Borneo Journal of Pharmacy
Vol 5 Issue 3 August 2022
Page 268 – 278
http://journal.umpalangkaraya.ac.id/index.php/bjop/article/view/3156
DOI: https://doi.org/10.33084/bjop.v5i3.3156
e-ISSN: 2621-4814

*Research Article*

# Taro (*Colosia esculenta*) Leaves Extract Inhibits *Streptococcus mutans* ATCC 31987

**Ayu Nala El Muna Haerussana***
**Angreni Ayuhastuti**
**Siti Fira Yuniar**
**Hana Alifah Bustami**
**Widyastiwi**

Department of Pharmacy, Poltekkes Kemenkes Bandung, Bandung, West Java, Indonesia

*email: ayunalaelmh@gmail.com

**Keywords**:
Antibacterial activity
*Colocasia esculenta*
*Streptococcus mutans*
Talas
Taro leaves extract

## Abstract

Dental caries was the most common disease in both adults and children. *Streptococcus mutans* is the main bacteria causing plaque formation and was the initiator of dental caries. Antibacterials derived from plants can be used to prevent plaque formation. Taro (*Colosia esculenta*) has been used in traditional medicine. Antibacterial compounds have been discovered in *C. esculenta* leaves. This study aimed to determine the ability of *C. esculenta* leaf ethanol extract to inhibit the growth of *S. mutans* ATCC 31987. Simplicia preparation, extract preparation, and phytochemical screening was carried out. Then, the antibacterial activity test was performed using the disc diffusion method to determine the zone of inhibition at various concentrations of 10, 20, 30, 40, 50, 60, and 70%. *Colosia esculenta* leaf ethanol extract contains alkaloids, flavonoids, triterpenoids, saponins, and produces an inhibition zone at each concentration variation. Very strong antibacterial activity was produced at a concentration of 70% at 21.11±0.46 mm, which was higher than the positive control.

*Received*: January 19th, 2022
*1st Revised*: July 7th, 2022
*Accepted*: July 20th, 2022
*Published*: August 31th, 2022

© 2022 Ayu Nala El Muna Haerussana, Angreni Ayuhastuti, Siti Fira Yuniar, Hana Alifah Bustami, Widyastiwi. Published by Institute for Research and Community Services Universitas Muhammadiyah Palangkaraya. This is an Open Access article under the CC-BY-SA License (http://creativecommons.org/licenses/by-sa/4.0/). DOI: https://doi.org/10.33084/bjop.v5i3.3156

## INTRODUCTION

The Global Burden of Disease Study reported that oral diseases afflicted about 2.3 billion people worldwide. Dental caries was the most common cause, accounting for 65% of infected permanent teeth and 15% of infected primary teeth[1]. Caries are caused by bacterial metabolism as a biofilm or plaque that changes sugars to acid, demineralizing the hard tissues of the teeth (enamel and dentine)[2]. Bacterial acid decreased the pH of tooth surfaces, causing demineralization from calcium ions and phosphate dentine release, which resulted in cavities[3]. The early stages of dental caries are frequently asymptomatic, but advanced stages can cause discomfort, infections, abscesses, and even sepsis. A severe condition in adults causes pain and infection, which may necessitate tooth extraction[4]. This caries might make children prone to infections in other parts of their bodies. The pain can make chewing meals excruciating, and getting enough nutrition can be problematic[5].

The most prevalent bacteria that cause caries were *Streptococci* and *Lactobacilli*. *Streptococci* lead to caries, and *Lactobacilli* contribute to further infection[6]. *Streptococcus mutans* was not only the basic bacterium engaged in the development of plaque but also in the commencement of dental caries. *Streptococcus mutans* have been linked to other extraoral pathologies such as cerebral microbleeds, IgA nephropathy, and atherosclerosis as a human pathogens[7,8].

Plaque treatment can be done mechanically or chemically. Brushing and flossing are mechanical techniques of plaque control, whereas mouthwash is a chemical plaque control treatment[9,10]. Mouthwash contains chemical compounds with antiseptic or antibacterial characteristics that aid in preventing plaque formation. On the other hand, regular utilization may cause tooth discoloration and temporary gustatory problems[11]. Pizzo *et al.*[12] stated another negative impact was that it induces mouth dryness, burning, and is harmful if ingested. Hence, plant components can be used as antibacterial agents.

**How to cite:** Haerussana ANEM, Ayuhastuti A, Yuniar SF, Bustami HA, Widyastiwi. Taro (Colosia esculenta) Leaves Extract Inhibits Streptococcus mutans ATCC 31987. Borneo J Pharm. 2022;5(3):268-78. doi:10.33084/bjop.v5i3.3156

According to a World Health Organization (WHO) report, approximately 80% of the world's population was treated using plants or plant-derived products[13]. Plants have long been employed as the primary human therapy source and have helped address the world's healthcare demands[14]. Medicinal plants have proven to be a remarkable source of newer and potent therapeutic agents and have taken the central stage in most research centers worldwide[15].

*Colocasia esculenta*, also known as taro (*talas* = Indonesian), was a nutritious plant widely consumed by the locals[16]. *Colocasia esculenta* tuber is used as ethnomedicine to treat diabetes, ringworm, cough, sore throat, wounds, asthma, arthritis, diarrhea, internal bleeding, neurological disorders, skin disorders, and it has been reported to have antihelminthic and anticancer properties[17,18]. Agyare *et al.*[19] stated that a decoction of the leaves was drunk to promote menstruation and was used to treat stomach problems and cysts. In addition, *C. esculenta* leaf extract has anti-inflammatory and antioxidant properties.

*Colocasia esculenta* biological properties were derived from natural products such as alkaloids, flavonoids, tannins, phytates, and minerals[17]. Elmosallamy *et al.*[20] showed that the significant constituents of *C. esculenta* leaves are flavonoids β-sitosterol and steroids. Anthocyanins, isoschaftoside, vicenin, apigenin, catechin, anthraquinones, β-amyrin, α-amyrin, riboflavin, and niacin were also reported in leaves[21,22]. The flavonoids in *C. esculenta* include orientin, isoorientin, vitexin, isovitexin, and luteolin-7-O-sophoroside[23]. Orientin flavonoids are reported to have antibacterial activity[24].

Wang *et al.*[25], in previous research, reported the presence of antimicrobial activity in tests using *C. esculenta*. The research of Singh *et al.*[26] stated that the water extract of *C. esculenta* leaves showed the diameter of the inhibition zone of *Staphylococcus aureus* MTCC 96 and *S. mutans* MTCC 890 at a concentration of 400 mg/mL was 8.50±0.09 mm and 15.33±0.13 mm, respectively. A different solvent produced a more significant inhibition zone, ethanol extract of *C. esculenta* leaves against *S. aureus* of 14.3±1.45 mm at a similar concentration[27].

The selection of the solvent and methods used for extraction was an important factor, so it must be fully considered[28]. The maceration extraction method has the advantage of being easy to use and requires less equipment. Since the maceration method was used at room temperature, thermolabile flavonoid compounds are appropriate[29]. Antimicrobial compounds are most commonly found in the phenolic compounds terpenoids but also found in flavonoids, saponins, and alkaloids classes[30-32]. Orientin was a flavonoid compound with low polarity, making it easily soluble in semipolar solvents. Since 96% ethanol was a semipolar solvent, it was chosen as the extraction solvent[33]. Based on the prior studies of 96% ethanol extract against *S. mutans* ATCC 31987, researchers intend to investigate the antibacterial activity of *C. esculenta* leaf ethanol extract against the growth of the primary bacteria responsible for dental caries.

## MATERIALS AND METHODS

### *Materials*

The materials used in this study were old fresh *C. esculenta* leaves, collected from Balai Penelitian Tanaman Sayuran (Research Center of Agriculture Ministry) Lembang, West Java, Indonesia. *Streptococcus mutans* ATCC 31987 isolates from Biology Department Universitas Indonesia, 96% ethanol, dimethyl sulfoxide (DMSO), chlorhexidine, 0.9% sterile physiological NaCl, Mueller Hinton Agar (MHA), blood agar, $H_2SO_4$, $BaCl_2$, 70% ethanol, HCl, acetic acid, *n*-hexane, Mg metal, Mayer reagent, Wagner reagent, aquadest, chloroform, $FeCl_3$, and blank disc. The instruments used in this study were an autoclave (Hirayama®), incubator (Memmert®), blender (Philips®), vacuum rotary evaporator (Buchi®), biological safety cabinet (Thermo Scientific®), oven (Memmert®), microscope (Leica®), spectrophotometer (Shimadzu®), analytic scale (Mettler Toledo®), waterbath (Electro-mag®), micropipette (Effendorf®), refrigerator, caliper, inoculation loop, and laboratory glassware.

### *Methods*

#### *Preparation of ethanolic extraction*

Preparations of simplicia adapted from Arnida *et al.*[34] including wet sorting, washing, chopping, drying, and dry sorting (**Figure 1**). The drying was done in an oven at 50±2°C. Dry leaves or simplicia could be crushed by hand into small pieces

with a water content of 10% or less. Gravimetric methods are used to determine the water content. **Equation 1** was used to calculate the yield extract:

$$\text{Water content (\%)} = \frac{(W1\text{-}W0)\text{-}(W2\text{-}W0)}{(W1\text{-}W0)} x100\% \quad \ldots [1]$$

W0 = weight of container (g)
W1 = weight of container + weight of moist simplicia (g)
W2 = weight of container + weight of dry simplicia (g)

Simplicia was ground to a powder with a blender and sieved at 60 mesh. *Colocasia esculenta* leaves simplicia were macerated in 96% ethanol. Simplicia powder was soaked in ethanol (1 : 10) for 24 hours at room temperature, stirring occasionally. This process was repeated until the pellucidity was achieved. Following filtration of the suspension through filter paper, *C. esculenta* leaf extract was evaporated at 50°C using a rotary vacuum evaporator and a waterbath (**Figure 1**). The crude extract was kept at 40°C until it was analyzed. **Equation 2** was used to calculate yield extract:

$$\text{Yield extract (\%)} = \frac{\text{crude extract weight (g)}}{\text{simplicia weight (g)}} x\ 100\% \quad \ldots [2]$$

**Figure 1.** *Colocasia esculenta* leaves before chopping (fresh) (**a**), drying process in the oven (**b**), and thickening extract process to evaporate the remaining solvent (**c**).

*Phytochemical analysis*[35,36]

Phytochemical compounds were identified by qualitative analysis. The extract's content of flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids was determined qualitatively. The extraction solvent affects the secondary metabolite content of the extract.

Alkaloid (Wagner and Mayer test): About 5 mg extract was dissolved in chloroform, then 0.5 mL of 1 M sulfuric acid was added and slowly shaken. The mixture was allowed to stand for a few moments until two layers formed. The transparent top layer was divided into two parts, one receiving 2-3 drops of Wagner's and the other receiving 2-3 drops of Mayer's reagent. The brown precipitate indicated the alkaloid compounds by Wagner's and the white precipitate by Mayer's reagent.

Flavonoid (Wilstater test): About 5 mg of extract was dissolved in 5 mL of hot water, boiled for 5 minutes, and then filtered. The filtrate was mixed with Mg metal, 1 mL of concentrated sulfuric acid, and 2 mL of 70% ethanol. The mixture was shaken vigorously and set aside. The presence of flavonoid compounds was indicated by the formation of a red, yellow, or orange color on the ethanol layer.

Tannins and Polyphenol ($FeCl_3$ test): About 0.5 g of the extract was dissolved in 2 mL of 70% ethanol, boiled in 10 mL of distilled water, and filtered in a test tube. Three drops of 0.1 % $FeCl_3$ were added, and a brownish-green or blue-black color formed indicating the presence of tannins and phenols.

Saponin (Foaming test): *Colocasia esculenta* extract was dissolved in 10 mL of hot water and allowed to cool. Once cool, the mixture was vigorously shaken vertically for 10 seconds. The presence of saponin compounds was indicated by a stable foam as high as 1 cm if the foam remains stable after adding one drop of 1% HCl.

Triterpenoids and Steroids: In a test tube, 0.5 mg of extract was placed. The mixture was then treated with 2 mL of anhydrous acetic acid and 0.5 mL of concentrated sulfuric acid. A blue or green color indicated the presence of steroids, while a brownish or purple ring indicated the presence of triterpenoids.

*Bacterial preparations*

The bacteria were obtained, and then Gram stained to examine cell morphology. Gram staining was tested by dripping the reagents in a specific order. The first reagent was gentian violet as a primary stain, followed by iodine solution as a mordant, alcohol as a decolorizer, and safranin as a counterstain. Gram-positive bacteria retain the first color, making them appear violet under a 100x magnification microscope. The bacteria were recultured by streaking a loop of bacteria in blood agar media and incubating it at 37°C for 18-24 hours before using the bacteria to make suspensions.

*Antibacterial activity*

McFarland standard 0.5 was made by combining 0.05 mL of 1% $BaCl_2$ with 9.95 mL of 1% $H_2SO_4$. The turbidity produced in the test tube was equivalent to $1.5x10^8$ CFU/mL of bacteria. The mixture of the two solutions was attempted for no more than 15 minutes to serve as a standard[37,39].

Recultured *S. mutans* in a sterile inoculation loop were suspended in a tube containing 10 mL of 0.9% physiological NaCl. The turbidity was then compared to McFarland 0.5 standard. Turbidity was measured with a spectrophotometer equal to 0.1 at 625 nm. The too high turbidity could be reduced by adding 0.9% physiological NaCl or bacterial colonies[40].

The test bacteria were planted in the solidified MHA media using the spread plate method. The prepared bacterial suspension was taken 100 µL with a micropipette and dripped on the surface of the solidified MHA media. The suspension droplets were spread using a sterile L-rod, with repeated rotation of the petri dish, to ensure that the test bacteria spread evenly. *Colocasia esculenta* leaf ethanol extract was prepared in various concentrations of 10, 20, 30, 40, 50, 60, and 70% dissolved in DMSO. The positive control was chlorhexidine, the negative control was DMSO solution, and the growth control was suspension only. Blank discs with a diameter of 6 mm were immersed in that test solution for 10 minutes to make sample discs. Sample discs were given to solid media mixed with the test bacteria. The samples were incubated at 37°C for 24 hours in an inverted position to prevent condensation from dripping onto the media[41].

Furthermore, the presence or absence of the formed inhibition zone was observed. The apparent diameter of the clear zone around the disc was measured using a caliper diagonally, vertically, and horizontally and averaged. The diameter of the clear zone was reduced by the diameter of the disc[37].

*Statistical analysis*

The inhibition zone values were calculated using IBM SPSS Statistic 25 for Windows and expressed as mean (n=3) per plate of three repetitions±standard deviations (SD). Suppose the data had a normal distribution ($p < 0.05$), the normality test was performed using Analysis of Variance (ANOVA) and Kruskal-Wallis if not. Then a Post Hoc ($p < 0.05$) analysis was performed.

## RESULTS AND DISCUSSION

*Ethanol extract preparations*

The water content of *C. esculenta* leaves was 7.07±0.05%, less than 10%. Microbes will easily overgrow in water with content greater than 10%. The simplicia was then mashed to facilitate the extraction process. A large surface area will increase the effectiveness of the solvent in breaking down plant cell walls, so it was expected that the extracted compounds would be maximal[42].

Antibacterial compounds such as orientin in *C. esculenta* leaves were extracted with 96% ethanol. Other compounds such as luteolin, apigenin, isoorientin, vitexin, and isovitexin have similar solubility. Previous studies show ethanol was the best choice because it contains the tremendous variety and extent of bioactive components in *C. esculenta* extract[20]. The resulting yield extract was 20.53%, which was calculated to determine the adsorption value of the solvent in extracting the extract.

*Phytochemical analysis*

Qualitative identification of compound groups was performed to determine the content of compound groups in *C. esculenta* leaf extract that may have antibacterial properties. The presence of alkaloids, flavonoids, triterpenoids, and saponins was detected in the qualitative test on *C. esculenta* leaf extract shown in **Table I**. Eddy[43] stated that the water extract of *C. esculenta* leaves contains saponins and tannins. Meanwhile, the ethanol extract of *C. esculenta* leaves, apart from containing saponins and tannins, also contains terpenoids, anthraquinones, flavonoids, and alkaloids. Different solvents can lead to the extraction of various phytochemical compounds. The precipitate formed in the alkaloid test was potassium-alkaloid because the alkaloid compound contains a nitrogen atom with a lone pair of electrons that can be used to form coordinate covalent bonds with metal ions. The alkaloids' nitrogen will react with the metal ions $K^+$ from potassium tetraiodomercurate (II) to form a precipitated potassium-alkaloid complex[44].

Flavonoid testing was carried out by adding HCl and Mg metal, which reduced the benzopyrone core contained in flavonoid compounds, resulting in the formation of red color in these compounds[45]. Meanwhile, the saponin test was performed by adding hot water to the ethanol extract. Saponins are polar in that they can dissolve in solvents such as water, though they are also non-polar in that they contain a hydrophobic group, an aglycone (sapogenin). The presence of glycosides, which can form foam in water and hydrolyze into glucose and other compounds, causes the foam produced in the saponin test[46].

Triterpenoid testing was carried out by adding acetic acid and sulfuric acid, which can produce a color change reaction to a brownish or purple ring. This can occur due to condensation or release of water and incorporation with carbocations. This reaction begins with the acetylation of the hydroxyl group using anhydrous acetic acid. Furthermore, the release of the hydrogen group and its electrons causes the double bond to move and experience resonance which acts as an electrophile or carbocation. The presence of a carbocation causes electrophilic addition, followed by the release of hydrogen. Then the hydrogen group and its electrons are removed. As a result, the compound undergoes conjugation extension, which shows the appearance of a brownish ring[47].

**Table I.** Phytochemical analysis of *C. esculenta* leaves extract

| Phytochemical analysis | Description | Result | Conclusion |
|---|---|---|---|
| Alkaloid | The color changed, but no white precipitate or white turbidity was formed in the Mayer's reagent, whereas a brick-red precipitate was formed in the Wagner's reagent. | | + |
| Flavonoid | Formation of red color on the top layer when compared to the blank | | + |
| Tannin | Same as blank, no brownish-green or blue-black color was formed. | | - |
| Triterpenoid | A concentrated dark color resulted, and the color of the blank was lighter than the test results. | | + |
| Saponin | The foam was formed and the foam that was formed was stableIya | | + |

Note: (+) presence; (-) absence

*Antibacterial activity*

The extract diffuses on the agar and inhibits the test microbes' growth, resulting in a clear zone formation. The zone of inhibition can be seen as the formation of a clear zone around the disc, with the visible diameter of the zone concluding as the extract's inhibition zone against the test microbes. Outside the zone, turbidity indicates the growth of microbes that are not inhibited by the extract. **Table II** shows the results of testing a 96 % ethanol extract of *C. esculenta* leaves against the caries-causing *S. mutans*. Each variation of the concentration of 96% ethanol extract of *C. esculenta* leaves has antibacterial activity, and a clear zone around the disc indicates an inhibition zone.

Tests revealed that a concentration of 70% can provide inhibition of 21.11±0.46 mm, indicating that it has a very strong antibacterial ability. Ponce *et al.*[48] classified the diameter of the inhibition zone into four categories: weak inhibition zone of 8 mm, moderate inhibition zone of 8-14 mm, strong inhibition zone of 15-19 mm, and very strong inhibition zone of >20 mm. The lowest concentration was in the weak category, and the highest was in the very strong category, according to the above categories. This could occur because the lower the concentration of an extract, the more dilute the extract produced and the smaller the inhibition zone formed to inhibit *S. mutans*.

The test revealed that chlorhexidine inhibited the microbes by 9.37±0.27 mm. Chlorhexidine was known to have bacteriostatic and bactericidal properties, and it can effectively inhibit and kill *S. mutans*. Chlorhexidine was an effective mouthwash in the treatment of dental caries[49]. The mechanism of chlorhexidine causes changes in the permeability of the bacterial cell membrane, resulting in the release of cytoplasm and cell components from within the cell through the cell membrane, resulting in bacterial death[50]. The 0.2% chlorhexidine concentration was chosen because it was the concentration commonly used as a mouthwash. Furthermore, increasing the concentration of chlorhexidine will make it toxic to dental cells.

The negative control showed no inhibition zones against the *S. mutans*. The negative control (10% DMSO), a solvent used in extract dilution, does not inhibit bacterial growth. DMSO was a neutral solvent capable of dissolving both polar and non-polar substances[51]. According to Trisia *et al.*[52], who examined the antibacterial activity test of the ethanolic extract using the disc diffusion method, DMSO had no inhibitory effect on the growth of *S. aureus* (Kirby-Bauer).

Previous research by Dutta and Aich[27] demonstrated that the diffusion of ethanol extract of *C. esculenta* leaves at a concentration of 400 mg/mL inhibited *S. aureus* by 14.3±1.45 mm. Our research yielded an inhibition of 7.33±0.64 mm at the same concentration (40%). Meanwhile, at a concentration of 20 mg/mL, the inhibition zones formed by *C. esculenta* leaves chloroform and methanol extract against *S. aureus* were 16.23±1.53 and 20±1.00 mm, respectively[53]. Singh *et al.*[26] tested the antibacterial activity of *C. esculenta* leaves aqueous extract against *S. mutans* and found an inhibition zone of 15.33±0.10 mm at 400 mg/mL.

The extract's phytochemical content influenced the antibacterial activity of *C. esculenta* leaf extract. Flavonoids, saponins, alkaloids, and terpenoids are found in *C. esculenta* leaves ethanol extract. Alkaloid group compounds can function as antibacterials by interfering with the peptidoglycan components in bacterial cells, causing the cell wall layer not to form entirely and causing the bacterial cell to die[54]. Furthermore, flavonoid compounds inhibit cell membrane function, DNA gyrase, and bacterial metabolism[55]. Other groups of compounds that are positive in the extract and have an antibacterial function are saponins and triterpenoids. Saponins can work as an antibacterial by disrupting the permeability of cell membranes so that the membrane becomes unstable and causes lysis[56]. Meanwhile, the mechanism of action of triterpenoids by damaging the bacterial cell membrane. Cell membrane damage can occur when active antibacterial compounds react with the active site of the membrane[54].

**Table II** showed that the test solution has a more significant zone of inhibition against *S. mutans* than the positive control (60 and 70%). The greatest concentration of 70% was twice as strong as the positive control, which had a concentration of 0.2%. According to the results of the tests, *C. esculenta* leaf extract can be utilized as an alternative to *S. mutans* inhibition.

**Table II.** Inhibition zone of *C. esculenta* leaves extract and control against *S. mutans*

| Sample | Inhibition zone (mm) | | | Average±SD (mm) |
|---|---|---|---|---|
| | P1 | P2 | P3 | |
| *C. esculenta* leaves extract (10%) | 3.97 | 3.73 | 3.37 | 3.69±0.3 |
| *C. esculenta* leaves extract (20%) | 5.6 | 4.87 | 4.57 | 5.01±0.53 |
| *C. esculenta* leaves extract (30%) | 6.3 | 5.07 | 6.70 | 6.02±0.85 |
| *C. esculenta* leaves extract (40%) | 8.07 | 6.9 | 7.03 | 7.33±0.64 |
| *C. esculenta* leaves extract (50%) | 9.1 | 7.07 | 8.46 | 8.21±1.04 |
| *C. esculenta* leaves extract (60%) | 12.13 | 12.97 | 11.13 | 12.13±0.93 |
| *C. esculenta* leaves extract (70%) | 20.93 | 21.63 | 20.77 | 21.11±0.46 |
| Chlorhexidine (0.2%) | 9.67 | 9.13 | 9.33 | 9.37±0.27 |
| DMSO (10%) | - | - | - | - |

Note: The diameter of the inhibition zone was reduced with the disc diameter (6 mm)

*Statistical analysisis*

All data on the antibacterial activity of the ethanol extract of *C. esculenta* leaves against *S. mutans* were analyzed using statistical tests. This study's variables were categorical and unpaired numeric variables and had more than two groups. Therefore, the statistical test that can be used is the ANOVA test with the condition that the data obtained must be normally distributed and have homogeneous data variances. The data obtained were first analyzed using the normality test, which aims to see whether the resulting data were normally distributed or not. The normality test results have two results: the Kolmogorov-Smirnov and the Shapiro-Wilk test. The normality value was seen in the Shapiro-Wilk test because the data obtained was less than 50. The normality value of each treatment was more than 0.05, so it can be concluded that the data obtained are normal.

After the normality test, the data from the antibacterial activity test results should be tested for homogeneity, which aims to see the homogeneity of the data variants. The homogeneity of variances test shows a p-value of >0.05. Therefore, it can be concluded that there are at least two groups that have significantly different data variances. Based on the normality and homogeneity test results, we used the ANOVA test because the data obtained were normally distributed, and the data's variance was homogeneous. Analysis was conducted Post hoc to find out which groups had significantly different data. **Table III** showed that the negative control significantly differed from the positive control. The overall activity data was significantly different from the negative control. Furthermore, the test group was significantly different from the positive control, significantly smaller or larger, with a p-value of <0.05.

**Table III.** Statistical analysis of antibacterial activity of *C. esculenta* leaves extract

| Samples | Negative control | Positive control | 10% | 20% | 30% | 40% | 50% | 60% | 70% |
|---|---|---|---|---|---|---|---|---|---|
| Negative control | - | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* |
| Positive control | | - | 0.000* | 0.000* | 0.000* | 0.001* | 0.040* | 0.000* | 0.000* |
| *C. esculenta* 10% | | | - | 0.022* | 0.000* | 0.000* | 0.000* | 0.000* | 0.000* |
| *C. esculenta* 20% | | | | - | 0.071 | 0.000* | 0.000* | 0.000* | 0.000* |
| *C. esculenta* 30% | | | | | - | 0.023* | 0.000* | 0.000* | 0.000* |
| *C. esculenta* 40% | | | | | | - | 0.113 | 0.000* | 0.000* |
| *C. esculenta* 50% | | | | | | | - | 0.000* | 0.000* |
| *C. esculenta* 60% | | | | | | | | - | 0.000* |
| *C. esculenta* 70% | | | | | | | | | - |

Note: (*) a significant difference with a 95% confidence level

## CONCLUSION

The results showed that the best inhibition zone was 21.11±0.46 mm at a 70% concentration of *C. esculenta*, which was more significant than the control. *Colosia esculenta* leaves had very strong antibacterial activity against *S. mutans*.

## ACKNOWLEDGMENT

The author would like to express gratitude to the Poltekkes Kemenkes Bandung for financial funding through the PDP research grant scheme.

## AUTHORS' CONTRIBUTION

**Ayu Nala El Muna Haerussana**: concept and design, research team leader and coordinator, validation, antibacterial assay, and article writing. **Angreni Ayuhastuti**: concept and design, validation, and article writing. **Siti Fira Yuniar**: sampling and simplicia preparation. **Hana Alifah Bustami**: extraction and phytochemical screening. **Widyastiwi**: data analysis.

## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to report regarding the present study.

## REFERENCES


1. Dye BA. The Global Burden of Oral Disease: Research and Public Health Significance. J Dent Res. 2017;96(4):361-3. doi:10.1177/0022034517693567

2. Chen X, Daliri EBM, Kim N, Kim JR, Yoo D, Oh DH. Microbial Etiology and Prevention of Dental Caries: Exploiting Natural Products to Inhibit Cariogenic Biofilms. Pathogens. 2020;9(7):569. doi:10.3390/pathogens9070569

3. Neel EAA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, et al. Demineralization-remineralization dynamics in teeth and bone. Int J Nanomedicine. 2016;11:4743-63. doi:10.2147/ijn.s107624

4. Siqueira Jr JF, Rôças IN. Microbiology and treatment of acute apical abscesses. Clin Microbiol Rev. 2013;26(2):255-73. doi:10.1128/cmr.00082-12

5. Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. J Nat Sci Biol Med. 2013;4(1):29-38. doi:10.4103/0976-9668.107257

6. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol. 2008;46(4):1407-17. doi:10.1128/jcm.01410-07

7. Naka S, Wato K, Misaki T, Ito S, Matsuoka D, Nagasawa Y, et al. Streptococcus mutans induces IgA nephropathy-like glomerulonephritis in rats with severe dental caries. Sci Rep. 2021;11(1):5784. doi:10.1038/s41598-021-85196-4

8. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The Biology of Streptococcus mutans. Microbiol Spectr. 2019;7(1):10.1128/microbiolspec.GPP3-0051-2018. doi:10.1128/microbiolspec.gpp3-0051-2018

9. Vyas T, Bhatt G, Gaur A, Sharma C, Sharma A, Nagi R. Chemical plaque control - A brief review. J Family Med Prim Care. 2021;10(4):1562-8. doi:10.4103/jfmpc.jfmpc_2216_20

10. James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 2017;3(3):CD008676. doi:10.1002/14651858.cd008676.pub2

11. Brookes ZLS, Bescos R, Belfield LA, Ali K, Roberts A. Current uses of chlorhexidine for management of oral disease: a narrative review. J Dent. 2020;103:103497. doi:10.1016/j.jdent.2020.103497

12. Pizzo G, Guiglia R, Imburgia M, Pizzo I, D'Angelo M, Giuliana G. The Effects of Antimicrobial Sprays and Mouthrinses on Supragingival Plaque Regrowth: A Comparative Study. J Periodontol. 2006;77(2):248–56. doi:10.1902/jop.2006.050116

13. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. doi:10.3389/fphar.2013.00177

14. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. 2013;10(5):210-29. doi:10.4314/ajtcam.v10i5.2

15. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites. 2019;9(11):258. doi:10.3390/metabo9110258

16. Ladeska V, Am RA, Hanani E. Colocasia esculanta L. (Talas): Kajian Farmakognosi, Fitokimia dan Aktivitas Farmakologi. J Sains Kesehatan. 2021;3(2):351-8. doi:10.25026/jsk.v3i2.441

17. Eleazu CO. Characterization of The Natural Products in Cocoyam (Colocasia esculenta) using GC–MS. Pharm Biol. 2016;54(12):2880–5. doi:10.1080/13880209.2016.1190383

18. Al-Kaf AG, Al-Deen AMT, ALhaidari SAA, Al-Hadi FA. Phytochemical Analysis and Antimicrobial Activity of Colocasia esculenta (Taro) Medicinal Plant Leaves Used in Folk Medicine for Treatment of Wounds and Burns in Hufash District Al Mahweet Governorate–Yemen. Univers J Pharm Res. 2019;4(2):29-33. doi:10.22270/ujpr.v4i2.254

19. Agyare C, Boakye YD. Antimicrobial and Anti-Inflammatory Properties of Anchomanes difformis (Bl.) Engl. and Colocasia esculenta (L.) Schott. Biochem Pharmacol. 2015;5:1. doi:10.4172/2167-0501.1000201

20. Elmosallamy A, Eltawil N, Ibrahim S, Hussein SAA. Phenolic Profile: Antimicrobial Activity and Antioxidant Capacity of Colocasia esculenta (L.) Schott. Egypt J Chem. 2021;64(4):2165–72. doi:10.21608/ejchem.2021.56495.3213

21. Pawar HA, Choudhary PD, Kamat SR. An Overview of Traditionally Used Herb, Colocasia esculenta, as a Phytomedicine. Med Aromat Plants. 2018;7:4. doi:10.4172/2167-0412.1000317

22. Alam S, Rashid MA, Sarker MMR, Emon NU, Arman M, Mohamed IN, et al. Antidiarrheal, Antimicrobial and Antioxidant Potentials of Methanol Extract of Colocasia gigantea Hook. f. Leaves: Evidenced from In Vivo and In Vitro Studies Along with Computer-aided Approaches. BMC Complement Med Ther. 2021;21:119. doi:10.1186/s12906-021-03290-6

23. Li HM, Hwang SH, Kang BG, Hong JS, Lim SS. Inhibitory Effects of Colocasia esculenta (L.) Schott Constituents on Aldose Reductase. Molecules. 2014;19(9):13212–24. doi:10.3390/molecules190913212

24. Lam KY, Ling APK, Koh RY, Wong YP, Say YH. A Review on Medicinal Properties of Orientin. Adv Pharmacol Sci. 2016;2016:4104595. doi:10.1155/2016/4104595

25. Wang S, Yao J, Zhou B, Yang J, Chaudry MT, Wang M, et al. Bacteriostatic Effect of Quercetin as An Antibiotic Alternative In Vivo and Its Antibacterial Mechanism In Vitro. J Food Prot. 2018;81(1):67–78. doi:10.4315/0362-028x.jfp-17-214

26. Singh B, Namrat, Kumar L, Dwivedi SC. Antibacterial and Antifungal Activity of Colocasia esculenta Aqueous Extract: An Edible Plant. J Pharm Res. 2011;4(5):1459–60.

27. Dutta S, Aich B. A Study of Antibacterial and Antifungal Activity of The Leaves of Colocasia esculenta Linn. Int J Pharm Sci Res. 2017;8(3):1184–7.

28. Zhang QW, Lin LG, Ye WC. Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review. Chinese Med. 2018;13:20. doi:10.1186/s13020-018-0177-x

29. Tiwari P, Kumar B, Kaur M, Gurpreet K, Kaur H. Phytochemical screening and Extraction: A Review. Int Pharm Sci. 2011;1:98-106.

30. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines. 2018;5(3):93. doi:10.3390/medicines5030093

31. Guimarães AC, Meireles LM, Lemos MF, Guimarães MCC, Endringer DC, Fronza M, et al. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. Molecules. 2019;24(13):2471. doi:10.3390/molecules24132471

32. Patra AK. An Overview of Antimicrobial Properties of Different Classes of Phytochemicals. Dietary Phytochem Microbes. 2012;1-32. doi:10.1007/978-94-007-3926-0_1

33. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. Front Microbiol. 2019;10:911. doi:10.3389/fmicb.2019.00911

34. Arnida A, Maulidia M, Khairunnisa A, Sutomo S, Faisal F. Standardization of Simplicia and Ethanol Extract of Purun Danau (Lepironia articulata (Retz.) Domin) Rhizome. Borneo J Pharm. 2021;4(4):273–82. doi:10.33084/bjop.v4i4.2794

35. Praptiwi P, Wulansari D, Fahoni A, Harnoto N, Novita R, Alfridsyah, et al. Phytochemical Screening, Antibacterial and Antioxidant Assessment of Leuconotis eugenifolia Leaf Extract. Nusantara Biosci. 2020;12(1):79–85. doi:10.13057/nusbiosci/n120114

36. Ngibad K. Phytochemical Screening of Sunflower Leaf (Helianthus annuus) and Anting-Anting (Acalypha indica Linn) Plant Ethanol Extract. Borneo J Pharm. 2019;2(1):24–30. doi:10.33084/bjop.v2i1.689

37. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in Vitro Evaluating Antimicrobial Activity: A Review. J Pharm Anal. 2016;6(2):71–9. doi:10.1016/j.jpha.2015.11.005

38. Díez-Aguilar M, Martínez-García L, Cantón R, Morosini MI. Is a New Standard Needed for Diffusion Methods for In Vitro Susceptibility Testing of Fosfomycin against Pseudomonas aeruginosa? Antimicrob Agents Chemother. 2015;60(2):1158-61. doi:10.1128/aac.02237-15

39. Saifudin A, Raharjo S, Eso A, Uji Aktivitas Antibakteri Ekstrak Metanol Rumput Laut (Kappaphycus alvarezii) pada Berbagai Tingkat Konsentrasi terhadap Pertumbuhan Bakteri Streptococcus mutans. Medula. 2015;3(1):185–91. doi:10.46496/medula.v3i1.2541

40. MaiaMRG, Marques S, Cabrita ARJ, Wallace RJ, Thompson G, Fonseca AJM, et al. Simple and Versatile Turbidimetric Monitoring of Bacterial Growth in Liquid Cultures Using a Customized 3D Printed Culture Tube Holder and a Miniaturized Spectrophotometer: Application to Facultative and Strictly Anaerobic Bacteria. Front Microbiol. 2016;7:1381. doi:10.3389/fmicb.2016.01381

41. Haerussana ANEM, Dwiastuti WP, Sukowati CA. Antibacterial Activity of Salam (Syzygium polyanthum) Leaves 70% Ethanolic Extract on Staphylococcus aureus and Staphylococcus epidermidis. J Trop Pharm Chem. 2021;5(4):375-80. doi:10.25026/jtpc.v5i4.352

42. Dewijanti ID, Mangunwardoyo W, Dwianti A, Hanafi M, Artanti N, Mozef T, et al. Antimicrobial Activity of Bay Leaf (Syzygium polyanthum (Wight) Walp) extracted using various solvent. AIP Conf Proceed. 2019;2175(1):020021. doi:10.1063/1.5134585

43. Eddy NO. Inhibitive and Adsorption Properties of Ethanol Extract of Colocasia esculenta Leaves for The Corrosion of Mild Steel in H2SO4. Int J Phys Sci. 2009;4(4):165–71. doi:10.5897/IJPS.9000319

44. Parbuntari H, Prestica Y, Gunawan R, Nurman MN, Adella F. Preliminary Phytochemical Screening (Qualitative Analysis) of Cacao Leaves (Theobroma cacao L.). Eksakta. 2018;19(2):40–5. doi:10.24036/eksakta/vol19-iss2/142

45. Zarmouh NO, Eyunni SK, Soliman KFA. The Benzopyrone Biochanin-A as a reversible, competitive, and selective monoamine oxidase B inhibitor. BMC Complement Altern Med. 2017;17(1):34. doi:10.1186/s12906-016-1525-y

46. Cheok CY, Salma HAK, Sulaiman R. Extraction and quantification of saponins: A review. Food Res Int. 2014;59:16-40. doi:10.1016/j.foodres.2014.01.057

47. Kareru PG, Keriko JM, Gachanja AN, Kenji GM. Direct detection of triterpenoid saponins in medicinal plants. Afr J Tradit Complement Altern Med. 2007;5(1):56-60. doi:10.4314/ajtcam.v5i1.31257

48. Ponce AG, Fritz R, Del Valle C, Roura SI. Antimicrobial Activity of Essential Oils on The Native Microflora of Organic Swiss Chard. LWT Food Sci Technol. 2003;36(7):679–84. doi:10.1016/S0023-6438(03)00088-4

49. Rajendiran M, Trivedi HM, Chen D, Gajendrareddy P, Chen L. Recent Development of Active Ingredients in Mouthwashes and Toothpastes for Periodontal Diseases. Molecules. 2021;26(7):2001. doi:10.3390/molecules26072001

50. Cheung HY, Wong MMK, Cheung SH, Liang LY, Lam YW, Chiu SK. Differential actions of chlorhexidine on the cell wall of Bacillus subtilis and Escherichia coli. PLoS One. 2012;7(5):e36659. doi:10.1371/journal.pone.0036659

51. Mi H, Wang D, Xue Y, Zhang Z, Niu J, Hong Y, et al. Dimethyl Sulfoxide Protects Escherichia coli from Rapid Antimicrobial-Mediated Killing. Antimicrob Agents Chemother. 2016;60(8):5054-8. doi:10.1128/aac.03003-15

52. Trisia A, Philyria R, Toemon AN. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kalanduyung (Guazuma ulmifolia Lamk.) terhadap Pertumbuhan Staphylococcus aureus dengan Metode Difusi Cakram (Kirby-Bauer). Anterior J. 2018;17(2):136–43. doi:10.33084/anterior.v17i2.12

53. Kubde MS, Khadabadi SS, Saboo SS, Ghorpade DS, Modi AJ. In Vitro Antimicrobial Activity of The Crude Extracts of Colocasia esculenta Leaves (Araceae). Int J Pharm Sci Res. 2010;1(8):88-91.

54. Khameneh B, Iranshahy M, Soheili V, Bazzaz BSF. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019;8:118. doi:10.1186/s13756-019-0559-6

55. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343-56. doi:10.1016/j.ijantimicag.2005.09.002

56. Sudji IR, Subburaj Y, Frenkel N, García-Sáez AJ, Wink M. Membrane Disintegration Caused by the Steroid Saponin Digitonin Is Related to the Presence of Cholesterol. Molecules. 2015;20(11):20146-60. doi:10.3390/molecules201119682