WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 8, 52-59 Research Article ISSN 2278 – 4357

PHYTOCHEMICAL SCREENING AND ANTI MICROBIAL ACTIVITY OF *n*-HEXANE, ETHYL ACETATE, AND METHANOL EXTRACT OF *ALOE VERA* L.

Shirly Kumala[1]* and Muhammad Andi Mahdiansyah[1]

[1]Faculty of Pharmacy, University of Pancasila.

[2]Srengseng Sawah, Jagakarsa, South Jakarta 12640 Indonesia.

Article Received on
28 May 2018,

Revised on 19 June 2018,
Accepted on 10 July 2018

DOI: 10.20959/wjpps20188-12078

***Corresponding Author**
Shirly Kumala
Faculty of Pharmacy,
University of Pancasila.

ABSTRACT

Utilisation of plant as traditional medicine has been well documented and has become a traditional method of medicinal therapy by the Indonesian community for many decades. One of those plants was"lidah buaya", which also known as *Aloe vera* (*Aloe vera* L.). Traditionally, *Aloe vera* leaves were applied in hair sculp to increase the hair growth and treatment for burns, sore throat, and diabetes. In this study, extraction of *Aloe vera* leaves were conducted by maceration. Three solvents, (n-hexane, ethyl acetate and methanol) were used in the extraction. The Extract was then used in antibacterial activity test with Agar diffusion methods using paper disk. Phytochemical screening by Farnsworth methods. The Phytochemical screening showed the presence of flavonoid, saponin, tannin,quinone, steroid, dan triterpenoid. The activity of antibacterial of ethyl acetate extract against *Salmonella typhi* (22.33±0.577mm), *Staphylococcus epidermidis* (19.67±0.577mm), *Proteus vulgaris* (23.67±0.577 mm). Antibacterial activity of the methanol extracts against (*Salmonella typhi*), *Staphylococcus epidermidis* and *Proteus vulgaris* 17±0, 19±0 and 18.33±.0.577 mm, respectively. *Aloe vera* L has broad spectrum antibacterial activity.

KEYWORDS: Antibacterial activity, Phytochemical screening, *Aloe vera* L.

INTRODUCTION

Aloe vera were medicinally beneficial plants within the Liliacea family. Almost all parts of the plant were nutritious, good for body care and for treatment of various diseases such as inflammation, fungal and bacterial infections as well as cell regeneration.

Furthermore, *Aloe vera* was also well documented for its effectiveness in lowering blood sugar levels in diabetic sufferes, controlling blood pressure and stimulate immune system in cancer patients. Chemical constituents such as flavonoids, tannins, steroid were known to be present within the *Aloe vera* plants.[1,2]

Recent study using plant product for the pharmaceutical purpose has been gradually increased. According to World Health Organisation, using medicinal plant could be as good raw material for drug. The plant extracts, with have anti microbial effect, can be the great significant in the treatment of various infection. Most of the studies were reported on antibacterial agents of *Aloe vera* was used as crude extract, but on the other hand they have different polarity property, when extracted with different solvent condition (n-hexane, ethyl acetate and methanol), variety of chemical constituents such as essential oils, steroids, triterpenoids, saponin, flavonoids can be extracted out. The current study, initially, aimed at extraction of *Aloe vera* plant using polar and non-polar solvent (n-hexane, ethyl acetate and methanol) then followed by phytochemical screening to verify the chemical composition of the plants and lastly, evaluate the extract againts *Candida albicans*, *Salmonella tyhpi*, *Staphylococcus epidermidis*, *Proteus vulgaris*[3,4, 5, 6] to determine its anti bacterial effect.

The aim of this study was to (i) extract dry powder of *Aloe vera* in various solvents; (ii) conduct phytochemical screening on the extracts (iii) assess antibacterial activity of the extracts.

MATERIAL AND METHODS

Materials. Plant identification was carried out in The Herbarium Bogoriense in Bogor. The extract used in this research where n hexane, Ethyl acetate and methanol extract. The test microbe used in this research were *Salmonella typhi*, *Staphylococcus epidermidis*, *Proteus vulgaris* and *candida albicans*.

METHOD

Preparing extract

Dry powder is extracted by maceration withn hexane, ethyl acetate and methanol solvent. The extract obtained is concentrated using rotary vacuum until a concentrated *n*- hexane, ethyl acetate and methanol extract are obtained.

Preparing bacterial suspension

Bacterial suspension were compared to Mc Farland standard 0.5 (1.5×10^8 CFU/ml) for

density uniformity and spread on to nutrient agar (NA) evenly using sterile cotton bud in a Petri dish until all surface was covered.

Phytochemical screening

Each extract n-hexane, ethyl acetate, and methanol were conducted to Phytochemical screening test. To determine the chemical content of each extract using the Farns worth method includes identification of alkaloids, flavonoid, saponins, tannin, quinones steroid /triterpenoids, coumarin and of essensial oils.[7]

Antimicrobial Activity Test

The activity test against *Candida albicans*were done using paper disc diffusion method with PDA (Potatoes Extract Agar) medium. Nystatin used as the positive control while for the activity test against *Salmonella typhi*, *Stapylococcus epidermidis*, *Proteus vulgaris*, used NA (Nutrient agar) medium and Chloramphenicol used as positive control.

The extract were diluted using a suitable solvent to obtain a concentration of 50%, 25%, 12.5%.

The suspension of *Staphylococcus epidermidis*, *Salmonella typhi*, *Proteus vulgaris and Candida albicans* after incubated at 35 °C -37 °C for 18-24 hours and for yeast incubated at 18°C - 20°C for 5 days. Each of these bacterial and yeast suspension were compared to McFarland standard 0.5 (1.5×10^8 CFU/ml) for density uniformity and spread on to Potatoes Dextrose Agar (PDA) and Nutrient Agar (NA) and evenly using sterile cotton bud in a Petri dish until all surface was covered.

Paper discs saturated with the n Hexane, EtOAc and methanol extract were placed on PDA, NA medium surface where Bacterial or yeast suspension were applied. After that were incubated at 35 °C -37 °C for 18-24 hours. And for the yeast18°C - 20°C for 5 days. The clear zone around the disc demontrated microbe inhibition zone area and were measured in millimeter (mm) scale.

RESULTS AND DISCUSSION

Rendemen value

The rendemen value of the extract showed in table I

Tabel I. The yield value of *Aloe vera* L.

Extract		Powder (g)	Thick extract (g)	Yield (%)
Aloe vera L	n-hexane	500.12	8.45	1.69
	Ethyl Acetate	500.12	8.65	1.73
	Methanol	500.12	43.12	8.63

Table I. showed the highest yield value was from methanol extracded.

Table I. Results of Phytochemical Screening of *Aloe vera* L.

Chemical content	Powder	*n*-Hexane extract	Ethyl acetate extract	Methanol extract
Alkaloid	-	-	-	-
Flavonoid	+	-	+	+
Saponins	+	-	-	+
Tannins	+	-	-	+
Quinone	+	-	+	+
Steroid	+	+	+	-
Triterpenoid	+	-	-	+
Essential oil	-	-	-	-
Coumarin	-	-	-	-

(+) positive reaction (-) negative reaction

The results obtained of phytochemical screening group of secondary metabolites contained in the leaves of *Aloe vera* (*Aloe vera* L) are flavonoids, saponins, tannins, quinones, steroids and triterpenoids.

Mechanism of action of flavonoids as antibacterial is a compound forming a complex with extracellular proteins and soluble so can damage the bacterial cell membrane and is followed by the release of intracellular compounds. While the mechanism of action of saponins as antibacterial is to lower the surface tension so that the resulting increase in permeability or leakage of intracellular cell and lead compounds will come out. The antibacterial mechanism of action of tannins is the reverse transcriptase enzyme and inhibit DNA topoisomerase that bacterial cells can not be formed.

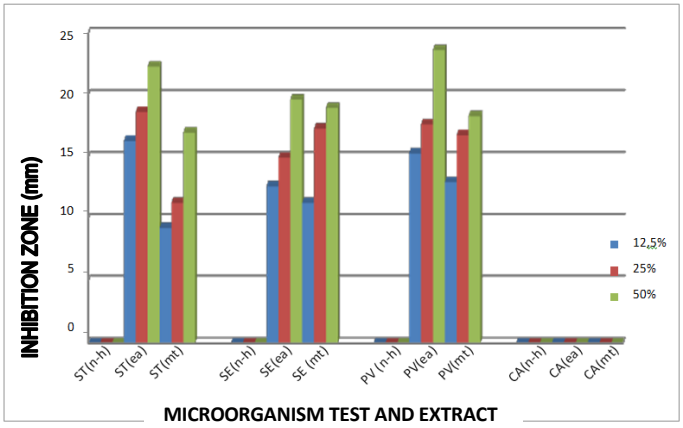

Figure 1: The diameter of inhibition zone *Aloe vera* L leaves (extract and concentration variable) on Staphyloccus epidermidis, *Salmonelal thypi, Proteus vulgaris, Candida albicans*.

Figure 1, showed inhibition zone of *Salmonella typhi* bacteria measuring 16.33, 18.67, and 22.33 mm by EtOAc extract concentration of 12.5%, 25%, and 50% respectively (a); 9.3 mm, 11.33 mm, and 17 mm by methanol extract concentration of 12.5%, 25%, and 50% respectively (b). Inhibition zone formed by the bacterium *Staphylococcus epidermidis* following exposure to ethyl acetate extract (12.5%, 25%, and 50%) measured 12.67, 15.00 and 19.67 mm respectively. Diameter of inhibition zone amounted to 11.33mm, 17.33mm, and 19 mm after exposure of the same bacteria to methanol extract. Bacteria *Proteus vulgaris* was inhibitied (imhibitory zone measured 15.33 mm, 17.67 mm, and 23.67 mm) after treatment by EtoAc extract and MeOH extract (observed inhibitory zone of 13 mm, 16.8 mm, and 18.33 mm).

It was surprising that yeast which was extracted from n-hexane, ethyl acetate and methanol did not have any inhibitory effect towards *Candida albicans*. Similarly, the n-hexane extract showed no effect on *Salmonelltyphi* bacteria, *Staphylococcus epidermidis,* and *Proteus vulgaris*. Thus it is clear that only ethyl acetate and methanol extracts of *Aloe vera* plant inhibit microbial growth. *Staphylococcus epidermidis*, *Salmonellatyphi*, and *Proteus vulgaris*, but not *Aloe vera* extract able to inhibit the growth of *Candida albicans*. Thus the highest inhibition zone formed by the ethyl acetate extract of the bacterium *Proteus vulgaris* at a concentration of 50%, while the methanol extract of the highest inhibition zone is *Staphyococcus epidermidis* at a concentration of 50%.

Extracts of *Aloe vera* can not inhibit the yeast *Candida albicans*, there were no inhibition at about paper disc, this is due to the formation of chlamydospores are asexual spores at the tips

of the hyphae that form the ends of thick making it difficult to penetrate antimicrobial compounds.

N-hexane extract the *Aloe vera* does not have antimicrobial activity due to the compounds contained in the n-hexane extract of *Aloe vera* can not inhibit the growth of microbes (8.9).

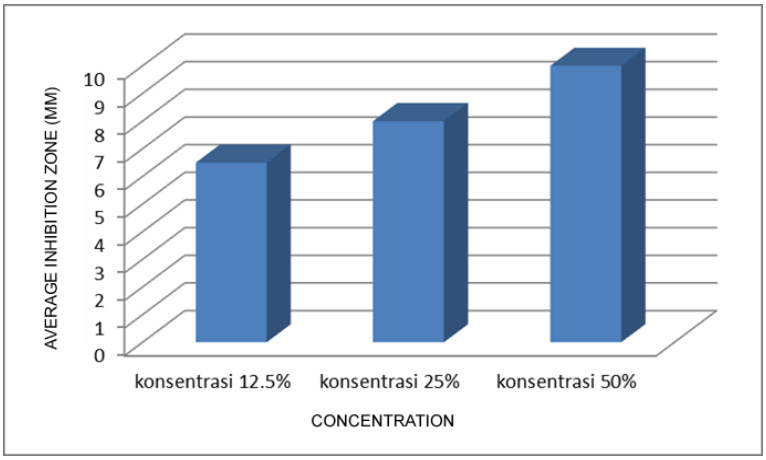

Figure 2. The average diameter of inhibition zone *Aloe vera* leaves (Variable concentration) on S*almonellatyphi, Staphylococcus epidermidis, Proteus vulgaris, dan Candida albicans*)

Figure 2 showed the concentration of n-hexane extract, ethyl acetate, and methanol that could inhibit the growth of the bacteria of *Salmonella typhi*, *Stahylococcus epidermidis*, and *Proteus vulgaris* is the concentration of 50%. In this study, the concentration of 50% is the maximum concentration of the extract works because it contains flavonoids and saponins most in these concentrations. The higher concentration of the extract, the higher of the extract in inhibiting microbial.

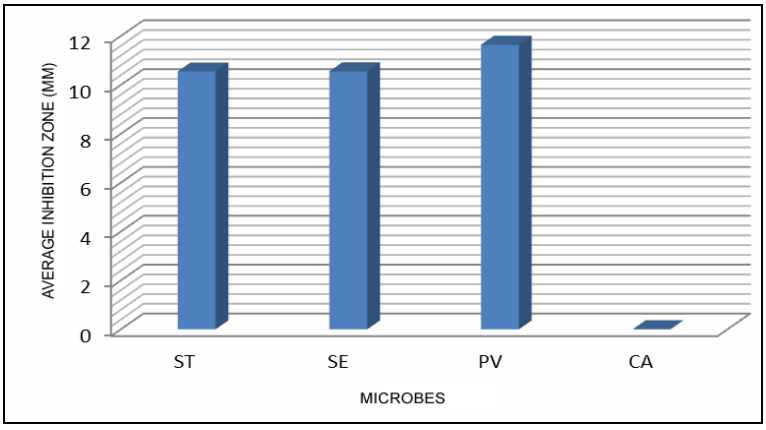

Figure 3: The average of diameter of inhibition zone *Aloe vera* leaves on *Salmonellatyphi Staphylococcus epidermidis, Proteus vulgaris, and Candida albicans*

Figure 3, showed that the inhibition zone formed in bacteria *Proteus vulgaris* extract had the greatest inhibition among *Salmonella typhi*, *Staphylococcus epidermidis*, and *Candida albicans*, due to the type of compound contained in *Aloe vera* such as flavonoids and saponins contained in *Aloe vera* is effective in killing of *Proteus vulgaris. Aloe vera L.* extracts have low inhitory power in the yeast *Candida albicans* due to form chlamydospores at the end of hyphae that become thicker and harder to penetrate the lead antimicrobial that section so that the antimicrobial is not effective in the yeast.

This results are also in line with the results by Irshad Saba *et al* that *Aloe vera* methanol extract could inhibit the growth of *Salmonella typhi* and *Staphylococcu epidermidis.*

This research is still necessary to do for further research to isolate the active compound which has antibacterial activity of leaves of *Aloe vera* L.

CONCLUSION

1. Phytochemical screening process verified that *Aloe vera* leaf powder does not contain alkaloids, essential oils and coumarin.
2. In Antimicrobial activity test against bacteria *Salmonellatyphi*, both EtoAc and MeOH extracts at 50% concentrations were active. They have inhibition zone of 22.33 mm and 17 mm respectively. Similarly with test against *Staphylococcus epidermidis*, with the same extract concentration, EtOAC and MeOH extracts formed inhibition zone of 19.67 and 19 mm respectively. Last but not least, when bacteria Proteus vulgaris was exposed to same extract treatment at the same concentration of 50%, inhibition zone observed was measuring 23.67 mm and 18:33 mm respectively.
3. Extract of *Aloe vera* does not have activity against *Candida albicans.*

REFERENCE

1. Thiruppathi S, Ramasubramanian V, Sivakumar T,and Thirumalai AV. Antimicrobial activity of *Aloe vera* (L) Burm,f. against pathogenic microorganisms. Journal of Biosciences Research, 2010; 1(4): 251-58.
2. Ranjbar R, Arjomandzadegan M, and Hosseiny H. Evaluation of Antioxidant activity and Growth control propertis of nonosclae structure produced from *Aloe vera* var. littoralis extract on clinical isolates of Salmonella. Scientia. Pharmaceutica, 2017; 85, 28. doi: 103390/scipharm85030028
3. Novelina, Surini, Etrina. Study manufacture of leaf *Aloe vera* drinks with the addition of

stabilizer to the quality of the product. Faculty of Agricultural Technology Unand; 2008; 1-8.

4. Syamsulhidayat SS, Hutapea JR. Inventory of Indonesian Medicinal Plant.(I) Ministry of Health Republic of. Indonesia 1991; 30-31.
5. Ariyanti NK, Darmayasa IBG, Sudirga SK. Inhibitory Power Leather Leaf Extract *Aloe Vera* (Aloe barbadensis miller) Against the growth of *Staphylococcus aureus* bacteria *Escherichia coli* ATCC 25923 and ATCC 25922, Biological Journal. 2012; 1-4.
6. Wibowo S, Dwi Putra RH, Noorhamdani US. Test Extract *Aloe Vera* Gel (*Aloe vera*) as Antimicrobials against Shigella bacteria dysentreriae In Invitro. Fakultas Medicine Brawijaya University, 2010; 4-5.
7. Farmsworth NN. Biological and phytochemicals Screenings of Plants. J Pharm Sci., 1966. 25-65.
8. T. Robinson High Content of Organic Plant, translated by Kosasih, Edition VI Bandung; Bandung Institute Of Technology, 1995; 72, 157, 198.
9. ZE Jawetz, Melnick JL, Adeberg EA. Mikrobiologi Medicine. Issue 20. Translated by Nugroho E, Maulany RF. Jakarta: publisher Kedokteran EGC; 1996; 627-629,236,238-241,243-6,160-169.
10. Irshad S, Butt M, and Younus H. *In-vitro* antibacterial activity of *Aloe barbadensis* Miller (*Aloe vera*). International Research Jornal of Pharmaceuticals, 2011; 01.02: 59-64.