

Original Research

INHIBITORY EFFECT OF ONION (*ALLIUM CEPA LINN*) AND SUGAR PASTE MIXTURE ON STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI BY IN VITRO

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Received: 16 November 2019 | Accepted: 14 December 2019

DOI: <http://dx.doi.org/10.36685/phi.v5i4.310>

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ABSTRACT

Background: Staphylococcus aureus and Escherichia coli are infection agents. The onion is known to have antibacterial properties. Meanwhile, sugar paste is effective to inhibit bacterial growth.

Objective: This study aimed to identify the antibacterial activity of onion extract (*Allium cepa* Linn) and sugar paste mixture on staphylococcus aureus and Escherichia coli by in vitro.

Methods: We used a post-test-only control group design with a completely random design. The onion extract with sugar paste mixture was divided into 4 concentrations; 25%, 50%, 75% and 100% with 3 repetitions. The data were analyzed by measuring inhibition zone diameter and tested using the One-Way Analysis of Variance (ANOVA) and Tukey test.

Results: Findings showed that on average, the antibacterial activity of onion extract and sugar paste mixture on staphylococcus aureus was 14.57mm, 17.44mm, 18.36mm, and 22.28mm, respectively 25%, 50%, 75%, and 100% concentrations. Meanwhile, on Escherichia coli, it was 17.27mm, 19.67mm, 20.31mm, and 21.62mm.

Conclusion: Onion extract and sugar paste mixture can inhibit the growth of Staphylococcus aureus and Escherichia coli.

Keywords: inhibition effect, onion extract, sugar paste, mixture, Staphylococcus aureus, Escherichia coli

BACKGROUND

Open wounds are susceptible to bacterial contamination (Biswas et al., 2010; Bradshaw, 2011). Meanwhile, bacteria are sources of infections and diseases (Praba & Kumaresan, 2014). Staphylococcus aureus is the gram-positive bacteria causing infections. Likewise, Escherichia coli is the gram-negative bacteria (Bradshaw, 2011; Nor, Indriarini, & Koamesah, 2018). Furthermore, infected wound requires longer healing time (Agra et al., 2013), while bad wound healing can trigger critical and

chronical ischemic (Biswas et al., 2010; Zaenal, 2016).

Various natural products have been empirically used as traditional medicine in wound healing (Zaenal, 2016). Onion (*Allium cepa* Linn) has been used in many countries as a traditional medicine and wound healing. Traditionally, onion has been used in Plethora, Sumeria, and Mesopotamia communities (Bakht, Khan, & Shafi, 2013; Sharifi-Rad et al., 2016; Teshika et al., 2019; Wang, Tian, & Ma, 2012). Besides,

the red onion has been used since 5,000 years ago in Egypt. The previous studies show that onion has antibacterial, anti-parasite, and anti-fungus properties ([Bakht et al., 2013](#)). The red onion bulbs contain kaempferol, flavonoid, β -sitosterol, ferulic acid, muriatic acid, and prostaglandin ([Shenoy, Patil, Kumar, & Patil, 2009](#)). These active compounds are antibacterial.

Sugar is the best topical for open wounds (especially contaminated wounds) ([Mathews & Binnington, 2002](#)). The use of sugars in the wound is one method that has been known for a long time ([Biswas et al., 2010](#)). The sugar paste has been used to heal the wound for a long time ([Topham, 2002](#)). In vitro, the sugar paste is effective in clearing infectious agents ([Naselli et al., 2017](#)). [Murandu, Webber, Simms, and Dealey \(2011\)](#) reported that in vitro, the granule sugar therapy on the slough and necrotic wound management can inhibit bacterial growth which can cause infection, namely *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Numerous studies have reported the use of red onion as an anti-microbe and wound healing. [Sidgwick, McGeorge, and Bayat \(2015\)](#) reported that onion extract is a natural medicine in wound healing. Furthermore, [Octaviani, Fadhli, and Yuneistya \(2019\)](#) showed that ethanol extract from red onion peeling has high antibacterial activity on *Staphylococcus aureus* and *Escherichia coli*. Likewise, [Surono \(2013\)](#) showed the inhibitory effect of red onion bulb extracts

Studies also have reported that sugar can inhibit bacterial growth ([Molan & Rhodes, 2015](#)). The sugar paste can accelerate the infectious wound healing process ([Murandu et al., 2011](#); [Naselli et al., 2017](#); O'Connell & Wardlaw, 2011; [Tanner, Owen, & Seal, 1988](#)). [Archer, Barnett, Irving, Middleton, and Seal \(1990\)](#) reported that sugar paste can be used on open wounds

To the best of our knowledge, there is no previous study about antibacterial activity of red onion with sugar paste mixture on *Staphylococcus aureus* and *Escherichia Coli*.

Therefore, this study aimed to identify the antibacterial activity of onion extract (*Allium cepa* Linn) and sugar paste mixture on *Staphylococcus aureus* and *Escherichia coli* by in vitro.

METHODS

Setting and Study Design

The study was conducted at Politeknik Bina Husada Kendari Laboratory. We used a post-test-only control group design with a completely random design.

Sample

This study used red onion bulb samples, sugar paste, *Staphylococcus aureus*, and *Escherichia Coli* bacteria.

Instruments

The instruments used in this study were rotary evaporator, glassware, autoclave, dark bottles, petri dishes, hot plate, incubator, calipers, transfer needles, cotton, disk paper, filter paper, oven, tweezers, micropipettes, drip plates, vortices, and analytical scales. While, the materials used in this study were red onion, icing sugar, and castor sugar obtained from one trader in Kendari, Southeast Sulawesi. The onion bulb was eventually extracted using the maceration method with 96% ethanol solvent ([Simaremare & Pryta, 2017](#)). Subsequently, Nutrient Agar (NA), disk, 96% ethanol, DMSO, physiological NaCl solution, aqua dest, chloroform, ammoniac chloroform, magnesium, Lieberman-Bouchard reactor, Mayer reactor, caster sugar, icing sugar, polyethylene glycol, and hydrogen peroxide. Bacteria used in this study were *Staphylococcus aureus* and *Escherichia Coli*.

Extraction

As much as 1 kg of red onion bulbs was dried for 7 days, peeled and cut roughly beforehand. The cut bulbs were out into glass tube and then soaked with 1 liter of 96% alcohol for 2 x 24 hours in tightly closed conditions. Maceration results were filtered using Buchner funnel and vacuum. The extract was then evaporated and concentrated under the low pressure at 5°C.

Subsequently, the extract was dried in a vaporizer plate on a water bath in order to obtain the thick extract. The thick extract was then poured into petri dishes and then an empty test disc was immersed in the extract for 15 – 30 minutes. Before use, the extract was stored in a refrigerator at 4°C and not exposed to direct sunlight ([Simaremare & Pryta, 2017](#); [Yunanda & Rinanda, 2016](#)).

Sugar Paste Making

The sugar paste was made using: Caster Sugar, Icing Sugar, Polyethylene Glycol, and Hydrogen Peroxide 30% ([Bhat et al., 2014](#)).

Antimicrobial Activity Test

The red onion extract with sugar paste mixture was made in various concentrations; 25%, 50%, 75%, and 100%. Furthermore, as much as 1 transfer needle of *Staphylococcus aureus* and *Escherichia Coli* were taken and mixed into 0.9% NaCl diluent solution and homogenized. Subsequently, the turbidity was compared with a standard solution of 0,5 McFarland. Bacterial suspension of *Staphylococcus aureus* and *Escherichia Coli* was then applied on a Muller Hinton jelly media used transfer needle. An empty test disc that had been soaked into onion extract was placed on the surface of the Muller Hinton jelly. The Muller Hinton jelly was then

incubated at 37°C for 24 hours. After that, we measured the clear zone diameter that was formed using a ruler.

Ethical Consideration

This study was approved by the ethics committee of the Association of Indonesian Public Health Expert Regional Management of Southeast Sulawesi.

Data Analysis

To analyze the antibacterial activity, the measurement of inhibition area diameter was made using caliper in each concentration. Furthermore, we applied a One-Way Analysis of Variance (ANOVA) and Tukey test using SPSS program.

RESULTS

Based on the measurement of the inhibition zone of the red onion extract with sugar paste mixture on *Staphylococcus aureus*, the widest diameter was found for 100% concentration in the 1st repetition, 22.87 mm. Meanwhile, for the same concentration, the 2nd and 3rd showed a diameter of 22.77mm and 21.20 mm, respectively (Table 1).

Table 1 Inhibition Effect of the red onion extract with sugar paste mixture on *Staphylococcus aureus* in various concentrations

| Concentrations | Inhibition zone of the red onion extract with sugar paste mixture on <i>Staphylococcus Aureus</i> (mm) | | |
|----------------|--|---------------|----------------|
| | Repetition I | Repetition II | Repetition III |
| 25% | 14.60 | 14.60 | 14.53 |
| 50% | 20.83 | 16.00 | 15.50 |
| 75% | 19.40 | 17.80 | 17.87 |
| 100% | 22.87 | 22.77 | 21.20 |

Table 2 Inhibition Effect of the red onion extract with sugar paste mixture on *Escherichia coli* in various concentrations

| Concentrations | Inhibition zone of the red onion extract with sugar paste mixture on <i>Escherichia coli</i> (mm) | | |
|----------------|---|---------------|----------------|
| | Repetition I | Repetition II | Repetition III |
| 25% | 17.50 | 17.50 | 16.80 |
| 50% | 18.50 | 18.50 | 22.00 |
| 75% | 19.17 | 19.10 | 22.67 |
| 100% | 20.83 | 20.05 | 23.53 |

Based on the measurement of the inhibition zone of the red onion extract with sugar paste

mixture on *Escherichia coli*, the widest diameter was found for 100% concentration in

the 3rd repetition, 23.53 mm. Meanwhile, for the 1st and 2nd repetitions, the widest diameter also

showed in the same concentration, 22.77mm and 21.20 mm respectively (Table 2).

Table 3 Antibacterial activity of the red onion extract with sugar paste mixture on Staphylococcus Aureus and Escherichia coli in various concentrations

| Variables | The average inhibition zone diameter (mm) | | | |
|---|---|-------|-------|-------|
| | 25% | 50% | 75% | 100% |
| The red onion with sugar paste mixture (SA) | 14.57 | 17.44 | 18.36 | 22.28 |
| The red onion with sugar paste mixture (EC) | 17.27 | 19.67 | 20.31 | 21.62 |
| Positive control (Smart Garlic Ointment) | 0 | 0 | 0 | 0 |
| Negative control (Aquadest) | 0 | 0 | 0 | 0 |

On average, the inhibition zone of the red onion with sugar paste mixture was wider on Staphylococcus aureus than Escherichia coli. While both positive and negative controls did

not show any inhibition zone (0 mm). This was shown at 100% concentration, 22.28 mm (Table 3).

Table 4 The comparison of antibacterial activity test of the red onion extract with sugar paste mixture on Staphylococcus Aureus and Escherichia coli

| Type of bacteria | Mean ± SD | 95% CI | p-value |
|------------------|-------------------|-------------------|---------|
| S. aureus | 18.1642 ± 3.18838 | 16.1384 – 20.1900 | 0.178 |
| E. coli | 19.7167 ± 2.18058 | 18.3312 – 21.1021 | |

The analytical test with One-Way ANOVA on the antibacterial activity of the red onion extract with sugar paste mixture on Staphylococcus Aureus and Escherichia coli showed a *p-value* = 0.178 (*p-value* > 0,05) indicating we failed to reject Ho. Thus, it can be concluded that there was no significant difference of the antibacterial activity of the red onion extract with sugar paste mixture on Staphylococcus

Aureus and Escherichia coli. However, the mean and standard deviation of the antibacterial activity of the red onion extract with sugar paste mixture on Staphylococcus Aureus and Escherichia coli were 18.1642 ± 3.18838 and 19.7167 ± 2.18058, respectively. Therefore, it implied that the average inhibition zone on Escherichia coli tended to be higher than Staphylococcus Aureus (Table 4).

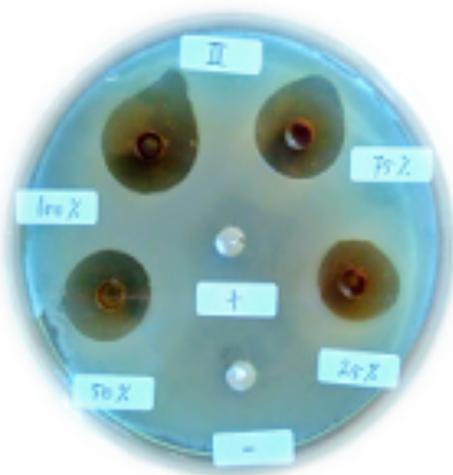


Figure 1 Antibacterial activity of the red onion extract with sugar paste mixture on Staphylococcus Aureus in various concentrations

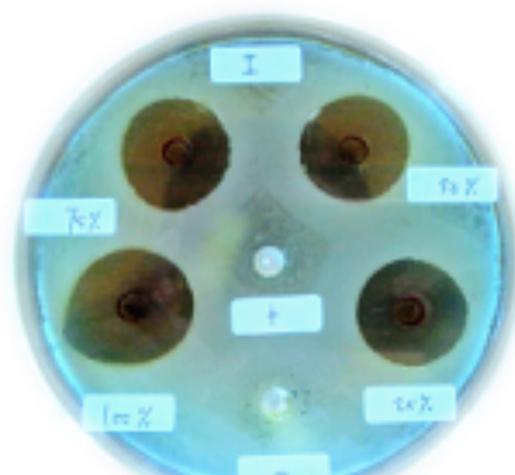


Figure 2 Antibacterial activity of the red onion extract with sugar paste mixture on Escherichia coli in various concentrations

DISCUSSIONS

This study aimed to identify the antibacterial activity of the red onion extract with sugar paste mixture on *Staphylococcus aureus* as positive gram bacteria and *Escherichia coli*, negative gram bacteria. This activity was observed by measuring the clear zone formed in the Muller Hinton medium that had been smeared with bacterial suspension. The zone was measured using a caliper. Subsequently, smart garlic ointment and aqua dest were used for positive and negative controls, respectively. The observation was repeated three times and then averaged. The average inhibition zone of the red onion with sugar paste mixture was compared to the positive and negative controls. All concentrations of the red onion with sugar paste mixture have a significant inhibition effect on bacteria. The previous studies have reported the anti-microbial properties on the red onion ([Octaviani et al., 2019](#); [Sidgwick et al., 2015](#); [Škerget, Majhenič, Bezjak, & Knez, 2009](#); [Surono, 2013](#)).

Previous studies on the effect of sugar paste to inhibit bacterial growth have also been reported. According to Archer et al. the sugar paste can be applied on open wound ([Archer et al., 1990](#)). To the best of our knowledge, there is no previous study addressed the antibacterial test of red onion extract with sugar paste mixture.

The use of the red onion extract with sugar paste mixture as anti-microbial for antibacterial activity test shows the existence of inhibition zone significantly on *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity of the 96% alcohol and red onion extract with sugar paste mixture at 100% concentration showed an average inhibition zone of 22.28 mm and 21.62 mm respectively for *Staphylococcus aureus* and *Escherichia coli*. These results is higher than previous study, whereas the inhibition zone of chloroform water of *Allium cepa* extract at 200 µg/0,1 mL concentration on *Staphylococcus aureus* was 18 mm and 21 mm on *Escherichia coli* ([Shenoy et al., 2009](#)), the ethanol extract of red onion at 50% concentration on *Staphylococcus aureus* was 16.03 mm and *Escherichia coli* was 7.77 mm

([Octaviani et al., 2019](#)), the red onion bulb extract at 80% concentration was 1.216 mm ([Surono, 2013](#)), the red onion extract was 8.33 mm ([Simaremare & Pryta, 2017](#)), the red onion peeling extract at 80% concentration on *Staphylococcus aureus* was 14.33 mm ([Misna & Diana, 2016](#)), and the red onion peeling extract has high antibacterial properties on bacteria growth such as *E. coli* ([Škerget et al., 2009](#))

The bioactive compounds of red onion have pharmacological characteristics, namely as antimicrobial, antioxidant, analgesic, antiinflammation, anti-diabetes, hypolipidemia, anti-hypertension, and immune-protective effects ([Teshika et al., 2019](#)). These compounds include allicin as antibacterial ([Surono, 2013](#)). Furthermore, the phytochemical test found flavonoids, tannin, and alkaloids ([Shenoy et al., 2009](#)). Sharifi-Rad, et al. showed antimicrobial effects of red onion ([Sharifi-Rad et al., 2016](#)).

The sugar paste has an effective antimicrobial activity on the infected wound ([Archer et al., 1990](#)). In vitro, the sugar paste effectively cleans the cause of wound infection ([Naselli et al., 2017](#)). Furthermore, the use of granule sugar therapy can inhibit the bacterial growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* ([Murandu et al., 2011](#)). The benefit of using sugar as wound dressing accelerates antibacterial mechanism, tissue granulation, and epithelization ([Mathews & Binnington, 2002](#)).

The inhibition zone of the red onion extract with sugar paste mixture on *Staphylococcus aureus* and *Escherichia coli* was due to the combination of the red onion bulb extract as antimicrobial and the sugar paste has a local osmotic effect. Misna and Diana reported that the red onion peeling extract is bacteriostatic and bactericidal on *Staphylococcus aureus* ([Misna & Diana, 2016](#)). The bactericidal activity is made by denaturing protein and destroys the cytoplasm membrane of a bacterial cell. Consequently, the selective permeability function, active transport, and structural control of bacterial cell protein are hampered. Bacterial cells loss their form and bacteriolysis ([Razak,](#)

Djamal, & Revilla, 2013). Furthermore, the onion extracts reduce the proliferation process of culture cell fibroblast, including matrix metalloproteinase-1 expression, suggesting a role in ECM re-modeling (Sidgwick et al., 2015). On the bactericidal process, the sugar has high osmolarity. The granule sugar has osmotic thermodynamic activities associated with water activity, pH, osmosis effects, bacterial and fungus competition to water, derivate tendency and membrane disruption of bacterial cell and fungi (Naselli et al., 2017). Stress due to high osmotic can damage cell wall permeability, and eventually, the bacteria will die (O'Connell & Wardlaw, 2011). The wound in moist conditions can prevent bacterial colonization (Ruhullah et al., 2013).

CONCLUSION

The study finding shows an average inhibition zone of antibacterial activity of the red onion extract with sugar paste mixture on *Escherichia coli* is wider than on *Staphylococcus Aureus*. The widest inhibition zone was found at 100% concentration in the 3rd repetition, 23.53 mm. Statistically, we found no significant difference in antibacterial activity test of the red onion extract with sugar paste mixture on *Staphylococcus aureus* and *Escherichia coli* (p -value = 0.178).

Acknowledgment

The present study was supported by the Research and Community Engagement Directory (DRPM) of the Republic of Indonesia.

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Cite this article as: Tahiruddin., Indriastuti, D. Inhibitory effect of onion (*allium cepa* linn) and sugar paste mixture on staphylococcus aureus and escherichia coli by in vitro. *Public Health of Indonesia*. 5(4), 131-137.