Jurnal Teknologi

Full Paper

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *RUTA ANGUSTIFOLIA* EXTRACT

Nur Azfa Shuib[a], Anwar Iqbal[b], Fatimatul Akmal Sulaiman[a], Izzatie Razak[a], Deny Susanti[c*]

[a]Department of Biotechnology, Kulliyyah of Science, International Islamic University of Malaysia, Kuantan Campus, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia
[b]School of Chemical Science, Universiti Sains Malaysia, 11800 Penang, Malaysia
[c]Department of Chemistry, Kulliyyah of Science, International Islamic University of Malaysia, Kuantan Campus, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia

Article history
Received
30 June 2015
Received in revised form
9 September 2015
Accepted
15 October 2015

*Corresponding author:
deny@iium.edu.my

Abstract

Ruta angustifolia was used in this study in order to evaluate the antimicrobial activity and antioxidant properties and its correlation with the polyphenolic content. Two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778) and two Gram–negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 8739) were used to determine the antibacterial activity. Aqueous maceration extract was used for antioxidant activities and methanolic maceration extract was used for antibacterial activity. The antioxidant properties and activities were evaluated by using total phenolic content (TPC), total flavonoid content (TFC), DPPH free radical scavenging activity and beta-carotene bleaching method. Whereas, the antibacterial activity was examined using disc diffusion method against selected microorganism at concentration 1.0 mg/disc. The results showed the phenolic content of *R. angustifolia* extract was 18.89 g GAE/100 g extract while the flavonoid content was 14.170 g QE/100 g extract. *R. angustifolia* exhibited good radical scavenging with IC50 value of 2.04 mg/ml. The result for disc diffusion method showed no inhibition zone against all the strains of bacteria at 1.0 mg/disc concentration of the extract. Based on the results, it can be concluded that the *R. angustifolia* aqueous extract has the antioxidant properties and there is correlation between polyphenolic content of the extract with its antioxidant activity. However, *R. angustifolia* methanolic extract did not show any antibacterial activity.

Keywords: Ruta angustifolia, antioxidant activities, antibacterial activity

© 2015 Penerbit UTM Press. All rights reserved

1.0 INTRODUCTION

Ruta angustifolia which commonly known as Garuda or Inggu grown as herb and can be easily found in Malaysia and Indonesia. *R. angustifolia* from the Rutaceae family is believed to have some therapeutic values. Although it has been used more extensively in early times, it is not a herb that typically suits modern tastes due to its scent. Therefore, *R. angustifolia* is largely unknown to the general public for its beneficial needs [13]. According to Elia [7], in small doses the infusion of *R. angustifolia* could be rubefacient. Nevertheless, excessive local application that mostly use as rubefacient will lead to side effects because some of the active substances in this herbal plant can cause hypersensitivity. To date, there is limited study on *R. angustifolia* for its antioxidant properties. There are several studies have been done on antibacterial agents of *R. angustifolia*. Some active constituents in *R. angustifolia* have been demonstrated to have promising antibacterial activity. Thus, investigation on *R. angustifolia* was conducted to prove the feasibility of its antioxidant properties as well as the determination of the correlation between the polyphenolic content. Two selected Gram-positive bacteria (*S. aureus* and *B.*

cereus) and two Gram-negative bacteria (*P. aeruginosa* and *E. coli)* were used in this study.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The plant *R. angustifolia* were obtained fresh from the Summer Nursery, Kuantan in September 2013. The plant sample was then weighted and dried in the oven at 37 °C for 1 week. The plant was ground into fine powder and sieved before kept in closed container and stored at room temperature until further used.

2.2 Sample Extraction

The ground sample was macerated using methanol with the ratio of 1:20 at room temperature. The beaker was covered with aluminium foil and constantly shaken using rotary shaker at 100 rpm. The extraction process was carried out in the dark for 3 days. Every 3 days, the new volumes of solvent were changed until the colour of the extract become colorless. The extract solution was then filtered by using filter papers. The total volume of the extract was recorded. Then, the extract solution was concentrated to dryness under vacuum and reduced pressure using rotary evaporator at 60 °C to obtain concentrated extracts.

2.3 Antioxidant Testing

2.3.1 Total Phenolic Content

Determination of total phenolic content (TPC) of *R. angustifolia* extract was examined by using the method of Folin-Ciocalteu with some modification [1]. In a test tube, 100 µl of sample or standard stock solution was transferred followed by addition of 750 µl of Follin-Ciocalteau reagent and were mixed gently. The mixtures were allowed to stand at room temperature for 5 minutes. Next, 750 µl of 6% sodium carbonate was added to the mixture and mixed gently and allowed to stand at room temperature for 90 minutes. The absorbance values were read using UV/Vis spectrophotometer at 725 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0.02 to 0.10 mg/mL). The TPC was expressed as grams of gallic acid equivalents (GAE) per 100 gram of extract (g GAE/100 g). The tests were examined in triplicate.

2.3.2 Total Flavonoid Content

Determination of total phenolic content of *R. angustifolia* extract was examined by using method as described by Chew *et al*. [7] with slightly modifications. Sample (250 µl) was transferred to test tube followed by the addition of 1250 µl of deionized water. Then, 75 µl 5% sodium nitrite solution was added followed by 150 µl of 10% aluminium chloride solution and the mixture was mixed gently. 500 µl of sodium hydroxide was added into mixtures and followed by 275 µl of deionized water. The absorbance values were read using UV/Vis spectrophotometer at 510 nm. The standard curve for total falvonoids was made using quercetin standard solution (0.05 to 0.50 mg/ml) under the same procedure as earlier discribed. The total flavonoids were expressed as grams of quercetin equivalents per 100 gram of extract (g QE/100 g). The tests were examined in triplicate.

2.3.3 DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity was determined according to Liyana and Shahidi with some changes [12]. In a test tube, 3000 µl of 0.004% DPPH was transferred followed by addition of 100 µl of serial dilution (0.0313, 0.062.5, 0.125, 0.25, 0.5, 1.0 mg/ml) of positive control and *R. angustifolia* extract separately. The mixture was mixed gently and kept in the dark within 30 minutes at room temperature. The absorbance values were taken at 517 nm using UV/Vis spectrophotometer. The control was prepared as above without any sample. The tests were examined in triplicate. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Scavenging effect (%)
= [(control absorbance – sample absorbance) / (control absorbance] x 100

2.3.4 Beta-Carotene Bleaching

Beta-carotene bleaching method was performed according to Othman *et al.* [19] with some modification. The positive controls used were ascorbic acid and BHT. 200 ml chloroform was dissolved with 20 mg/ml of beta-carotene and then mixed with 200 µl of Tween 20 as well as 20 µl of linoleic acid. At 40 °C, the chloroform was removed by using a rotary evaporator. Next, distilled water (50 ml) was added into the mixture and then shaken in vigorous to form emulsion. 4000 µl of emulsion was then transferred into the test tube. Then, 100 µl of ascorbic acid, BHT and *R. angustifolia* extract was added into the emulsion separately. The test tubes were placed in a water bath at 50 °C. The absorbance values were read at 470 nm using UV/Vis spectrophotometer at initial time and every 20 minutes intervals for 120 minutes. All determinations were performed in triplicate. The antioxidant activity was calculated using formulation:

Antioxidant activity (%AOA)
= [1- (A0 – At) / (A °0 -A°t)] x 100

A0 = Measurement of absorbance at 0 minute for sample
A°0 = Measurement of absorbance at 0 minute of time for control
At = Measurement of absorbance value at specific minute of time for standard or sample
A°t = Measurement of absorbance value at specific minute of time for control

2.5 Antibacterial Testing

2.5.1 Disc Diffusion Method

Disc diffusion method was done according to Shryock *et al.* [20] with some changes. The blank discs were filled with concentration of 1.0 mg/disc of extracts. The test for each sample was done in triplicate. Then, the plates were incubated at 37 °C or 24 hours. The diameter of inhibition zone around each disc was then measured. The antibacterial activity was determined by the diameter of inhibition zones around the disc on the agar surface.

2.6 Statistical Analysis

All the antioxidant tests were done in triplicate and data were analyzed by using Microsoft Office Excel 2007.

3.0 RESULTS AND DISCUSSION

3.1 Total Phenolic Content

The total phenolic content of *R. angustifolia* extract was 18.89 g GAE/100 g of extract. The formation of blue intensity indicates the presence of phenolic compounds. Dejian *et al.* [3] expressed that Folin-Ciocalteu's reagent can oxidize phenolic compounds thus reducing the chromogens to a blue complex that can be quantified by visible-light spectrophotometry. *R. angustifolia* extract also presented weak blue colour intensity as compared to the gallic acid. The previous study done by Ekiert *et al.* [6] showed that, HPLC detection of *R. graveolens,* which was the same genus exhibit high free phenolic acid and furanocoumarin content. Thus, the low phenolic content of *R. angustifolia* might be due to the low total content of phenolic acid metabolites that were depend on the species variants.

3.2 Total Flavonoid Content

The total flavonoid content of aqueous extract of *R. angustifolia* was 14.17 g QE/100 g of extract. It has been reported that flavonoid contains hydroxyl group that are responsible for radical scavenging effect of most plants. Harborne [9] emphasized that plants give yellow to orange colours as the markers of flavonoid content. Kanes *et al.* [11] reported that, flavonoid compound one of the main compositions of Rutaceae species. Thus, there was contradiction with the finding of flavonoid content in *R. angustifolia*. Nauman *et al.* [17] further supported contains high amount of flavonoid compared to *R. angustifolia.* The low content of flavonoid content of *R. angustifolia* might be due to the type of species variants.

3.3 DPPH Free Radical Scavenging Activity

The antioxidant activity of the extract *R. angustifolia* was determined based on the IC_{50} values and scavenging effect at the lowest concentration (0.0312 mg/ml) for the positive control and extract. Figure 1 shows the scavenging effect for the positive control and extract. Based on the results in Table 1, ascorbic acid exhibited the higher scavenging effect with the value of IC_{50} value of 0.22 mg/ml compared to the *R. angustifolia* extract, which was 2.04 mg/ml.

Table 1 IC_{50} values and percentage inhibition of DPPH scavenging activity

Sample	IC_{50} (mg/ml)	% Inhibition at 0.0313 mg/mL
Ascorbic acid	0.22	23.29
***R. angustifolia* extract**	2.04	23.75

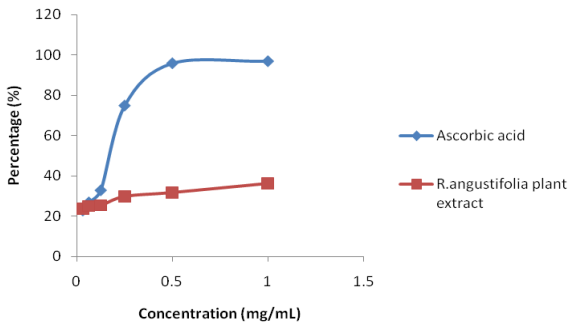

Figure 1 Scavenging effect of *R. angustifolia* extract and ascorbic acid (control)

3.4 Beta Carotene Bleaching Assay Activity

Figure 2 shows the absorbance values of antioxidant activity (AOA) for the extract, BHT, and control at 470 nm. Figure 3 shows the bleaching of beta-carotene for the extract and BHT. The findings in Table 2 shows the ascorbic acid possessed the highest antioxidant activity as a whole followed by BHT and extract. Antioxidant activity (%AOA) of ascorbic acid, BHT and extract of *R. angustifolia* were 83.11, 45.86, 26.38%,

respectively, after 60 minutes incubation, which signify the maximal antioxidant activity.

Based on the results shows in Table 2, *R. angustifolia* recorded the lowest antioxidant activity after 60 minutes incubation, which was 26.38% when compared to BHT and ascorbic acid. Antioxidant agent in plant extract bleached the yellow colour of beta-carotene gradually and retained its activity starting on the 120th of incubation time.

According to Jayaprakasha *et al.* [10] the extent of beta-carotene bleaching was hindered by the existence of antioxidant agent, which neutralizes the free radical that was generated in the system. Ascorbic acid was relatively more polar and acts as the hydrophilic antioxidant, while BHT was known as the lipophilic antioxidant. Based on the result in Figure 3, ascorbic acid showed the highest antioxidant activity for beta-carotene bleaching assay followed by BHT and *R. angustifolia* extract. It can be deduced that, *R. angustifolia* can act as the hydrophilic antioxidant well than the lipophilic antioxidant due to solubility reactions.

Table 2 Antioxidant activity of beta carotene bleaching assay within incubation time

	Antioxidant activity (%AOA)		
	Min-20	**Min-60**	**Min-120**
BHT	78.67%	45.86	40.78
Ascorbic	87.70%	83.11	78.00
***R. angustifolia* extract**	54.71%	26.38	22.09

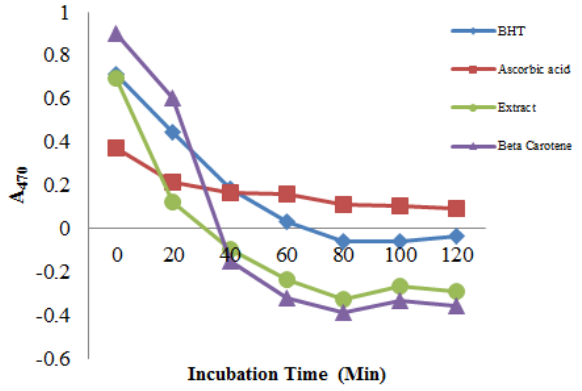

Figure 2 The antioxidant activity of *R. angustifolia* (extract), BHT, ascorbic acid and beta carotene (negative control)

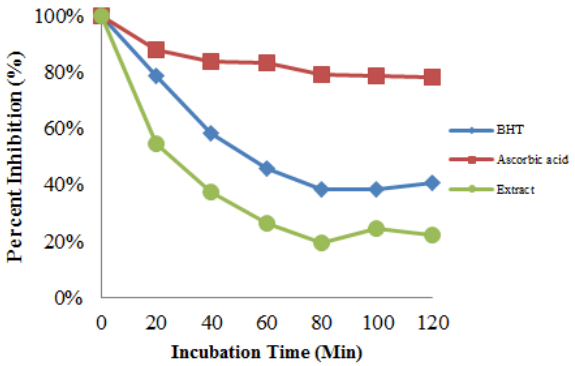

Figure 3 Beta-carotene bleaching of *R. angustifolia*(extract), BHT and ascorbic acid activity

3.5 Correlation between TPC and TFC with Antioxidant Activities

Figure 4 shows the correlation between total phenolic and total flavonoid contents with scavenging effect and antioxidant activity. The findings show that, TPC and TFC of *R. angustifolia* extract had a high positive correlation of antioxidant activity with DPPH as compared to the beta-carotene. Besides, total phenolic content contributed more to *R. angustifolia* antioxidant activities. Barros *et al.* [2] explained, the antioxidant activity in the plant has the relationship between beta-carotene bleaching assay and the phenolic compound.

According to Diwan *et al* [4], there should be correlation between total phenolic content and antioxidant activity. The results proved that, phenolic content of *R. angustifolia* contributed to the scavenging effect. A study done by Diwan *et al.* [4] stated that, the *in vitro* cultures of *R. graveolens* showed strong scavenging effect attributed to the flavonoid and phenolic content. *R. angustifolia* has the potential to act as hydrophilically antioxidant due to same family species with *R. graveolens.*

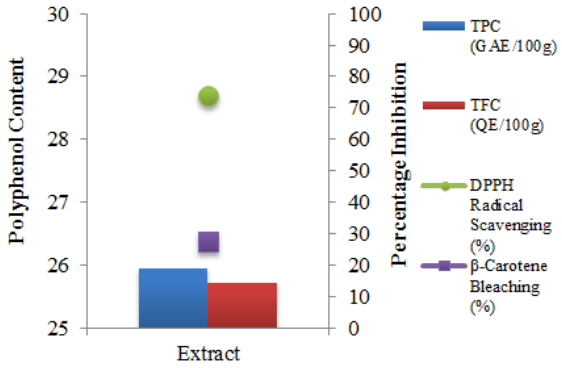

Figure 4 Correlation between TPC and TFC with antioxidant activity

3.6 Disc Diffusion Method

R. angustifolia methanolic extract showed no inhibition zone against four selected bacteria at concentration 1.0 mg/disc. This result supported by Farah *et al*. [8] and the result was probably due to the abundance of ketones, which is the mainly active constituents that hindered the antibacterial activity. Contradictly, Mohd Hassan *et al.* [14] stated that, alkaloid of *R. angustifolia* extract showed antibacterial activity against all the four identical bacteria with MIC ranged between 62.5 and 1000 µg/ml. Furthermore, Mohd Kamal *et al.* [15] proved that, MIC of *R. angustifolia* ranged between 0.01 and 0.025 mg/ml. However, both studies have used different methods of extraction and solvent. The maceration with absolute methanol might conceivably unable to extract larger concentration of active constituents from this plant.

4.0 CONCLUSION

The natural antioxidant of *R. angustifolia* has positive correlation with antioxidant activity of DPPH free radical scavenging activity compared to beta-carotene bleaching assay. *R. angustifolia* has the potential as the alternative antioxidant with respect to its hydrophilic properties. However, *R. angustifolia* did not exhibit any antibacterial activity against tested *organisms.* Thus, further studies on *R. angustifolia* have to be performed in order to evaluate the antibacterial activity.

Acknowledgement

We would like to thank Kulliyyah of Science, International Islamic University Malaysia for funding this research.

References

[1] Azim, S. H., Hassan, A. M., and Abdel-Wahhab, M. A. 2011. Dietary Supplementation with Whey Protein and Ginseng Extract Counteracts the Oxidative Stress and DNA Damage in Rats Fed Aflatoxins-Contaminated Diet. *Journal of Mutation Research*. 723: 65-71.

[2] Barros, L., Baptista, P., and Ferreira, R. 2007. Effect of Lactarius piperatus Fruiting Body Maturity Stage on Antioxidant Activity Measured by Several Biochemical Assays. *Journal of Food Chemistry.* 45: 1731-1737.

[3] Chew, K. K., Ng, S. Y., Thoo, Y. Y., Khoo, M. Z., Wan Aida, W. M. And Ho, C. W. 2011. Effect of Ethanol Concentration, Extraction Time and Extraction Temperature on the Recovery of Phenolic Compound and Antioxidant Capacity of *Centella asiatica* Extracts. *International Food Research Journal.* 18: 571-578.

[4] Dejian, H., Boxin, O & Ronald, R. P. 2005. The Chemistry Behind Antioxidant Capacity Assays. *Journal Agricultural Food Chemical.* 53: 1841-1856.

[5] Diwan, R., Shinde, A., Malpathak, N. 2012. Phytochemical Composition and Antioxidant Potential of *Ruta Graveolens L*. in-vitro Culture Lines. *Journal of Botany.* 12: 6.

[6] Ekiert, H., Szewczyk, A., and Kus, A. 2009. Free Phenolic Acids in *Ruta graveolens* L. In Vitro Culture. *Journal of Pharmacy*. 64(10): 694.

[7] Elia, S. M. 2003. Rue *(Ruta* L., Rutaceae) in Traditional Spain: Frequency and Distribution of Its Medicinal and Symbolic Applications. *Journal of Economic Botany*. 57(2): 231-244.

[8] Farah, H., Tarik, M. C., Yosr, Z., Riadh, K., Amina, A., Abdelhafid, B. 2013. Chemical Composition and Antimicrobial Activity of the Essential Oils From Four *Ruta* Species Growing in Algeria. *Journal of Food Chemistry*. 141(1), 1: 253-258.

[9] Harborne, J. B. 1994. *The Flavonoids: Advances in Research since 1986.* Chapman & Hall. London, UK.

[10] Jayaprakasha, G. K., Singh, R. P., and Sakariah, K. K. 2001. Antioxidant Activity of Grape Seed (*Vitis Vinifera*) Extracts on Peroxidation Models in-vitro. *Journal of Food Chemistry.* 73: 285-290.

[11] Kanes, K., Tisserat, B., Berhow, M., and Vandercook, C. 1993. Phenolic Composition in Various Tissues of Rutaceae species. *Journal of Phytochemistry*. 32: 967-974.

[12] Liyana, C. M., and Shahidi, F. 2005. Antioxidant Activity of Commercial Soft and Hard Wheat (*Triticum Aestivum* L) as Affected by Gastric ph Conditions. *Journal of Agricultural Food Chemistry.* 53: 2433-2440.

[13] Maskolis. 2010. Inggu, the Medical Plant Efficacious. Retrieved May 29, 2010, from http://natudays.blogspot.com/2012/02/rue.html.

[14] Mohd Hassan, Norazian and Abdol Latif, Fatimah and Tuan Aziz, Wan Hazni and Mohd Noor, Siti Sarah and Mohd Kamal, Laina Zarisa and Darnis, Deny Susanti. 2012. Antimicrobial Activity of Extracts and Alkaloids from Callus and Shoot Cultures of *Ruta angustifolia (L.) Pers.* In: *International Seminar on Natural Product Medicines 2012: Current Issues on Future Researchers and Applications of Natural Product Medicines, 22-23 Nov 2012, Bandung, Indonesia.*

[15] Mohd Kamal, Laina Zarisa and Mohd Hassan, Norazian and Md Taib, Nurhaya and May Khin, Soe. 2010. Bioassay-guided Isolation of Antimicrobial Active Alkaloids from *Ruta angustifolia (L.) Pers.* In: *3rd International Conference on Biotechnology for the Wellness Industry & Exhibition, 8-9 October 2010, Putra World Trade Centre, Kuala Lumpur, Malaysia.*

[16] Mukherjee, A., Basu, S., Sarkar, N., and Ghosh, A. 2001. Advances in Cancer Therapy with Plant Based Natural Products. *Journal of Current Medicinal Chemistry*. 8: 1467-1486.

[17] Nauman, I. T., Hamad, M. N., and Hussain, S. A. 2012. Comparative Study of the Analgesic Activity of Two Iraqi Medicinal Plants, *Ruta graveolens* and *Matricaria chamomilla* extracts. *Journal of Intercultural Ethnopharmacology*. 1(2): 79-83.

[18] Nucbe, N. S., Afolayan, A. J., and Okoh, A. I. 2008. Assessment Techniques of Antimicrobial Properties of Natural Compounds oant Origin: Current Methods and Future Trends. *Journal of Biotechnology*. 7(12): 1797-1806.

[19] Othman, A., Amin, I., Nawalyah, A. G. and Ilham, A. 2007. Antioxidant Capacity and Phenolic Content of Cocoa Beans. *Journal of Food Chemistry*. 100: 1523-1530.

[20] Shryock, T. R., Apley, M., Jones, R. N., Lein, D. N., Thornsberry, C., Walker, R. D., Watts, J. L., White, D. G., and Wu, C. C. 2002. *NCCLS. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard*– Second Edition. USA: NCCLS.

[21] Singh, J. 2012. Maceration, Percolation And Infusion Techniques of Extraction of Extraction of Medicinal and Aromatic Plants. Central Institute of Medicinal and Aromatic Plants. Lucknow, India.