ANTIBACTERIAL ACTIVITY OF *Chromolaena odorata* (L) King LEAVES WITH BIOAUTOGRAPHY

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ABSTRACT

Chromolaena odorata (L) *King, commonly referred as kirinyuh, is a traditional medicinal plant from Asteraceae. It has been reported that C. odorata scientifically possessed antimicrobial activity. The aim of this study is to obtain active antibacterial substances from 70% etanolic extract of C. odorata against Staphylococcus aureus. Separation of active substances was carried out by Thin Layer Chromatography (TLC) techniques. The separated substances were tested for their antibacterial activity by bioautography assay using S. aureus. Separation of 70% etanolic extract C. odorata produced six spots with retention factor (Rf) values are 0.9, 0.8, 0.7, 0.6, 0.5, and 0.3. The diameters of inhibition zone of those spots were 35, 27, 27, 20, 31, and 14 mm, respectively. Based on the TLC profiles, the compounds with Rf of 0.9, 0.6, and 0.5 were identified as flavonoids and the compounds with Rf of 0.8, 0.7, and 0.3 were identified as terpenoids. It is concluded that the spot with the most potent antimicrobial activity was flavonoids with of Rf 0.9.*

Key words*: antibacterial, bioautography, Chromolaena odorata.*

Introduction

Chromolaena odorata (L) King, commonly referred kirinyuh, is a weed from Asteraceae. These plants can reduce yields of cultivated plants such as rubber, oil palm, coconut, and cashew. However, this plant also functions as organic fertilizers, bio-pesticides, and herbicides (Zachariades et al., 2009). Constituents of chemical compounds of kirinyuh that have been reported are tannin, saponin, flavonoids, beta cyanins, quinones, glycosides, cardioglycosides, terpenoids, phenols, coumarins, steroids, and alkaloids (Vijayaraghavan et al., 2013).

In the several country, *C. Odorata* was also use as medicinal properties. *C. odorata* is being used traditionally for external uses as in wounds, skin infections, and inflammation (Vaisakh and Pandaey,

2011). *C. odorata* leaves have demonstrated having antioxidant (Akinmoladun et al., 2007; Parameswari & Suriyavathana, 2012; Vijayaraghavan et al., 2013); nematicidal (Thodes et al., 2007), larvacidal (Sukhthankar et al., 2014), hemostatic (Akomas and Ijioma, 2014), antibacterial (Vital and Rivera, 2009; Kigigha and Zige, 2013; Stanley et al., 2014). In this study, *C. odorata* extracted with ethanol 70%, then tested for antibacterial activity with bioautography assay using *Staphylococcus aureus*. The aims of this study is to obtain active antibacterial substances from 70% etanolic extract of *C. odorata* against *S. aureus*.

Methods

Materials and Equipment

Ethanol 70%, n-hexan, ethyl acetat, TLC Plate silica gel F_{254}, *S. aureus* collected from Biomedic Laboratory, Medicine Faculty, University of Muhammadiyah Malang, nutrient agar, nutrient broth, aquadest sterile, ampicillin, petri dish, tube, micropipette, nippers, ose needle, autoclave, oven, incubator, Laminary Air Flow, frezer, rotavapour, vernier calipers.

Plant Material

C. odorata leaves were obtained and identified by Materia Medica Center, Batu, Malang. Leaves were cleaned with running tap water, and dried under shade at room temperature for 7 days. The dried leaves were finely ground.

Preparation of Extracts

Two hundred and fifty grams of powder of *C. odorata* leaves were extracted using maceration method with 1.3 l of ethanol 70% for 24 hours (3 times). The filtrate was collected and concentrated by evaporation with a vacuum rotary evaporator at 50 °C to obtain a viscous extract. The extract then dried in an oven at 40 °C. The crude ethanolic extract was stored in the refrigerator at 5 °C until required for use.

Sample Preparation

Aliquot 50 mg of 70% etanolic extract of *C. odorata* dissolved into 70% ethanol ad 1.0 ml.

Bacteria Preparation

One ose from original culture of *S. aureus was* cultivated on 9 ml nutrient broth, and then incubated for 24 hours at 37 °C. Aliquot 1 ml of bacteria culture is suspended and diluted in nutrient broth to obtain colony density of 10^6 CFU/ml. Bacteria suspension then

inoculated on nutrient agar and incubated for 24 hours at 37 °C.

Antibacterial Activity of C. odorata Leaves with Bioautography Assay

Aliquot 5 µl sample were spotted (3-5 mm diameters) on TLC plate. The plates were developed in n-hexane-ethyl acetat (4:6 v/v) and dried for 1 hour. The TLC plate was overlayed on the surface of nutrient agar inoculated with *S. aureus* for 30 minutes. The plate was removed and the media inoculated with *S. aureus* were incubated for 24-36 hours at 37 °C. This study was performed for three times. Ampicillin 20 µg/disc was used as positive control. The presence of an inhibition zone indicated the existence of antibacterial substances (Choma and Grzelak, 2010).

Phytochemical Identification

To identify the components of chemical compounds in the crude ethanolic extract, TLC and precipitation reactions were applied.

Results and Discussion

Extract of *C. odorata* leaves was viscous and deep green. Phytochemistry screening showed that it contained saponins, polyphenols, tannins, flavonoids, and terpenoids. Saponins were shown by positive result of froth test. Polyphenols and tannis were shown by positive result of reactions with $FeCl_3$ and gelatine test (Harborne, 1987; Sampietro et al., 2009). TLC chromatogram profile showed black spot with Rf of 0.2, 0.7, and 0.8, indicated the presence of polyphenols (Figure 1).

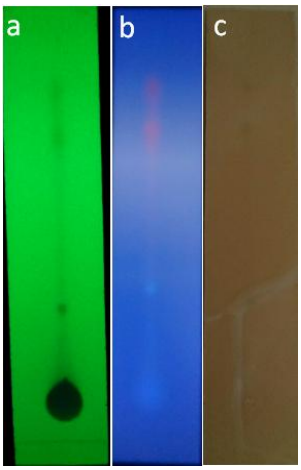

Figure 1. TLC chromatogram profile developed on silica gel F_{254} with chloroform-ethyl acetate-formic acid (0.5:9:0.5 v/v) as mobile phase. Visualization on (a) UV 254; (b) UV 366; (c) after derivatization with $FeCl_3$.

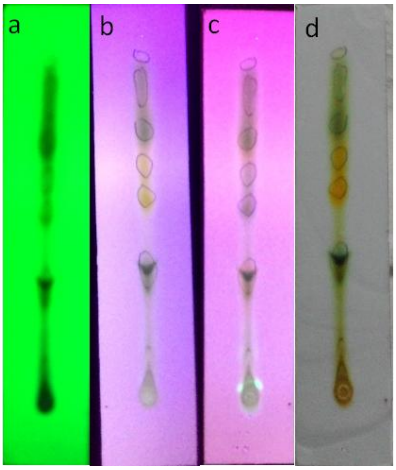

Figure 2. TLC chromatogram profile developed on silica gel F_{254} with n-hexane-ethyl acetate (4:6 v/v) as mobile phase. Visualization with (a) UV 254; (b) UV 366; (c) after derivatization with 10% H_2SO_4 on UV 366; (d) after derivatization with 10% H_2SO_4 on visible light.

Flavonoids were identified by yellow spots at Rf of 0.5, 0.6, and 0.9, while terpenoids were shown as green spot at Rf of 0.8, 0.7, and 0.3 (Figure 2). (Harborne, 1987; Debenedetti, 2009).

The identified compounds were separated and tested for their antibacterial activity with bioautography assay using *S. aureus*. Activities of the separated compounds were comparable to those of ampicillin, a standard antibacterial agent used as positif control. Antibacterial activity of *C. odorata* is presented in Figure 3 and Table 1.

Table 1. Antibacterial activity of *C. odorata* leaves

Sample	Rf	Zone Inhibition (mm)
Separated compounds from ethanolic extracts of *C. odorata* (250 µg)	0.9	35 ± 1
	0.8	27±0.5
	0.7	27±1
	0.6	20±0.5
	0.5	31±1
	0.3	14±1
Ampicillin (20 µg)		14±0.5

Among the separated compounds, flavonoids at Rf of 0.9 showed the highest antimicrobial activity. Flavonoids are known to demostrate a variety of biological activities, including anti-inflammatory, antispasmodic, antiviral, antifungal, antibacteria, antitumoral, and diuretic properties. Antibacterial activities of the flavonoids have been reported (Reichling, 2009). Mori et al. (1987) reported antibacterial activity, a structure-activity relationship and the effects of several flavonoids (e.g. flavones, flavonols, flavanones, flavanonols and catechins) on DNA and RNA syntesis in *S. aureus*. Furthermore, Bernard et al. (1997) described, for the first time, a DNA topoisomerase inhibitor specific for topoisomerase IV.

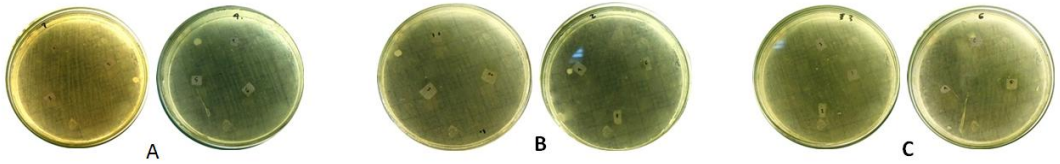

Figure 3. Bioautography assay of ethanolic extract of *C. odorata* leaves against *S. aureus.* (A) replication 1; (B) replication 2; (C) replication 3.

Conclusions

Separation of 70% etanolic extract *C. odorata* produces six spots with retention factor (Rf) values are 0.9, 0.8, 0.7, 0.6, 0.5, and 0.3. Bioautography assay shows that the diameter of inhibition zone of those spots are 35, 27, 27, 20, 31, and 14 mm, respectively. Based on the TLC profiles, the compounds with Rf of 0.9, 0.6, and 0.5 were identified as flavonoids and the compounds with Rf of 0.8, 0.7, and 0.3 were identified as terpenoids. It is concluded that the spot with the most potent antimicrobial activity was flavonoids with of Rf 0.9.

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