



Phytochemical screening and antibacterial activity of different fractions of Indonesian *vinca rosea* leaves (*Catharanthus roseus* L. G. Don)

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ABSTRACT

The phytochemical and antibacterial activity of different fractions (hexane, ethyl acetate and methanol) obtained from leaves of tapak dara (*Catharanthus roseus* L.G. Don) has been investigated as a part of a new searching on antibacterial compounds. The phytochemical screenings showed that the hexane fraction contains terpenoids and steroids, while both the ethyl acetate and methanol fractions contain alkaloids and flavonoids. The antibacterial activity was determined by disc agar-diffusion method against *E. coli*. The ethyl acetate fraction showed the highest inhibitory zone than hexane or methanol. It represents an important step for the search and development of a new antibacterial agent.

Keywords: *Catharanthus roseus* L.G. Don, *E. coli*, antibacterial, phytochemical screening

INTRODUCTION

Plants are sources of interesting chemicals. One of which is *Vinca rosea* (*Catharanthus roseus* L.G. Don). In Indonesia, the plant known as *Tapak Dara*. It is a very important ornamental plant that has been used in all around the world including Indonesia in traditional medication to cure asthma, diabetics, hypertension, and injure [1]. This plant is an outstanding source of natural chemicals, one of which is vinblastine and vincristine, the very famous anticancer natural drugs. The leaves rich alkaloids and carbohydrates. It has been known to contain 150 useful alkaloids among other pharmacologically active compounds [2].

Previous research have reported that this plant showed interesting bioactivities. It has been reported that the ethanol extract of the plant showed antioxidant activity [3], antimicrobial [4], antitumor, antimalarial, antihypercholesterol, antifungal, antibacterial, and antiviral [5]. Gadir reported the cytotoxicity of the ethanol extract of the leaves and it has been shown that the leaves has antidiabetic and anticancer activity [6]. Ibrahim *et al* evaluated the antibacterial and antidiabetic activity of the whole plant extract against fourteen bacterial strains [7].

Over the past several years, the necessity for the development of new antibacterial agents from natural product rises up caused of the emergence of new infections and the increase of bacteria drug-resistance [8 – 10].

This study aims to investigate the phytochemical and antibacterial activity of some fractions (hexane, ethyl acetate, and methanol) obtained from the leaves of Indonesian tapak dara (*Catharanthus roseus* L.G. Don) as part of a new searching on natural antibacterial compounds.

EXPERIMENTAL SECTION

Materials

Leaves of *Catharanthus roseus* L.G. don were collected from Malang region in East Java Province – Indonesia. For the extraction, it have been used methanol, chloroform, hexane, acetone, ethyl acetate, silica gel 60, silica gel GF254, Dragendorff reagent, Mayer reagent, Lieberman Burchard, HCl 5%, aqueous NaOH 20%, NA, NB, aquadest, ethanol 70%. *E. coli* collected from the Biology Department, Faculty of Mathematics and Science, Universitas Negeri Malang (State University of Malang), DMSO, chloramphenicol.

Instrumentation**Procedure****Extraction Method**

The leaves are dried for 5 days in room temperature. The dried leaves are then powdered using blender. The powdered of the leaves are macerated using methanol for 3x 24 hours. The extract was then filtered and evaporated by rotary evaporation under reduced pressure at temperatures below 60°C. The resulting crude extract was then fractionated by liquid-liquid fractionation by hexane, ethyl acetate, and methanol, respectively. The different fraction is then assayed for the phytochemical screenings and antibacterial activity.

Phytochemical screening

The hexane, ethyl acetate, and methanol fractions of *Catharanthus roseus* L.G. Don were subjected to phytochemical tests for plant secondary metabolites in accordance with Trease and Evans¹¹, Tiwari¹², & Harborne¹³ with little modification.

Antibacterial Assay Method

The antibacterial activity of the fraction was evaluated against *E. coli* by disc-diffusion agar method. 1 mg of the each fraction was diluted in 1 mL 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 dilute 1.3 g in 100 mL of aquadest. After 24 hours, 200 µL of the bacteria are ready to inoculated in the NA media made of 2,3 g in 100 mL of aquadest. The activity was shown by the inhibitory zone of each fraction. The assay was done in three times for each fraction.

RESULTS AND DISCUSSION

Extraction and Phytochemical Screening

The extraction and fractionation by liquid-liquid fractionation produced three different fractions, i. e. hexane, ethyl acetate, and methanol fraction. The each fraction was then tested for the phytochemical screening and the results shown in Table 1.

Table 1 Phytochemical Screening of Various Fraction of the Leaves of *Catharanthus roseus*

Secondary Metabolites	Hexane Fraction	Ethyl acetate Fraction	Methanol Fraction
Alkaloid	–	+	+
Flavonoid	–	+	+
Terpenoid	+	–	–
Steroid	+	–	–

Antibacterial activity

The antibacterial activity of the various fractions showed varied degree of zones of inhibition against the tested bacterial pathogen, i.e. *E. coli*. The results were illustrated in Table 2.

Table 2 Antibacterial activity of fraction A, B, dan C from Tapak dara against *E. coli*

Sampel	Zone of inhibition (mm)				Classification of inhibition*
	Test 1	Test 2	Test 3	average	
Fraction A (n-hexane)	12	9	9	10	medium
Fraction B (ethyl acetate)	14	9	9	10.7	strong
Fraction C (methanol)	8	8	7	7.7	medium
chloramphenicol (+)	12	14	11	12.3	strong
DMSO (-)	0	0	0	0	Inactive

* = based on Greenwood (1995)

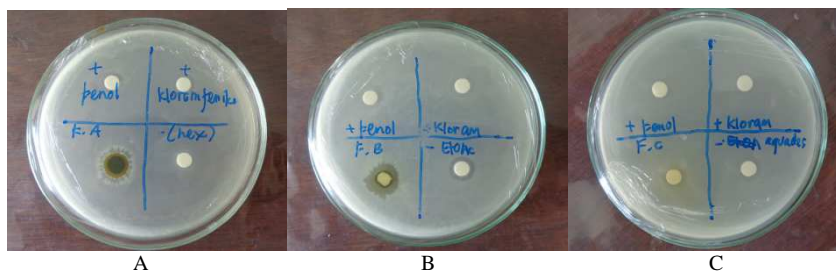
Zone of inhibition < 5 : weak

Zone of inhibition 5 – 10 : medium

Zone of inhibition 10 – 20 : strong

Zone of inhibition > 20 : very strong

The ethyl acetate fraction of *Catharanthus roseus* L.G Don showed the highest antibacterial activity against *E. coli* among the two fractions with the zone of inhibition of 10,7 mm. It indicated that the ethyl acetate fraction is the source of the natural antibacterial compounds from *Catharanthus roseus* L.G. Don.



Picture 1 The antibacterial activity results against *E. coli*: (a) fraction n-hexane, (b) fraction ethyl acetate, (c) methanol-water fraction

CONCLUSION

The study showed that the ethyl acetate fraction of the *Catharanthus roseus* L.G. Don leaves is the most active one against *E. coli* bacteria with the zone of inhibition of 10,7 mm. The phytochemical screening showed that the ethyl acetate fraction contains flavonoids and alkaloids. As part of new searching on antibacterial compounds, it is recommended to explore the ethyl acetate fraction to gain new antibacterial compounds from natural products.

Acknowledgements

The authors gratefully thanks to DP2M Dikti Republic of Indonesia for funding the research via the Hibah Bersaing Grant.

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