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Original Research Article

Antibiotic Effect of Leaf, Stem, and Root Extracts in *Smallanthus sonchifolius* H. Robinson

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Abstract - This study was conducted with the aim of evaluating the antibiotic effects of leaves, stems, and roots in yacon (*Smallanthus sonchifolius*). Antibacterial activity of leaf extract by disk diffusion method with *Bacillus subtilis* and *Escherichia coli* respectively showed 13.3 and 13.75mm diameters of clear zone. There was no significant difference between the stems and leaves. The minimum inhibitory concentration of leaves' heating and agitation extraction showed a restrain of strain at 1mg/ml, but the stems and root extract did not appear. Yacon is a functional antibacterial material, and methanol extraction is more effective than water. This research was to investigate the growth stage of collection has the most effective antibacterial effects. It has collected yacon's leaves from June to October, which is an appropriate time for collection right before reaping. Yacon leaf has antibacterial effects on *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecium*, and *Salmonella enteritifis*. There were no significant differences by the growth stage of collection. Leaves collected in July are high in phenol which helps in sulfating activity works well considering the high scavenging capability of DPPH. Leaves collected in September are high in total flavonoid.

Key words - Antibiotic effect, Leaf, *Smallanthus sonchifolius* H. Robinson, Yacon

Introduction

Recently, yacon has become more popular for its many benefits. As a result, related research has been increasing, along with its utility value as food resource (Kim, 2012). Yacon is a perennial bulbous crop that belongs to Asteraceae. The yacon's native habitat is Ecuador and Peru in South America and it was delivered in Korea at an agricultural experimental station in 1985. The leaves grow big in size with an arrowhead shape and saw shape edge. The stem grows until 1.5-2m showing green or purple color with many hairs and it transforms to a hollow in maturation period. The flower is yellow and blooms in late October. The root of yacon is similar to sweet potato, and as it grows, 1-6kg tuberous roots form per week. Though they are similar in shape, sweet potatoes have starch which yacon doesn't have; the latter also has a crunchy texture like pear.

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The distinctive effect of yacon contained in roots called fructo-oligosaccharides is not being achieved in the body and relieving constipation (Chen *et al*., 2000). Yacon is well-known to decrease blood lipid and blood glucose, to prevent diabetes, and to give benefit in a diabete patient's food (Yang and Tsai, 1993). Fructose can be used as a natural sweetener, and the substance slows intestinal absorption to prevent obesity and arteriosclerosis (Kim, 2005). According to the study of fermented *Chrysanthemum* extract (Hyeon, 2010), yacon has high polyphenol and flavonoid contents that bring antioxidant effects and no inhibition effect. Yacon is effective in addressing cell destruction caused by free radical, inflammation-related disease, and cardiovascular disease. By virtue of its various effects, yacon is broadly used as a health tea product (Shin *et al*., 2015; 2007). Yacon has high feasibility as a natural food additive due to its various beneficial components. The study of its stem is more insufficient than its leaves and roots, and its stem that grows to 1.5~2.0 m is actually thrown away. This study is conducted to carry out the possibility of a yacon becoming a natural food preservative,

which is an alternative to chemical food preservatives.

In addition, upon referring to the *Chrysanthemum*'s antioxidant substances and bioactive research (Woo, 2008), it was found that the moisture content of the plant and extraction yield declines as the harvest was delayed. The result of the research shows that the transition of content depends on the growth period. According to the research on the antimicrobial effect of the leaves, stems, and roots (Park, 2014), the root and stem had less antibacterial effects but the leaves showed meaningful result. By this research, this study also conducted an experiment by using leaves. Not only antibacterial effect, the study also investigated identifying the diversity of yacon by knowing the difference in antioxidant activity based on growth period.

Materials and Methods

Plant material and microorganisms

The yacon leaves, stems, and bulbs were studied at the Sunchon National University farm beginning April 30, 2015. The leaves were collected on October 23, 2015 and the stems and roots on November 5, 2015. The following strains were used in this experiment: gram-positive bacteria (1 species) and gram-negative bacteria (1 species). For the growing media of bacteria, nutrient broth (Difco, USA) was used. The other yacon leaves for the experiment are from the Sunchon National University farm (June to October of 2016) and they were collected once in about 30 days.

Extraction methods and media

Agitated extraction and heat extraction were used to extract the samples. Extraction solvents were water and methanol and extracted by three replicated samplings. Agitated extraction was made by stirring 10 g of the extract sample and 100 ㎖ of the solvent mixture for 24 hours at room temperature. Extracts by heating were made at boiling point for 30 minutes. The collected extract was filtered (Whatman No. 2), vacuum evaporated it in 50℃ by vacuum rotary evaporator (N-1100, Eyella, Japan), and it was kept in cold storage at 4℃. The sample was diluted in a consistency of 50 ㎎/㎖ by DMSO (Junsei, Japan) and was kept in cold storage at 4℃. The strain used in yacon's antimicrobial experiment was pre-cultivated in medium of a nutrient broth for 18 hours and activated strain was mainly incubated for 5 hours.

The agitated extraction was used to extract the other samples. Extraction solvents were water and methanol and extracted by three replicated samplings. Agitated extraction was made by stirring 10 g of the extract sample and 100 ㎖ of solvent mixture for 24 hours at room temperature. Extracts by heating were made at boiling point for 30 minutes. For the extract of the sample, agitated extraction used methanol and distilled water. Ten grams of sample and 100 ㎖ of methanol and distilled water were mixed by agitated extraction for 24 hours at room temperature. This was repeated three times. The collected extract was filtered (Whatman No. 2) and vacuum evaporated by a rotary vacuum evaporator (N-1100, Eyella, Japan) at 50℃. The sample was diluted in a consistency of 50 ㎎/㎖ by DMSO (Junsei, Japan) and was kept in cold storage at 4℃. $MgCl_2$ and $6H_2O$ 10 ㎎/L and $CaCl_2$ and $2H_2O$ 20 ㎎/L were filtered using Sterilized Mueller Hinton Brothin and were manufactured by mixture.

Analysis of antibiotic effect

Manufacture of plate culture for antibacterial effect research was made by division per 20 ㎖ of Nutrient agar (Difco, USA) in petri dish and coagulation. Five hour-main-cultivated strain (Table 1) was adjusted to turbidity by MC standard 0.5 (1×10^8 CFU/㎖) and 100 ㎕ smear was taken with sterilized swabs. The strain was fixed with 6 ㎜ paper disk, and extract was injected per 20 ㎕ that was diluted to 50 ㎎/㎖. Petri dish was incubated in 35℃ for 18 hours with the diameter (㎜) measured in clear zone.

The tube method stated in CLSI (2012) was carried out in the experiment. The sample was prepared in 5 concentration stages by diluting the sample in DMSO and mixed with

Table 1. List of strains used for experiments

	Stains	Stain number
Gram (+)	*Bacillus subtilis*	KCCM 11730
	Pseudomona aeruginosa	CCARM 2202
	Staphylococcus aureus	CCARM 3A048
	Enterococcus faecium	CCARM 5202
Gram (-)	*Salmonella enteritidis*	KCCM 12021
	Escherichia coli	KCCM 41427

nutrient broth medium. This 1 ㎎/㎖ of the mixed sample is double-diluted and made into 5 stages of concentration until 0.0612 ㎎/㎖. The used strain was adjusted to turbidity by MC standard 0.5 (1×10^8 CFU/㎖) and used after dilution to 1×10^6 CFU/㎖. The 1 ㎖ of diluted strain and 1 ㎖ of sample was injected to 13×100 ㎜ size tube and it was incubated in 35℃ incubator for 18 hours. After cultivation, the increased strain was visible and the minimum inhibition concentration was determined where the concentration was not turbid.

Another yacon antimicrobial experiment was pre-cultivation in a medium of nutrient broth for 18 hours and activated strain was incubated for 4 hours using disk diffusion method. The manufacture of plate culture for antibacterial effect research was made by a division per 20 ㎖ of nutrient agar (Difco, USA) in petri dish and coagulation. 4 hours-main-cultivated strain was adjusted to turbidity by 0.5 MC standard and smeared with sterilized swabs. The strain was fixed with 8 ㎜ paper disk and 1.6 ㎎/disk of the sample was injected. It was incubated in the incubator of 37℃ for 18hours and the diameter (㎜) was measured in clear zone. The tube method stated in CLSI (2012) was carried out in the experiment. The sample was prepared in five concentration stages and the sample was mixed with the manufactured CAMHB medium. This 1 ㎎/㎖ of the mixed sample is double-diluted and made to 5 stages of concentration until 0.032 ㎎/㎖. The used strain was adjusted to turbidity by MC farmland standard 0.5 (1×10^8 CFU/ml) and diluted to 1×10^6 CFU/㎖. The 1 ㎖ of diluted strain and 1 ㎖ of sample was injected to 13×100 ㎜ size tube and incubated in 37 ℃ incubator for 18 hours. After cultivation, the increased strain was visible and the minimum inhibition concentration where the concentration was not turbid could be determined.

This test was conducted to determine the active antioxidant in the total polyphenol content by Folin-Dennis law. The sample was made by mixture of 0.1 g of sample and 20 ㎖ of MeOH extracted for 24 hours in a shaking bath. The extracted sample was under centrifugation for 10 minutes in 13, 000 rpm. For each 1 ㎖ of supernatant and 3 ㎖ of distilled water, 1 ㎖ of Folin and Dennis regent was put on conical tubes and mixed. 1 ㎖ of saturated sodium carbonate was added and left for 1 hour at room temperature. After then, 1 ㎖ was moved to micro tube and measured optical density with a spectrophotometer by 640 ㎚. This process was repeated 3 times. Control group measured the Ferulic acid concentration with a total of 5 ㎖ in 100 ppm, 75 ppm, 50 ppm, 25 ppm, 1 ppm, and 0 ppm. To investigate the antioxidant ability, radical elimination method was done by DPPH. The sample was made by 0.1 g of sample and 20 ㎖ of MeOH extracted in shaking bath for 24 hours. The extracted sample was under centrifugation for 10 minutes in 1, 3000 rpm.

Results and Discussion

Disk diffusion

In the result (Figs. 1 and 2) pertaining to the antimicrobial activities of water and methanol extracts of yacon leaf, stem, and root against *Bacillus subtilis* and *Escherichia coli* revealed that there were no significant differences. No meaningful difference was found between *Bacillus subtilis* and *Escherichia coli*. The distilled water extract of yacon's leaves, stem, and root did not have antibacterial effect. There was a large clear zone in yacon's leaves in both *Bacillus subtilis* and *Escherichia coli* and they were better in agitated extraction than in heat extraction. The clear zone of the root

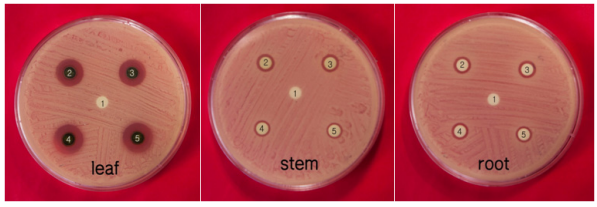

Fig. 1. Antimicrobial activities of methanol extracts of yacon leaf, stem, root against *Bacillus subtilis.* 1: control, 2, 3: 1 ㎎/disk methanol agitation extract, and 4, 5: 1 ㎎/disk methanol heating extract.

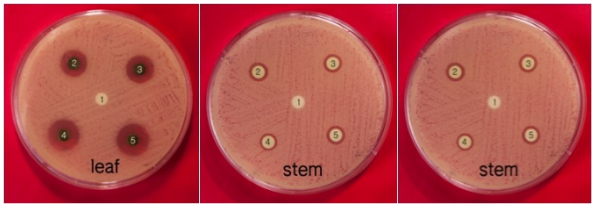

Fig. 2. Antimicrobial activities of methanol extracts of yacon leaf, stem, root against *Escherichia coli subtilis*. 1: control, 2, 3: 1 ㎎/disk methanol agitation extract, and 4, 5: 1 ㎎/disk methanol heating extract.

Table 2. Antimicrobial activities of methanol extracts of yacon leaf

Stains	Clear zone on plate (㎜) ø[z] (Methanol extract)	
	Agitation	Heating
Bacillus subtilis	11.96[b]	13.31[a]
Escherichia coli	11.65[b]	13.76[a]

[z]Diameter of clear zone.
In: 1 ㎎/disk.
*in a row, means followed by a common letters are not significantly different at the 5% level by DMRT.

Table 3. Antimicrobial activities of methanol extracts of yacon stem

Stains	Clear zone on plate (㎜) ø[z] (Methanol extract)	
	Agitation	Heating
Bacillus subtilis	7.81	7.11
Escherichia coli	8.01	7.66

[z]Diameter of clear zone.
In: 1 ㎎/disk.

and stem was smaller than that of the leaves. The above result is the same as that of Kim (2005) which evaluated that yacon K-23 has antifungal activity against food poisoning bacteria and that most of its strain has antifungal activity. Also this result is the same as that of Yun who found that antibacterial effect didn't appear in the moist extract and methanol extract of wasong leaves, stems, and roots (Yoon *et al*., 2009). According to the result of the experiment, yacon has the possibility of whole food preservative. The following are the measure of the diameter in the clear zone.

As for yacon's leaves, the diameter of clear zone is shown in Table 2. In *Bacillus subtilis,* the agitation extraction turned out to be 11.96 ㎜, heat extracts were shown as 13.3 ㎜, and in *Escherichia coli*, the agitation extraction was - 11.65 ㎜, and heat extraction was identified as 13.76 ㎜. The heat extract had a broader clear zone in both *Bacillus subtilis* and *Escherichia coli* heating, rather than the agitation extract. There is significant difference between heat and agitation extraction.

As for the *Bacillus subtilis,* agitation extraction was 7.81 ㎜, heat extracts were shown as 7.11 ㎜ and in *Escherichia coli* the agitation extraction was 8.01 ㎜, and heat extraction was identified as 7.66 ㎜. The agitation extract had a broader clear zone slightly in both *Bacillus subtilis* and *Escherichia coli* and no significant statistical difference (Table 3).

Table 4. Antimicrobial activities of methanol extracts of yacon root

Stains	Clear zone on plate (㎜) ø[z] (Methanol extract)	
	Agitation	Heating
Bacillus subtilis	7.66	7.61
Escherichia coli	7.11	7.11

[z]Diameter of clear zone.
In: 1 ㎎/disk.

As for the *Bacillus subtilis,* agitation extraction was 7.66 ㎜, heat extracts were shown as 7.61 ㎜, and in *Escherichia coli*, the agitation extraction was 7.11 ㎜, and heat extraction was identified as 7.11 ㎜. The agitation and heat extract had a similar size of clear zone in both *Bacillus subtilis* and *Escherichia coli* and there was no meaningful statistical difference (Table 4).

The minimum inhibition concentration

The result of the minimum inhibition concentration of the antibacterial effect appeared only in yacon leaves on both *Bacillus subtilis* and *Escherichia coli*. Also, bacterial effect appeared in concentration of 1 ㎎/㎖ in both agitation and heat extraction.

As for the extract of stem and root, minimum inhibition concentration didn't appear, so growth restraints against strain were judged to be very weak. A similar study by Yoon *et al*. (2009) also mentioned that the minimum inhibition concentration of extract only appeared on wasong leaves rather than stem and root which didn't show. According to Kang (1994), the antimicrobial activity of extracts of song with cuts in the minimum inhibition concentration appears only in the stem, while there was none in roots and leaves. For natural food preservation, the leaves of yacon can be more effective than its stems and roots.

Based on the result of the minimum inhibition concentration of yacon leaves monthly, the antibacterial effect appeared in every 1.0 ㎎/㎖ concentration of *Bacillus subtilis* and

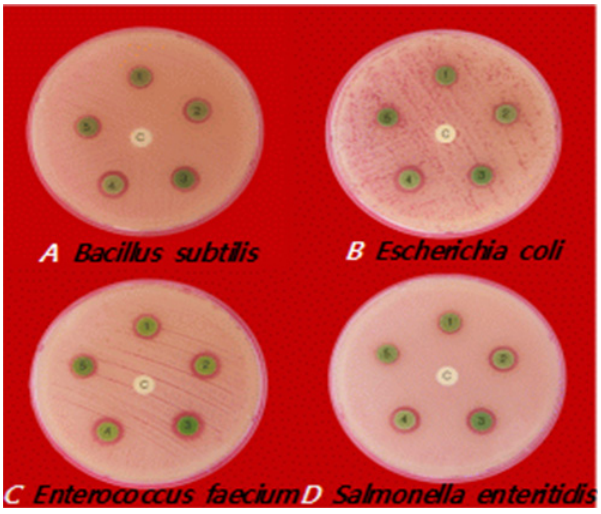

Fig. 3. Antibacterial effect of methanol extract of yacon leaf. C: Control, 1: June, 2: July, 3: August, 4: September, and 5: October.

Escherichia coli, *Enterococcus faecium*, and *Salmonella enteritidis*. As for the *taphylococcus aureus* and *Pseudomona aeruginosa*, minimum inhibition concentration didn't appear, so growth restrains against the strain was judged to be very weak. Showing the effect on four strains in disk diffusion method and minimum inhibition concentration can lead to a conclusion that yacon leaves have antibacterial effect on microbe.

There was a difference in a clear zone's size but the minimum inhibition concentration is shown as similar to 1.0 ㎎/㎖. This means that yacon leaves have no difference in antibacterial effect as regards the growth period. This can be proven by the result of the research name yacon as processed food (Kim, 2010). Yacon's utility value for food depends on the safety research of this study that yacon's jam and can were considered safe in microbe.

Antibacterial effect of the methanol extract of yacon leaf

Comparing the antibacterial effect of yacon's leaves by agitation extraction with methanol and distilled water, distilled water extract was not effective but methanol extracts showed meaningful effect by period. The experiment was made based on this result using methanol extract (Fig. 3).

Among six bacteria, *Bacillus subtilis, Enterococcus faecium, Escherichia coli,* and *Salmonella enteritidis* had antibacterial

Table 5. Antimicrobial activities of methanol extracts of yacon leaf

Stains	Clear zone on plate (㎜) ø[z]				
	6	7	8	9	10
Bacillus subtilis	9.04	9.20	9.35	11.13	9.20
Escherichia coli	8.97	9.12	9.43	11.63	8.97
Enterococcus faecium	9.12	10.05	9.66	10.59	9.20
Salmonella enteritidis	8.70	11.13	11.01	11.21	8.85

[z]Diameter of clear zone.
In: 1 ㎎/disk.

effects which did not appear in other bacteria. The period of the effect was high in July to September especially in September. There was more than 10 ㎜ size of clear zone in four species of bacteria. According to the antibacterial effect of the *Camelia* leaves (Kwon, 2003), there were antibacterial effects in Staphylococcus aureus, the extract of methanol gram-positive bacteria, and *Salmonella typhimurium* and *Escherichia coli*, gram-negative bacteria. In yacon leaves, there were antibacterial effects on *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecium*, and *Salmonella enteritidis (*Table 5).

An antioxidant activity that plant phenolic compounds have is flavonoid and mostly lipid aqueous system or lipid system with a strong anti-oxidation activity. They have various antioxidants based on chemical structure, which are active in various plant materials (Huang, 1992). For the result of leaves' anti-oxidation activity by growth period, the total phenolic content of July was 23.2 ㎎/g DW which was relatively high. The flavonoid content of September was 15.00 ㎎/g DW, and DPPH was 25.14% which were all relatively high (Table 6). The Antioxidant active increased in June and July, decreased in August, increased again in August-September, and decreased in October. This was because of the phenolic compounds it contained, depending on the materials and amount and presenting various aspects according to maturity, etc. (Ho, 1992; Hui, 1991). Regarding the research period, the amount of the compounds was under the influence of the environment and the amount of material can be different. The three contents' amount was different and it depended on the period.

According to the anti-oxidation activity research of chrysanthemum, an Asteraceae same as yacon (Woo, 2008),

Table 6. Total phenolic, total flavonoid contents, DPPH radical at growth stage of yacon leaf

Sample	Sampling period	Total phenolic compound contents (mg/g DW)	Total flavonoid contents (mg/g DW)	DPPH scavenging activity (%)
Yacon leaf	June	20.3^{b*}	10.92^{bc}	22.74^{ab}
	July	23.2^{a}	11.40^{b}	25.14^{a}
	August	17.6^{b}	8.55^{c}	14.68^{c}
	September	18.2^{b}	15.00^{a}	18.62^{bc}
	October	12.1^{b}	10.07^{bc}	15.94^{c}

*Means within a column were analyzed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

the delay of the harvest made the scavenging activity low. However, the anti-oxidation activity of yacon leaves happening apparently in July, polly-phenolic compounds and flavonoid in September, and DPPH in July had a meaningful difference. Anti-oxidation activity was mostly high in July and September so it would be the most effective. Anti-bacterial effect and anti-oxidation activity made commercial sales and value of food utility grow rapidly.

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