



***Catharanthus roseus*: A plant Source for Antifungal Activity Against Selected Plant Diseases**

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ABSTRACT

It is important to search for an effective and environmentally safe compound in controlling fungal pathogens. In addition, the plant that is suitable to be used as a source of bio-pesticide should be able to propagate easily and free of diseases and pests. Thus, *in vitro* antifungal activity screenings of *Catharanthus roseus* stems extract against fungal pathogens of crops were studied. *C. roseus* is an important medicinal plant used in traditional and modern medicine. Even though *C. roseus* contains various antimicrobial compounds, the plant is not being used as bio-pesticide. Six pathogenic fungi : *Colletotrichum gleosporioides*, *Fusarium oxysporum*, *Fusarium solani*, *Ganoderma philippii*, *Phellinus noxius* and *Rigidoporus microporus* were tested with different dichloromethane (DCM), acetone, ethanol, methanol and water extracts of *C. roseus*. Four different concentrations of extracts (5, 10, 15 and 20 mg/mL) were also tested. *C. roseus* DCM extract at 20 mg/mL showed the most effective activity against *R. microporus* and *F. oxysporum* with the inhibition zone diameters of 11.2 and 8.0 mm respectively. *C. roseus* is a bio-pesticide source that should be explored further.

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Keywords: *Catharanthus roseus*; Antifungal

INTRODUCTION

Catharanthus roseus L. belongs to the family Apocynaceae (Figure 1). In Malaysia, the local name is Kemunting cina. This shrub is commonly found in sub-tropical and tropical countries. It is also easily propagated by seed and stem cutting, and not many diseases and pests have been



recorded on *C. roseus* in Malaysia (Gaby, 2008). In traditional medicine, every plant part of *C. roseus* is very useful. The whole plant is used as a household remedy in many countries (Don, 1999). Srivinas et al. (2003) also reported that, the flowers and leaves are used to control diabetes in India. Meanwhile, in traditional Chinese medicine, the plant extract from roots and shoots have been used against numerous diseases, including diabetes, malaria, and Hodgkin's lymphoma (Schmelzer, 2007). In Malaysia, *C. roseus* has numerous medicinal properties (Anonymous, 2012). *C. roseus* is also used in modern medicine especially to produce anticancer drugs for the treatment of acute leukemia and Hodgkin's disease (Indu & Ng, 2000).

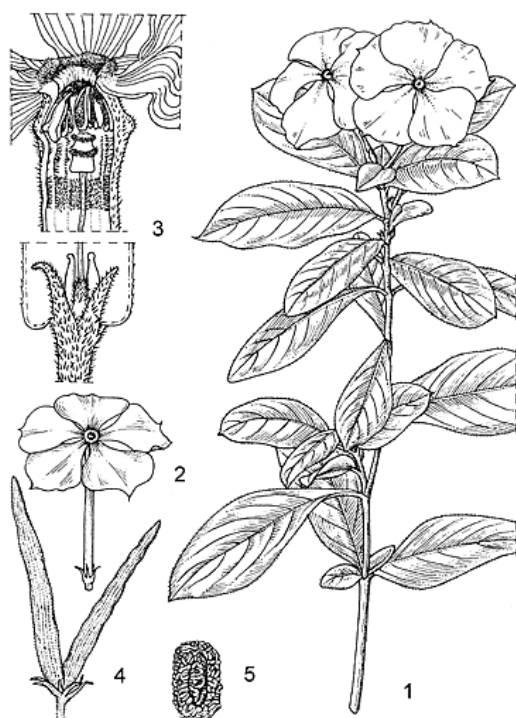


Figure 1: *Catharanthus roseus* morphology.

1, Flowering twig; 2, flower; 3, base and top corolla tube in longitudinal section; 4, Fruit; 5, Seed of *C. roseus*. (Source: Schmelzer, 2007).

C. roseus is a medicinal plant with potential antifungal activity (Balaabirami & Patharajan, 2012). For example, *C. roseus* extract of leaves was active against *A. fumigatus*, *C. albicans*, *P. chrysogenum*, and *A. niger* (Balaabirami & Patharajan, 2012). Flower extracts of the plant was also effective against *Aspergillus niger*, *C. albicans*, and *C. lipolytica* (Eufrocino et al., 2002). According to Moreno et al. (1994a), the leaves of *C. roseus* contains phenolic compound acting as antifungal agents against *Phytium aphaniderma*. In addition, *C. roseus* methanol extract of leaves inhibited the growth of *C. albicans* with a MIC value 4.0 µg/mL (Wakhede et al., 2013). The methanol extract of *C. roseus* leaves was also effective against *A. niger* and *Curvularia* sp. with the inhibition zone of 1.0 and 8.0 mm, respectively. The extract contained alkaloids, glycosides, and phytosterols (Shalini & Prema, 2012). Meanwhile, the flower of *C. roseus* methanol extracts was effective against *Curvularia* sp. with an inhibition zone of 7.0 mm (Shalini & Prema, 2012). The extract contained terpenoids, tannins, coumarin glycosides, phytosterols, alkaloids, flavonoids, and phenolics (Shalini & Prema, 2012).



Very few research on antifungal activity on *C. roseus* extract against plant diseases is available in literature. Several fungal diseases affect crops, resulting in losses and decreasing the quality and safety of agricultural products. For example, *Ganoderma philippii*, *Phellinus noxius*, and *Rigidoporus microporus* are responsible for root disease in forest plantations (Mohd Farid, 2010). These pathogens have induce a high mortality rates in crops (Eyles et al., 2008). *Fusarium oxysporum* causes high mortality on germinating seeds and seedlings (Mesheram & Soni, 2011). *Colletotrichum gleosporioides* infects *Capsicum* spp. (Than et al., 2008), guava fruit (Amusa et al., 2005), and coffee (Beer et al., 1998).

Chemical crop protection has a vital role in securing food supplies for the growing global population. However, a challenge in crop protection management is to protect the agricultural commodities from harmful agrochemicals (Janna, 2008; Wayne, 2013). Some fungi have evolved to resist pesticides hence the need for higher doses of chemical fungicides which indirectly increases environmental pollution and creates ecological disturbances (Chan et al., 1991; Jahanshir & Dzhililov, 2010). Therefore, the production of new fungicidal products for crop protection should be effective in reducing pest populations and should have low toxicity to humans and other mammals (Janna, 2008). In order to achieve this, it is important to search for an effective and environmentally safe compound in controlling plant fungal pathogens. Maria *et al.* (2007) stated that organic fungicides from some medicinal plants contain alkaloids, terpenoids, phenolics, essential oils, and flavonoids which are effective against fungal pathogens. These compounds provide a pool of rich biologically active compounds in agrochemical research. Thus, focus on medicinal plants especially *C. roseus* that contain antifungal active compounds is needed to replace the existing chemical fungicides. In addition, *C. roseus* stem extract efficacy in controlling plant pathogens has also yet to be tested. Thus, this study aims to screen the antifungal activity of *C. roseus* with different solvent and concentration against *G. philippii*, *P. noxius*, *R. microporus*, *F. oxysporum*, *F. solani*, and *C. gleosporioides* fungi. The positive results of antifungal activity from the *C. roseus* extract, should be explored a new plant source as bio-pesticide for further study.

MATERIAL AND METHODS

Sample collection and preparation of extracts: *C. roseus* with pink in color of flower samples were collected from Peninsular Malaysia, and about 500 g of stem of the samples were collected. The stems of plant samples were air-dried and ground to a powder using a grinder. About 200 g powder samples were soaked in 1 liter of five different solvents for three days. The solvents are dichloromethane (DCM), acetone, ethanol, methanol and water. The samples were shaken at 200rpm for one hour using a table orbital shaker. The samples were filtered with Whatman no. 1 filter paper. The filtrates were concentrated under pressure at 40°C using a rotary evaporator (Diagonal model, 115VAC) to remove the solvents.

Antifungal test: A total of 360 cultures of the 6 fungal species were equally prepared in the Pathology laboratory, Institute of Forest Research Malaysia (FRIM) prior to bioassay. The dried crude extracts of *C. roseus* stem were dissolved again in the same solvents to prepare for 5, 10, 15, and 20 mg/mL concentrations each. For bioassay, a 6 mm diameter of a 6-day-old fungal disc made using puncher was placed in the center of PDA plate. A sterile Whatman No.1 filter paper disc of 6 mm diameter was soaked in the respective extracts (Oumadevi et al., 2007). The filter paper disc was immediately placed on a new sterile filter paper to remove excessive solvents in the open. The treated filter paper disc with the plant extract was then placed approximately 3 cm away from the 6-



day old fungal disc. For control, the study was carried out using filter paper discs soaked in solvents without *C. roseus* extract. This filter paper was also placed 3 cm away from the fungal disc, but in opposite direction with the filter paper disc treated with *C. roseus* extract. The petri dish plate was immediately sealed and incubated at $25 \pm 2^\circ\text{C}$ temperature in the laboratory for 6 days observation (Figure 2). The experiment was arranged according to the Complete Randomized Design (CRD) with 3 replications. After six days of incubation, the PDA plates were examined for the presence of the fungal inhibition zone. The inhibition zone (mm) of antifungal activity was measured with a caliper-under stereo-microscope (Figure 2).

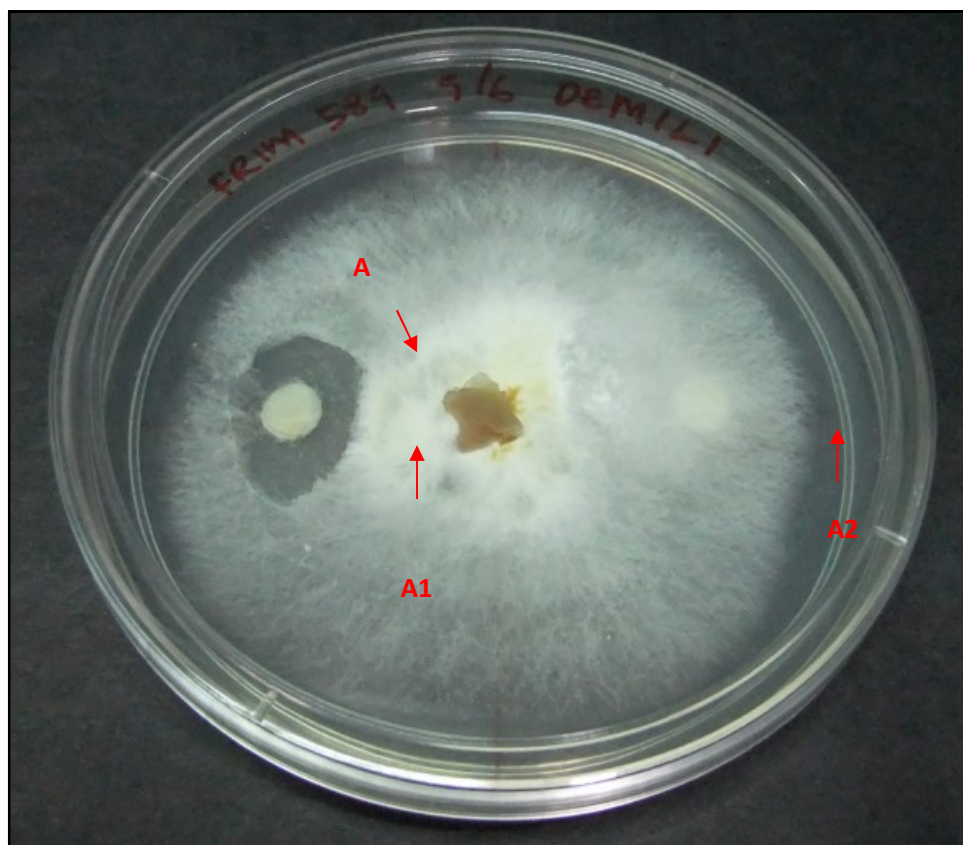


Figure 2. Filter paper was placed on the PDA plate containing a fungal culture. (A) inhibition zone area; (A1) Filter paper (6 mm) with plant extract; (A2) Filter paper (6 mm) without plant extract used as a control (solvent only of DCM, acetone, ethanol, methanol and water).

RESULTS

The results of antifungal activity of *C. roseus* extracted with five different polarity solvents; DCM, acetone, ethanol, methanol and water solvents with four different concentrations of 5, 10, 15 and 20 mg/mL against six fungi species are shown in Table 1 (Supplementary Material). From this study, it was found that the DCM stem extract of *C. roseus* was the most effective toxin against *R. microporus* and *F. oxysporum* with value of 11.2 mm and 8.0 mm respectively, compared to acetone, ethanol, methanol, and water extracts. *C. roseus* DCM extract at concentration of 20 mg/mL showed



highest activity against the fungal pathogens *R. microporus*, *F. oxysporum*, and *G. philippii* compared to other extracts (15, 10 and 5 mg/mL). The acetone extract showed high and effective antifungal activity against *R. microporus* with value of 9.10 mm.

DCM extracts of *C. roseus* were the best in controlling the growth of the *R. microporus*, *F. oxysporum*, and *G. philippii* compared to other extract solvents. DCM extract is a non-polar extract which might contain medium polar compound such as aglycone terpenoids and flavonoids. Terpenoids compounds can destroy the membrane cells of fungi (Gurja et al., 2012) while flavonoids are also effective in controlling fungi due to their ability to form complex with extracellular and soluble proteins, and bind with fungal cell walls (Narayana et al., 2011).

Acetone extracts of *C. roseus* stems were also active against *R. microporus*, *G. philippii*, and *C. gleosporioides* compared to other extracts. The extract showed the strongest inhibitory activity effect against *R. microporus*, *G. philippii*, and *C. gleosporioides* at 20 mg/mL compared to other extract solvents and concentrations. In a previous study, *C. roseus* was active against microbial pathogens (Prajakta & Jai, 2010). Acetone extract of *C. roseus* leaf extract also showed 90.76% inhibition percentage of antifungal activity against *F. oxysporum* (Neela et al., 2014). The acetone extracts may contain the most active biologically active compounds against plant fungal or microbial pathogens. Masako & Eloff (2006) reported that acetone is better than more polar solvents because the polar solvents give a larger spectrum, whereas non-polar solvent yields more lipophilic components. Acetone is usually preferred because it extracts polar and non-polar components.

Ethanol extracts of stems were active against the all fungal pathogens except *C. gleosporioides* and *P. noxius*. Their effectiveness was much less compared to acetone and methanol extracts especially at 20 mg/mL concentration. Both fungi did not show any inhibition zone. However, the leaves of *C. roseus* ethanol extract showed excellent activity against *F. moniliforme* with the inhibition zone of 2.0 mm (Kratika & Sharmita, 2013). The effective antifungal activity of compounds present in this extract against the pathogens might possibly depend on microbial resistance.

Methanol extract possessed antimicrobial activities against *R. microporus*, *F. oxysporum*, and *F. solani*. Extract from a culture of *C. roseus* callus also showed a very strong antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Marfori et al., 2002). However, in this investigation, this extract showed the lowest antifungal activities against compared to non-polar and semi-polar solvents.

Water extracts of *C. roseus* stem was not active against the fungal pathogens, unless against *R. microporus* and *F. oxysporum*. Their effectiveness was much lower compared to DCM, acetone, ethanol and methanol extracts especially at 20 mg/mL concentration. The production cost of the water extract is economical.

CONCLUSION

C. roseus stems DCM extract at 20 mg/mL showed the highest antifungal activity compared to other extracts in this study. This plant extract can be used as an antifungal agent which may help to reduce the severity of plant diseases. In addition, identification of potent and pure compounds requires further extensive study.



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DECLARATION OF CONFLICT OF INTEREST

No conflict of interest is associated with this research work.

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Supplementary Material

Table 1. Mean diameter of inhibition zone (mm) of *C. roseus* extracts against plant pathogenic fungi

Fungal Species	Concentration mg/mL																			
	5 mg/ml					10 mg/ml					15 mg/ml					20 mg/ml				
	DC M	AC N	ET N	ME OH	WA T	DC M	ACN	ETN	MEO H	WAT	DC M	ACN	ETN	MEO H	WA T	DCM	ACN	ETN	MEO H	WA T
<i>Rigidoporus microporus</i>	3.49 ± 0.82	2.78 ± 0.26	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.26 ± 0.22	6.38 ± 0.31	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	7.81 ± 0.21	7.30 ± 0.77	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	11.29 ± 0.29	9.20 ± 0.66	0.40 ± 0.04	0.00 ± 0.00	0.60 ± 0.02
<i>Ganoderma philippii</i>	2.00 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.09 ± 0.22	0.00 ± 0.00	0.70 ± 0.13	0.46 ± 0.21	0.00 ± 0.00	3.83 ± 0.12	2.69 ± 0.27	0.91 ± 0.15	0.84 ± 0.17	0.00 ± 0.00	4.09 ± 0.34	3.20 ± 0.25	0.97 ± 0.12	1.16 ± 0.22	0.00 ± 0.00
<i>Fusarium oxysporum</i>	0.50 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.81 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.09 ± 0.31	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.01 ± 0.34	0.08 ± 0.01	0.12 ± 0.02	0.41 ± 0.02	0.29 ± 0.16
<i>Fusarium solani</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.12 ± 0.02	0.13 ± 0.02	0.00 ± 0.00
<i>Ganoderma philippii</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.51 ± 0.18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.02	2.7 ± 0.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>Phellinus noxius</i>	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Note. DCM (dichloromethane), ACN (acetone), ETN (ethanol), MEOH (methanol), WAT (water)