

**ANTIBACTERIAL EFFICACY of BELUNTAS (*Pluchea indica* L.) LEAVES
AQUEOUS EXTRACT against *Staphylococcus aureus* and *Escherichia coli*
Which CAUSE SUBCLINICAL MASTITIS in DAIRY COW**

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ABSTRACT

The purpose of this research was to examine the effect of Beluntas (*Pluchea indica* L.) leaves aqueous extract as herbal antibacterial against bacteria which causes subclinical mastitis in dairy cow. *Staphylococcus aureus* and *Escherichia coli* from mastitis milk with score +3 were used in this research. The research method was experimental using Completely Randomized Design (CRD) design with five treatments and four replications. The treatments consisted of 20% (P1), 40% (P2), 60% (P3), and 80% (P4) beluntas leaves extract, while iodips® 10% as control (P0). The variable measured was diameter of inhibition zones. Results showed that beluntas leaves aqueous extract had highly significant effect ($P < 0.01$) on diameter of inhibition zones of *Staphylococcus aureus* and *Escherichia coli*. The research can be concluded that 40% beluntas leaves extract had equal capability to 10% iodips for inhibiting the growth of *Staphylococcus aureus*, while 80% beluntas leaves extract had equal capability to 10% iodips on growth inhibition *Escherichia coli*. Beluntas leaves aqueous extract were more effective as herbal antibacterial against *Staphylococcus aureus* than *Escherichia coli*. 80% Beluntas leaves extract give best result in inhibiting *Staphylococcus aureus* and *Escherichia coli*.

Keywords: California mastitis test, inhibition zones and iodips

**EFEKTIVITAS ANTIBAKTERI EKSTRAK DAUN BELUNTAS (*Pluchea indica* L.)
DENGAN PELARUT AQUADEST TERHADAP
Staphylococcus aureus dan *Escherichia coli*
PENYEBAB MASTITIS SUBKLINIS PADA SAPI PERAH**

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ABSTRAK

Tujuan dari penelitian ini adalah untuk mengetahui pengaruh antibakteri ekstrak daun beluntas dalam menghambat pertumbuhan bakteri *Staphylococcus aureus* dan *Escherichia coli* penyebab mastitis subklinis pada sapi perah. Materi yang digunakan meliputi bakteri *Staphylococcus aureus* dan *Escherichia coli* dari hasil isolasi susu mastitis subklinis skor CMT

+3 yang berasal dari peternakan Jabung, ekstrak daun beluntas dan iodips 10%. Metode yang digunakan adalah percobaan dengan Rancangan Acak Lengkap (RAL) pola tersarang dengan 5 perlakuan dan 4 kali ulangan. Perlakuan terdiri dari ekstrak daun beluntas konsentrasi 20% (P1), 40% (P2), 60% (P3), dan 80% (P4), 10% iodips sebagai kontrol (P0). Variable yang diamati adalah luas diameter zona hambat antibakteri. Hasil penelitian menunjukkan bahwa ekstrak daun beluntas dengan pelarut aquades berpengaruh sangat nyata ($P < 0.01$) dapat menghambat pertumbuhan *S. aureus* dan *E. coli*. Kesimpulan penelitian ini bahwa ekstrak daun beluntas 40% memiliki kemampuan setara dengan iodips 10% dalam menghambat pertumbuhan *S. aureus*, sedangkan ekstrak daun beluntas 80% memiliki kemampuan setara dengan iodips 10% dalam menghambat *E. coli*. Ekstrak daun beluntas lebih efektif menghambat *S. aureus* dibandingkan dengan *E. coli*. Ekstrak daun beluntas konsentrasi 80% memiliki kemampuan terbaik dalam menghambat pertumbuhan *S. aureus* dan *E. coli*.

Kata kunci: California mastitis test, zona hambat dan iodips

INTRODUCTION

Mastitis in dairy cow is udder inflammation which causes in declining milk production. Mastitis can be divided into two types: clinical it can be seen directly due to appearance damage of udder in outer part, while subclinical inflammation can only be known when tested on laboratory. Subclinical mastitis is most often attack on dairy cows (Ariyanti and Supar, 2008).

Abrar, Wibawan, Priosoeryanto, Soedarwanto and Pasaribu (2012) reported that the incidence of subclinical mastitis in East Java, Central Java, West Java and Yogyakarta about up to 67% and clinical mastitis up to 30%. Surjowardojo, Suyadi, Hakim and Aulaniam (2011), also confirmed that subclinical mastitis reduced milk production about 28.4% to 53.5% per cow.

Staphylococcus aureus and *Escherichia coli* are infectious bacteria especially on subclinical mastitis in dairy cows. Ariyanti and Supar (2008) reported that isolation result from subclinical mastitis cow consisted of 18.1% *Staphylococcus aureus* and 5% *Escherichia coli*. *Staphylococcus*

aureus can be isolated from various parts of the body, such as the abdominal skin, udder skin, tail and the floor of the housing and the tools around livestock. Different with *Escherichia coli* are known as coliforms in the intestine of cattle that often found in feces cause udder infection through via teat canal (Siegrist, 2015). Disease transmission through contamination by hand worker, milking apparatus and the water used (Subronto, 2008).

Teat dipping is common mastitis prevention method to minimize incidence of subclinical mastitis. Iodips is commonly used in dairy cow industries. However improper use of chemical substance will increase chemical residue in milk production even resistant mutant bacteria in the environment. Hence, an alternative by using herbal antibacterial is currently popular to treat mastitis infection.

Beluntas is a small herbaceous plant, grow upright and reach approximately 2 m. In rural area beluntas leaves only used as a bush plant or as a vegetable (Rukmana, 2010). Sulistyarningsih (2009) reported that Beluntas

leaves contained chemicals compound, among others alkoloid, saponins, polyphenols, fat, tannins, sterols, amino acids, chlorogenik acids, essential oils, potassium, magnesium, aluminum, phosphorus, iron, vitamin A, vitamin C and flavonoids.

Andarwulan, Batari, Sandrasari, Bolling and Wijaya (2010) reported that the highest percentage of substances is flavonoid in Beluntas leaves. Flavonoid is highest content which include phenolic compound to inhibit the activity of *Escherichia coli* and *Staphylococcus aureus* (Susanti, 2007). Lathifah (2008) mentioned that flavonoid can be extracted by using aquadest, etanol and methanol. This research was conducted to examine efficacy of Beluntas leaves extracted with aquadest as herbal antibacterial against both *Staphylococcus aureus* and *Escherichia coli* causes subclinical mastitis.

MATERIAL and METHODS

Material and tools:

Materials used in this research were Simplisia of Beluntas leaves, aquadest steril, *Staphylococcus aureus*, *Escherichia coli*, Iodips® solution with 10% concentration, media Nutrient Agar (NA), Alcohol 70%, Mannitol Salt Agar (MSA), Nutrient Broth Agar, Beluntas leaves extract.

The tools used to extract Beluntas leaves were Scales analytic, beaker glass, busher, shaker incubator, media glass, rotary evaporator, vacuum pump, filter paper and stirrer. Tools used to test the inhibition of antibacteria were Petri dish, test tube, Bunsen, autoclave, incubator, erlenmeyer 250 ml, cotton, beaker glass, micro pipet, pinset, jangka sorong, stirrer L glass, aluminium foil, plastic wrap, paper label and tissue.

Extraction Procedure of Beluntas Leaves:

Beluntas leaves extracted by maceration method with aquadest. The extraction process based on methods Nurhalimah, Widyaningsih and Wijayanti (2015) which reported that the optimal ratio between the materials with a solvent was 1:10 to obtain the optimum yield. Extraction procedure (Ismi, Ratnawati and Yudi, 2010):

- a. Simplisia of beluntas leaves weighed as much as 100 grams.
- b. Beluntas leaves poured into 1 litre size of erlenmeyer.
- c. Maceration was done by added 1000 ml of aquadest and then homogenized with a shaker incubator for 24 hours.
- d. The solution beluntas leaves were filtered by vacuum pump until the residue did not drip and the filtrate obtained.
- e. The filtrate evaporated with rotary evaporator at a temperature of 80 ° C until the solvent evaporated completely in order to obtain a concentrated extract of beluntas leaves.

Preparation of MSA Media

The Mannitol Salt Agar media (MSA) used to isolate, and identify the *Staphylococcus aureus*. MSA media can be used to select the bacteria *Staphylococcus aureus* or other members of the genus *Staphylococcus*. Suryanto, Irmayanti and Lubis (2007), reported that there is growth of *Staphylococcus aureus* characterized by a color changed from red to yellow in media due to mannitol fermentation carried *Staphylococcus aureus*. MSA media 28 gram dissolved with 250 ml aquadest, then stirred with hot stirrer and covered with aluminum foil. MSA media sterilized with autoclave at

121° C temperature 2 atm of pressure for 15 minutes. The media poured into each petri dish each 20 ml, and left until and allowed until solid.

Preparation of Nutrient Broth Media

The Nutrient Broth (NB) used to isolate, and identify *Escherichia coli*, then incubated in an aerobic state at a temperature of 37° C for 24 hours (Indrawani, Sartika, and Sudiarti, 2005). Preparation the nutrient broth media according to Gunawan, Sarwiyono, and Surjowardojo (2013) reported that NB media 1.3 gram dissolved with 100 ml aquadest, then stirred with hot stirrer and covered with aluminum foil. MSA media sterilized with autoclave at 121° C temperature 2 atm of pressure for 15 minutes. The media poured into each petri dish each 20 ml, and left until and allowed until solid.

RESULT and DISCUSSION

Beluntas leaves aqueous extract 22 gram obtained in this research. Beluntas leaves extract that obtain in form solid with dark green color. The research about Beluntas leaves aqueous extract that's mean extraction using pure water or aquadest as solvent. Lathifah (2008) reported that the weight of extract product influenced by the solvent polarity, polar solvents produced higher extract product weight than nonpolar solvents. It is indicated that aquadest is polar solvent and it is capable to extract polar compounds in Beluntas leaves. Poernomo (2001) reported that flavonoid and tannin are part of the polar compounds, which are contained in Beluntas leaves.

Inhibition Zone of Beluntas Leaves Aqueous Extract toward *Staphylococcus aureus*

Statistical analysis result showed that Beluntas leaves aqueous extract in concentrations of 20% to 80% highly significant effect ($P < 0.01$) on diameter of inhibition zones of *Staphylococcus aureus* (Table 1).

Table 1. The average diameter of inhibition zones Beluntas leaves extract on *Staphylococcus aureus*

No.	Treatment	Diameter of inhibition zones (mm)
1.	P0 iodip	4.725±0.056 ^b
2.	P1 20 %	2.505±0.052 ^a
3.	P2 40 %	4.710±0.06 ^b
4.	P3 60%	6.228±0.126 ^c
5.	P4 80%	8.193±0.051 ^d

Description: Different superscript indicated that treatments highly significant difference ($P < 0.01$) toward size diameter of inhibition zones of *Staphylococcus aureus*.

Increasing the concentration of Beluntas leaves extract increased the inhibition zones diameter. Beluntas leaves extract 20% lower than iodips 10% on capability antibacterial agent, while Beluntas leaves extract 40% equal with iodips 10% on capability antibacterial agent against *Staphylococcus aureus*. Beluntas leaves extract 60%, and 80% were more effective than 10% iodips. Furthermore Beluntas leaves extract 80% showed the highest average of inhibition zone diameter (Figure 1).

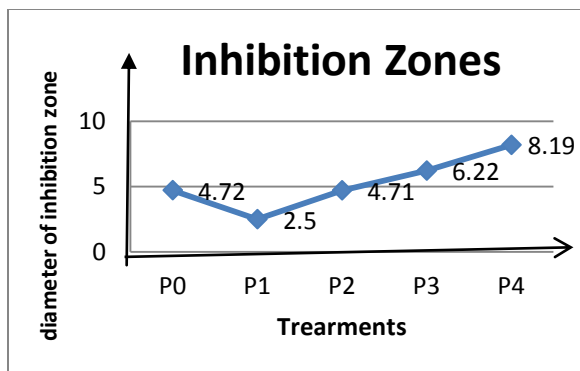


Figure 1. Graphic diameter average of inhibition zones of *S. aureus*

Inhibition zone diameter indicated that the active compound in Beluntas leaves can inhibit the bacteria activity. Nisaa' and Darjono (2011) reported that the clear zone formed around the holes showed the antibacterial activity from Beluntas leaves is capable to inhibit the activity of *Staphylococcus aureus*. Average inhibition zones diameter resulted by Beluntas leaves aqueous extract toward *Staphylococcus aureus* (Figure 1) with ranging 2.50 to 8.19 mm, this is more higher than research before according to Ardiansyah et al. (2003) reported that average inhibition zone diameters of Beluntas leaves extracted with hexan at concentration 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% toward *Staphylococcus aureus* only ranging 4.10-5.77 mm, that is because hexan is non polar solvent that cannot extraction polar active compound from Beluntas leaves which can penetrate cell wall bacteria.

Susanto *et al.* (2012) reported that antibacterial activity categorized as strongest function if the diameter of inhibition zones reached more than 20 mm. it will be classified as medium strength if the diameter of inhibition zone about 6-10 mm and low category with inhibition zone diameter less

than 5 mm. The results showed that, inhibition zone diameter of Beluntas leaves aqueous extract concentrations of 20% to 40% and 10% iodips® categorized as low strength of antibacterial activity against *Staphylococcus aureus*, caused only achieved the inhabitation zones diameter about 2.505 mm to 4.725 mm (below 5 mm). Beluntas leaves extract concentration 60% and 80% categorized as medium strength due to inhibition zones diameter reached about 6.228 to 8.193 mm.

Inhibition Zone of Beluntas Leaves Aqueous Extract toward *Escherichia coli*

Statistical analysis result showed that Beluntas leaves aqueous extract in concentrations of 20% to 80% highly significant effect ($P < 0.01$) on diameter of inhibition zones of *Escherichia coli* (Table 2).

Table 2. The average diameter of inhibition zones of Beluntas leaves aqueous extract on *Escherichia coli*

No.	Treatment	Diameter inhibition zone (mm)
1.	P0 iodip	2.090±0.062 ^d
2.	P1 20 %	0.965±0.121 ^a
3.	P2 40 %	1.185±0.021 ^b
4.	P3 60%	1.708±0.095 ^c
5.	P4 80%	2.188±0.092 ^d

Description: Different superscript indicated treatments highly significant difference ($P < 0.01$) toward diameter size of inhibition zone of *Escherichia coli*.

Increasing the concentration of Beluntas leaves extract increased the inhibition zones diameter. Beluntas leaves aqueous extract in concentration of 20% to 80% gave smaller than iodips 10% on inhibition zones diameter. While Beluntas leaves extract 80% equal with iodips 10% in inhibited growth of *Escherichia coli*. Furthermore, Beluntas leaves extract 80%

showed the highest average of inhibition zone diameter. The diameter average of inhibition zones was resulted by Beluntas leaves aqueous extract (Figure 2) ranging 0.96 to 2.18 mm.

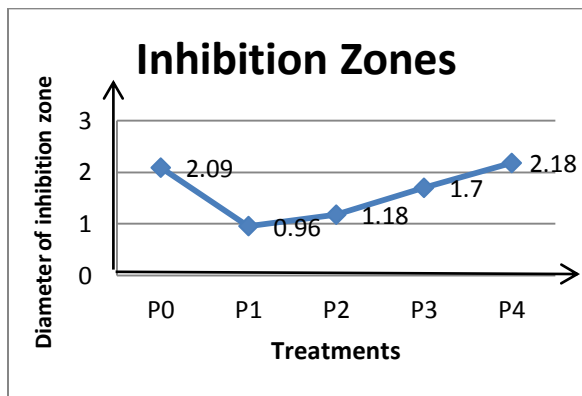


Figure 2. Graphic diameter average of inhibition zones of *E. coli*

The diameter average of inhibition zones was resulted by Beluntas leaves aqueous extract (Figure 2) ranging 0.96 to 2.18 mm, that is lower than result research according to Ardiansyah et al. (2003) reported that inhibition zone diameter of reported that average inhibition zone diameters of Beluntas leaves extracted with hexan at concentration 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% toward *Escherichia coli* about 3.57-5.60 mm. Hexan is non polar solvent which can extract non polar compound from Beluntas leaves. Different with aquadest is polar solvent that only can extract polar compound of Beluntas leaves. Cell walls of *Escherichia coli* high contain non polar compound which more easily can be penetrated by non polar compound of Beluntas leaves, so can inhibit bacteria growth more effective used hexan than aquadest.

This research showed that the highest concentration of 80% Beluntas leaves extract had the best capability to inhibit *Escherichia*

coli. This is appropriate according to Ulfa (2009) reported that the best capability of Beluntas leaves ethanolic extract toward *Escherichia coli* was the highest concentration (100% of Beluntas leaves ethanolic extract). This is explaining that higher concentration of extract high contain of active compound that can inhibit bacteria growth.

Susanto *et al.* (2012) reported that antibacterial activity categorized as antibacterial low strength if only reached inhibition zone diameter smaller than 5 mm. The results showed that, inhibition zone diameter of Beluntas leaves aqueous extract in concentrations of 20 to 80% and iodips 10% categorized as low antibacterial activity against *Escherichia coli*, caused only achieved the inhibition zones diameter about 0.965 mm to 2.188 mm (below 5 mm).

Effectiveness Comparison Beluntas Leaves Aqueous Extract as Antibacterial toward *Staphylococcus aureus* and *Escherichia coli*

Statistical analysis result showed that type of bacteria give highly significant effect ($P < 0.01$) on size diameter of inhibition zones. *Staphylococcus aureus* produced diameter of inhibition zones larger than *Escherichia coli*. *Staphylococcus aureus* produced inhibition zones diameter about 5.27 ± 1.932^b mm and categorized as medium strength antibacterial, different with *Escherichia coli* which only produced inhibition zones diameter about 1.62 ± 0.501^a and categorized as weak strength antibacterial (Figure 3).

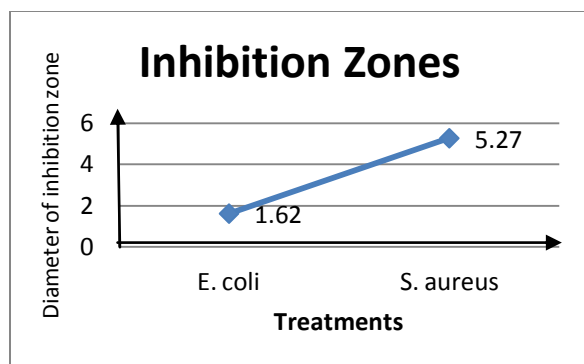


Figure 3. Graphic diameter average of inhibition zones of *E. coli* and *S. aureus*

Type of bacteria can influence magnitude of inhibition zones diameter. This research showed that average inhibition zones diameter of *Staphylococcus aureus* higher than *Escherichia coli*. That is appropriate according to Ardiansyah et al. (2003) reported that Beluntas leaves extracted with hexan at concentrations of 10%, 20%, 30%, 40%, 50%, 60% and 70% that more sensitive antibacterial effect toward *staphylococcus aureus* than *Escherichia coli*. It's related with cell wall structure of bacteria and penetrates of active compound from Beluntas extract

Mechanism of inhibition related with interaction between active compounds from Beluntas leaves extract with bacteria cell. Chuakul, Soonthornchareonnon and Wiwat (2012) reported that gram-positive bacteria are more susceptible to antibacterial compounds than gram-negative bacteria. It reported that inhibition zones diameter of *Staphylococcus aureus* higher than *Escherichia coli*. Pukumpuang, Thongwai and Tragoolpua (2012) reported that level of sensitivity of gram positive and gram negative towards treatment with plant extract due to differences in morphology. Gram-negative bacterial cell wall structure is more complex than the gram-

positive bacteria, gram-negative bacteria so that the cells may not be damaged by the active compounds from extracted plant. Pelczar and Chan (2008) reported that the structure of the cell wall of gram-negative bacteria plated three (multi-layer) with high lipid content of 11-22%, it make active compound of Beluntas leaves is difficult to access into bacteria cell. It explained that inhibition zones diameter produced by *Escherichia coli* in small size.

Mechanism of Beluntas leaves extract inhibit bacteria growth initially with damaged the cell wall. Bacterial cell wall is a thick layer so that when there a shock from outside the cell wall remains strong to protect contained therein. In the opinion of Dinda (2008) reported that the inhibitory activity the bacteria activity due to interaction of phenolic compounds and their derivatives with a bacterial cell, wherein the phenol compound into the bacterial cell pass through the bacterial cell wall and cytoplasmic membrane, in a bacterial cell phenolic compounds cause protein denaturation constituent of protoplasm and damaged the bacterial cell cytoplasm. Nurhalimah et al. (2014) reported that tannin compound damage the cell membrane, so that in such circumstances the metabolism become inactive and can inhibit bacteria activity. It is reported that Beluntas leaves aqueous extract have capability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*.

CONCLUSIONS AND SUGGESTIONS

Conclusion

Based on these results it can be reported as follows:

1. Beluntas leaves aqueous extract have capability to inhibit *Staphylococcus aureus* with diameter average of

inhibition zone ranging from 2.505 to 8.193 mm and *Escherichia coli* ranging from 0.965 to 2.188 mm.

2. 40% Beluntas leaves extract and 80% are equal with iodips 10% to inhibit growth of *Staphylococcus aureus* (4.71±0.06 mm) and *Escherichia coli* (2.18±0.09 mm) respectively.
3. 80% Beluntas leaves extract give best result in inhibiting *Staphylococcus aureus* and *Escherichia coli*.
4. Beluntas leaves extract were more effective as herbal antibacterial toward *Staphylococcus aureus* with average diameter of inhibition zones 5.272 mm compared with *Escherichia coli* which only reach diameter of inhibition zones 1.627 mm.

Suggestions

Based on the research recommended use Beluntas leaves aqueous extract in concentration of 80% as herbal antiseptic to teat dipping in dairy cow. Also recommended further research about efficacy of Beluntas leaves extract toward other bacteria causes subclinical mastitis.

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