*., 1998).

Biologically active compounds from natural sources have always been a great interest for scientists working in infectious diseases. The potential for developing antimicrobial from higher plants appears rewarding as it will result to the development of a phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Further studies will be needed to purify the bioactive compounds of the methanol extracts of Korean camellia mistletoe and characterize their phytochemical mode of action.

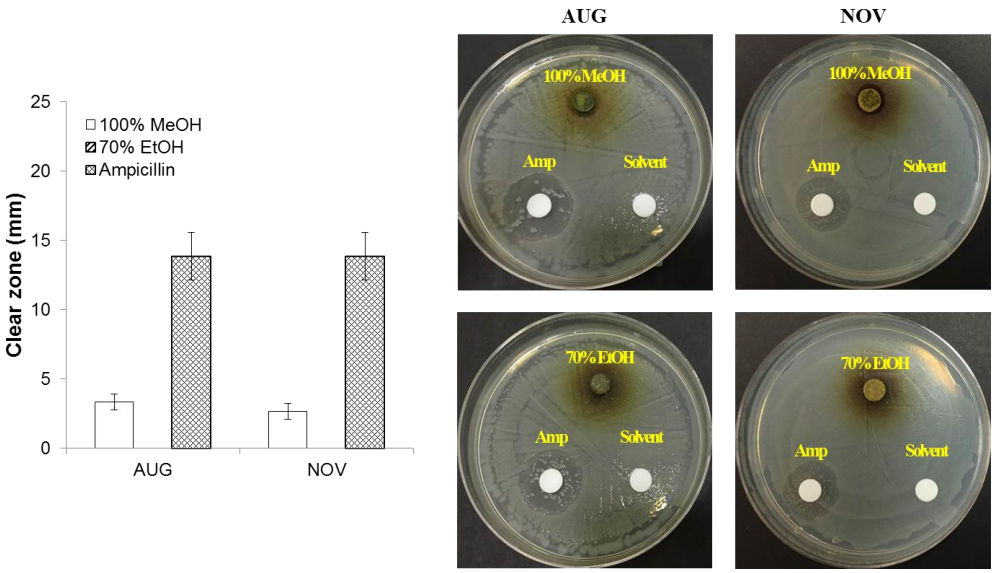

Fig. 3: Average diameter (mm) of microbial free zone area of methanol and ethanol extracts of Korean camellia mistletoe (*Korthalsella japonica* (Thunb.) Engl.) harvested in August and November of 2015, on *K. pneumonia*. *Each* value is expressed as mean ± S.D. (n = 3).

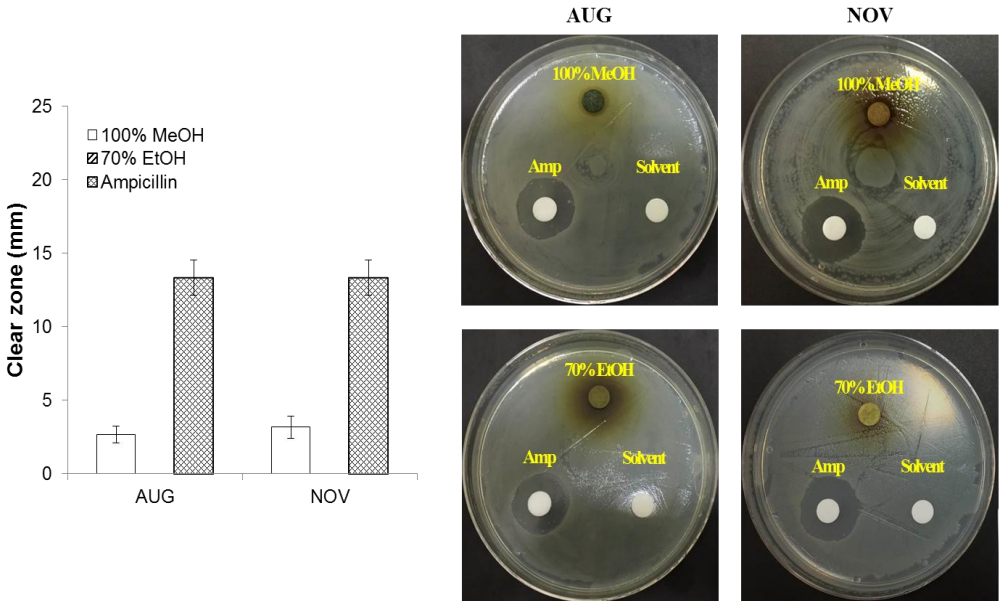

Fig. 4: Average diameter (mm) of microbial free zone area of methanol and ethanol extracts of Korean camellia mistletoe (*Korthalsella japonica* (Thunb.) Engl.) harvested in August and November of 2015, on *E. coli*. Each value is expressed as mean ± S.D. (n = 3).

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Conflict of Interests: There are no conflicts of interest.

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