# Evaluation of Antimicrobial Activities of Rhubarb Extracts on Putrefactive Microorganisms Related to Soybean Curd (Doobu)

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# 두부 부패 미생물에 대한 대황(Rhubarb) 추출물의 항균 활성 평가

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## 국문요약

대황(Rhubarb)은 중국과 일본에서 혈액 순환제, 진통제, 신장치료제 등으로 전통적으로 사용되고 있는 약용식물이다. 이 연구에서는 한국산 *R. undulatum* L.의 뿌리 추출물, 미국산 *R. rhabarbarum* L.과 중국산 *R. palmatum* L.의 줄기 추출물의 두부 부패에 관여하는 미생물에 대한 항균 활성을 평가하였다. Rhubarb의 최적 추출을 위해서 각각 50%, 70%, 80% 에탄올을 용매로 사용하였으며, 항균 활성은 Kirby-Bauert test, minimum inhibitory concentration (MIC)과 minimum bactericide concentration (MBC)에 의해서 평가되었다. Kirby-Bauert test 결과, *R. undulatum* L.의 뿌리 추출물은 대부분 두부 부패균에 대해서 항균 활성을 가지는 것으로 나타났으며, *R. rhabarbarum* L.의 줄기 추출물의 경우는 20$\mu$g/disc의 농도에서는 항균 활성이 거의 나타나질 않았으나, 고농도로 갈수록 높은 항균 활성을 나타내었다. 또한 *R. palmatum* L.의 줄기 추출물은 고농도로 갈수록 항균 활성이 높아졌으나, *Pseudomonas aeruginosa*에 관해서는 항균 활성이 나타나지 않았다. MIC와 MBC에 의한 항균 활성 평가실험에서는, 세 종류의 rhubarb 추출물 중에서 phenolic 화합물이 가장 많이 함유되어 있는 *R. undulatum* L.의 뿌리 추출물보다 *R. rhabarbarum* L.의 줄기 추출물이 MIC와 MBC 값이 낮았다. 이는 phenolic 화합물의 양 이외에 낮은 pH가 항균 활성에 영향을 준 것으로 판단된다. 본 연구를 통해서 rhubarb 추출물은 두부 부패에 관여하는 미생물에 대해서 항균 활성을 가지는 것으로 나타났으며, 천연 항균제로써 식품에 적용 가능성이 있는 것으로 평가되었다.

**Key Words** : 항균 활성, 대황추출물, 두부, phenolic compounds, MIC, MBC

## I. INTRODUCTION

Recently, antimicrobial activity has been reported for phenolics from several sources, such as fruits and vegetables, including apples, coffee beans, grapes, potatoes, prunes, and tea leaves, medicinal plants, and spices (Baron *et al.* 1997; Friedman 1977; Groffoths & Bain 1977; Lathia & Frentzen 1980; Lu & Foo 1999; Spanos *et al.* 1990).

Phenolic compounds, one of the widely distributed plant constituents, have been used as antimicrobial agents in an array of products, including food, paint, leather, metal working fluids, textiles, and pertroleum either (Marouchoc 1979). Antimicrobial activity of phenolic compounds including ferulic acid, tea catechins, oleuroein, ellagic acid, and *p*-coumaric acid to inhibit the growth of bacteria such as *Salmonella enteritidis, Staphylococcus aureus, and Listeria monocytogenes*, and fungi has been reported in several studies (Payne *et al.* 1989; Rosenthal *et al.* 1977; Schaller *et al.* 2000; Tassou & Nychas 1944; Tassou & Nychas 1995). Especially, the substitution of synthetic food additives by naturally derived antimicrobial agent is considered desirable, although the safety of natural and alternative additives must be assessed prior to utilization (Hattori *et al.* 1998).

Rhubarb belongs to the family of Polygonaceae. It is an important medicinal origin plant which has been used as Chinese and Japanese traditional herbal constitutional medicine. Rhubarb is well known to have pharmacological activities, such as purgation, analgesic effects, curing mental and renal disorders, antibiosis, antitumor and antimutagenicity (Matsuda *et al.* 2001).

Unexceptionally, there are so many variations of rhubarb differed in origin or quality and there must be difference in the concentration of active components or pharmacological

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activity according to varieties. Especially, the rhizome of *Rheum undulatum* L., which has been cultivated in Korea, is used as a remedy for the blood stagnation syndrome (Oketsu syndrome in Japanese) as well as a purgative agent. This rhubarb is considered to have a lesser purgative effect but more potent effect on the blood stagnation syndrome than other rhubarbs such as *R. palmatum* (Matsuda *et al.* 2001).

Meanwhile, Doobu (Soybean curd, Tofu) is very liable to be putrefactive because it has a high protein and high moisture content and near neutral pH. Therefore, Doobu has been linked to several food-borne bacteria (Tuiemwong & Fungdmfh 1991; Ashraf *et al.* 1999). A number of studies reported that microorganisms, such as *Acinetobacter calcoaceticus; Klebsiella pneumoniae, Bacillus cereus,* and *Escherichia coli* and so on, were related to putrefaction of Doobu (Joo *et al.* 1998; Shin *et al.* 1992).

Therefore, it was the purpose of this article to evaluate antimicrobial activities of extracts from stalks of *Rheum rhabarbarum* L. and *Rheum palmatum* L., and roots of *Rheum undulatum* L. against microorganisms related to putrefaction of Doobu and to try deciding concentration of the above extracts required to inhibit growth of microorganisms for the possible use as a food additive.

## II. MATERIAL AND METHOD

### 1. Materials

#### 1) Plant materials and chemicals

The fresh roots of *Rheum undulatum* L. were purchased, which was cultivated in 2002 in Uisung, Kyongbuk, Korea. The fresh stalks of *Rheum rhabarbarum* L. (U.S.A) and the dried stalks of *Rheum palmatum* L. (China) were provided by "S" Chemical Company. Then, the fresh stalks and roots of rhubarb deposited in a freezer at -20°C, and then immediately lyophilized in Freeze Dry System (Model 77530-13, Labconco Co., Kansas City, MO, U.S.A.) for the following experiments. HPLC grade solvents, such as methanol, ethanol, and water, were purchased from Duksan Pure Chemical Company (Ansan, Korea), and chemical reagents from Sigma Co. (St. Louis, MO, USA), unless otherwise stated.

#### 2) Preparation of the extract of rhubarbs

The freeze-dried and powdered stalks of *R. rhabarbarum* L. and *R. palmatum* L., and roots of *R. undulatum* L. were extracted by using the method described by Julkunen-Titto (1985) and Kähkönen *et al.*(1999) with some modification. Each sample (5 g) was defatted with hexane (3×24 hr) at room temperature and subsequently extracted with 50% of aqueous ethanol for *R. rhabarbarum* L., 70% for *R. palmatum* L., and 80% for *R. undulatum* L. (3×24 hr) at room temperature (Kim & Suh 2005). The solutions were filtered with Whatman No.1 filter paper and centrifuged for 10 min at 1500×g and then the clear supernatants were evaporated to a volume of about 10 mL under vacuum at 45 °C. These concentrated samples were lyophilized to powder forms and stored in a freezer at -20°C.

#### 3) Test microorganisms and cultures

*Acinetobacter calcoaceticus* (IMSNU 10305T), *Klebsiella pneumoniae group pneumoniae* (IMSNU 10104), *Enterobacter coloaccae* (IMSUN 10070), *Salmonella typhimurium* (IMSUN 10251), and *Photorhabdus luminescens* (KCCM 40878) were purchased from Microbial Resources Center (Seoul, Korea) and Korea Culture Center of Microorganisms (Seoul, Korea). *Bacillus cereus* (KACC 10097), *Pseudomonas putida* (KACC 10192), *Pseudomonas aeruginosa* (KACC 10186), *Listeria monocytogenes* (KACC 10550), and *Escherichia coli* (KACC 10005) were provided by Korean Agricultural Culture Collection (Suwon, Korea). Nutrient agar and Nutrient broth were purchased from Difco (Sparks, MI., U.S.A.). Tryptone soya broth and Mueller Hinton agar and broth were purchased from Oxoid Ltd (Basingstone, Hampshire, England). Trypicase soy agar was purchased from BBL (Cockeysville, MD., U.S.A.).

### 2. Determination of antimicrobial activity of rhubarb extracts by Kirby-Bauert test

Antimicrobial tests were measured by a method as Kirby-Bauer test described by Mau *et al.*(2001). Each culture was streaked onto a Trypicase soy agar or Nutrient agar to obtain the isolated colonies and incubated at 35°C for 24 hr. The 4 or 5 well-isolated colonies were selected with an inoculating loop and transferred into a tube of a sterile saline for growth. The bacterial suspension was compared to the 0.5 McFarland standards against a sheet of white paper. The swab was streaked over the entire surface of the medium three times after adjusting the turbidity of the inoculums suspension in 15 min. The extracted amounts of 0.05 to 0.75 g from stalks of *R. rhabarbarum* L., stalks of *R. palmatum* L., and roots of *R. undulatum* L. were dissolved in 50 mL of distilled water. Distilled water was used as control. As a standard, penicillin G antibiotic absorbed disks (10 units) were used. Sterile filter paper (Whatman No. 1, diameter = 6mm) was impregnated

with the individual extract (20 μL) and placed in the center of the agar plate and the prepared agar plate were incubated at 35°C for 16 hr.

### 3. Determination of the minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) by microdilution assay

Antimicrobial activity was evaluated according to the method described by Aligiannis *et al.*(2001) and Cruz *et al.*(2001). The selected microorganisms were incubated for 24 hr under favorable conditions for proliferation in media. Then, the concentrated extracts were added to sterilized tubes containing Mueller Hinton medium (MHm) to reach final concentrations of $6\times10^4$, $3\times10^4$, $1.5\times10^4$, $7.5\times10^3$, $3.75\times10^3$, and $1.875\times103$ μg of extract/mL. The strain cultures in MHm were diluted to give a final concentration in the test tube of ~ $10^8$ colony forming units (CFU $\times10^8$/mL). The bacterial suspension was compared to the 0.5 McFarland standards. The tubes treated with each sample in various amounts were incubated at 37°C and were checked for the presence of turbidity after 24 hr. MIC was determined as the lowest extract concentration at the tubes in which no growth was observed when spotted on to MHm agar plates. MBC corresponded to the lowest extract concentration at which fewer than five colonies were obtained.

## III. RESULTS AND DISCUSSION

### 1. Evaluation of antimicrobial effect of rhubarb extracts by Kirby-Bauert test

In previous studies, it was reported that the phenolic compounds present in extracts of medicinal plants inhibited the growth of bacteria by prolonging the lag phase and the concentration of phenols was related to inhibiting growth of bacteria (Estrada-Munoz *et al.* 1988; Sunen 1988). Rhubarb extracts were tested for their antimicrobial activity against the selected ten strains of microorganisms and compared among them.

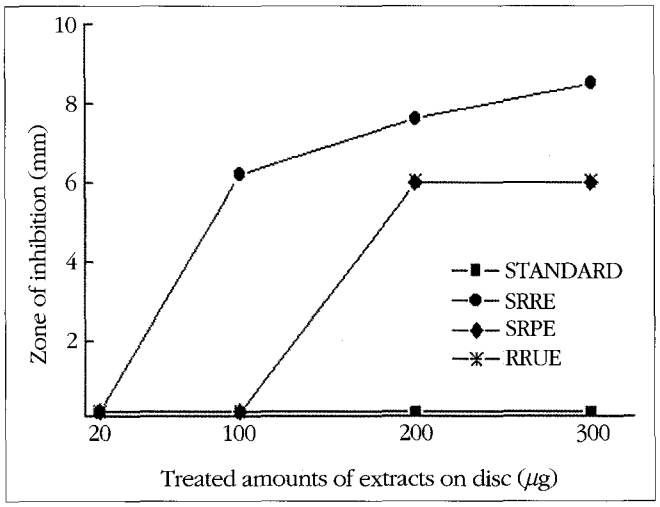
The results by Kirby-Bauer test in this study list in <Table 1>, and <Figure 1 to 4> illustrate activity of extracts of stalks of *R. rhabarbarum* L., extracts of stalks of *R. palmatum* L., and extracts of roots of *R. undulatum* L. against the tested microorganisms. Inhibition zone of 6 and 10 mm are considered as indicators for good and better inhibitory effects, respectively (Mau *et al.* 2001). Extracts (20μg/disc) from stalks of *R. rhabarbarum* L., stalks of *R. palmatum* L., and roots of *R. undulatum* L. possessed extensive inhibitory effect on growth tested microorganisms. Extracts of stalks of *R. rhabarbarum* L. had good inhibition against *Pseudomonas putida* and *Listeria monocytogenes* among tested microorganisms (2/10 good), extracts of stalks of *R. palmatum* L. had good inhibition against *Bacillus cereus, Photorhabdus luminescens, Enterobacter coloacae, Salmonella typhimurium*, and *Escherichia coli*, and better

<Table 1> Antimicrobial Activity of *R. rhabarbarum* L., *R. palmatum* L., and *R. undulatum* L. extracts (20-300 μg/disc) by Kirby-Bauer Test

| Extracts | Microorganisms | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | SRRE[1] | SRPE | RRUE | Standard |
| 20 μg/disc | *Acinetobacter calcoaceticus* | - | -[2] | 9.5 | 15.8 |
| | *Klebsiella pneumoniae* | - | - | - | - |
| | *Bacillus cereus* | - | 6.0 | 8.3 | 9.9 |
| | *Photorhabdus luminescens* | - | 6.5 | 10.7 | 10.8 |
| | *Pseudomonas putida* | 7.8 | 10.0 | 8.5 | - |
| | *Pseudomonas aeruginosa* | - | - | 6.0 | 6.0 |
| | *Enterobacter coloacae* | - | 9.3 | - | - |
| | *Salmonella typhimurium* | - | 6.0 | 9.3 | 13.8 |
| | *Listeria monocytogenes* | 6.0 | - | 9.0 | 19.7 |
| | *Escherichia coli* | - | 7.5 | 9.5 | - |
| 100 μg/disc | *Acinetobacter calcoaceticus* | 9.3 | - | 9.3 | 15.8 |
| | *Klebsiella pneumoniae* | 6.2 | - | - | - |
| | *Bacillus cereus* | 9.0 | 9.7 | 10.3 | 9.9 |
| | *Photorhabdus luminescens* | 8.5 | 9.3 | 11.3 | 10.8 |
| | *Pseudomonas putida* | 11.3 | 14.0 | 9.4 | - |
| | *Pseudomonas aeruginosa* | 10.0 | - | 9.8 | 6.0 |
| | *Enterobacter coloacae* | 7.3 | 9.0 | - | - |
| | *Salmonella typhimurium* | 7.8 | 10.7 | 10.3 | 13.8 |
| | *Listeria monocytogenes* | 7.0 | 8.8 | 8.8 | 19.7 |
| | *Escherichia coli* | 7.9 | 8.3 | 9.8 | - |
| 200 μg/disc | *Acinetobacter calcoaceticus* | 8.0 | 13.0 | 9.7 | 15.8 |
| | *Klebsiella pneumoniae* | 7.6 | 6.0 | 6.0 | - |
| | *Bacillus cereus* | 10.5 | 9.3 | 12.0 | 9.9 |
| | *Photorhabdus luminescens* | 11.8 | 11.9 | 12.6 | 10.8 |
| | *Pseudomonas putida* | 10.8 | 14.3 | 11.5 | - |
| | *Pseudomonas aeruginosa* | 10.9 | - | 10.5 | 6.0 |
| | *Enterobacter coloacae* | 9.5 | 9.7 | 10.5 | - |
| | *Salmonella typhimurium* | 9.3 | 13.0 | 10.7 | 13.8 |
| | *Listeria monocytogenes* | 10.4 | 11.0 | 13.3 | 19.7 |
| | *Escherichia coli* | 8.7 | 11.0 | 9.3 | - |
| 300 μg/disc | *Acinetobacter calcoaceticus* | 8.2 | 12.3 | 9.8 | 15.8 |
| | *Klebsiella pneumoniae* | 8.5 | 6.0 | 6.0 | - |
| | *Bacillus cereus* | 9.5 | 10.3 | 14.5 | 9.9 |
| | *Photorhabdus luminescens* | 13.7 | 11.5 | 15.5 | 10.8 |
| | *Pseudomonas putida* | 8.0 | 14.0 | 14.3 | - |
| | *Pseudomonas aeruginosa* | 11.8 | - | 12.0 | 6.0 |
| | *Enterobacter coloacae* | 9.1 | 9.7 | 11.3 | - |
| | *Salmonella typhimurium* | 10.8 | 13.3 | 10.3 | 13.8 |
| | *Listeria monocytogenes* | 12.0 | 11.3 | 17.7 | 19.7 |
| | *Escherichia coli* | 9.0 | 11.2 | 9.7 | - |

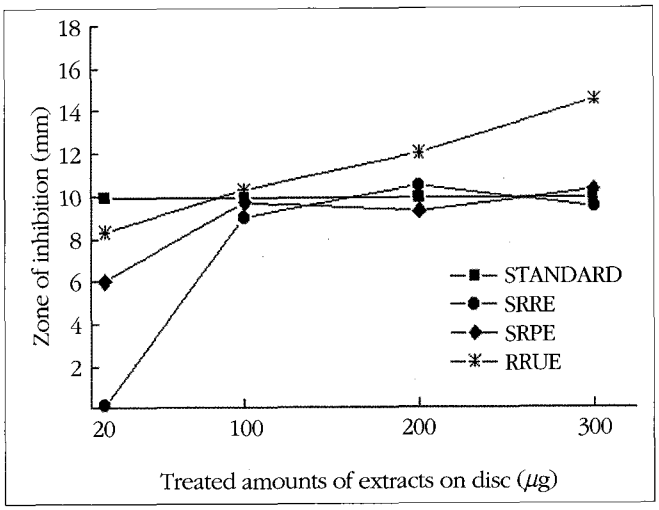
[1] SRRE: stalks of *R. rhabarbarum* L.; SRPE: stalks of *R. palmatum* L.; RRUE: roots of *R. undulatum* L.; Standard: penicillin G (10 units) was used as a standard antibiotic disc (Oxoid).

[2] (-): indicates no inhibition zone; the numbers in the Table indicate the diameters of inhibition zones (mm).



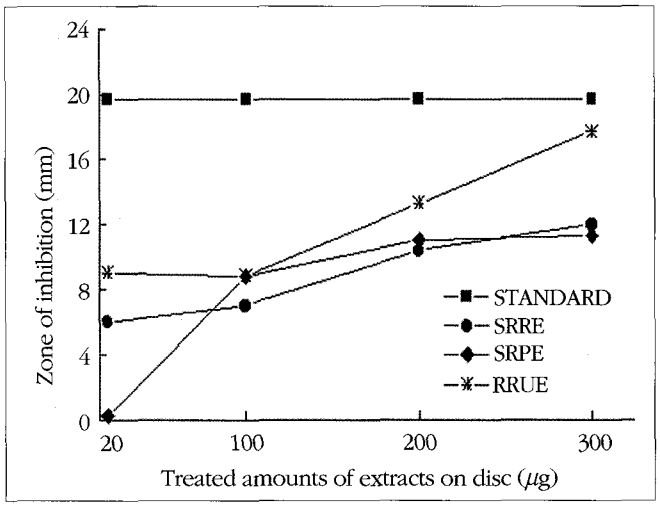

<Figure 1> Antimicrobial activity of ethanol extracts from stalks of *R. rhabarbarum* L., *R. palmatum* L., and roots *R. undulatum* L. against *Klebsiella pneumoniae*

(SRRE, stalks of *R. rhabarbarum* L.; SRPE, stalks of *R. palmatum* L.; RRUE, roots of *R. undulatum* L. and Standard: penicillin G (10 units)).



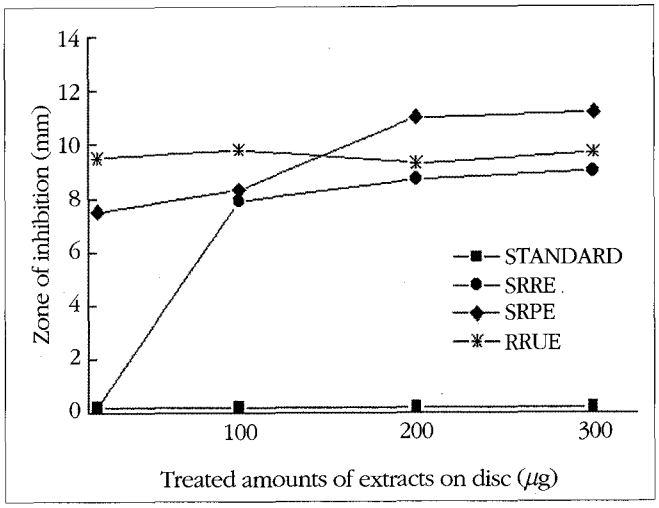

<Figure 2> Antimicrobial activity of ethanol extracts from stalks of *R. rhabarbarum* L., *R. palmatum* L., and roots of *R. undulatum* L. against *Bacillus cereus*

(SRRE, stalks of *R. rhabarbarum* L.; SRPE, stalks of *R. palmatum* L.; RRUE, roots of *R. undulatum* L. and Standard: penicillin G (10 units)).




<Figure 3> Antimicrobial activity of ethanol extracts from stalks of *R. rhabarbarum* L., *R. palmatum* L., and roots of *R. undulatum* L. against *Listeria monocytogenes*

(SRRE, stalks of *R. rhabarbarum* L.; SRPE, stalks of *R. palmatum* L.; RRUE, roots of *R. undulatum* L. and Standard: penicillin G (10 units)).




<Figure 4> Antimicrobial activity of ethanol extracts from stalks of *R. rhabarbarum* L., *R. palmatum* L., and roots of *R. undulatum* L. against *Escherichia coli*

(SRRE, stalks of *R. rhabarbarum* L.; SRPE, stalks of *R. palmatum* L.; RRUE, roots of *R. undulatum* L. and Standard: penicillin G (10 units)).

inhibition against *Pseudomonas putida* (5/10 good, 1/10 better), and extracts of roots of *R. undulatum* L. had good inhibition against *Ancinetobacter calcoaceticus, Bacillus cereus, Pseudomonas putida, Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes* and *Escherichia coli*, and better inhibition against *Photorhabdus luminescens* (7/10 good, 1/10 better). One hundred micrograms per disc (100 μg/disc) of extracts of stalks of *R. rhabarbarum* L. had significant antimicrobial activity even though 20 μg/disc of extracts of stalks of *R. rhabarbarum* L. did not have prominent antimicrobial activity. One hundred micrograms per disc (100 μg/disc) of extracts of stalks of *R. palmatum* L. also had antimicrobial activity enhanced against *Listeria monocytogenes* comparing with 20 μg/disc of extracts of stalks of *R. palmatum* L. and was more effective on *Pseudomonas putida* and *Enterobacter coloacae* than other rhubarbs extracts. However, extracts of stalks of *R. palmatum* L. still indicated no inhibition zone against *Acinetobacter calcoaceticus, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Moreover, extracts of roots of *R. undulatum* L. reported as containing sufficient phenolic

compounds among the tested samples had overall significant antimicrobial activity except *Klebsiella pneumoniae* and *Enterobacter coloacae* (Kim & Suh 2005). Over 200 μg/disc of each extract had overall better inhibition against most of microorganisms tested. Especially, each extract had more significant antimicrobial activity against *Klebsiella pneumoniae, Photorhabdus luminescens, Pseudomonas putida, Enterobacter coloacae,* and *Escherichia coli* comparing with penicillin G used as standard. However, extracts of stalks of *R. palmatum* L. did not have antimicrobial activity against *Pseudomonas aeruginosa* although using 300 μg/disc of extract.

## 2. Evaluation of the minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) by microdilution assay

All extracts tested in this study showed both inhibitory and bactericidal activities. MIC values of microorganisms tested with extracts of stalks of *R. rhabarbarum* L., extracts of stalks of *R. palmatum* L. and extracts of roots of *R. undulatum* L. were shown in <Table 2>. The highest inhibitory action on microbial growth corresponded to extracts of stalks of *R. rhabarbarum* L. with MIC values ranging from 7.5 mg/mL-15 mg/mL, in comparison with extracts of stalks of *R. palmatum* L. (10.5 mg/mL-50 mg/mL) and extracts of roots of *R. undulatum* L. (15-20 mg/mL). Extracts of stalks of *R. rhabarbarum* L. were proved that it had the maximum inhibitory activity against *Acinetobacter calcoaceticus, Bacillus cereus, Photorhabdus luminescens, Pseudomonas putida,* and *Listeria monocytogenes,* and they were the most sensitive microorganisms toward the assayed extracts. *Klebsiella pneumoniae* and *Bacillus cereus* were also the most sensitive against extracts of stalks of *R. palmatum* L. Even though the total phenolic content of extracts of roots of *R. undulatum* L. was the highest among samples, MIC value of extracts of stalks of *R. rhabarbarum* L. was higher than extracts of stalks of *R. rhabarbarum* L. or extracts of roots of *R. undulatum* L. These results supposed to have more inhibitory action on microorganisms by low pH of extracts of stalks of *R. rhabarbarum* L (Kim & Suh 2005). Moreover, the stability of the phenolic compounds strongly depends not only on the pH and storage time but also on the structure of the phenolic compound (Friedman & Jrugens 2000). In this study, MBC values determined were higher than those required to inhibit growth as shown in <Table 3>.

<Table 2> MIC values of *R. rhabarbarum* L., *R. palmatum* L., and *R. undulatum* L. extracts against microorganisms tested in microdilution assay

| Microorganisms | MIC[1] (mg/mL) | | | |
|---|---|---|---|---|
| | SRRE[2] | SRPE | RRUE | Standard |
| *Acinetobacter calcoaceticus* | 7.5 | 12.5 | 15.0 | NT[3] |
| *Klebsiella pneumoniae* | 15.0 | 10.5 | 50.0 | $7.4\times10^{-3}$ |
| *Bacillus cereus* | 7.5 | 10.5 | 50.0 | NT |
| *Photorhabdus luminescens* | 7.5 | 30.0 | 15.0 | NT |
| *Pseudomonas putida* | 7.5 | 50.0 | 25.0 | NT |
| *Pseudomonas aeruginosa* | 10.0 | 50.0 | 25.0 | $8.6\times10^{-3}$ |
| *Enterobacter coloacae* | 15.0 | 50.0 | 25.0 | $8\times10^{-3}$ |
| *Salmonella typhimurium* | 12.5 | 40.0 | 20.0 | NT |
| *Listeria monocytogenes* | 7.5 | 50.0 | 25.0 | NT |
| *Escherichia coli* | 12.5 | 40.0 | 20.0 | $9.8\times10^{-3}$ |

[1] MIC: minimum inhibitory concentration.
[2] SRRE: stalks of *R. rhabarbarum* L.; SRPE: stalks of *R. palmatum* L.; RRUE: roots of *R. undulatum* L., extracts and Standard: Netilmicin (30μg).
[3] NT: not tested.

<Table 3> MBC values of *R. rhabarbarum* L., *R. palmatum* L., and *R. undulatum* L. extracts against microorganisms tested in microdilution assay

| Microorganisms | MIC[1] (mg/mL) | | | |
|---|---|---|---|---|
| | SRRE[2] | SRPE | RRUE | Standard |
| *Acinetobacter calcoaceticus* | 30 | 15 | 20 | NT[3] |
| *Klebsiella pneumoniae* | 15 | 20 | 60 | $7.4\times10^{-3}$ |
| *Bacillus cereus* | 22.5 | 15 | 60 | NT |
| *Photorhabdus luminescens* | 30 | 40 | 20 | NT |
| *Pseudomonas putida* | 25 | >60 | 30 | NT |
| *Pseudomonas aeruginosa* | 25 | >60 | 30 | $8.6\times10^{-3}$ |
| *Enterobacter coloacae* | 30 | >60 | 30 | $8\times10^{-3}$ |
| *Salmonella typhimurium* | 50 | 50 | 25 | NT |
| *Listeria monocytogenes* | 30 | >60 | 30 | NT |
| *Escherichia coli* | 30 | 50 | 25 | $9.8\times10^{-3}$ |

[1] MBC: minimum bactericide concentration.
[2] SRRE: stalks of *R. rhabarbarum* L.; SRPE: stalks of *R. palmatum* L.; RRUE: roots of *R. undulatum* L., extracts and standard: Netilmicin (30μg).
[3] NT: not tested.

# IV. CONCLUSION

The results of antimicrobial activity by Kirby-Bauer test indicate that extracts of stalks of R. rabarbarum L. over 100 μg/disc had significant antimicrobial activity while extracts of stalks of R. rabarbarum L. under 20 μg/disc did not have prominent antimicrobial activity except *Pseudomonas putida* and *Listeria monocytogenes*. Extracts of stalks of *R. palmatum* L. were more effective on *Pseudomonas putida* and *Enterobacter coloacae* than other extracts in low concentration. However, extracts of stalk of *R. palmatum* L. had no antimicrobial activity against *Pseudomonas aeruginosa* even in high concentration. Extracts of roots of *R. undulatum* L. containing sufficient

phenolic compounds among the tested samples had significant overall antimicrobial activity, but 100 μg/disc of extracts of roots of *R. undulatum* L. had no antimicrobial activity against *Klebsiella pneumoniae* and *Enterobacter coloacae*. Over 200 μg/disc of each extract had overall better inhibition against most of microorganism except *Pseudomonas aeruginosa*. Especially, each extract had more significant antimicrobial activity against *Klebsiella pneumoniae, Photorhabdus luminescens, Pseudomonas putida, Enterobacter coloacae*, and *Escherichia coli* than penicillin G used as standard.

All extracts tested in this study showed both inhibitory and bactericidal activities. Extracts of stalks of *R. rhabarbarum* L. were shown to have the more maximum inhibitory activity although there is some difference according to a variety of microorganism. Especially, extracts of stalks of *R. rhabarbarum* L. was proved that it had the maximum inhibitory activity against *Acinetobacter calcoaceticus, Bacillus cereus, Photorhabdus luminescens, Pseudomonas putida*, and *Listeria monocytogenes*. Even though the total phenolic content of extracts of roots of *R. undulatum* L. was the highest among samples, MIC value of extracts of stalks of *R. rhabarbarum* L. was higher than extracts of stalks of *R. rhabarbarum* L. or extracts of roots of *R. undulatum* L. These results supposed to have more inhibitory action on microorganisms by low pH of extracts of stalks of *R. rhabarbarum* L.

The results indicate that rhubarb extracts containing phenolic compounds can be used as an antimicrobial agent to extend shelf-life of Doobu and especially, roots of *R. undulatum* L. cultivated in Korea have significant antimicrobial activity against microorganism tested in this study. Further research will be done which step might be applied the rhubarb extracts during Doobu processing. However, the safety of natural and alternative additives must be assessed prior to application toward food.

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