**Antibacterial Activity of Vacuum Liquid Chromatography**

**Fractions of Tapak Dara Stem (*Catharanthus roseus*)**

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**Abstract.** Tapak dara is known as one of the most outstanding plants in cancer and many diseases treatment. Here, the atibacterial activity of this plant is evaluated. This research is objected to isolate compounds having the antibacterial activity. As part of the searching of natural antibacterial compounds from tapak dara*,* it is reported the fractionations and antibacterial activity of the Tapak Dara stem using vacuum liquid chromatography. The methanol extract of the plant were fractionated using mixture of hexane:acetone with various composition to give 14 fractions. The concentrated fractions were tested for their antibacterial activity against *E. coli*. Fraction E from the hexane:acetone 7:3 eluent exhibited the highest activity. The preliminary phytochemical screening of the fraction showed the presence of alkaloids and terpenoids.

**INTRODUCTION**

Natural products have been a rich source in providing leads for the development of drugs for the treatment of bacterial infections1. One of which is *Catharanthus roseus* L. G. Don. This plant commonly known as “Periwinkle”, “Madagascar periwinkle”, or “Vinca” belonging to the family Apocynacea. It is an ornamental plant widely known as important medicinal plant used to treat many diseases around the world. It is also one of the most outstanding plants in cancer treatment. This plant is cultivated mainly for its alkaloids, which are used as cancer drug. The two classes of active compounds in *Tapak Dara* are alkaloids and tannins2. More than 100 alkaloids and related compounds have so far been isolated and characterized from the plant3. It also produces more than 100 monoterpenoids indole alkaloids in different organs3. Moreover, this plant has a variety of medicinal properties,

such as antimicrobial4,5,6,7, antifungal5, antidiabetics, antidiarrheal and antioxidant4.

In Indonesia, this plant is known as “Tapak Dara”, used traditionally to cure some diseases. The leaves is used to cure hypertension, diabetes mellitus, leukemia, asthma, bronchitis, and wound8. The root is used in asthma and malaria treatment, while the herb is used parotitis8.

In order to overcome the emergence of new infections and the increase of bacteria drug-resistance as well as the failure of available antibacterial to treat many infectious diseases9, it is necessary to develop new antibacterial agents from natural products. Muhammad et al7 reported the antibacterial activity in crude extracts of different parts (*viz.,* leaves, stem, root and flower) of *C. roseus* against clinically significant bacterial strains. However, the isolation of pure compounds are rarely performed yet.

As a part of searching on new antibacterial compounds from natural products using bioassay-guided isolation, this study has been carried out to isolate the fractions having the antibacterial activity of methanol extract of stem of *Catharanthus roseus* L. G. Don against *Escherichia coli*.

**EXPERIMENTAL SECTION**

**Material**

The stem of “Tapak Dara” *Catharanthus roseus L. G. Don* were collected from Malang region, East Java, Indonesia. For the extraction, it have been used methanol, chloroform, hexane, acetone, ethyl acetate, silica gel 60, silica gel GF*254*, Dragendorff reagent, Mayer reagent, HCl 5%, aqueous NaOH 20%, NA, NB, aquadest, ethanol

70%, H2SO4, FeCl3, and DMSO, chloramphenicol. *E. coli* collected from the Biology Department, Faculty of

Mathematics and Science, State University of Malang,.

**Extraction**

The stems are dried under the sun for 5 days. The dried stems are then powdered. The powdered were macerated using methanol for 3x 24 hours to give extract solution. The extract was then filtered using Buchner funnel and evaporated by rotary vacuum evaporation at temperatures below 600C. The dark brown resulting crude extract was then subjected to Vacuum Liquid Chromatography using silica gel 60 G from Merck. A 60 mm column was dry- packed by using suction to make a 4–5 cm bed onto which the sample powder was added10. The silica gel was compressed under vacuum in order to achieve a uniform layer in order to get a better separation11. The column was eluted with hexane, hexane:acetone 9:1; 8:2; 7:3; 6:4; 1:1; 3:7; ethylacetate, ethylacetate:methanol 9:1; 8:2, and

100% methanol respectively in numbered 150 ml Erlenmeyer flasks. The each fraction is then assayed for antibacterial activity. The fractions with high antibacterial activity were then subjected to phytochemical screening.

**Antibacterial Assay**

The antibacterial activity of the each fraction was evaluated against *Escherichia coli* ATCC 25922 by disc- diffusion agar method. The each fraction was diluted in DMSO to give 1000 ppm of solution. The starter was prepared by inoculated the bacteria in the NB media. The NB media was prepared by diluting 1.3 g of NB agar in

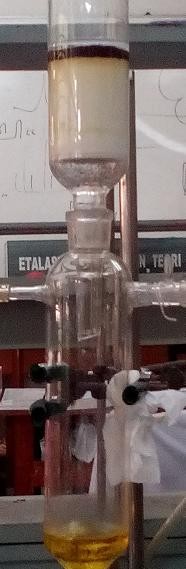
100 mL of aquadest. The NA media, used as medium for the test, made of 2,3 g in 100 mL of aquadest. The media and all of the apparatus are autoclaved for 15 minutes at 1210C at 15 Pa. After 24 hours, 200 µL of the bacteria are ready to inoculated to each filter paper disc Whatman No.1 (5 mm dia.) in the NA media. The activity was shown by the inhibitory zone of each fraction. The assay was performed two times (duplo) for each fraction and the plates were inoculated at 37oC for 24 hour.

**Phytochemical Screening**

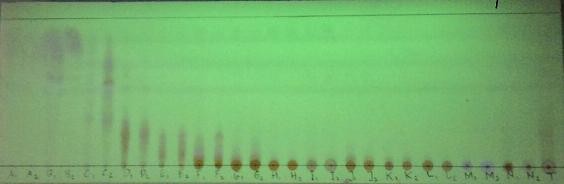
The most active fraction(s) were then subjected to preliminary phytochemical screening following the standard methodologies12 to investigate the phytochemicals in each fraction. The procedure of each test is described below.

**RESULTS AND DISCUSSION**

The vacuum liquid chromatography fractionation of the dark brown methanol extract or the crude extract of the stem of Tapak dara yielded 14 fractions. The Vacuum Liquid Chromatography shown on **FIGURE 1** below.



(a) (b)



**FIGURE 1.**Vacuum Liquid Chromatography of the Methanol Extract of Tapak dara Stem; (b) chromatogram of the VLC

fractions under UV254 lamp

The each fraction are subjected to be assayed its antibacterial activity. The data of the antibacterial assay showed on **TABLE 1** below.

**TABLE 1. The antibacterial activity of Vacuum Liquid Chromatography Fractions**

**Eluent**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Fractions** | | **(m** | **m)** | **Classification of** | |
| **Code** |  | **1** | **2** | **average** | **activity\*** |
| 1 | Hexane:acetone 9:1 | 6.95 | 6.75 | 6.85 | Medium |
| 2 | Hexane:acetone 8:2 | 7.00 | 7.00 | 7.00 | Medium |
| 3 | Hexane:acetone 7:3 | 7.90 | 8.00 | 7.95 | Medium |
| 4 | Hexane acetone 7:3 | 7.90 | 7.85 | 7.875 | Medium |
| 5 | Hexane:acetone 6:4 | 13.55 | 11.90 | 12.725 | Strong |
| 6 | Hexane:acetone 5:5 | 9.70 | 10.40 | 10.05 | Strong |
| 7 | Hexane:acetone 3:7 | 8.75 | 10.30 | 9.525 | Medium |
| 8 | Ethyl acetate | 9.65 | 8.75 | 9.20 | Medium |
| 9 | Etyhlacetate:methanol 9:1 | 10.00 | 10.20 | 10.10 | strong |
| 10 | Ethylacetate:methanol 8:2 | 9.45 | 9.45 | 9.45 | Medium |
| 11 | Ethylacetate:methanol 8:2 | 10.00 | 6.50 | 8.25 | Medium |
| 12 | Methanol | 6.50 | 6.50 | 6.50 | Medium |
| 13 | Methanol | 9.60 | 9.60 | 9.60 | Medium |
| 14 | Methanol | 8.70 | 8.55 | 8.275 | Medium |
| + | chloramphenicol | 12.30 | 12.56 | 12.43 | Medium |

**Inhibition zone**

\* = based on Greenwood (1995)

Zone of inhibition < 5 : weak

Zone of inhibition 5 – 10 : medium Zone of inhibition 10 – 20 : strong Zone of inhibition > 20 : very strong

The antibacterial activity showed that the fractions possesing the antibacterial character are fractions 5, 6, and 9 which are soluble in medium polar solvent, i.e. hexane:acetone 6:4 and hexane:acetone 5:5 as well as ethyl

acetate:methanol 9:1 respectively. It indicates that the compounds possessing the antibacterial character are present in various polarity. Based on this data, it can be guessed that the group of compounds are terpenoids, phenolics or alkaloids. The most active fraction, fraction 5, is then evaluated for its phytochemistry. The phytochemical screening were performed to identify the class of the compounds in the most active fraction. The result of the phytochemical screening is shown below.

**TABLE 1**. The Phytochemistry of the most Active Fractions of VLC, Fraction 5

**Phytochemical screening Terpenoids**

**Saponins Alkaloids Glycosides Phenolics Flavonoids**

Terpenoids   – –  

The phytochemical screening of the most active fractions showed that fraction 5 containing terpenoids and flavonoids. As we know that compounds with hydroxyl groups exhibit antibacterial activities. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity13. The terpenoids are also exhibit antibacterial activity The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds.

**CONCLUSION**

It can be concluded that among the fraction from Vacuum Liquid Chromatography, the most active antibacterial fraction is fraction 5, indicated with its high zone of inhibition, 12.725. The phytochemical screening of the fraction indicated that the fracton is a mixture of terpenoids and flavonoids.

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