ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF RUTA ANGUSTIFOLIA EXTRACT

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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

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 Folin-Ciocalteu 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 flavonoids was made using quercetin standard solution (0.05 to 0.50 mg/ml) under the same procedure as earlier described. 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, 0.0625, 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 (%) = \left( \dfrac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \right) \times 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) = \left[1 - \frac{A_0 - A_t}{A_0^0 - A_0^t}\right] \times 100

A_0 = Measurement of absorbance at 0 minute for sample
A_0^0 = Measurement of absorbance at 0 minute of time for control
A_t = Measurement of absorbance value at specific minute of time for standard or sample
A_0^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 IC50 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 IC50 value of 0.22 mg/ml compared to the R. angustifolia extract, which was 2.04 mg/ml.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (mg/ml)</th>
<th>% Inhibition at 0.0312 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>0.22</td>
<td>23.29</td>
</tr>
<tr>
<td>R. angustifolia</td>
<td>2.04</td>
<td>23.75</td>
</tr>
<tr>
<td>extract</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Figure 1](image1.png) 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 shown 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

<table>
<thead>
<tr>
<th>Antioxidant activity (%AOA)</th>
<th>Min-20</th>
<th>Min-60</th>
<th>Min-120</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>78.67%</td>
<td>45.86%</td>
<td>40.78%</td>
</tr>
<tr>
<td>Ascorbic</td>
<td>87.70%</td>
<td>83.11%</td>
<td>78.00%</td>
</tr>
<tr>
<td>R. angustifolia extract</td>
<td>54.71%</td>
<td>26.38%</td>
<td>22.09%</td>
</tr>
</tbody>
</table>

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 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

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 main active constituents that hindered the antibacterial activity. Contrarily, 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

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References


