

Search for biological activities from an invasive shrub species rose myrtle (*Rhodomyrtus tomentosa*)

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Abstract. Kusuma IW, Ainiyati N, Suwinarti W. 2016. Search for biological activities from an invasive shrub species rose myrtle (*Rhodomyrtus tomentosa*). *Nusantara Bioscience* 8: 55-59. Research into the potential of diversity, ethnobotany and ethnopharmacology and bioactivity of Indonesia plants is essential. In continuation of our search into biologically-active substances from plant sources, the ethanol extract of fruit, leaves, twig and stem of masisin or rose myrtle (*Rhodomyrtus tomentosa*) were evaluated for their antioxidant and antimicrobial properties and toxicity. Antioxidant property was evaluated by DPPH free radical scavenging activity. Antimicrobial activity was examined by agar well diffusion against *Salmonella typhi*, *Bacillus cereus*, *Propionibacterium acnes* and *Candida albicans*. Toxicity of the plant was determined by brine shrimp lethality test. The plant ethanolic extracts showed the occurrences of flavonoid, triterpenoid and carbohydrate in the phytochemical analysis. In the antioxidant assay, the plant extracts exhibited 90-93% of DPPH radical scavenging activity at 50 ppm. Ascorbic acid, a standard compound showed 96-98% activity at the same concentration tested. In the antimicrobial assay, the activities against *B. cereus* and *C. albicans* were displayed by the fruit and leaves of *R. tomentosa* with activity index (AI) of 0.42 and 0.35, respectively. Leaves, stem, twig and fruit of the plant showed activity against *S. typhi* and *P. acnes* with AI of 0.19-0.50 in comparison to that of reference compound, chloramphenicol. In the brine shrimp lethality test, leaves and fruit showed cytotoxicity with LD₅₀ of 43.4 and 8.5 µg/mL. The stem and twig ethanolic extracts were shown to be cytotoxic inactive. The present results showed potential of *R. tomentosa* extracts as natural antioxidant, antimicrobial and cytotoxic agents.

Keywords: Antimicrobial activities, cytotoxic, phytochemicals, *Rhodomyrtus tomentosa*, rose myrtle

INTRODUCTION

Globally, research into biologically active natural products from plants has attracted many natural product chemists. Various plants have been examined for their biological activities and in some cases active substances have been isolated and identified. We believe in Indonesia most research activity into natural products is still limited to the inventory of folkloric information and utilization of various plants and trees, meaning that obtaining scientific proof for their biological activity is still challenging.

Rose myrtle (*Rhodomyrtus tomentosa* (Aiton) Hassk.), locally known as *masisin*, is an ornamental, evergreen shrub grows up to four meters and plant-invading species native to Southern and Southeastern Asia. The popularity in cultivation, ability to form dense monocultures, dispersion of fruits by bird and mammal, saline condition tolerance, high seed production and germination, aggressive growth rate, and ability to resprout following fire events, make this species a serious invader (Winotai et al. 2005; Csurhes and Hankamer, 2011). The Dayak and the Paser, two local tribes at East Kalimantan, Indonesia use the root decoction of *R. tomentosa* to treat diabetes, while the leaves of the plant is used to soften meats during cooking. Furthermore, the plant is also traditionally used as anti-diarrhea, anti-wound, stomachache, and also used to formulate skin-whitening, antiaging, and skin beautifying

agents (Miyake and Nojima 2006; Sutomo et al. 2010). Ethanolic extract and an acylphloroglucinol compound isolated from the *R. tomentosa* leaves have been reported to profound antibacterial activity against several Gram-positive pathogenic bacteria (Saising et al. 2008; Limsuwan et al. 2009; Voravuthikunchai et al. 2010). However, further reports on the biological activities and phytochemicals of other parts of *R. tomentosa* are limited. Here we reported the phytochemicals and potential of biological activities from *R. tomentosa* leaves, stem, twig and fruit as antioxidant, antimicrobial and cytotoxic agents.

MATERIALS AND METHODS

Plant materials and chemicals

Leaves, stem, twig and fruit of *R. tomentosa* were collected from nearby Samarinda, Indonesia, in June 2013. The plant was kindly identified by a taxonomist of Mulawarman University, Samarinda, Indonesia. Voucher specimens were deposited in the Laboratory of Forest Products Chemistry, Faculty of Forestry, Mulawarman University. The plant materials were shade dried for 3 days and ground with a blender. DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DMSO (dimethyl sulfoxide), sulfuric acid, hydrochloric acid, acetic anhydride, potassium iodide and

peptone were purchased from Merck (Darmstadt, Germany). Ascorbic acid, 1-naphthol and bismuth (III) nitrate were obtained from Sigma (St. Louis, MO, USA). Nutrient agar was obtained from Difco (Detroit, MI, USA). Other chemicals were of HPLC grade or the highest purity commercially available.

Procedures

Extraction

Ground *R. tomentosa* leaves (12 g), stem (85 g), twig (5 g) and fruit (64 g) were extracted with 95% ethanol at room temperature with continuous shaking on a shaker (7400 Tübingen; Edmun Buchler, Germany) for 24 hours. This process was then repeated. Following filtration of the suspension through Whatman filter paper no. 2 (Maidstone, UK), the crude alcohol extract was rotoevaporated at 40°C and put in a vacuum oven to near dryness to yield the extract of leaves (1.6 g, 16.2% dry weight, DW), stem (7.6 g, 9.8% DW), twig (0.4 g, 9.7% DW) and fruit (1.5 g, 2.9% DW).

Phytochemical analysis

One gram of the plant ethanol extracts was dissolved in 100 mL ethanol and subjected to qualitative phytochemical screening following a previous method (Senthilmurugan et al. 2013).

Antioxidant assay

The sample was first dissolved in DMSO and used at a 30 times dilution for the actual experiment. Antioxidant by means of DPPH radical scavenging assay was performed according to the method previously reported by Kusuma et al. (2011). UV absorption was measured on a Shimadzu UV-VIS 1240 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Antimicrobial analysis

Antimicrobial assay was conducted using the agar disc diffusion method as previously reported (Kusuma et. 2011). *Bacillus cereus*, *Salmonella typhi*, *Propionibacterium acnes*, and *Candida albicans* were used in all experiments. Nutrient agar was used in antimicrobial assay, respectively. Twenty-milliliter aliquots of sterile media were transferred to Petri dishes and allowed to solidify. The media plates were inoculated with 10 µL of microbial suspension spread uniformly on the surface of the plates. A seven-mm well were cut using a sterile cork borer and 10 µL solution containing 100 µg/well of ethanolic extracts were added to the well. Chloramphenicol was used as a positive control at the concentration of 10 µg/well. The plates were incubated in the dark at 32°C for 24 hours. Zones of inhibition around the well were measured in mm. Activity index (AI) was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug (Kumar et al. 2013).

Cytotoxicity assay

Cytotoxicity of *R. tomentosa* extracts was determined by brine shrimp lethality test according to the modified method of Bückner et al. (2013). *Artemia salina* eggs were hatched in a tank containing sea water. Five milligram of

each extract was dissolved in 1 mL DMSO. Aliquots of the extract solution were taken in separate vials to prepare 25, 50, 100, 250 and 500 µg/mL of *R. tomentosa* extract in 5 mL sea water. After 48 hrs of hatching period of *A. salina*, ten nauplii were moved to vials containing the plant extracts or gallic acid as the positive control. Ten brine shrimp were transferred to each vial using adequate pipettes. The control vial contained DMSO and sea water only. Each test consisted of exposing groups of ten brine shrimp to various concentrations of the extract of *R. tomentosa*. The toxicity was determined after 24 h (nauplii in instar II/III) of exposure. The numbers of survivors were counted and percentage of deaths was calculated. Analysis of the mortality and LC₅₀ data was performed by probit analysis on StatPlus 2009 for Windows (AnalystSoft, Vancouver, Canada).

RESULTS AND DISCUSSION

Plant extracts and phytochemicals

Leaves, stem, twig and fruit of *R. tomentosa* were macerated by ethanol at room temperature. The maceration of the leaves, stem, twig and fruit of *R. tomentosa* yielded 16.2%, 9.8%, 9.7% and 2.86% of extracts on the basis of sample dry weight. Results of the phytochemical analysis were listed in Table 1. Qualitative phytochemical analysis showed the occurrence of flavonoid, triterpenoid and carbohydrate in the leaves, stem, twig and fruit. Alkaloid, steroid and saponin were not detected during the analysis.

Antioxidant activity

The antioxidant activity of leaves, stem, twig and fruit of *R. tomentosa* was given in Fig 1. The leaves extract of *R. tomentosa* demonstrated 65%, 85%, 88% and 90% of DPPH radical scavenging activity at 6-50 µg/mL. The stem extract exhibited 44%, 70%, 81% and 93% at 6-50 µg/mL. Furthermore, twig extract inhibited 63%, 86%, 91% and 92% of radical formation at 6-50 µg/mL. The fruit extracts caused 73%, 85%, 89% and 93% of radical scavenging activity at the same extract concentration tested. The antioxidant properties of *R. tomentosa* extracts at 50 µg/mL is comparable to that of reference, ascorbic acid that displayed 96-98% activity at the same concentration. The results of antioxidant assay by mean of DPPH radical scavenging activity suggested a concentration-dependent activity.

Antimicrobial activity

Results of antimicrobial tests of the plant extracts were listed in Table 2. At 100 µg/disk, the extracts of fruit and leaves of *R. tomentosa* showed good activity with activity indexes (AI) of 0.42 and 0.35 to *Bacillus cereus* relative to chloramphenicol, a standard drug. Furthermore, the leaves and the fruit of the plant displayed growth inhibitory activity against *S. typhi* with AI of 0.47 and 0.50, respectively. The twig and stem extracts possessed low activity against the same microbial strains tested. In antiacne activity assay, whole plant parts tested showed good activity against *P. acnes* with AI of 0.37-0.44.

Activity of the plant extracts against *C. albicans* was displayed by the fruit and leaves extracts with AI of 0.37-0.39 at 100 µg/well, while stem and twig extracts did not show any activity. In general, fruit and leaves extracts of *R. tomentosa* displayed more antimicrobial activity than stem and twig.

Cytotoxicity

Results of brine shrimp lethality test on *R. tomentosa* extracts were estimated by a probit analysis using mortality data as listed in Table 3. From the calculation, the fruit and

leaves extracts revealed cytotoxicity effect to brine shrimp after 24 h with LC₅₀ at 8.50 µg/mL and 43.38 µg/mL. This suggested that the extract could contain compounds that are cytotoxic, as the LC₅₀ value was low at 24 hours. The stem and twig extracts showed low cytotoxicity with LC₅₀ higher than 500 µg/mL. Gallic acid used as the positive control for the experiment showed LC₅₀ at 4.20 µg/mL. While the fruit and leaves extracts showed cytotoxicity to brine shrimp, the results have proved that fruit extract had a comparable cytotoxic properties to that of gallic acid.

Table 1. Phytochemicals analysis of ethanolic extracts of *R. tomentosa*

Plant parts	Phytochemicals					
	Alkaloid	Flavonoid	Triterpenoid	Steroid	Saponin	Carbohydrate
Leaves	-	+	+	-	-	+
Stem	-	+	+	-	-	+
Twig	-	+	+	-	-	+
Fruit	-	+	+	-	-	+

Note: Positive (+) indicated the presence and negative (-) indicated the absence of the phytochemicals

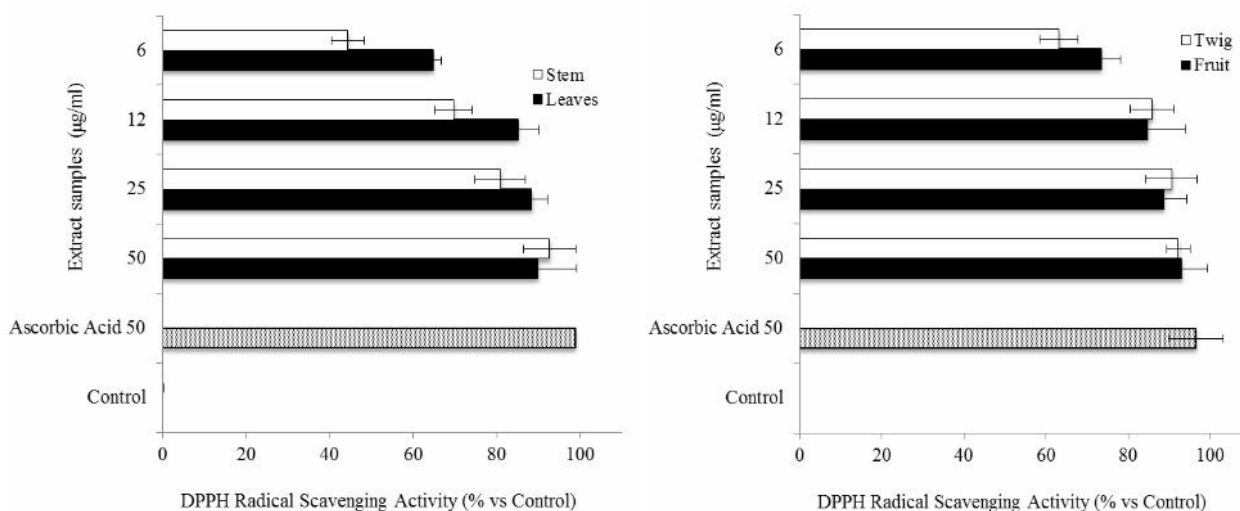


Figure 1. DPPH-radical scavenging activity of ethanolic extracts of *R. tomentosa*

Table 2. Antimicrobial activity of *R. tomentosa* against four microbial strains

Sampel tested	Microorganism tested							
	<i>B. cereus</i>		<i>S. typhi</i>		<i>P. acnes</i>		<i>C. albicans</i>	
	Inhibition zone (mm)*	Activity index (AI)**	Inhibition zone (mm)	Activity index (AI)	Inhibition zone (mm)	Activity index (AI)	Inhibition zone (mm)	Activity index (AI)
Leaves	11 ± 0.8	0.35	15 ± 1.6	0.47	11 ± 0.5	0.43	10 ± 0.1	0,39
Stem	0	0	6 ± 0.1	0.19	11 ± 1.9	0,44	0	0
Twig	0	0	3 ± 0.3	0.09	10 ± 1.1	0,37	0	0
Fruit	13 ± 0.7	0.42	16 ± 0.9	0.50	10 ± 0.8	0,38	10 ± 0.3	0.37
CHP	31 ± 1.7	1.00	32 ± 2.1	1.00	26 ± 0.8	1.00	26 ± 0.7	1.00

Note: *Sample concentration was 100 µg/well; Inhibition zones (IZ) were presented as mean of triplicates ± SD and include the well diameter (7 mm); **AI = IZ of test sample divided by the IZ of a standard drug; CHP=chloramphenicol, a standard drug used as a positive control.

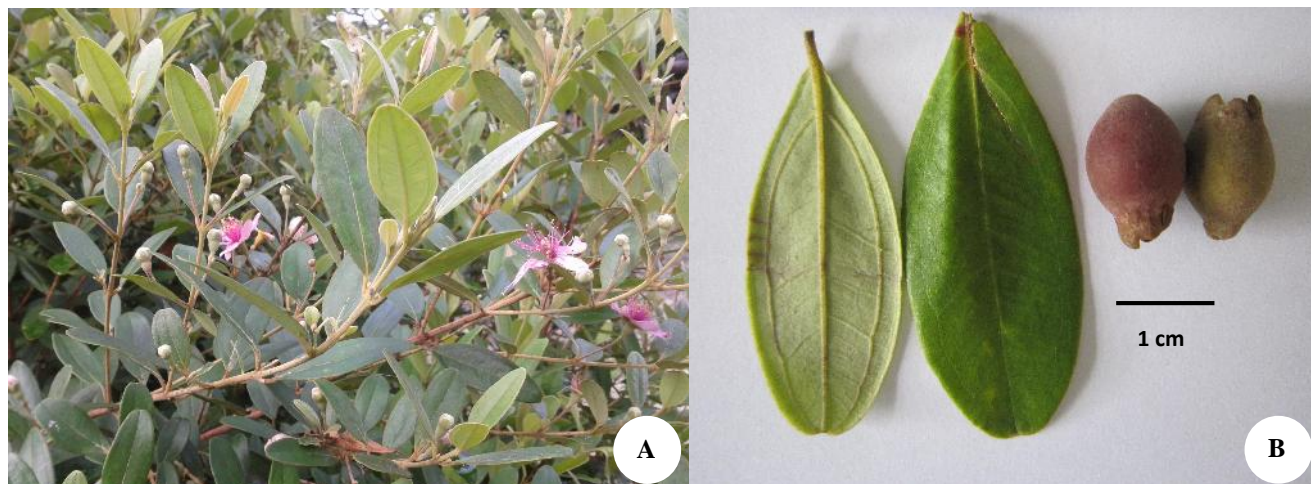


Figure 2. Plant (A) and leaf and fruit (B) of *Rhodomyrtus tomentosa*

Table 3. Cytotoxicity of *R. tomentosa* extracts in brine shrimp lethality test

Sampel	LC ₅₀ (µg/mL)*
Leaves	43.38
Stem	>500
Twig	>500
Fruit	8.50
Gallic acid	4.20

Note: *LC₅₀ value was calculated from triplicates by a probit analysis

Discussion

In recent years, many researchers try to discover novel and effective natural products from plants, microbes and other living organisms to treat various diseases. In continuation of our search into biological activities from tropical medicinal plants, phytochemicals, antioxidant and antibacterial activities and cytotoxicity of *R. tomentosa* grown in Indonesia were reported.

Phytochemical screening of the plant extracts as presented in Table 1 revealed that the crude extracts contained flavonoid, triterpenoid and carbohydrate. Alkaloid, steroid and saponin were not detected during the analysis. The phytochemicals tested are known to exhibit medicinal and physiological activities. Flavonoids have been reported to possess antibacterial, antioxidant, antinociceptive, anti-inflammatory, anticancer and acetylcholinesterase activities (Okoth et al. 2013; Cao et al. 2013; Rauf et al. 2016). Triterpenoids have been reported to possess various important bioactivities, including antibacterial, anticancer, antiviral and cytotoxic properties (Mathabe et al. 2008; Watanabe et al. 2011; Safe et al. 2012). The presence of biologically important phytochemicals in the *R. tomentosa* extracts, as tested in this study, contributes to the medicinal value, and therefore, point to potential sources for useful drugs. Further phytochemical and pharmacological investigations of the active compounds from *R. tomentosa* should be

conducted given their diverse and extensive traditional uses and potential therapeutic applications.

Antioxidant activity of *R. tomentosa* extracts was evaluated by DPPH radical scavenging mechanism. DPPH is a free radical compound that has widely been used to test the free radical scavenging abilities of various types of samples. The method offers advantages of being rapid, simple and inexpensive and provides first hand information on the overall antioxidant capacity of the test system (Kedare and Singh 2013; Naik et al. 2008). The results were shown as the relative activities against standard ascorbic acid (Figure 1). The activities of the leaves, stem, twig and fruit of the plant at 50µg/mL were similar to that of ascorbic acid at the same concentration. Investigation into the discovery of natural resources as potent antioxidant has been in increase to substitute the available synthetic antioxidants.

Result of antimicrobial activity assay against four pathogenic microbial strains was presented in Table 2. The fruit and leaves of *R. tomentosa* possessed activity against *B. cereus*, *S. typhi* and *C. albicans*. Potential activity against *B. cereus* and *S. typhi* informed that the extracts may be applied as antiseptic agents. Good activity of the plant extracts against *Candida albicans* suggesting the possibility that the extracts may be useful for the treatment of vaginal yeast infection, skin and diaper rash, and other *Candida* infection-caused diseases. Furthermore, in antiacne activity assay, whole plant parts tested showed good activity against *Propionibacterium acnes*. The results suggested the possibility that the extracts maybe used for treating acne lesion and acne-related diseases. The development of microbial bacterial resistance to presently available antibiotics leads the search for new antimicrobial agents (Parekh et al. 2006). Due to the problem of microbial resistance to antibiotics, attention is given towards biologically active components isolated from plant species commonly used as herbal medicine, as they may offer a new source of antibacterial, antifungal and antiviral activities.

Plant extracts tested for cytotoxicity using the brine shrimp lethality test are presented in Table 3. *R. tomentosa* stem and twig extracts were shown to be low cytotoxicity ($LD_{50} > 500$ mg/mL), while the fruit and leaves extracts showed significant activity (LC_{50} 8.50 μ g/mL and 43.38 μ g/mL, respectively). Therefore, the fruit and leaves of *R. tomentosa* have potential to be candidates for investigation as cytotoxic compounds. Cytotoxicity against brine shrimp shows strong correlation with cytotoxicity towards 9KB (nasopharynx cancer), P388 (murine leukemia), and other cancer cells (Ghisalberti 2008). Therefore, cytotoxic properties of the leaves and fruit of *R. tomentosa* may open possibilities of these plant extracts possessing anticancer activity.

The present work has proven that leaves, stem, twig and fruit ethanolic extract showed partial activities in terms of antioxidant and antimicrobial properties. Cytotoxicity assay displayed that the leaves and fruit extracts of the plant possessed potential as an anticancer agent.

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