The effect of ethanol extracts of pegagan (*Centela asiatica)* urban in inhibiting the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* that caused pneumonia

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Abstract. *Pegagan (Centela asiatica [L] Urban) contains alkaloid, flavonoid, saponin, triterpenoid and tannin that have antibacterial activity. Staphylococcus aureus and Klebsiella pneumoniae are most common of bacteria that cause pneumonia. This study conducted t odetermine the effect of ethanol extracts of pegagan in inhibiting the growth of S. aureus and K. pneumoniae that caused pneumonia. The type of this study is a laboratory experiment using a completely randomized design (CRD). Testing of inhibitory growth effect was measured by Kirby Bauer disc diffusion method. The results showed that ethanol extracts of pegagan at 12.5%, 25%, 50% and 75% concentrations formed inhibition zones on the growth of S. aureus, on average respectively of 7.00 mm, 9.20 mm, 13.20 mm, and 14.50 mm, whereas on the growth of K. pneumoniae, it didn't form any inhibition zone. The results of ANOVA and Duncan (α=1%) tests showed that ethanol extracts of pegagan at all concentrations made a significant difference in inhibiting the growth of S. aureus compared to negative and positive control. The ability of ethanol extracts of pegagan to inhibit the growth of S. aureus at 12.5% and 25% concentrations categorized as no inhibitory growth effect, whereas 50% and 75% concentrations categorized as weak inhibitory growth effect. While the ethanol extracts of pegagan at all concentrations of the tests categorized as no inhibitory growth effect for K. pneumoniae. It can be concluded that ethanol extracts of pegagan inhibit the growth of S. aureus, but do not inhibit the growth of K. pneumoniae.*
Key words: *Centela asiatica, Staphylococcus aureus, Klebsiella pneumoniae.*

Introduction

The incidence of pneumonia is still quite high in some countries and being the main cause of death in developing countries. This happens because the lack of availability of drugs and the emergence of the problem of resistance due to the use of antibiotics in the Community (Zampini, 2009). The development of drug resistance and the emergence of a variety of unwanted side-effects of certain antibiotics have led the research should be directed to find new antimicrobial substances from other sources. The plant became the main choice of researchers in search of antimicrobial substance from another source because it is easy to get it and used by various ethnic groups in treatment (Arora and Kaur, 2007).

Pegagan (*Centela asiatica* [l.] Urban) is one of the herbs that are used as a traditional medicine in the form of fresh ingredients, dry, or already in the form of the herb (herbs) (Lasmadiwati *et al*., 2003). Pegagan contains alkaloids, flavonoids, saponins, tannins and triterpenoid (Winarto and Surbakti, 2003; Santoso and Gunawan, 2004; Kristina et al., 2009). These compounds have the effect of Pharmacology, one of which is the antibacterial effects (Pittella *et al.,* 2009). Dash *et al.* (2011) prove that the antibacterial effect and pegagan effects of antifungal active against pathogens in humans. The result obtained is the ethanol extract pegagan shows antibacterial activity and antifungal drag growth zones by establishing 15-19 mm against microorganisms tested.

Pegagan in many studies have demonstrated antibacterial effect, however the antibacterial effect against bacteria research isolates clinic apparently has never been done. In connection with the reality of the above research is done to identify the effect of

inhibiting the growth of bacteria in pegagan *s. aureus* and *Klebsiella pneumoniae* was isolated from the sputum of pneumonia sufferers.

Materials and Methods

Tools And Materials

Memmert incubator, autoclave, ovens, scales (Ohaus), electric stove, spectrophotometer, kuvet Erlenmeyer flask, glass tube, measure, test tubes, racks of test tubes, petri dish, a light spritus, matchboxes, glass objects, ruler, tweezers, spuit 3 ml, ose, autoclave, cotton rib, paper labels, gloves, microscopes. Maceration, knives, tool racks painting to slide, mikropipet 100 µg -1000 µg and timer. The materials used are plants pegagan from the Kecamatan Jangka Buya Kabupaten Pidie Jaya Term "Buya, bacterial isolates stock cultures *s. aureus* and *Klebsiella pneumoniae* from sputum sufferers pneumoniae has terdiagnosa from the laboratory of Microbiology Regional General Hospital Dr. Zainoel Abidin, akuades sterile, sterile oil emersi, NaCl, blank paper discs, amoxicillin and gentamicin antibiotic discs, paper, paper labels, lens tissue, MacConkey media in order (MAC), M ler Hinton agar (MHA), Blood agar, Nutrient agar, 96% alcohol, ethanol 96%, fresh blood, Crystal violet, safranin, is test for reagent lugol's and phytochemicals.

Extraction and phytochemical test

Pegagan fresh cleaned and cut into small, then anginkan-dried in the open air for 20 days produces 500 grams pegagan dry. Pegagan dry and then dimaserasi with ethanol 96%. The filtrate is obtained dipekatkan with a Rotary evaporator until the concentrated extract obtained in and separate from the pelarutnya. Ethanol extract pegagan diluted with sterile akuades at concentrations of 75%, 50%, 25% and 12.5%. Phytochemical analysis is conducted to determine active compounds contained in the extract pegagan. Tests performed on fresh pegagan phytochemicals and also on the ethanol extract.

Manufacture of Bacterial Suspension

Colonies of s. aureus and k. pneumoniae age 24 hours from the Nutrient agar plate taken one ose and put into test tubes containing NaCl 0.9% sterile, then whipped until it formed a suspension with a certain cloudiness (Lay, 1994). Furthermore the bacterial suspension was taken using a micro pipette as much as 850 l and put into special containers, then performed a reading by using a Spectrophotometer at a wavelength of 620 nm absorbance 0.08 degrees reached so-0.1 equivalent to 1.5 x 107 CFU/ml **(Wande, 2011).**

Effect of ethanol extract pegagan test

Testing is using methods diffusion discs kirby bauer. Testing for each bacteria uses 5 petri dishes containing MHA. A petri dish that contains the MHA given a code b c, d, and e. Then the mha that has been evenly spread suspension bacteria laid discs containing extracts ethanol pennywort 75 %, by concentration 50 %, 25 %, 11.5-12.5 %, a sterile containing akuades and disk antibiotics. Then the mha media diinkubasi at temperatures 350c 24-hour obstruent zone and observed the growth of bacteria in any group by measuring the diameter clear zones formed by the mizzen. Compact discs containing extracts ethanol pennywort various concentrations made by immersing blank paper discs sterile into a solution by concentration pennywort different drained for 30 minutes on the tube for 10 minutes (poeloengan *et al.* , 2007).

Results and Discussion

Extraction result

The extraction yield 500 grams of dried pegagan with maceration method using solvent ethanol 96% as much as the 3 L 3 x during the 24 hours obtained extract pure pegagan 53.4 GMS. The extract was greenish, like pasta with typical smell and berkonsistensi.

Phytochemical test result

Test results of fresh pegagan phytochemicals and ethanol extract demonstrate pegagan compounds alkaloids, saponins, steroids and tannins (table 1).

Table 1. Comparison of Test Results with Fresh samples Phytochemicals Ethanol Extract Centella

No	Phytochemical test		fresh samples	ethanol extract
1.	Flavanoid		-	-
2.	Tanin		-	++
3.	Saponin		+	++
4.	Steroid		++	+
5.	Terpenoid		-	-
		Wagner	-	+
6.	Alkaloid	Dragendroff	+	+
		Mayer	+	+

Remarks: no (-), slight (+), many (+ +)

Differences in the results of the secondary metabolite compounds may be caused by many factors such as differences in climate, altitude, soil type and biological influence of insects, worms, fungi, bacteria or cattle to the plants (Dalimartha, 2008).

Results test influence extract ethanol pegagan against *Staphylococcus aureus*

Results test influence extract ethanol pegagan against s. Aureus look that extracts ethanol concentration 12.5 %, 25 %, 50 %, and 75 % yield flattened **?** flattened diameter zone obstruent successive: 7,00 mm, 9,20 mm, 13,20 mm and 14,50 mm. while diameter average zone obstruent akuades sterile as a control negative, amoksisilin 25 & amp; # 956; g as a control positive each is 0,00 mm and 28,80 mm table (2).

Table 2. The Diameter of inhibiting zones on the growth of s. aureus that are formed on each group Testing

group	Diameter of inhibiting zones on each repetition (mm)					Mean of Ø of inhibiting zones (mm)	classification based on Greenwood	CLSI
	A	B	C	D	E			
P_{s-0}	0	0	0	0	0	0,00	No inhibition	
P_{s-1}	8	6	7	7	7	7,00	No inhibition	-
P_{s-2}	10	9	9	9	9	9,20	No inhibition	-
P_{s-3}	13	14	12	14	13	13,20	weak	-
P_{s-4}	14,5	15	13	15	15	14,50	weak	-
P_{s-5}	30	28	27	30	29	28.80	-	Sensitive

Results of data analysis using the ANAVA (α=1%) to the diameter of the zones of drag s. aureus acquired results F_{hitung} = 729,20 and F_{tabel} = 2.62 (Appendix 8). These results indicate that F_{hitung} is greater than F_{tabel} in extent 1% so that proves that there is a very real effect of ethanol extract of granting pegagan against the growth of s. aureus. Next to get the results more thoroughly, then further testing should be used is the test of Duncan (α = 1%).

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Table 3. Test results of Duncan test of diameter of inhibiting zones on the growth *S. aureus* formed at each group testing

treatment	**Mean ± SD**
Ps-$_0$ (Aquadest)	0,00^a ± 0,00
Ps-$_1$ (extract concentration 12,5%)	7,00^b ± 0,70
Ps-$_2$ (extract concentration 25%)	9,20^c ± 0,45
Ps-$_3$ (extract concentration 50%)	13,20^d ± 0,84
Ps-$_4$ (extract concentration 75%)	14,50^e ± 0,87
Ps-$_5$ (amoxicillin 25 µg)	28,80^f ± 1,30

Description: Different Superscript letters indicate significant differences (P <0.01)

Based on tables above look that group treatment ps-1, ps-2, ps-3 ps-4 and show differences less-obvious metrics between any group treatment and also shows the difference when compared to the control group negatively (p0) and the control group positively (p5) in pursuing growth of s. Aureus. It showed that extracts ethanol pennywort was influential into inhibits bacterial growth s. Aureus but not amounting to influence amoxicilin.

According to Greenwood (1995), power divider drag growth of bacteria from natural ingredients there are four categories of the diameter of the clear zone is more than 20 mm including strong, clear zone diameter 16-20 mm diameter, medium zone category nodes 10-15 mm in diameter and weak zones category nodes are less than 10 mm categories include drag no power against the growth of bacteria. Based on the classification of the drag power according to Greenwood (1995), ethanol extract pegagan on the concentration of 12.5% and 25% is classed as category no drag power, whereas the concentration of 50% and 75% to the category is weak. The results of this research in accordance with the opinion of the Dash et al. (2011), that have the effect of pegagan antibacterial and antifungal effects which are active in the fight against the pathogen in humans. Similar results were reported by **Jagtap** *et al*. (2009), that the ethanol extract pegagan has power of drag against the growth of s. aureus NCIM 2086 at concentrations of 125 & amp; # 956; g/ml, 250 & amp; # 956; g/ml, 500 & amp; # 956; g/ml, and 1000th & amp; # 956; g/ml respectively 8 mm, 12 mm, and 20 mm General mm. similar Results were also obtained by Arumugam *et al.* (2011) the methanol extract pegagan, that have antibacterial activity at doses of 10 mg/ml, 20 mg/ml, 50 mg/ml and 100 mg/ml against s. aureus MTCC-7443 by forming a zone drag each of 24 mm, 26 mm, 27 mm and 28 mm.

Test results of ethanol extract pegagan drag power against k. pneumoniae that treatment looks Pk-1, Pk-1, Pk-Pk-3, and 4 with each concentration% u2013masing 12.5%; 25%; 50% and 75% form drag zone with an% u2013rata the same diameter i.e. 0.00 mm. in the meantime, Pk-12 (sterile as akuades negative controls) and Pk-5 (gentamicin 10 & amp; # 956; g as a positive control) form drag zones with an average diameter of each $ 0.00 mm and 25.60 mm (table 4)

Table 4. Growth inhibition zone diameter of K. pneumoniae were formed in each test group

group	**Inhibition zone diameter on each repetition (mm)**					**Mean of Ø inhibiting zones (mm)**	**Greenwood clasification**	**CLSI**
	A	B	C	D	E			
P_{k-0}	0	0	0	0	0	0,00	No inhibition	
P_{k-1}	0	0	0	0	0	0,00	No inhibition	-
P_{k-2}	0	0	0	0	0	0,00	No inhibition	-
P_{k-3}	0	0	0	0	0	0,00	No inhibition	-
P_{k-4}	0	0	0	0	0	0,00	No inhibition	-
P_{k-5}	25	25	26	26	26	25,60	-	Sensitif

Description: NI = No inhibition, S = Sensitive

Hese results are in accordance with the results of the classification ability of drag power pegagan upon Greenwood (1995), namely the ability to extract the ethanol concentration testing on all pegagan in inhibiting the growth of k. pneumoniae categorized into no drag power (table 4).

The results of such research in accordance with the results of the study, Malik et al. (2011) in Pakistan who did research on the antibacterial activity of plants extracted by methods which pegagan maceration using ethanol solvent, water and n-hexan, pegagan that extracts with the third type of solvent is not capable of inhibiting the growth of k. pneumoniae ATCC 10031.

From the results of this research are visible distinction ability of ethanol extract pegagan in inhibiting the growth of Gram positive bacteria than gram-negative. It is thought to be due to differences in the structure of the cell wall between the two groups of the bacteria. This is in accordance with the opinion of Brooks et al. (2005), that the difference in the structure of the cell wall of a bacterium to determine penetration, bonding and antibacterial activities of compound.

Conclusions

Based on the research that has been done can be concluded that:

1. Centella asiatica extract ethanol concentration of 12.5%, 25%, 50% and 75% effect in inhibiting the growth of S. aureus, but had no effect on the growth of K. pneumoniae.
2. The higher concentration of ethanol extract of Centella asiatica (*Centela asiatica* [L.] Urban), the greater the bacterial growth inhibition zone diameter S. aureus produced.
3. Centella asiatica extract concentration of 50% ethanol and 75% have inhibitory effects on the growth of S. aureus were categorized in a weak category.

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