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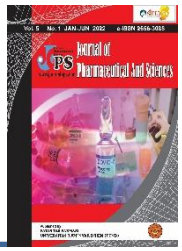

ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF JERINGAU (Acorus Calamus L.) RHIZOME AGAINST Staphylococcus aureus AND Escherichia coli

AKTIVITAS ANTIBAKTERI EKSTRAK ETANOL RIMPANG JERINGAU (*Acorus calamus* L.) TERHADAP *Staphylococcus aureus* DAN *Escherichia coli*

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ABSTRACT
Jeringau rhizome (Acorus calamus L.*) has the potency to be an antibacterial. This research aimed to find the activity of the ethanol extract of jeringau rhizomes on the growth of Staphylococcus aureus and Escherichia coli bacteria. This research utilized an experimental method of maceration with ethanol solvent, phytochemical screening, and activity testing with concentrations of 2,5; 5; 10; 15, and 20% using a suitable diffusion method. The phytochemical screening shows that the extract contains compounds of alkaloids, tannins, flavonoids, saponins, and triterpenoids. The activity test has demonstrated that the jeringau rhizome extract could obstruct the growth of bacteria from the lowest concentration of 2,5% on 6,86 mm of S. aureus and 7,36 mm on E. coli. The largest restrictive zone was at 20% concentration with a 10,73 mm diameter of S. aureus and 12,63 mm of E. coli.*

Keywords: *Rhizome, Jeringau, Acorus calamus, Phytochemicals screening, Staphylococcus aureus, Escherichia coli*

ABSTRAK
Rimpang jeringau (*Acorus calamus* L.) berpotensi sebagai antibakteri. Penelitian ini bertujuan untuk mengetahui aktivitas ekstrak etanol rimpang jeringau terhadap pertumbuhan bakteri *S.aureus* dan *E.coli*. Penelitian menggunakan metode eksperimental, ekstraksi secara maserasi, skrining fitokimia dan uji aktivitas pada konsentrasi 2,5; 5; 10; 15 dan 20% dengan metode difusi agar sumuran. Hasil skrining fitokimia menunjukkan rimpang jeringau memiliki kandungan senyawa alkaloid, tanin, flavonoid, saponin dan triterpenoid. Ekstrak rimpang jeringau menghambat pertumbuhan bakteri mulai dari konsentrasi terkecil yaitu 2,5%, terhadap *S. aureus* 6,86 mm dan *E. coli* 7,36 mm. Zona hambat terbesar pada konsentrasi 20% dengan diameter 10,73 mm terhadap *S. aureus* sedangkan pada *E. coli* 12,63 mm.

Kata kunci: *Rimpang, Jeringau, Acorus calamus L., Skrining fitokimia, Staphylococcus aureus, Escherichia coli.*

INTRODUCTION

Indonesia has abundant plant resources that could be utilized as medicinal ingredients (Hapsoh & Hasanah, 2011). Medicinal herb has become an alternative treatment in current society, one of which is to treat the infection. Infection occurs when a pathogen is contaminated; common pathogens are *Staphylococcus aureus* and *Escherichia coli* (Anisah *et al.*, 2014). Antibiotic administration is a form of therapy done so far. However, irrational antibiotics could trigger resistance (Permenkes, 2011).

Jeringau (*Acorus calamus* L.) from the family of Acoraceae has the potential to become a medicinal ingredient. Jeringau grows in damp areas such as rice fields, shallow waters, and lakes or riversides (Wahyuni *et al*., 2016). The jeringau rhizome is used as a treatment for dysentery, expectorant, and bronchitis (Paithankar *et al*., 2011) and as an antibacterial (Kim *et al*., 2005). In Indonesia, especially Pontianak, jeringau rhizome is used to treat worm infection, dysentery, and diarrhea (Anisah *et al*., 2014). Meanwhile, Aceh is empirically used to treat coughing, vitality disorder, fever, toxication, ulcers, diarrhea, pre- and post-birth treatment, hemorrhoids, headaches, and mythical utilities (Widyastuti *et al*., 2019).

Figure 1. Jeringau (*Acorus calamus* L.)

Rita *et al.* (2019) research shows that jeringau contains compounds of terpenoids, steroids, alkaloids, flavonoids, and phenol. Li *et al.* (2017) found alpha-asarone and beta-asarone compounds. Mahboubi *et al.* (2015) found flavonoids and phenolics.

Anisah *et al.* (2014) found that the ethanol extract of a jeringau rhizome has an obstacle zone diameter on the 25, 50, 75, and 100% of concentrations in a row for the *E. coli* bacteria of 2,20; 2,39; 2,48, and 2,75 cm respectively. For the *S. aureus* bacteria of 2,36; 2,42; 2,60; and 2,98 cm respectively. Novaryatiin *et al.* (2018) found that the ethanol extract of jeringau leaves has an obstacle zone with 1, 5, 10, and 15% concentration on *Staphylococcus aureus* in a row of 22,3; 32,3; 26,5; 13,1 mm respectively.

Based on the completed research, jeringau rhizome has potency as an antibacterial. However, there has yet to be related information on the phytochemical screening and antibacterial activity test of jeringau extracts from Gayo Lues Aceh on the *S. aureus* and *E. coli* bacteria.

RESEARCH METHOD

Sample Collecting Method

The jeringau sample was collected from Gayo Lues, Aceh, through *purposive sampling,* as determined by LIPI (No: B-284/IV/DI.01/2/2021).

Equipment

The research utilizes the following equipment; Laminar Air Flow Cabinet (Microbiology Safety Cabinet), Spectrophotometer UV-VIS (Orion Aquameter 8000), Incubator (Memmert), Rotary Evaporator (BUCHI Labortechnik AG 9230**)**, and Microscope (Olympus).

Materials

The materials used for this research are the jeringau rhizome, Nutrient Agar (NA) medium, Mueller Hinton Agar (MHA) medium, amoxicillin, gentamicin, and ethanol 96%.

Bioindicator Bacteria

Bioindicator microorganisms are *S. aureus* and *E. coli* bacteria.

Simplicia Preparation and Extraction

Ten kg of fresh jeringau rhizome were wet sorted and washed in running water. It was then thinly sliced to speed up the drying process. The sample was wind-dried while protected from sunlight. The samples were mashed to obtain coarse powder (Menkes RI, 2009). The model was macerated into 7,5 parts of 10 solvent ethanol parts.

Then, it was left for five days, being stirred occasionally. The re-maceration was done with the remaining 2,5 parts of the solvent for two days with the same method. The sample was concentrated using the rotary evaporator at a temperature of not more than 50^{o}C until a thick extract was obtained (Anief, 2010).

Macroscopic and microscopic sample

The microscopic observation utilizes the microscope (40x), which includes the shape, smell, and color of the fresh sample and Simplicia powder.

Phytochemical Screening

Phytochemical screening consists of flavonoids, tannins, saponins, and steroids or triterpenoids (Harbone,1987).

Antibacterial Activity

Antibacterial testing utilizes the suitable diffusion method, the *E. coli* and *S. aureus* suspension used was equivalent to the McFarland standard of 0,5 (1-9 x 10^{8} CFU/mL) (WHO, 2003). The testing concentration is 2,5; 5; 10; 15, and 20%. Each well on the MHA medium was dripped with 30 µL of extract, amoxicillin, gentamicin, and ethanol. It was later incubated at 37^{o}C for 24 hours (Yusriana *et al*., 2014). It was done three times. The clear zone formed was measured with vernier callipers.

RESULT AND DISCUSSION

Simplicia was collected with a yielding value of 21,68%. The jeringau rhizome ethanol extract for 59,58 g with the section yielded a value of 7,4%. The higher the yielding value shows the increasing compound of secondary metabolite content of the quote (Nur *et al*., 2019). The collected jeringau rhizome ethanol extract is dark brown with a dense concentration and smelting of jeringau rhizomes.

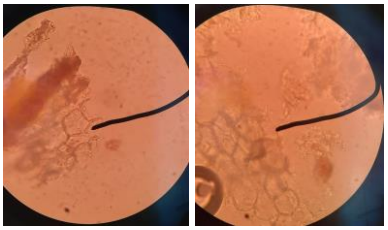
Macroscopic and Microscopic

The specific characterization of the sample of the macroscopic observation of the jeringau rhizome with a length of 20-70 cm and width of 1-5 cm, the stem is segmented and porous, the rhizome skin is dark green, and the inside is white, when it was broken apart, it exerts a particular smell. Saman (2014), jeringau have a ±75 cm long rhizome and ±5 cm width that exerts a stinging smell. The sample powder has the characteristics of being coarse powder, brown, harsh smell, as well as being spicy and bitter. Paka (2019), the jeringau rhizome sample has a spicy and bitter taste, is slightly late, and has a stinging smell. The microscopic observation of the jeringau rhizome can be seen in Image 2.

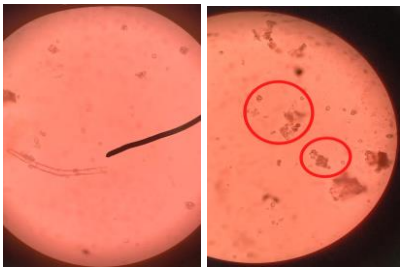
a **b**

Figure 2. (a) rhizome (b) sample powder

The recognizing fragments on the sampilicia powder can be seen microscopically in Figure 3.



a b



c d

e

Figure 3. Microscopic sample powder (a) parenchyma tissue (b) cork cell (c) trichome (d) starch grains (e) essential oil

Paka (2019), the jeringau familiar fragments consist of parenchyma tissue, trichome, and starch.

Phytochemical Screening

Jeringau rhizome ethanol extract contains alkaloids, flavonoids, tannins, saponins, and triterpenoids. Test results are observed based on the colour formed with the reagents used by Harbone (1987) (Table 1.)

Antibacterial Activity Test

The antibacterial activity test of ethanol extract of jeringau rhizomes on *S. aureus* and *E. coli* bacteria can be seen in Table 2.

Table 1. Phytochemical testing result of the jeringau rhizome ethanol extract.

No.	Secondary Metabolite	Reagent	Observation	Exp.
1.	Alkaloids	Mayer	Yellow sediment	+
		Bouchardat	Brown sediment	+
		Dragendorf	Yellow-orange sediment	+
2.	Flavonoids	Powdered Mg + Thick HCl	Yellow-orange liquid	+
3.	Tannins	$FeCl_3$ 10%	Dark-green liquid	+
4.	Saponins	H_2O + HCl 2N	Bubbling (1,2 cm)	+
5.	Steroid/Triterpenoid	CH_3COOH + Thick H_2SO_4	- / red liquid	-/+

Explanation:
(+) : with secondary metabolite
(-) : with no secondary metabolite

Table 2. Activity test on *S. aureus* and *E. coli* bacteria

Jeringau Rhizome Ethanol Extract Concentration (%)	Average Diameter of Resistance Zone (mm) ± SD			
	S. aureus	Category	*E. coli*	Category
2,5	6,86±0,05	Weak	7,36±0,40	Weak
5	7,43±0,20	Weak	8,66±0,64	Weak
10	7,8±0,4	Weak	9,63±0,40	Weak
15	10,33±0,30	Weak	12,06±0,72	Average
20	10,73±0,41	Weak	12,63±0,56	Average
Positive Control	22,43±0,20	Sensitive	23,43±0,15	Sensitive
Negative Control	0±0	No Activity	0±0	No Activity

Explanation:
Positive control: *S. aureus*: Amoxicillin 1%
E. coli : Gentamicin 4%
Negative control: Ethanol solvent

The extract of jeringau rhizomes could resist the growth of bacteria from the minor concentration of 2,5%, with 6,88 mm on *S. aureus* and 7,36 mm on *E. coli*. The resisting zone with a concentration of 20% of 10,73 mm on *S. aureus* and 12,63 mm on *E. coli*. The resisting zone category refers to Morales (2003). Conversely, Anisah *et al.* (2014) showed that the result at the minor concentration of 25% jeringau rhizomes diameter formed 22 mm on *E. coli* and 23.6 mm on *S. aureus*. In contrast, the largest zone at 100% concentration was 27.5mm and 29.8mm for *E. coli* and *S. aureus.*

The positive control restrictive zone of gentamicin is about 23,43 mm, while amoxicillin is about 22,43 mm. Gentamicin has a sensitivity of $\geq$ 15 mm, while amoxicillin of $\geq$ 29 mm (CLSI, 2018).

The increased extract concentration increases the restrictive zone because it was infected with the amount of secondary metabolite within it (Sari *et al*., 2012). Lingga *et al.* (2016), the concentrated extract used increases the penetration of the compound into the bacteria cells and causes lysis on the cells.

The result was different from the research done by Anisah *et al.* (2014). In this research, the smallest concentration extract could halt the growth of bacteria. A similar compound creates a different resisting zone.

The different amounts of active compounds could happen on a similar herb. Active compounds on an herb could be affected by the location, age, time of harvest, the part used, the extraction method, and the solvent (Depkes RI, 2000). Astuti et al. (2014), temperature, climate, light, air moisture, rainfall, nutrition, and soil characteristics could affect the secondary metabolite.

The collected activity results showed a more extensive zone on *E. coli* that a sequential peptide chain could cause and are closely arranged, causing the cell walls of *S. aureus* bacteria to break down harder. The essential oil in the jeringau rhizome could break down the peptide chain that builds the peptidoglycan to lysis the cell walls. The peptide chain in *E. coli* is random and loose, so the essential oil from the jeringau rhizome could penetrate more accessible into the cell walls (Winarti et al., 2009).

The role of secondary metabolite compounds on jeringau rhizomes like alkaloids, tannins, flavonoids, saponins, and triterpenoids could resist the growth of *S. aureus* and *E. coli* bacteria. A secondary metabolite compound could resist the growth of bacteria with different mechanisms.

Alkaloids disturb the synthesis of peptidoglycan (Juliantina et al., 2008). Flavonoids disturb the integrity of the cell membrane (Sari and Ernawati, 2015). Other than those, flavonoids resist the connecting of glycan chains to cross into the peptidoglycan of membrane cells to weaken the structures (Sulastrianah *et al.*, 2014). Tannins denaturized the bacteria protein cells (Roslizawaty, 2013). Saponins reduced the bacteria cell surface tension (Kusumawati et al., 2015). Triterpenoids broke down the porins (Budifaka, 2014).

CONCLUSION

Jeringau rhizome ethanol extract contains *alkaloids, tannins, flavonoids, saponins, and triterpenoids.* The extract could be activated to resist the growth of *S. aureus* and *E. coli* bacteria.

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