

Screening of Leaves of Higher Plants for Antibacterial Action

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Abstract—The methanol and benzene extracts of the leaves of 55 higher plants in Korea were tested for their antibacterial activity against three Gram positive bacteria, *Streptococcus mutans*, *Staphylococcus aureus* and *Bacillus subtilis*, and one Gram negative bacterium *Escherichia coli*. Among them, the methanol extract of the leaves of *Liriodendron tulipifera* showed remarkably potent antibacterial activity against both Gram positive and negative bacteria.

Keywords—*Liriodendron tulipifera* • antibacterial activity • *Streptococcus mutans* • *Staphylococcus aureus* • *Bacillus subtilis* • *Escherichia coli* • paper disk method

Natural products still provide an important source of the world's pharmaceuticals and folkloric medicines. According to Philipson,¹⁾ only 5 to 10% of the world's plant resources have been evaluated for pharmacological activity. Especially, the studies on antimicrobial activities of the extracts or the components of plants growing or cultivating in Korea were reported,²⁻⁹⁾ but systematic investigations have not been carried out. For the purpose of developing of antimicrobial agents, we have attempted firstly the screening tests on the leaves of 55 higher plants in Korea. The present investigations were solely restricted to the evaluation of the antibacterial activity being carried out by means of *in vitro* assays, utilizing three Gram positive and one Gram negative bacteria.

Experimental

Preparation of Extracts

For the methanol and benzene extracts, 3 g of dried leaves of each sample was extracted

with 30 ml methanol or benzene for 2 hours, refluxing in 60° water bath, respectively. The hot extract was filtered and concentrated. The resulting extract was prepared for a test solution (10 mg/ml).

Test Organisms

Gram positive bacteria: *Streptococcus mutans* ATCC OMZ 176, *Staphylococcus aureus* 57, *Bacillus subtilis* ATCC 6633

Gram negative bacterium: *Escherichia coli* NIHJ-JC2

Antibacterial Activity

Each strain was cultured in liquid Brain Heart Infusion (BHI, BBL Co.) broth at 37° overnight prior to testing, and subcultured again for 6 hours. The turbidity of bacterial cell suspensions was adjusted with the same sterile broth to 0.07 optical density (OD) unit at 550 nm and then used for the tests. For the paper disk method, 0.6 ml of the bacterial cell suspensions was poured uniformly into the plate made of BHI as media. The paper disks containing 100 µg of methanol or benzene extracts were carefully

45 <i>Gnaphalium affine</i>	—	—	—	—	—	—	—	—
46 <i>Inula helenium</i>	—	—	—	—	—	—	—	—
47 <i>Xanthium strumarium</i>	—	—	+	‡	—	—	—	+
48 <i>Eupatorium fortune</i>	—	—	—	—	+	+	—	+
49 <i>Ligularia fischeri</i>	—	—	—	—	—	—	—	—
50 <i>Syneilesis palmata</i>	+	+	+	+	+	+	+	+
51 <i>Achillea sibirica</i>	—	—	—	—	+	—	—	+
52 <i>Siegesbekia glabrescens</i>	—	—	—	—	+	+	—	+
53 <i>Bidens bipinnata</i>	—	—	—	—	—	—	—	—
54 <i>Ixeris dentata</i>	+	—	—	+	+	—	—	—
55 <i>Chrysanthemum sibiricum</i>	—	—	—	—	+	+	—	+

The antibacterial activity is represented as follows: —, no inhibitory zone was formed by adding 100 μg of methanol or benzene extracts of each samples per filter paper disk; +, inhibitory zone was formed with 8.5~11.0 mm in diameter; †, 11.1~13.0 mm; ‡, 13.1~15.0 mm; ‡‡, 15.1~17.0 mm in diameter.

placed on the seeded Petri dishes. The cultivation was carried out at 37° overnight. Filter paper was 8 mm in diameter (Toyo Co.).

Results and Discussion

According to the paper disk method,¹⁰⁾ the antibacterial activity of the methanol or benzene extracts are represented on the basis of the following criterion, an inhibitory zone below 8.5

mm in diameter is negative in antibacterial action and that over 8.5 mm positive. The latter is further represented by four grades based on their activity. Table 1 shows antibacterial activity of the leaves of 55 higher plants against three Gram positive and one negative bacteria. The antibacterial activity of the methanol and benzene extracts shows generally same pattern.

Among 55 species, the methanol extract of the leaves of *Liriodendron tulipifera*, Magnoliaceae, shows the most potent antibacterial activity and its inhibitory zones were 16.5, 14.0, 16.0 and 15.2 mm against *S. mutans*, *S. aureus*, *B. subtilis* and *E. coli*, respectively (Table 1 and Fig. 1). The benzene extract of *L. tulipifera* also shows a potent activity and its inhibitory zones were 12.0, 14.0, 12.0 and 9.0 mm in diameter against *S. mutans*, *S. aureus*, *B. subtilis* and *E. coli*, respectively. The methanol and benzene extracts of the leaves of *Magnolia kobus*, *M. obovata*, *Acanthopanax sieboldianum* and *Syneilesis palmata* show the activity against the strains. The methanol extract of *Xanthium strumarium* shows a potent activity against a Gram negative bacterium, *E. coli*. Twenty six species including *Convallaria keiskei* have activities against certain strains but have not an activity against the other strains. No inhibitory zone was formed in 23 species including *Dryopteris crassirhizoma*.

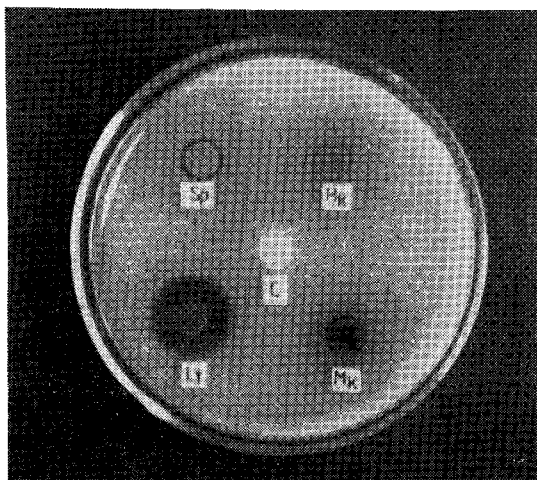


Fig. 1. Antibacterial activities of methanol extracts against a cariogenic bacterium *Streptococcus mutans*. Each disk contains 100 μg methanol extract.

C: Control, Lt: *Liriodendron tulipifera*, Sp: *Syneilesis palmata*, Bk: *Broussonetia kazinoki*, Mk: *Magnolia kobus*

The methanol extract of the leaves of *L. tulipifera* has been fractionated into petroleum ether, ether, chloroform and water fractions for the isolation of active components, and the ether fraction has been clarified to be the most active one. The active components shall be reported in the later paper. We are also checking on the seasonal variation of active components for reasonable collecting period of sample.

As reported in the previous paper,¹¹⁻¹²⁾ magnolol and honokiol had been identified as the main components of antibacterial activity, isolated from the stem bark of *Magnolia obovata* Thunb., Magnoliaceae. The compounds are found in the flower, stem, leaf and root of *M. obovata* and *M. kobus*,¹³⁾ but the amount of two compounds in the leaves are supposed to be less than the stem from comparing of the antibacterial activity of methanol extracts.

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