

## Extracts of *Oxalis triangularis* shows broad spectrum antibacterial activity in acidic condition.

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### Abstract

Antimicrobial effects of various plants have been widely used to prevent diseases. Antibacterial activity in the extracts of *Oxalis triangularis* against five Gram-positive bacteria and three Gram-negative bacteria was investigated. The extracts of *O. triangularis* were tested the antibacterial activity against several Gram (+) and Gram (-) bacteria. The antibacterial activity was tested in the heat-treated extracts from leaves of *O. triangularis* as well as in the extracts of the various pH by NaOH addition. The extracts from leaves, stems, roots, and buds of *O. triangularis* showed broad spectrum antibacterial activity against all bacteria tested including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium*. The heat-treated extracts from leaves of *O. triangularis* showed the similar antibacterial activity. However, the antibacterial activity dramatically disappeared by pH change of the *Oxalis* extracts. These results indicated that the broad spectrum antibacterial activity in *O. triangularis* is from water soluble, heat-stable substances active only in acidic pH condition. Therefore, the antibacterial substances would be oxalic acid or oxalate compounds which are abundant in *Oxalis* family plants.

**Keywords:** Antibacterial activity, *Oxalis triangularis*, Oxalic acid, pH, Food-born pathogen.

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### Introduction

Penicillin was discovered by Sir Alexander Fleming in the study of influenza virus in *Staphylococcus aureus* by accident that the environment surrounding the blue fungus (*Penicillium chrysogenum*) in the incubator was aseptic [1]. Hans has developed a special staining method [2] and divided bacteria into two groups which were differed in their cell wall structure later.

Several food-born pathogenic bacteria are well identified [3-9]. Among them *Staphylococcus aureus* is Gram-positive (+) bacteria and causes serious illness including osteomyelitis, meningitis and neuralgia, vomiting, diarrhea in humans and animals [3]. *Staphylococcus aureus* is infected mainly via nose and respiratory system caused food poisoning. Other severe food-born Gram (+) bacteria is *Bacillus cereus* which is large bacilli which arranged in the form of a chain [4]. Because it can cause food poisoning by forming strong spores on heat, it is necessary to sterilize by high temperature. An environment in which the bacterium can grow well and when it is above 10<sup>6</sup> CFU/g, it causes diarrhea and vomiting. One of the severe food-born Gram-negative (-) bacteria is *Salmonella typhimurium* which is rod-shaped bacteria and infects mainly in the digestive tract of people and warm-blooded animals causing typhoid fever and enteritis [5]. Recently, problems of pathogenic *Escherichia coli* have been reported [6]. *E. coli*

discovered in 1885 by Theodore is easily reproduced, genetically comparatively simple and easy to manipulate [7]. Therefore, *E. coli* is the most studied prokaryotes and is greatly contributed to biotechnological progress. Most *E. coli* strains are not harmful often seen in the intestines of warm-blooded animals and benefits to the human body for vitamin K generation [8]. Some pathogenic *E. coli* cause food poisoning including urethral infection caused by elderly people or patients who have lowered immunity after surgery and traveller's diarrhea [9]. One of the well-studied pathogenic *E. coli* is *E. coli* O157:H7 which produces shiga-like toxins [6].

*Oxalis triangularis* is a perennial grass native in tropical Mexico and an ornamental plant in purple color which can be decorated for salads or foods. It is resistant to severe environment of high temperature and draught due to bulbous roots [10]. *Oxalis corniculata*, so called cat eaten plant in Korea, is different in color in green and smaller size compared to *O. triangularis*. The organic compound oxalic acid is isolated by François, Switzerland in 1773 from the wood-sorrel including plant *O. corniculata* and its crystal structure is revealed recently [11]. The oxalic acid is highly acidic which its pKa is 1.25 and 4.14 because it contains two carboxylate moieties [12]. Some plants such as parsley, chives, purslane, cassava as well as wood-sorrel (*oxalis*) contain high concentration of oxalic acid over 1% [13]. The *oxalis*

substitution over banana leaves has largely eliminated the food poisoning of bongkreng in Indonesia because the *oxalis* leaves contribute to decrease of pH which prevents bacterial overgrowth [14]. The kidney stone is usually caused by calcium oxalate, one of the common oxalate compounds [15]. Color-pigmented plants such as carrot, pepper, paprika etc. harbor rich antioxidants and vitamins which help health to maintain a harmonious diet and a healthy lifestyle.

In this research, from some ethnobotanical evidences of *oxalis* plants treated in local traditional medicine, antimicrobial activity against various pathogenic bacteria was studied in the extracts of *O. triangularis* in heat-treatment or various pH value. The antimicrobial substance of *O. triangularis* was not previously suggested to oxalic acid or oxalate compounds which are heat-stable and affected by pH.

## Materials and Method

### Plant samples

The plant samples of pepper, garlic, blueberry, ginseng, paprika, ginger, cinnamon, rosemary and *O. triangularis* were purchased from local market in Jeju, Korea. The *O. triangularis* was grown in the plant growth chamber at 25°C with a diurnal cycle of 12 h light/12 h darkness [16].

### Antimicrobial activity in plant extracts

Eight bacteria [(*S. aureus* (KCTC 1621), *B. cereus* (KCTC 1021), *B. sphaericus* (KCTC 1185), *S. pyogenes* (KCTC 3096), *S. mutans* (KCTC 3065), *E. coli* (KCTC 1682), *S. typhimurium* (KCTC 2057) and *E. aerogenes* (KCTC 2190)] were ordered from Korean Collection for Type Cultures (KCTC) and inoculated in LB broth (Sigma-Aldrich, Germany) and incubated at 37°C for 24 h. The bacteria were spread out on the LB agar (Sigma-Aldrich, Germany) evenly using triangular glass rod sterilized by flame. The plant samples of pepper, garlic, blueberry, ginseng, paprika, *O. triangularis*, ginger, rosemary and cinnamon in a -80°C freezer were crushed and the liquid of supernatant from the crushed samples was extracted after centrifuge at 6,000 rpm for 5 min. The leaves, stems, roots, and buds from *O. triangularis* were divided by blade and the extracts were collected after centrifuge at 6,000 rpm for 5 min. Antibacterial activity of each extract was tested by disk diffusion assay that the extracts were dropped on the bacterial lawn after spreading followed by drying [17,18]. Antibiotics such as ampicillin (Sigma-Aldrich, Germany, 25 µg/ml) or kanamycin (Sigma-Aldrich, Germany, 25 µg/ml) were used as positive controls. The diameter of the clear inhibition zone was measured after 24 h incubation.

### Heat treatment for antibacterial substances

The extracts of mashed *O. triangularis* leaves have been prepared with distilled water and divided in two tubes, and then one tube was heated with boiling water bath at 100°C for 1 min [19]. The antibacterial activity of the heat treated extracts was assayed as the non-treated extract as a control.

### Antibacterial assay on neutralization reaction

The 200 µl extracts of *O. triangularis* leaves have been prepared with distilled water and divided into eight tubes, and then added 0 µl, 1 µl, 2 µl, 3 µl, 5 µl, 10 µl, and 20 µl of 10 N NaOH solution (Sigma-Aldrich, Germany) to each tube. The 20 µl of the NaOH-treated extract was dropped to the medium containing the target bacteria and incubated for 24 h [19]. The 20 µl of a solutions made by mixing 0 µl, 1 µl, 2 µl, 3 µl, 5 µl, 10 µl, and 20 µl of 10 N NaOH and 200 µl of water were dropped into each medium cultured by bacteria as negative controls.

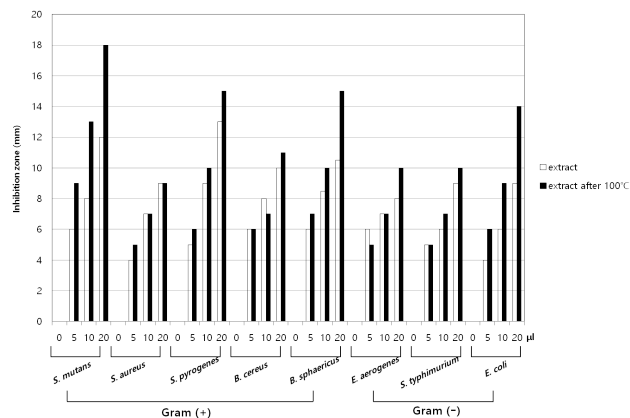
## Results and Discussion

The antimicrobial activity was shown in the extracts of garlic and *O. triangularis* among pepper, garlic, blueberry, ginseng, paprika, *O. triangularis*, ginger, rosemary and cinnamon against *E. coli*, *S. mutans* and *S. aureus*. The antimicrobial activity of *O. triangularis* was further investigated in detail because the activity of garlic is well documented but not in *O. triangularis* [20].

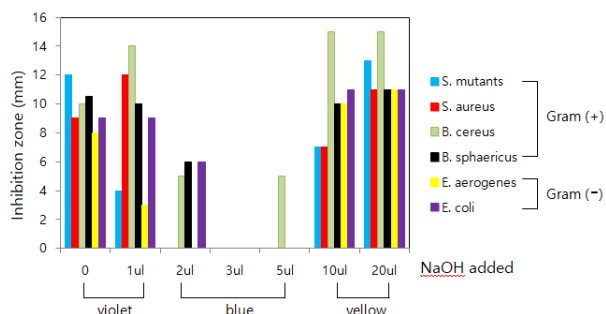
The extracts of *O. triangularis* from leaves showed the broad spectrum antimicrobial activity against Gram (+) bacteria such as *S. aureus*, *B. cereus*, *B. sphaericus*, *S. pyogenes*, *S. mutans* and Gram (-) bacteria such as *E. coli*, *S. typhimurium*, *E. aerogenes* (Figure 1). It is interesting that the results differ from that of *O. corniculata* which the extracts of *O. corniculata* showed no antibacterial activity against *S. aureus* [21]. However, other report with methanolic extract of *O. corniculata* showed antimicrobial activity against *S. aureus* and various *Shigella* strains [22]. The extracts of *O. triangularis* from stems, roots, or buds showed the similar antimicrobial activity against Gram (+) and Gram (-) bacteria.

To elucidate the nature of antibacterial substances, the antimicrobial activity was measured after 100°C heat treatment or ethanol extraction and compared to the water extraction without heat treatment. The heat-treated extracts of *O. triangularis* leaves showed similar broad spectrum antimicrobial activity and seemed to show stronger antibacterial activity against the target Gram (+) and Gram (-) bacteria compared to that of no heat treatment (Figure 1). It is supposed that the antibacterial substances in the extract were concentrated by the heat treatment due to reduction in total volume and were not in the nature of protein which is easily denaturated by heat. The ethanol extracts of *O. triangularis* leaves showed the similar antimicrobial activity against the target bacteria. It is suggested that the antibacterial substances are dissolved in ethanol as well as water. Therefore, the nature of the antibacterial substances would be assumed to be oxalic acid or oxalate compounds which are abundant in *Oxalis* family plants [23]. To confirm the hypothesis, the experiment for antibacterial assay using neutralization reaction was designed and performed. The extracts of *O. triangularis* leaves in violet color (acidic pH) gradually turned to blue (approx. neutral pH) and further to yellow (in basic pH) as NaOH added. It seems that this color change is due to anthocyanins

abundant in *O. triangularis* [24]. The antibacterial activities in the extracts of *O. triangularis* leaves were showed in the extracts in violet color with the acidity of below pH 3.7. However, the extracts in blue color showed dramatically decreased antibacterial activities against the target Gram (+) and Gram (-) bacteria (Figure 2). The antibacterial activities were re-appeared in the extracts in yellow color. The fact that the antibacterial activity comes from the nature of NaOH added was confirmed from the control experiment which the activity was measured in the water added different volume of NaOH.



**Figure 1.** Antibacterial activity in the water extracts of *Oxalis* leaves after heat treatment. The diameter (mm) of the inhibition zone was graphed in the extracts (open square) and the extracts after 100°C treatment (closed square) over the extracts added (µl).



**Figure 2.** Antibacterial activity of *Oxalis* extracts on neutralization reaction. The color of *O. triangularis* extracts by NaOH addition was designated on the x-axis. The target bacteria are shown in colors in *S. mutans* (blue), *S. aureus* (red), *B. cereus* (green), *B. sphaericus* (black), *E. aerogenes* (yellow) and *E. coli* (violet), respectively.

These results indicated that the extracts of *O. triangularis* show broad spectrum antibacterial activity in acidic pH. The further experiment with pure oxalic acid was performed to confirm the hypothesis that the substances of the antibacterial activity would be oxalic acid or oxalate compounds. The antibacterial activity was shown in the concentration of pure oxalic acid with above 71 mM.

It is not excluded that other biologically active compounds in bactericidal activity rather than oxalic acid may be existed in the extracts of *O. triangularis* plants. The previous report

revealed that the isolated antibacterial substances in the extracts of *O. corniculata* were 5-hydroxy-6, 7, 8, 4-tetramethoxyflavone and 5, 7, 4'-trihydroxy-6, 8-dimethoxyflavone [21]. It could be predicted that the concentration and pH effects by oxalic acid or oxalate salts would be affected to other biological activities such as antifungal or insecticidal effects [21]. The other viewpoint in the plant physiology is how the *Oxalis* plant cells maintain and resist in such a strong acidity. Based on the discovery of the natural antimicrobial effect derived from plants against both Gram (+) and Gram (-) bacteria, it can be used for prevention and treatment of various food poisoning, tooth decay or livestock or crop diseases caused by pathogenic bacteria, It is also applied to reduction of odor in piggery, barn or henhouse.

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