Phytochemical Studies of *Rhodomyrtus tomentosa* Leaves, Stem and Fruits as Antimicrobial and Antioxidant Agents

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ABSTRACT *Rhodomyrtus tomentosa*, or kemunting (local name) is a well-known medicinal plant used to treat various sickness. *R. tomentosa* is native to southern and south-eastern Asia. The chemical constituents from leaves, stems and fruits of *R. tomentosa* was determined. The antibacterial and antioxidant activity of the crude extract and isolated compounds was performed. Screening and assay-guided isolation technique was performed upon the plant extract against Gram-positive and -negative bacterial strain, namely *Escherichia coli* and *Staphylococcus aureas*. In vitro antioxidant activity of the extract will also be assessed by 2,2-diphenylpicrylhydrazyl (DPPH) assay. The successful isolated compounds were also evaluated for their antibacterial and antioxidant activity.

KEYWORDS: *Rhodomyrtus tomentosa*; assay-guided isolation; antibacterial activity; antioxidant activity; DPPH assay; *E. coli*; *Staphylococcus aureas*.

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INTRODUCTION

R. tomentosa is a member of the Myrtaceae plant family native to Indian Sub-continent, China and South-East Asia. The plant is mainly growing along coastal shores, wetlands and riparian zones, from sea level up to2400 m elevation (Csurhes & Hankamer, 2016). The plant can grow up to 1-2 m tall, and sometimes 3 m in height. The leaves are oval in shape, and rounded tips. The flowers have five bright pink petals that fade to pale pink as they age. It has five sepals at the base and numerous stamens in the centre. The fruits are edible, oblong-shaped berry, and crowned by the persistent sepals (Navie, 2013). Previous studies on this plant had show antibacterial activity of ethanolic leaves extract against *Streptococcus pyogenes* (Limsuwan *et al*., 2012; Mordmuang *et al*., 2015). *R. tomentosa* extract possesses potential anti-inflammatory and antiulcer activity, and can serve as a potent antioxidant (Geetha *et al*., 2010; Lavanya *et al*., 2012; Jeong *et al*., 2013). Studies conducted on *R.tomentosa* fruits had show antioxidant properties due to present of phenolic compounds (Lai *et al*., 2012; Cui *et al*., 2013; Lai *et al*., 2014). There are lacks of studies in details on stems and fruits of *R. tomentosa*, further studies on other parts of the plants should be performed. Therefore, the aims of this study are to investigate the chemical constituents from leaves, stems and fruits of *R.tomentosa* and to evaluate the antibacterial and antioxidant activity of the crude extracts and the isolated compounds *via* assay-guided isolation.

METHODOLOGY

General Methods

Assay guided isolation was performed on *R. tomentosa* leaves, stems and fruits extract to determine the compounds which possessed potent antimicrobial and antioxidant activity. Leaves,

fruits and stems samples were soaked with methanol, and then the crude extracts were collected. Acetonitrile extraction/partitioning were performed on the extract. Then, the samples were partition into hexane, ethyl acetate and methanol extracts. Disk diffusion and DPPH scavenging activity of crude and partition extract were performed. The best partition extracts then were further isolated using column chromatography. Fractions collected were tested for antimicrobial and antioxidant activity.

Plant Material

Plant sample was collected in Lundu, Sarawak area. The samples were cleaned, air-dried and ground to powder form. 1123 g of leaves, 185 g of fruits and 963 g of stems powder samples were collected.

Extraction and Isolation

The samples were successfully extracted at room temperature with MeOH (thrice for each extract; soaked for 3 days). Then the extracts were dried using rotary evaporator. Acetonitrile extraction/partitioning was performed for each extract according to method reported by Díez *et al*., (2006) with some modification, to remove fatty acids and sugar. The crude extract were mixed with acetonitrile (10g of crude; 10ml of acetonitrile; 1g of NaCl), the upper layer was collected and dried. Then, the extract were extracted with dH_2O and petroleum ether (1:1), the upper layer was collected and dried. The extracts then were partition with hexane, ethyl acetate and MeOH respectively.

DPPH Radical Scavenging Assay

The measurement of the DPPH radical scavenging activity was performed according methodology reported Garcia, *et al*., (2012) and Maskam, *et* al., (2014), with some adjustment. The samples were reacted with stable DPPH radical in a methanol solution. Ascorbic acid was used as control. 1 mg of sample was dissolved in 1 mL of methanol and 20 mg of DPPH was dissolved in 100 mL of methanol. The stock solution then was prepared with different concentration by transferring 10, 30, 50, 100, 300, 500 µL of sample into vials. 1 mL of methanol was added into each vial. The antioxidant test was conducted by mixing 0.5 mL of sample, 3 mL of methanol and 0.3 mL of DPPH solution. Methanol was served as the blank. The control solution was prepared by mixing 3.5 mL methanol and 0.3 mL DPPH radical solution. When DPPH reacted with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color were read at 517 nm after 100 min of reaction using a UV-VIS Spectrophotometer. The scavenging activity analysis percentage was determined based on equation shown below.

$$Percentage\ of\ inhibition, \% = \frac{Abs.of\ control - Abs.of\ Sample}{Abs.of\ control} \times 100\%$$

Antimicrobial Assay

The antimicrobial assay was conducted based on Kirby-Bauer disk-diffusion method. All the test compounds were evaluated for antibiotics activity against *S. aureus* (gram positive) and *E. coli* (gram negative). Mueller-Hinton agar was used as the media. A dilution of the broth culture of *E. coli and S. aureus* was performed to achieve McFarland 0.5 standard. The plate was swap using cotton bud to ensure uniform spreading. 5 mm of filter paper disk was dipped with appropriate concentration of *R. tomentosa* samples. 4 samples discs were applied on the inoculums with sterile forceps. The samples were incubated at 37°C for 24 hours. The antimicrobial properties of the samples were determined by measuring the zones of inhibition of growth around the disc.

Isolation

Methanol partition extract of leaves, fruits and stems showed good antioxidant and antimicrobial activity. Leaves methanol extract was chromatographed on silica gel column with gradient of 20% EA/Hex (LM1-4), 50% EA/Hex (LM5), 50% EA/DCM (LM6-7), 20% MeOH/EA (LM8-10), 50% MeOH/EA (LM11), and MeOH (LM12-13) respectively. Fruits methanol extract was chromatographed with gradient of 17% EA/Hex (FM1-3), 25% EA/DCM (FM4), and 17% MeOH/DCM (FM5) respectively. Stems methanol extract was chromatographed with gradient of 25% EA/Hex (SM1), 17% EA/DCM (SM2), 50% EA/DCM (SM3), 17% MeOH/DCM (SM4), and 50% MeOH/DCM (SM5) respectively.

RESULT AND DISCUSSION

The result of the antimicrobial and antioxidant activity of *Rhodomyrtus tomentosa* leaves, stems and fruits were shown in the **Table 1-3**.

Table 1. Inhibition zone and EC_{50} of leaves extracts

Samples	*S. aureus* (mm)	*E. coli* (mm)	EC_{50}
Crude extract	11	10	262.1
Hexane extract	6	8	567.3
Ethyl Acetate Extract	7	8	321.4
Methanol Extract	10	10	144.2

Table 2. Inhibition zone and EC_{50} of fruits extracts

Samples	*S. aureus* (mm)	*E. coli* (mm)	EC_{50}
Crude extract	9	9	189.7
Hexane extract	13	11	782.1
Ethyl Acetate Extract	10	9	64.6
Methanol Extract	16	12	106.9

Table 3. Inhibition zone and EC_{50} of stems extracts

Samples	*S. aureus* (mm)	*E. coli* (mm)	EC_{50}
Crude extract	11	12	189.2
Hexane extract	7	9	77.7
Ethyl Acetate Extract	6	7	53.8
Methanol Extract	10	13	43.7

Table 1-3 shows the inhibition zone and EC_{50} value of leaves, fruits and stems extracts respectively. For leaves extract, methanol extract shows strongest inhibition properties against *E.coli* (10 mm) and *S.Aureas* (10 mm), followed by ethyl acetate and lastly hexane extract. For fruits extract, the order of inhibition activity for different extract is methanol>hexane>ethyl acetate, with methanol inhibition zone were 12 mm (*E. coli*) and 16 mm (*S. aureas*). For stems extract, the order of inhibition activity is methanol>hexane>ethyl acetate, with methanol inhibition zone were 13 mm (*E.coli*) and 10 mm (*S. aureas*).

Figure 1 shows the ability of *R. tomentosa* leaves, fruits and stems extract to scavenge DPPH radicals compared with the ability of ascorbic at different concentrations. Increasing the concentrations of *R. tomentosa* extracts resulted in the increase of the radical scavenging activities.

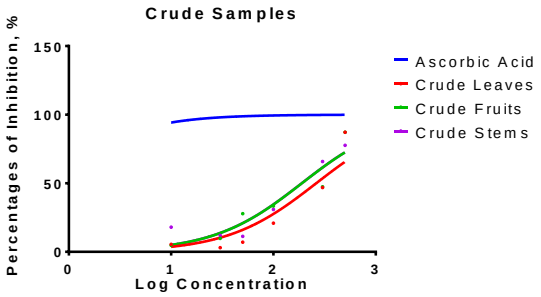


Figure 1. Antioxidant activity of Leaves, Fruits and Stems crude extract.

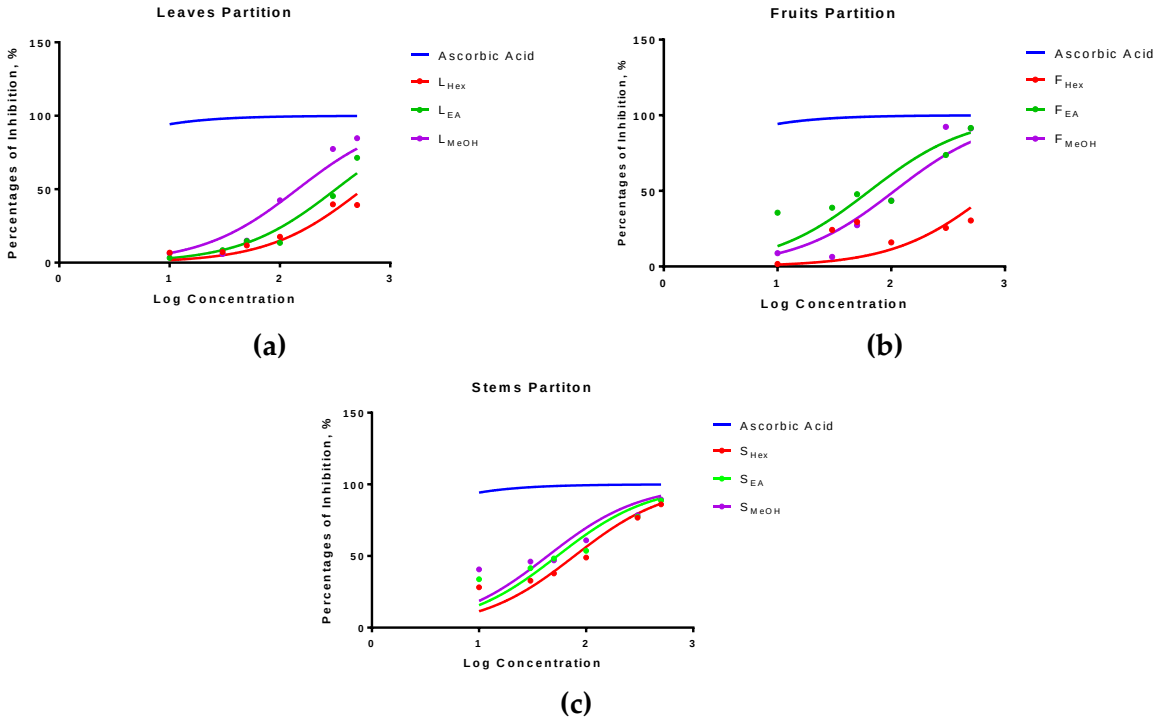


Figure 2. Antioxidant activity of **(a)** leaves, **(b)** fruits, and **(c)** stems partition extracts.

Table 4-6 shows the inhibition zone and EC_{50} value of leaves, fruits and stems fractions respectively. For leaves fractions, LM2, LM3 and LM4 show strong inhibition against *E.coli* and *S.Aureas*, as well strong DPPH scavenging activity. For fruits and stems fractions, FM2 and SM1 shows strong antimicrobial and DPPH scavenging activity.

Table 4. Inhibition zone and EC_{50} of leaves fractions

Fractions	*E. coli* (mm)	*S. aureus* (mm)	EC_{50}
1	6	6	141.20
2	8	9	28.35
3	7	7	38.40
4	7	7	98.41
5	13	15	257.70
6	15	20	1529.00
7	20	23	1482.00
8	26	28	1498.00
9	13	13	810.80
10	24	22	944.80
11	27	26	950.50
12	14	14	658.10
13	19	18	907.30

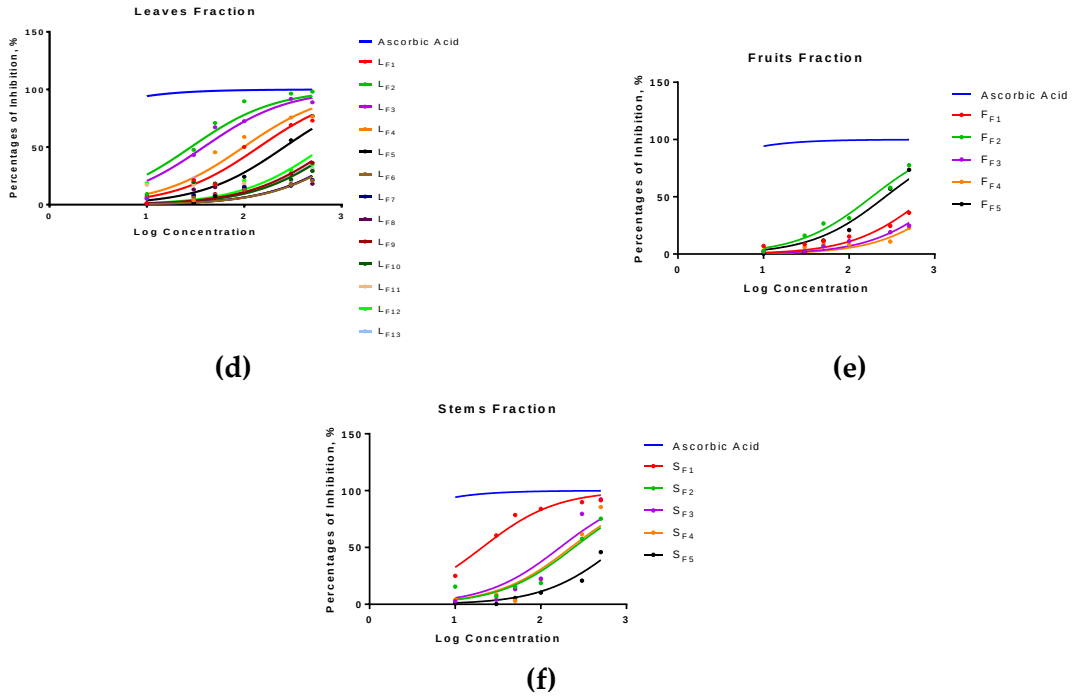
Table 5. Inhibition zone and EC_{50} of fruits fractions

Fractions	*E. coli* (mm)	*S. aureus* (mm)	EC_{50}
1	11	10	813.70
2	7	9	184.20
3	10	8	1332.00
4	11	9	1746.00
5	6	8	264.20

Table 6. Inhibition zone and EC_{50} of stems fractions

Fractions	*E. coli* (mm)	*S. aureus* (mm)	EC_{50}
1	11	8	20.69
2	11	10	243.80
3	7	10	167.80
4	11	15	223.80
5	10	10	786.60

Figure 3 shows the ability of *R. tomentosa* leaves, fruits and stems fractions to scavenge DPPH radicals. From the fractions results, the antioxidant activity are higher compare to partition extracts. Some of the fractions show strong antimicrobial activity but mild scavenging properties. The main reason was the chromatography techniques will isolate by fraction the compounds which possessed strong antioxidant and antimicrobial properties from other compounds which show mild properties. Therefore, the fractions which showed potent properties should be selected for further analysis *via* assay-guided isolation.



(d) **(e)** **(f)**

Figure 3. Antioxidant activity of **(d)** leaves, **(e)** fruits, and **(f)** stems methanol fractions.

CONCLUSION

In conclusion, *R. tomentosa* leaves, stems and fruits showed antimicrobial activity. Strong antioxidant activity were showed in LM2, LM3, LM4 in leaves, FM2 in fruits and SM1 in stems methanol extract. Further studies will be conducted on selected fractions of *R. tomentosa* leaves, stems and fruits extracts.

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REFERENCES

[1] Csurhes, S. & Hankamer, C. (2016). *Ceylon Hill Cherry (Downy Rose Myrtle): Rhodomyrtus tomentosa*. Queensland Goverment .

[2] Cui, C., Zhang, S., You, L., Ren, J., Luo, W., Chen, W. & Zhao, M. (2013). Antioxidant capacity of anthocyanins from *Rhodomyrtus tomentosa* (Ait.) and identification of the major anthocyanins. *Journal of Food Chemistry*, **139**(2013), 1-8.

[3] Díez, C., Traag, W. A., Zommer, P. & Atienza, J. (2006). Comparison of an acetonitrile extraction/partitioning and "dispersive solid phase extraction" method with classiacal multi-residue methods for the extraction of herbicide residues in barley samples. *Journal of Chromatography A*. **1131**(2006), 11-23.

[4] Garcia, E. J., Oldoni, T. L. C., Alencar, S. M., Reis, A., Loguericio, A. D. & Grande, R. H. M. (2012). Antioxidant Activity by DPPH Assay of potential Solution to be Applied on Bleached Teeth. *Brazilian Dentist Journal*, **23**(1), 22-27.

[5] Geetha, K. M., Sridhar, C. & Murugan, V. (2010). Antioxidant and Gastroprotective activities of *Rhodomyrtus tomentosa* (Ait.) Hassk. *International Journal of PharmTech Research*, **2**(1), 283-291.

[6] Jeong, D., Yang, W. S., Yang, Y., Nam, G., Kim, J. H., Yoon, D. H., Noh, H. J., Lee, S., Kim, T. W., Sung, G. & Cho, J. Y. (2013). *In vitro* and *in vivo* anti-inflammatory effect of *Rhodomyrtus tomentosa* methanol extract. *Journal of Ethnopharmacology*, **146**(2013), 205-213.

[7] Lai, T. N. H., André, C., Rogez, H., Mignolet, E., Nguyen, T. B. T. & Larondelle, Y. (2014). Nutritional composition and antioxidant properties of the simfruit (*Rhodomyrtus tomentosa*). *Journal of Food Chemistry*, **168**(2015), 410-426.

[8] Lai, T. N. H., Herent, M., Quentin-Leclerq, J., Nguyen, T.B. T., Rogez, H., Larondelle, Y. & André, C. M. (2012). Piceatannol, a potent bioactive stilbene, as major phenolic component in *Rhodomyrtus tomentosa*. *Journal of Food Chemistry*, **138**(2013), 1421-1430.

[9] Lavanya, G., Voravuthikunchai, S. P. & Towatana, N. H. (2012). Acetone Extract from *Rhodomyrtus tomentosa*: A Potent Natural Antioxidant. *Evidence-Based Complementary and Alternative Medicine*. 10.1155/**2012**/535479.

[10] Limsuwan, S., Kayser, O. & Voravuthikunchai, S. P. (2012). Antibacterial Activity of *Rhodomyrtus tomentosa* (Aiton) Hassk. Leaf Extract against Clinical Isolates of *Streptococcus pyogenes*. *Evidence-Based Complementary and Alternative Medicine*, 10.1155/**2012**/697183.

[11] Maskam, M. F., Mohamad, J., Abdulla, M. A., Afzan, A. & Wasiman, I. (2014). Antioxidant activity of *Rhodomyrtus tomentosa* Fruits and Its effect on Lipid Profile in Induced-cholesterol New Zealand White Rabbits. *Sains Malaysiana*, **43**(11), 1673-1684.

[12] Mordmuang, A., Shankar, S., Chethanond, U. & Voravuthikunchai, S. P. (2015). Effect of *Rhodomyrtus tomentosa* Leaf Extract on Staphylococcal Adhesion and Invasion in Bovine Udder Epidermal Tissue Model. *Journal of Nutrients*, **2015**(7), 8503-8517.

[13] Navie, S. (2013). *Ceylon Hill Cherry (Rhodomyrtus tomentosa)*. Nerang: Technigro Australia.