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Antibacterial Activity of Chayote (*Sechium edule* Swartz) Squash Extracts and Their Phytochemical Constituents

Nur Indah Sulistiyani[1], Ahmad Ainurrofiq[2], and Venty Suryanti[1,*]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia
[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia

**Corresponding author: venty@mipa.uns.ac.id*

Abstract
Antibacterial activity of chayote (*Sechium edule* Swartz) squash extracts against pathogenic bacteria has been evaluated. Dried chayote squash powder was extracted using methanol which was then extracted successively using hexane, chloroform, ethyl acetate, and butanol. The antibacterial activities of the extracts were tested by the hole diffusion method. Methanol extract of chayote squash had antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* but did not have antibacterial activity against *Enterobacter aerogenes*, *Shigella dysenteriae*, and *Salmonella typhi*. Ethyl acetate extract had the highest antibacterial activity against *P. aeruginosa* and *E. coli*. The ethyl acetate extract contains phenolics, condensed tannins, flavonoids, and terpenoids. The ethyl acetate extract had a MIC of 50 mg/ml for *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*. Different bioactivities of tested extracts were found due to various chemical compositions of extracts as a result of different polarities of the solvent.

Keywords: antibacterial activity, chayote squash, phytochemical constituents, *Sechium edule* Swartz.

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Introduction

Indonesia is a tropical country that is famous for its diversity of plant species. Studies have been conducted on many plants regarding their compounds and bioactivities (1-5). Indonesian people have used medicinal plants in an effort to overcome health problems. Chayote (*Sechium edule* Swartz) is one of the Indonesian medicinal plants from the Cucurbitaceae family. The squash can be consumed as vegetables and sweets. Chayote squash is efficacious in facilitating urination, reducing fever, and reducing high blood pressure (6). Chayote squash contains phenolics, flavonoids, condensed tannins, alkaloids, saponins, vitamin A and amino acids (7-9). Composition of chayote squash per 100 g are water (92.30 g), protein (0.6 g), fat (0.1 g), carbohydrates (6.7 g), Ca (14 mg), P (25 mg), Fe (0.5 mg), vitamin B1 (0.02 mg) and vitamin C (18 mg) (6).

Several plants belonging to the Cucurbitaceae have been studied for their bioactive. Methanol extract of Lagenaria breviflora fruit has antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Pseudomonas aeruginosa* (11). Hexane extract of Coccinia grandis L. leaves has antibacterial activity against *S. aureus, E. coli, P. aeruginosa*, and *Salmonella typhi* (12). Methanol extract of *Momordica charantia* L. has antibacterial activity against *Enterobacter aerogenes* (13). The ethanol extract of bitter melon leaves containing saponins, flavonoids, and triterpenoids has antibacterial activity against *S. aureus* (14). In this study, chayote squash extracts were examined for antibacterial activities and determined for phytochemical constituents.

Materials and Methods

Materials

Chayote squash was obtained from a local traditional market in Surakarta, Indonesia. Bacteria used were obtained from Universitas Gadjah Mada, Indonesia such as *B. subtilis* FNCC 0059, *S. aureus* FNCC 0047, *E. coli* FNCC 0091, *P. aeruginosa* FNCC 0063, and *S. thypi* FNCC 0050. Other bacteria, such as *E. aerogenes* and *S. dysenteriae* were obtained from Indonesian Institute of Sciences, Bandung. Chemicals are purchased from Sigma-Aldrich.

Extraction of Chayote Squash Simplicia Powder

Chayote squash was peeled, removed of seed, cut into thin strips, and air-dried for 15 h. Then, the sample was further dried at 55°C for 72 h. The dried squash flesh was grounded to powders. Dried chayote squash powders (1 kg) were extracted by maceration using methanol for 1 x 48 h, then continued for a further 2 x 24 h by changing the methanol. The filtrate was collected, the solvent was evaporated, and concentrated methanol extract was obtained. Maceration was continued using different solvent polarities. Chayote squash methanol extract (150 g) was dissolved in a mixture of methanol: water (4:1). The solution was placed was then extracted with hexane. The top layer (hexane solution) was evaporated to obtain hexane extract. The bottom layer solution was extracted with chloroform. The bottom solution (chloroform solution) was evaporated to obtain chloroform extract. The top layer solution was extracted with ethyl acetate. The top layer (ethyl acetate solution) was evaporated to obtain ethyl acetate extract. The bottom layer solution was extracted with butanol. The top layer (butanol solution) and the bottom layer (water solution) were separated and evaporated to obtain butanol extract and water extract, respectively.

Antibacterial Activity Test of Extracts

The antibacterial activity of extracts was tested using the diffusion method. The bacterial suspension (100 µl) was put into a petri dish and mixed with 15 ml of nutrient agar. The mixture was homogenized and then allowed to solidify. Then, holes were made using a 6 mm diameter perforator. One hole was filled with DMSO (20 µl) as a control, and other holes were filled with sample extracts in DMSO. Petri dishes were incubated for 20 h at 37°C. The diameter of inhibition zone was then measured, which was shown by the clear area around the hole.

Phytochemical Screening of Extracts

- Phenolics Test

  The presence of phenolics in extracts were tested by addition of $FeCl_3$ (1%) in water into extracts. Extracts contain phenolics if there is a color changes into green, red, purple, blue or black (15).
- Flavonoids Test

  The extract (20 mg) was dissolved with hexane, where the hexane phase was then discarded. The procedure was repeated until the hexane solution was colorless. The residue was dissolved with ethanol, then divided into 2 parts, namely A and B. Part A was used as a blank. Part B was added with HCl, then warmed on a water bath for 15 mins and observed for color changes. The formation of a strong red or violet color indicates the presence of flavonoids (16).
- Tannins and Polyphenols Test

  Extract (25 mg) was dissolved in hot distilled water, stirred and cooled. Five drops of 10% NaCl were added and filtered. The filtrate was divided into 3 parts, namely A, B and C. Part A was used as a blank, part B was added with gelatin solution, and part C was added with 3 drops of 1% $FeCl_3$ reagent. The solution changes were observed. If filtrate B is formed a precipitate indicates the presence of tannins. If filtrate C is formed blue-black color indicates the presence of hydrolyzed tannins and brownish green color indicates the presence of condensed tannin compounds, in addition to the color above indicates the presence of polyphenolic compounds (16).
- Terpenoids Test

  Extract (10 mg) was placed on a plate and then added with vanillin and concentrated H_2SO_4. The test is positive if a purple color is formed (15, 17).
- Saponins test

  Extract (15 mg) was dissolved with 15 ml of distilled water, shaken, then allowed to stand. If a foam is formed that does not disappear for 30 mins, indicating plant extract contains saponins (16).
- Alkaloids Test

  Extract (20) mg was added with 2M HCl, heated on a water bath, stirred, cooled to room temperature. Then, NaCl powder was added and the mixture was stirred and filtered. The filtrate was added with 2M HCl. The filtrate was divided into 2 parts, namely A and B. Part A was used as a blank, part B was reacted with Wagner's reagent. Positive

results are indicated by the formation of a precipitate (16).

Minimum Inhibitory Concentration (MIC) Determination

MIC determination was evaluated for the highest antibacterial activity to determine the lowest concentration of the extract that could inhibit the bacterial growth. The determination of MIC was carried out by varying extract concentration, such as 750, 300, 100, and 30 mg/ml as well as 200, 100, 50, and 25 mg/ml.

Results and Discussion

A total of 37.5 kg chayote squash can be produced into 8.29 kg air-dried sample. Dried Simplicia powder was obtained in 1.2 kg. Simplicia powder (1 kg) was extracted with methanol. More antibacterial compounds can be extracted with methanol than other polar organic solvents (18). The thick brownish methanol extract was obtained in 182.37 g or 18.2% (w/w) yield. Antibacterial activity testing of methanol extract was carried out with concentrations of 20, 15, and 10 mg/well against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *S. typhi*, *E. aerogenes*, and *S. dysenteriae* (Table 1). As the concentration of chayote squash methanol extract increases, the concentration of antibacterial active compound increases so that their abilities for inhibiting bacterial growth was also greater. The ability of antimicrobial agents in inhibiting or killing microorganisms depends on the concentration of the antimicrobial agents (19). The results showed that the methanol extract of chayote squash had antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*. Further studies focused on these four bacteria, since methanol extract of chayote had no antibacterial activity against *E. aerogenes*, *S. dysenteriae* and *S. typhi*.

Methanol extract of chayote squash was subsequently extracted with increasing polarity solvents, such as hexane, chloroform, ethyl acetate, and butanol which aims to obtain compound from plant extracts based on their polarities (Table 2) (20).

Antibacterial activity of hexane, chloroform, ethyl acetate, butanol, and aqueous extracts were conducted using extract concentration of 15 mg/hole in 6 mm of diameter holes. This concentration was used because it had greater antibacterial activities than the 10 mg/hole methanol extract, so it was expected that the extracts would obtain clearer antibacterial activity and could represent the qualitative test results of the extracts. Antibacterial tests were only performed for *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli* (Table 3).

The results revealed that the hexane and butanol extracts only provided antibacterial activity against *B. subtilis*, while the aqueous extract did not provide antibacterial activity against all bacteria. Ethyl acetate extract had the highest antibacterial activity against *P. aeruginosa* and *E. coli*, whereas chloroform extract had the highest antibacterial activity against *S. aureus* and *B. subtilis*.

Table 1. Antibacterial activities of chayote methanol extract.

Bakteria	Antibacterial activities		
	Concentration of chayote squash methanol extract (mg/well)		
	10	15	20
S. aureus	+	+	+
B. subtilis	+	+	+
P. aeruginosa	+	+	+
E. coli	+	+	+
E. aerogenes	-	-	-
S. dysenteriae	-	-	-
S. thypi	-	-	-

(+) = active; (–) = no activity

Table 2. Extraction of chayote squash methanol extract in different polarity of solvents.

Extract Types	**Weight (g)**	**Extract Colour**
Hexane extract	3.2	Dark green
Cloroform extract	4.1	Dark green
Ethyl acetate	3.0	Dark brown
Butanol extract	2.5	Brown
Water extract	99.5	Dark brown

Table 3. Antibacterial activity test of extracts.

Bacteria	Inhibition Zone (mm)				
	Hexane Extract	Chloroform Extract	Ethyl Acetate Extract	Butanol Extract	Aqueous Extract
S. aureus	6.00 ± 0.00	13.04 ± 0.52	12.92 ± 0.14	6.00 ± 0.00	6.00 ± 0.00
B. subtilis	8.87 ± 0.24	12.52 ± 0.44	12.33 ± 0.44	11.09 ± 0.59	6.00 ± 0.00
P. aeruginosa	6.00 ± 0.00	12.70 ± 0.06	13.52 ± 0.04	6.00 ± 0.00	6.00 ± 0.00
E. coli	6.00 ± 0.00	11.78 ± 0.23	13.48 ± 0.64	6.00 ± 0.00	6.00 ± 0.00

ANOVA statistical test revealed a significance value of <0.05, meaning that antibacterial activity had a significantly different effect between the tested bacteria and the extracts. The Least Significance Different (LSD) follow-up test was performed to determine the effect of antibacterial activity between one extract and another on each tested bacterium. Ethyl acetate extract had the greatest antibacterial activity compared to hexane, chloroform, butanol, and aqueous extracts against *P. aeruginosa* and *E. coli*. The results of the LSD follow-up test showed that the difference was significant (sig.<0.05). In contrast, it was an insignificant difference, although the antibacterial activity of the ethyl acetate extract was lower than that of the chloroform extract against *S. aureus* and *B. subtilis*. It can be considered that the antibacterial activity of ethyl acetate extract and chloroform extract against *S. aureus* and *B. subtilis* was almost the same.

The ethyl acetate extract of chayote squash with the highest antibacterial activity against *P. aeruginosa* and *E. coli* was further studied for phytochemical screening and determination of MIC. Other antibacterial active extracts were only studied for phytochemical screening (Table 4). The results of phytochemical screening showed that the methanol and chloroform extracts contained phenolics, flavonoids, condensed tannins, terpenoids, saponins, and alkaloids. Ethyl acetate extract contains phenolics, flavonoids, condensed tannins, and terpenoids. Butanol extract contains phenolics, condensed tannins, and alkaloids. Hexane extract contains only terpenoids. Antibacterial compounds are generally found in the phenolic, flavonoid, tannin, terpenoid, saponin, and alkaloid groups (18, 21-22).

Methanol extract of chayote squash contains polar compounds such as phenolics, flavonoids, condensed tannins, terpenoids, saponins, and alkaloids. Compounds obtained in methanol extract can be separated according to their polarities using hexane, chloroform, ethyl acetate, and butanol. It was revealed that hexane extract contains only terpenoids. Hexane extract contained polar compounds because polar compounds also have non-polar parts forming van der Waals interactions with non-polar hexane (23-24).

Chloroform and ethyl acetate extracts contained a different type of flavonoids. More polar flavonoids were isolated in ethyl acetate than those flavonoids in chloroform. For example, 3-methoxy-luteolin has four hydroxyl groups, and hispiludin has three hydroxyl groups (25). Condensed tannins can be extracted by ethyl acetate and butanol because they have hydroxyl groups, oxygen atoms, and polar double bonds. However, ethyl acetate and butanol extracted the different types of condensed tannin. As examples, catechins and epicatechins are extracted with ethyl acetate, whereas procyanidin B1, trimer procyanidins, and pentamer procyanidins are extracted with butanol. The reason is the number of hydroxyl groups of catechins and epicatechins are smaller than that of procyanidin B1, trimer procyanidins, and pentamer procyanidins (26).

Terpenoids in hexane extract played a role in inhibiting the growth of *B. subtilis*. Phenolics, flavonoids, condensed tannins, terpenoids, saponins, and alkaloids in methanol and chloroform extracts played roles in inhibiting the growth of *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli*. Phenolics, condensed tannins, and alkaloids in butanol extract played a role in inhibiting the growth of *B. subtilis*.

Chayote squash contains phenolics and condensed tannins. Phenolics cause protein denaturation through an adsorption process involving hydrogen bonds. At low levels, protein-phenol complexes are formed with weak bonds and immediately undergo decomposition, followed by penetration of phenolics into cells and causing precipitation and protein denaturation. At high levels, phenolics cause protein coagulation and cell membrane lysis, changing the permeability of bacterial membranes (27). Condensed tannins have antibacterial activity because they bind to

bacterial cell walls, lead to inhibiting cell growth and protease activity (18).

Chayote squash contains flavonoids. Flavonoids have antibacterial activity because they can form complexes with extracellular proteins, soluble proteins, and complexes with cell walls (18). Flavonoids isolated from Artemisia, namely 6-methoxylapigenin or methoxy-6 trihydroxy-5,7,4' flavone (6MAPI) and 6-methoxyluteolin or methoxy-6 tetrahydroxy-5,7,3',4' flavone (6MLU) can interact with the enzyme dihydrofolate reductase (DHFR) in *E. coli*. The DHFR enzyme plays a role in synthesizing nitrogenous bases in the bacterial cell nucleus. It causes the bacterial cell nucleus not formed so that the bacteria die. Terpenoids in chayote are antibacterial by damaging bacterial cell membranes (18). Terpenoids in the rhizomes of Hedychium species play a role in inhibiting the growth of *S. aureus* and *E. coli* (28). The terpenoids phytadiene and 1,2-seco-cladiellan contained in Meniran herb are antibacterial active against *S. aureus* and *E. coli* (29). Terpenoid of caurenoic acid from *Pseudognaphalium vira vira* damages the cell membrane of *S. aureus* by forming hydrogen bonding between carboxylic group of the caurenoic acid with the phosphoryl oxygen atom of the cell membrane (30).

Table 4. Phytochemical Screening Results of Active Antibacterial Extracts.

Phytochemical Screening	Active Antibacterial Extracts				
	Methanol Extract	**Hexane Extract**	**Chloroform Extract**	**Ethyl Acetate Extract**	**Buthanol Extract**
Phenolics	+	-	+	+	+
Flavonoids	+	-	+	+	-
Condensed tanins and poliphenols	+	-	+	+	+
Terpenoids	+	+	+	+	-
Saponins	+	-	+	-	-
Alkaloids	+	-	+	-	+

(+) = contains the tested compounds; (-) = do not contains the tested compounds

The active extract of chayote squash contains saponins. These compounds act as emulsifiers to reduce cell surface tension, destroying bacteria (31). Erylosides is one example of saponins that have antibacterial activity against *E. coli* and *B. subtilis* (32). Chayote squash contains alkaloids. Alkaloids interfere the cross-bridges formation of peptidoglycan components in bacterial cells so that the cell wall layer is not properly formed, which causes cell death (33). Lupanine (2-oxosparteine) and S-calycotomine are alkaloids that have antibacterial activities (34).

MIC is the lowest concentration of antibacterial compounds, which can still inhibit bacterial growth (35). This study determined MIC for ethyl acetate extract because it had the highest antibacterial activities against *P. aeruginosa* and *E. coli*. The extract concentration variations used were 750, 300, 100, and 30 mg/ml (Table 5)**.** Ethyl acetate extract with 30 mg/ml concentration had no antibacterial activity. The inhibition zone of ethyl acetate extract with a concentration of 100 mg/ml was 7.26-10.75 mm, indicating that the ethyl acetate extract had antibacterial activities. The concentration range of 30-100 mg/ml is still far enough so that further testing needs to be done at various concentrations of 200, 100, 50, and 25 mg/ml to determine the more precise MIC of ethyl acetate extract (Table 6).

Table 6 revealed that MICs of ethyl acetate extract against *B. subtilis*, *P. aeruginosa*, *E. coli*, and *S. aureus* were the same at a concentration of 50 mg/ml. ANOVA statistical test showed an effect of antibacterial activity of ethyl acetate extract at a concentration of 100 mg/ml (sig.<0.05). Further LSD test showed that there was a significant difference in antibacterial activity of 100 mg/ml ethyl acetate extract on *B. subtilis* compared to *E. coli* and *S. aureus*. In contrast, the antibacterial activity of 200 mg/ml ethyl acetate extract was only significantly different against *B. subtilis* in comparison to *S. aureus*. LSD results showed that there was a significant difference in antibacterial activity among ethyl acetate extract at concentrations of 200 mg/ml compared to concentrations of 100, 50, and 25 mg/ml against *P. aeruginosa* and *E. coli*. There was also a significant difference among the ethyl acetate extract with a concentration of 200 mg/ml compared to a concentration of 50 and 25 mg/ml against *S. aureus* and *B. subtilis*.

Table 5. Inhibition zone of ethyl acetate extract at 30-750 mg/ml concentrations.

Ethyl Acetate Extract Concentration (mg/ml)	Inhibition Zone (mm)			
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E. coli***
750	14.02 ± 0.41	12.69 ± 0.48	12.75± 0.38	13.69± 0.35
300	9.07 ± 0.40	9.48 ± 0.28	10.69 ± 0.24	11.52 ± 0.07
100	7.26 ± 0.13	8.28 ±0.16	8.99 ± 1.19	10.75 ± 0.59
30	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

Table 6. Inhibition zone of ethyl acetate extract at 25-200 mg/ml concentrations.

Ethyl Acetate Extract Concentration (mg/ml)	Inhibition Zone (mm)			
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E. coli***
200	9.06 ± 0.74	11.02 ± 0.62	10.43 ± 0.27	9.82 ± 0.28
100	8.48 ± 0.47	9.86 ± 0.37	9.18 ± 0.14	8.97 ± 0.01
50	7.68 ± 0.08	8.10 ± 0.80	8.24 ± 0.34	8.54 ± 0.37
25	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00

Conclusion

Methanol extract of chayote squash had antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli*. Ethyl acetate extract of chayote squash contains phenolics, condensed tannins, flavonoids, and terpenoids and had the highest antibacterial activity against *P. aeruginosa* and *E. coli*. MIC value of ethyl acetate extract for *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli* was 50 mg/ml. Chayote squash shows good antibacterial properties. However, the mechanism of antibacterial inhibition of chayote squash chemical compounds against tested bacteria is not yet studied in this research.

Conflict of Interest

All authors declare no conflicts of interest in this research.

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