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IN VITRO ANTIBACTERIAL OF ETHANOLIC EXTRACT AFRICAN LEAF (*VERNONIA AMYGDALINA*)

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ABSTRACT

African leaves (*Vernonia amygdalina*) is traditionally used in Indonesia to treat various diseases; e.g diabetes mellitus and infection diseases. The aim of this research is to obtain phytochemicals compounds and determine antibacterial activity from ethanolic extracts of African leaves. Phytochemical screening was carried out using qualitative methods: color reaction and precipitation. Antibacterial activity were checked using contact bioautography and agar diffusion methods. This study used *Escherichia coli* as representative of Gram negative and *Staphylococcus aureus* as representative of Gram positive bacteria with 3 replications. The results showed ethanolic extract of African leaves contains bioactive compounds of flavonoids, phenols, saponins, tannins, alkaloids, and steroids. Antibacterial activity was not found in the contact bioautography method, but it could be seen in the agar diffusion method. African leaf ethanol extract had a strong inhibition of bacterial growth against *S. aureus* than *E. coli* ($p<0.05$). The minimum inhibitory concentration (MIC) was 1.56 mg/mL for *S. aureus* and 12.5 mg/mL against *E. coli*. The active fraction in the mobile phase used chloroform:methanol (9:1). Further research is being carried out to isolated antibacterial compound from the active fraction using Gas chromatography-mass spectroscopy (GC-MS) and Liquid chromatography-mass spectroscopy (LC-MS).

Keywords: *Bioactive compounds, Antibacterial, Ethanolic extract , Vernonia amygdalina*

INTRODUCTION

African leaves (Vernonia amygdalina) have been widely used by Indonesian people as medicinal herbs to treat diabetes mellitus and infectious diseases. The taste of African leaf water decoction which is very bitter is considered effective for treatment due to high glucose levels in the blood and kills disease -causing bacteria . (1) The Covid-19 pandemic situation increases the consumption of multivitamins so that the body's immunity is maintained. Consumption of various multivitamins and drugs which are synthetic chemicals can cause side effects to health.

The transition from the use of synthetic drugs to herbal-based medicines has made basic research on traditional medicines very essential as a reference for the production of standardized herbal medicines. African leaves (V. amygdalina) are known to contain bioactive compounds of flavonoids, tannins, saponins and terpenoids; alkaloids, steroids, coumarins, alkaloids, phenolic acids, xanthones, and anthraquinones. These secondary metabolites include antibacterial and antioxidant properties. (2).

The bioactive compounds of African leaves that grow in Indonesia are not yet known. Likewise with its ability to inhibit the growth of pathogenic bacteria. The research was conducted to determine the bioactive compounds of the ethanol extract of African leaves grown in Indonesia and their antibacterial activity.

RESEARCH METHODS

The research conducted was an experimental research. The research was conducted at the Chemical Analysis Laboratory of the Pharmacy Diploma Program, Pelita Harapan University and the Microbiology Laboratory of the Department of Pharmacy and Food Analysis, Health Polytechnic, Ministry of Health, Jakarta II.

Preparation of African Leaves

African leaves were obtained from African plant plantations in Nanggerang Village, Cicurug Sukabumi District. The sampling technique used is purposive sampling ; The leaves used for the research were sorted into 6-8 cm long and green in color. The leaf samples were then washed using tap water and then air-dried. (3).

The dried leaf samples were then ground using a grinder and sieved using a 40 mesh sieve. The powdered leaves to be further analyzed is then weighed (grams). Samples were authentication at the Bogor Botanical Gardens National Agency for Research and Innovation (BRIN) to find out the species name.

Extraction

500 g of powdered leaves was extracted by the maceration method using 1.500 mL of 96% ethanol, for 3x24 hours, stirring occasionally and every 24 hours a new solvent was replaced in the simplicia . (4) The liquid extract was then filtered and concentrated using a rotary evaporator (Eyela) at 40ºC. The concentrated extract was then weighed (grams) before further analysis.

Phytochemical Screening

Identification of the class of active compounds contained in ethanolic extract includes testing the class of compounds of alkaloids, flavonoids, saponins, steroids, tannins, and terpenoids. (5)

Antibacterial Activity

The ethanol extract was prepared in five concentration; 50, 100, 200, 400 and 500 mg/mL. Antibacterial activity was determined using contact autobiography and agar diffusion methods. The test was carried out with three replication. The test bacteria used were *Staphylococcus aureus* and *Escherichia coli.* All cultures is a collection of the Microbiology Laboratory of the Department of Pharmaceutical and Food Analysis. The test cultures were maintained on Soy Broth Tripticase media (Merck).

Contact autobiography using mobile phase chloroform:ethanol (7:3), Mueller Hinton Agar (Merck) medium (MH), and MacFarland 1.5 test culture suspension. (6) The agar diffusion method was carried out by incorporating 20 µL of samples with different concentrations into MH media that had been inoculated with the 1.5 MacFarland test culture. All cultures were incubated at 37 ºC for 24 hours, then the diameter of the inhibition zone formed was observed and measured . (7) The negative control used was DMSO (Smartlab) while the positive control used was tetracycline antibiotics (Oxoid). The inhibition zone is an area where there is no growth of the test bacteria (clear) due to the treatment of the sample in the culture. Measurement of the minimum inhibitory concentration (MIC) was carried out using the dilution methods. (8)

Data on the diffusion antibacterial activity were analyzed using analysis of variance using Minitab 20 software. The analysis was carried out to determine whether there were significantly different extract treatments on the growth of the test bacteria.

RESULTS AND DISCUSSION

The tested sample was found to be African leaf (*V. amygdalina*) based on the results of the determination by BRIN through letter number B.466/IV/D1.01/3/2022. The sample was then extracted which resulted in an extract yield of 24.91%. The yield results obtained were almost 2x from previous studies . (9) The yield is greater, presumably because the bioactive compounds are attracted to the solvent used more . (10) The extract obtained was then subjected to phytochemical screening. The results of the phytochemical screening are in table 1.

Table 1 Bioactive compounds of African leaves (*V. Amygdalina*)

Bioactive Compound	Results
Flavonoids	+
Saponins	+
Phenol	+
tannins	
- $FeCl_3$	+
- gelatin	-
- Steasny	-
Alkaloids	+
Steroids	+
Triterpenoids	-
Quinone	-

African leaves in this study were detected to contain a different class of compounds from Papua region . (11) African leaves from Papua was not contain saponins and alkaloids. This is presumably due to geographical differences causing differences in the content of secondary metabolites. (12) The results of the phytochemical screening showed the presence of antibacterial bioactive compounds. Therefore, the measurement of anti-bacterial activity was carried out using contact autobiography and agar diffusion methods. The results of antibacterial research using the contact aubiography method are shown in Figure 1.

The results of the antibacterial activity using the autobiographical method showed that no spots were detected that could inhibit the growth of the test bacteria. The negative results of the contact autobiography method are thought to be due to the inaccurate composition of the mobile phase. (13) Further analysis found that the composition of chloroform:methanol (9:1) had antibacterial activity in the agar diffusion methods. The results of antibacterial activity using the diffusion methods in table 2.

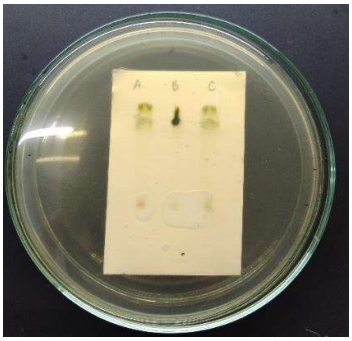

Figure 1 Antibacterial activity was not detected in the contact autobiography methods

Table 2 Antibacterial activity of African leaves (*V. amygdalina*

Extract concentration (mg/mL)	Inhibition zone (mm)	
	Escherichia coli	***Staphylococcus aureus***
50	8.33±0.58[a]	16.00±1.00[d]
100	9.00±0.00[ab]	17.33±0.58[de]
200	9.67±0.58b[b]	18.00±0.00[e]
400	13.67±1.15[c]	20.00±0.00[f]
500	14.00 ± 0.00[c]	22.33±0.58[g]

note: different letters represent significant differences ($p<0.05$)

The ethanol extract of African leaves was able to inhibit all the test bacteria. The ethanol extract was more effective in inhibiting the growth of *S. aureus* than *E. coli* ($p<0.05$). The smallest concentration of African leaf ethanol extract; 50 mg/mL has very weak activity against inhibiting the growth of *E. coli*; which is indicated by the diameter of the inhibition of less than 9 mm. On the other hand, the concentration was classified as partially active against *S. aureus*. The highest concentration in this study; 500 mg/mL is classified as partially active on *E. coli* and very active on *S. aureus*. (14)

The results indicated that the ethanol extract of African leaves was more effective in inhibiting the growth of Gram positive bacteria . (15) The composition of the bacterial wall greatly influences the mechanism of action of the antibacterial compounds. Bioactive compounds of flavonoids ethanol extract of African leaves have a mechanism of destroying peptidoglycan bonds (the main compound that makes up cell walls), this is because Gram positive bacteria have more peptidoglycan composition than Gram negative bacteria. Gram positive also does not contain lipopolysaccharide as a peptidoglycan coating (different from Gram negative). As a result of the destruction of peptidoglycan bonds, the cell wall becomes lysis. (16)

The smallest concentration of the extract capable of inhibiting the growth of the tested bacteria can be determined by the minimum inhibitory concentration (MIC) using the dilution methods. The results of the minimum inhibitory concentration are in table 3.

Table 3 showed that the minimum inhibitory concentration (MIC) for *E. coli was* 12.50 mg/mL and for *S. aureus it* was 1.56 mg/mL. MIC against *S. aureus* was 8x smaller than *E. coli*; this indicated that the ethanol extract of African leaves is very effective in inhibiting the growth of *S. aureus*. However, the MIC in previous studies was lower than this study. (17)

Table 3 MIC of African leaves (*V. amygdalina*)

Extract concentration (mg/mL)	Growth	
	Escherichia coli	***Staphylococcus aureus***
25	-	-
12.50	-	-
6.25	+	-
3.13	+	-
1.56	+	-
0.78	+	+

note: (+): growth; (-): not growth

CONCLUSION

The ethanol extract of African leaves (*V. amygdalina*) contains bioactive compounds of flavonoids, saponins, tannins, phenols, alkaloids and tannins. African leaf ethanol extract was able to inhibit the growth of *S. aureus* and *E. coli*. The (MIC) value for *E. coli* was 12.5 mg/mL and for *S. aureus* was 1.56 mg/mL. The research results will be continued using the *Liquid*

Chromatography-Mass Spectroscopy (LC-MS) and *Gas Chromatography-Mass Spectroscopy* (GC-MS) to identified antibacterial compound.

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