BILINGUAL PUBLISHING CO.
Pioneer of Global Academics Since 1984

Veterinary Science Research
https://ojs.bilpublishing.com/index.php/vsr

ARTICLE

Antibacterial and Antioxidant Activity of *Rhodomyrtus Tomentosa* and *Cinnamomum Zeylanicum* Crude Extracts

Tran Thi Quynh Lan* **Vu Manh Khiem** **Nguyen Van Tin**

Departement of Veterinary Biosciences, Animal Science and Veterinary Medicine, Nong Lam University, HCM City, Vietnam

ARTICLE INFO

Article history
Received: 27 November 2020
Accepted: 20 April 2021
Published Online: 16 May 2021

Keywords:
Antibacterial and antioxidant activity
Minimal inhibitory concentrations (MICs)
Crude extracts

ABSTRACT

The aim of this study was to investigate the extraction method for *R. tomentosa* and *C. zeylanicum* leaves and the evaluation of antibacterial and antioxidant activities of crude extracts. The results of the study showed that the active ingredients of crude extracts were clearly separated by Thin-layer chromatography and the presence of rhodomyrtone in *R. tomentosa* crude extract and cinnamaldehyde in *C. zeylanicum* crude extract. *R. tomentosa* crude extract was antibacterial activity against *Staphylococcus aureus* with 13.1 mm of inhibition zone, but is not effective against *Salmonella* Typhimurium. *C. zeylanicum* leaf extract did not show antibacterial activity on both *S. aureus* and *S*. Typhimurium. At a dilution of 1/2 of the *R. tomentosa* crude extract can completely inhibit *S. aureus* growth. This study also indicated the presence of antioxidant compounds such as flavonoids, tannins, phenols and terpenoids in *C. zeylanicum* and *R. tomentosa* crude extracts. The results showed that *R. tomentosa* and *C. zeylanicum* crude extracts should be used as a biotherapy alternative to antibiotic therapy. However, further study would be needed to investigate the antibacterial activity of crude extracts *in vivo*.

1. Introduction

The use of herbs and medicinal plants as the first medicines. In recent years, multiple drug resistance in human and animal pathogenic microorganisms has developed due to indiscriminate use and commercial antibacterial drugs commonly used in treatment. This situation encouraged scientists for searching new alternative substances from various sources like medicinal plants which are the good sources and novel antimicrobial chemotherapeuticagents. Likewise, antioxidants play an important role in protecting cellular damage by reactive oxygen species. The medical plant is the most important targets to search for natural antioxidants from the point of view of safety.

R. tomentosa and *C. zeylanicum* have long been used in Oriental medicine. In human medicine, *R. tomentosa* leaves have long been used to treat diarrhea, relieve pain, stop bleeding wounds, or some diseases of the urinary tract [7, 8]. Meanwhile, *C. zeylanicum* leaves are effective for flatulence, indigestion, nausea, abdominal pain, diarrhea, gastrointestinal spasm and gastrointestinal disturbances [6]. *R. tomentosa* leaf extract and *C. zeylanicum* leaf extract were reported to have good antibacterial, anti-inflammatory, anti-fungal and antioxidant activity [5].

The present study was, therefore, aimed at evaluating the antimicrobial and antioxidant activity of *R. tomentosa*

**Corresponding Author:*
Tran Thi Quynh Lan,
Departement of Veterinary Biosciences, Animal Science and Veterinary Medicine, Nong Lam University, HCM City, Vietnam;
Email: lan.tranthiquynh@hcmuaf.edu.vn

DOI: https://doi.org/10.30564/vsr.v3i1.2623

and *C. zeylanicum* leaf extracts against some pathogenic microbes. The results of research should be applied to the prevention and treatment for animal diseases.

2. Materials and Methods

2.1 Herbal Plants Preparation and Extraction

R. tomentosa and *C. zeylanicum* leaves were collected from Phu Yen province – South Central Coast Vietnam. The leaves were then washed under running tap water and shade dried at 60 °C for 48 hours, then grinded into powder.

A total of 50g of powder was dissolved with 200 ml of ethanol solvent and then centrifuged (5000 rpm / 15 minutes). The supernatant was then evaporated at 60 °C for 30 minutes [8], and screened for antimicrobial and antioxidant activity.

2.2 Analysis of Active Ingredients of Crude Extracts

Crude extracts of *R. tomentosa* and *C. zeylanicum* were loaded on a thin plate of aluminum backed silica gel 60 F254 (Merck, Germany) on the semi-automatic thin plate chromatography system (Camag, Switzerland), the blasted plate was dried naturally at room temperature, then placed in a 20 x 10 cm twin trough chamber (Camag, Switzerland) containing the developing solvent which is toluene: ethyl acetate (93: 7) [16].

The interpretation of separated active compounds was observed by UV chamber (Camag, Switzerland) with 256 nm wavelength [1].

2.3 Identification of Pharmacological Active Ingredient (Rhodomyrtone for *R. tomentosa* and Cinnamaldehyde for *C. zeylanicum*)

Rhodomyrtone was determined by comparing movement coefficient (Rf) of chromatographic streak corresponding to *R. tomentosa* leaf extract sample with Rf of positive control rhodomyrtone (rh) (SMB00114, purity ≥ 95%, Sigma, USA).

Cinnamaldehyde was determined based on the comparison of the Rf of the chromatographic streak corresponding to the *C. zeylanicum* extract with the Rf of cinnamaldehyde according to the study research has been published [16].

2.4 Antibacterial Activity

The antimicrobial activities were done by using bacteria strain like *Salmonella* Typhimurium (ST) and *Staphylococcus aureus* (SA). The antimicrobial activity was determined by disc diffusion method. The Mueller Hinton agar plates were inoculated with a bacterial suspension (adjusted to 1-3 x 10^8 CFU /ml). 20 µl of extracts were loaded onto sterile paper disks and placed on the culture plates. 20 µl of amoxicillin + clavulanic acid (Nam Khoa Company) was used as control. Then the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, the diameter of inhibition zones around the discs was measured.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

Standard bacteria suspension *S. aureus* at 600 nm (OD = 600) (equivalent to 10^5 CFU/ ml).

The crude extract was diluted into 3 concentration levels: undiluted (1), diluted 1/2 and 1/4. Determine the minimum inhibitory concentration of *R. tomentosa* extract using the 96-well microplate described by Sultanbawa et al. [15].

2.6 Determination of the Antioxidant Activity

The presence of antioxidant compounds such as flavonoids, tannins, phenols and terpenoids in *C. zeylanicum* and *R. tomentosa* crude extracts were determinated by chemical reactions [6].

3. Results and Discussion

3.1 Analysis of Active Ingredients of Crude Extracts

The results of the separation on the thin plates of crude extract of *R. tomentosa* and *C. zeylanicum* leaves (Figure 1) showed that the number of chromatographic streaks separated from the *R. tomentosa* leaf extract sample was 8 streaks and the *C. zeylanicum* leaves were 12 streaks. Thus, the number of chromatographic streaks separated from *C. zeylanicum* leaf extract is higher than *R. tomentosa*. Furthermore, the streaks appearing in the chromatogram of the sample was *C. zeylanicum* darker and clearer than that of *R. tomentosa* leaf extract. This shows that the leaf extract of *C. zeylanicum* contains more active ingredients than the *R. tomentosa*.

3.2 Determination of the Presence of Rhodormyrtone and Cinnamaldehyde

Research results from Figure 2 show the ability to detect the presence of rhodormyrtone and cinnamaldehyde in extracts of *R. tomentosa* and *C. zeylanicum* by TLC. Rhodomyrtone in *R. tomentosa* leaf extract, used experimentally in many different studies, has been shown to reduce the invasion and adhesion of *S. aureus* in the subcutaneous tissue of bovine udders, which is an important

 DOI: https://doi.org/10.30564/vsr.v3i1.2623

property in the treatment of mastitis in dairy cows in clinical and subclinical form [7,8]. Meanwhile, the active ingredient cinnamaldehyde in *C. zeylanicum* leaves has also been shown to be resistant to many foodborne pathogens [6].

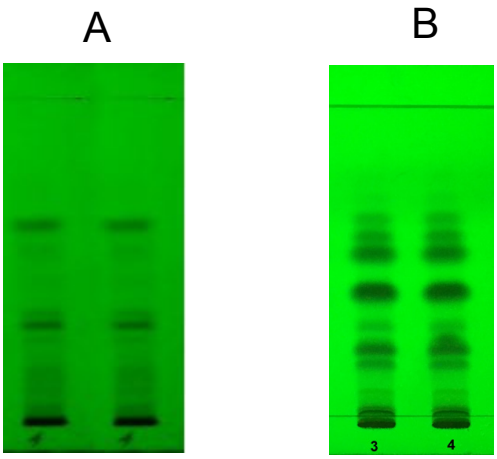

Figure 1. Results of separation of active ingredients in *R. tomentosa* and *C. zeylanicum* leaf extracts observed with UV (λ= 254 nm). A: *R. tomentosa*; B: *C. zeylanicum*

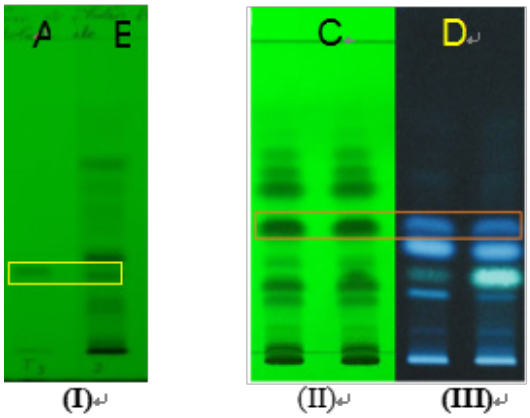

Figure 2. Results of separation of active ingredients with pharmacodynamic activity from *R. tomentosa* leaf (I), *C. zeylanicum* leaf (II) observed with UV (λ= 254 nm) and *C. zeylanicum* leaves according to Wagner et al. [16] (III). A: standard rhodomyrtone; B: *R. tomentosa* leaf extract; C: *C. zeylanicum* leaf extract; D: *C. zeylanicum* leaf extract [16].

3.3 Determination of Antibacterial Ability from *R. tomentosa* and *C. zeylanicum* Leaf Extracts

Table 1. Results of antibacterial ring diameter (mm) of *R. tomentosa* and *C. zeylanicum* leaf extracts for *S. aureus* and S. Typhimurium

Extract (20 µl)	*Staphylococcus aureus*	*Salmonella* Typhimurium
R. tomentosa	13.1 ± 0.8	0
C. zeylanicum	0	0
AMC	33	13

For *R. tomentosa* leaf extract, presence of rhodomyrtone was confirmed through a chromatographic streak on a thin plate with standard rhodomyrtone. For *C. zeylanicum* leaf extracts, the determination of cinnamaldehyde was based on the movement coefficient Rf (= 0.40) and chromatograms of Wagner et al. [16].

From the results of separation of active ingredients and the presence of two active ingredients with pharmacological activity: rhodormyrtone and cinnamaldehyde *R. tomentosa* and *C. zeylanicum* leaf extracts showed the applicability of TLC in the detection of valuable active ingredients in pharmacology.

Testing the antibacterial susceptibility assay by disk diffusion test on agar showed that the inhibition zone of *R. tomentosa* leaf extract against *S. aureus* strain was average at 13.1 ± 0.8 mm compared with the control inhibition zone of amoxicillin + clavulanic acid (AMC) at 33 mm (Table 1). According to Mordmuang et al. [4], paper plate containing 2.5 mg crude extract of *R. tomentosa* leaf extract showed an inhibition diameter of 8.7-15.5 mm for *S. aureus* in the study of antibacterial activity.

However, the antimicrobial of *R. tomentosa* leaf extract has not been observed when tested with *Salmonella* Typhimurium. In a study by Kusuma [4], *R. tomentosa* leaf extract for the inhibition zone of *Salmonella* typhi is about 15 mm in diameter. However, there have not been many more clear tests on the antibacterial activity of *R. tomentosa* leaf extract to *Salmonella typhi*murium.

While *R. tomentosa* leaf extract was effective against *S. aureus*, in contrast, *C. zeylanicum* leaf extract did not show the ability to inhibit both *Staphylococcus aureus* and *Salmonella typhi*murium. *C. zeylanicum* leaf extract had no effect on *Salmonella typhi*, but had good effects on *Escherichia coli, Bacillus subtilis, Candida albicans, Klebsiella pneumoniae* [2].

3.4 Minimum Inhibitory Concentration (MIC) of *R. tomentosa* Leaf Extract for *S. aureus*

Table 2. Minimum inhibitory concentration of *R. tomentosa* leaf extract against *S. aureus*

Active elements	Minimum inhibitory concentration (MIC)		
	Time (hour)		
	18	24	48
R.tomentosa extract (dilution)	½	½	½
Amoxicillin (µg /ml)	12.5	12.5	12.5
AMC (µg /ml)	6.25	6.25	6.25
Ceftiofur (µg /ml)	6.25	6.25	6.25

Control: concentration of the antibiotic is 200 μg /ml

DOI: https://doi.org/10.30564/vsr.v3i1.2623

Based on the antibacterial activity of *R. tomentosa* leaf extract against *Staphylococcus aureus*, the study continued to determine the minimum inhibitory concentration (MIC) of *R. tomentosa* leaf extract against *S. aureus* (Table 2). *R. tomentosa* crude extract at 1 and at 1/2 dilution gave the ability to completely inhibit *S. Aureus* according to the method of Sultanbawa et al. [15]. The use of crude extracts has advantages such as an easy access to *R. tomentosa* leaves, fast extraction process, and the extract can be used immediately after extraction. According to research by Saising et al. [11], the minimum inhibitory concentration of *R. tomentosa* leaf extract ranged from 512 µg /ml for *S. Aureus* isolated from the field. As for bacteria strain *S. Aureus* ATCC 25923, the value is 32 µg / ml [11]. Meanwhile, according to Mordmuang et al. [8], the MIC value of *R. tomentosa* leaf extract for *S. Aureus* isolated from mastitis cows in Canada was 16 **µ**g /ml.

MIC results of ceftiofur and amoxicillin + clavulanic acid (AMC) for *S. Aureus* were lower than amoxicillin (6.25 µg /ml versus 12.5 µg /ml) after 3 times of investigation. This shows that *S. Aureus* were more sensitive to ceftiofur and AMC than amoxicillin. *R. tomentosa* leaf extract at 1/2 dilution gave antibacterial abilities equivalent to antibiotics at a concentration of 12.5 µg /ml. The latest research by Mordmuang et al. [7, 8] was conducted to test the injection of *R. tomentosa* leaf extract on the mammary glands of the rat population with the *S. Aureus*. Results of the study showed that *R. tomentosa* leaf extract with a concentration of 300 µg /ml was injected directly into the mammary gland to help reduce the concentration of bacteria. *S. Aureus* is an important pathogenic bacteria in veterinary medicine and particularly the main cause of mastitis in cows - the most costly economic disease in dairy industry in the world.

The results of this study showed that the crude extract of *R. tomentosa* has good antibacterial activity against *S. Aureus*. Therefore, using *R. tomentosa* leaf extract can be an alternative to antibiotics in the treatment of diseases caused by *S. Aureus*. To do this, it is necessary to have follow-up studies in vivo to test the effectiveness of *R. tomentosa* leaf extract.

3.5 Determination of the Antioxidant Activity

The result shows the presence of flavaniods, tannins, terpenoids and phenol in both *R. tomentosa* and *C. zeylanicum* leaf extracts. The research of Hasibuan et al. [3], also showed similar results with the very high content of terpenoids and phenols in the extract of *R. tomentosa*.

According to study results of Mazimba et al. [6], in the extracts of *C. zeylanicum* leaves are rich in flavonoids, terpenoids, tannins and phenols. The presence of these active ingredients explains the medicinal properties of *R. tomentosa* and *C. zeylanicum* such as: antibacterial, anti-inflammatory, anti-allergic, diabetes treatment, pain relief as well as central nervous system support.

4. Conclusions

In conclusion, the active ingredients in *R. tomentosa* and *C. zeylanicum* leaf extracts good by TLC. The antimicrobial activity against *S. Aureus* of rhodomyrtone in *R. tomentosa* leaf extract and the presence of flavaniods, tannins, terpenoids and phenol in both *R. tomentosa* and *C. zeylanicum* leaf extracts should be applied in therapy fields such as bovine mastitis, dermatis, respiratory diseases.

The future study should investigate the antimicrobial activity against *S. Aureus* of rhodomyrtone in *R. tomentosa* leaf extract *in vivo* and the side effects of the active ingredients in *R. tomentosa* and *C. zeylanicum* leaf extracts.

References

[1] Eloff, JN., Ntloedibe, DT., & Van Brummelen, R. (2011). A *R. tomentosa*plified but effective method for the quality control of medicinal plants by planar chromatography. African Journal of Traditional, Complementary and Alternative Medicines, 8 (5S).

[2] Joshi, K., Shyam, A., Payal B., Sameer, W., Rajesh, G., Swati, J., Sushma, S., Sarang, B., & Padalkar AS. (2010). Cinnamomum zeylanicum extract inhibits proinflammatory cytokine TNF $\propto$: in vitro and in vivo studies. Research in Pharmaceutical Biotechnology 2(2): 14-21.

[3] Hasibuan, R., Syafruddin, I., & Saleha, H., (2015). Effect of leaf extract Haramonting (Rhodomyrtus tomentosa) to lower blood sugar levels in mice induced by alloxan. Int.J. PharmTech Res. 2015,8(6): 284-291.

[4] Kusuma Irawan Wijaya (2016). Search for biological activities from an invasive shrub species rose myrtle (Rhodomyrtus tomentosa). Nusantara Bioscience 8(1):55-59.

[5] Manosi Das, Mandal Suvra, Mallick Budhimanta, Hazra Jayram (2013). Ethnobotany, phytochemical and pharmacological aspects of Cinnamomum zeylanicum blume. International Research Journal of Pharmacy 4(4), 58-63.

[6] Mazimba, O., Wale, K., Tebogo, E., Tebogo, E., & Kwape Shetonde (2015). Cinnamomum verum: Ethylacetate and methanol extracts antioxidant and antimicrobial activity. OJJMPS 3 (3), 28-32.

[7] Mordmuang, A., Shankar, S., & Voravuthikunchai, S.

 DOI: https://doi.org/10.30564/vsr.v3i1.2623

(2015). Effects of *R. tomentosa* leaf extract on staphylococcal adhesion and invasion in bovine udder epidermal tissue model. Nutrients, 7 (10), 8503-8517.

[8] Mordmuang, A., & Voravuthikunchai, SP (2015). *R. tomentosa* (Aiton) Hassk. leaf extract: An alternative approach for the treatment of staphylococcal bovine mastitis. Research in science, 102, 242-246.

[9] Mubarack, HM, Doss, A., Dhanabalan, R., & Venkataswamy, R. (2011). Activity of some selected medicinal plant extracts against bovine mastitis pathogens. J. Anim. Vet. Adv, 10 (6), 738-741.

[10] Rubin, JE, Ball, KR, & amp; Chirino-Trejo, M. (2011). Antimicrobial susceptibility of *Staphylococcus aureus* and Staphylococcus pseudintermedius isolated from various animals. The Canadian Veterinary Journal, 52 (2), 153.

[11] Saising, J., Ongsakul, M., & Voravuthikunchai, SP (2011). *R. tomentosa* (Aiton) Hassk. ethanol extract and rhodomyrtone: a potential strategy for the treatment of biofilm-forming staphylococci. Journal of medical microbiology, 60 (12), 1793-1800.

[12] Singh, M., Singh, N., Khare, PB, & Rawat, AKS (2008). Antimicrobial activity of some important Adiantum species used traditionally in indigenous systems of medicine. Journal of ethno-pharmacology, 115 (2), 327-329.

[13] Sinulingga, SE, Hasibuan, PAZ, & Suryanto, D. (2018). Antibacterial activity of Karamunting (*R. tomentosa* (Aiton) Hassk) leaf extract and fractions. Asian J Pharm Clin Res, 11 (3), 163-165.

[14] Sirisha, N., Sreenivasulu, M., Sangeeta, K., & Chetty, CM (2010). Antioxidant properties of Ficus species – a review. Int J PharmTech Res, 2 (4), 2174-2182.

[15] Sultanbawa, Y., Cusack, A., Currie, M., & Davis, C. (2009). An innovative microplate assay to facilitate the detection of antimicrobial activity in plant extracts. Journal of Rapid Methods & Automation in Microbiology, 17 (4), 519-534.

[16] Wagner, H., Rudolf, B., Dieter, M., Pei-Gen Xiao and Anton Staudinger (2011). Chromatographic Fingerprint Analysis of Herbal Medicines - Thin-layer and High-Performance Liquid Chromatography of Chinese Drugs, Second Revised and Enlarged Edition, Vol. 2. SpringerWienNewYork. p 991.

DOI: https://doi.org/10.30564/vsr.v3i1.2623