Antioxidant Activity Of Four Selected Herbal Plants Used In Herbal Rice Preparation By Siamese Community Of Pasir Puteh, Kelantan, Malaysia

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ABSTRACT

Herbal plants are widely used as alternative medicine and daily dietary food in many communities by means of consuming ‘herbal rice’ cooked with herbal plants. Historically, consuming herbal rice is regarded as the best practice that has nutritional and medicinal values. It is traditionally reported to have treated fever cough, stomach pain, diarrhoea, control blood sugar level and blood pressure. Herbal rice is a traditional cuisine among the Siamese community of Pasir Putih, Kelantan, Malaysia, in which the plants such as Clitoria ternatea, Cassia alata L., Citrus hystrix L. and Morinda citrifolia are used as some of its main ingredients. The present study aimed at determining the total phenolic and flavonoid content and antioxidant capacity of aqueous extracts prepared from the selected plants used in herbal rice preparation. The TPC of the prepared extracts was determined through Folin-Ciocalteu colorimetric method and TFC via aluminium chloride colorimetric method. The antioxidant activity of the plant extracts was studied via DPPH (2, 2'-diphenyl-1-picrylhydrazyl hydrate) assay and Ferric Reducing/Antioxidant Power (FRAP) assay. The TPC value was from 3.993 to 130.035 mg GAE/g dry extract and flavonoid content was from 60.67 to 187.33 mg QE/g dry extract. The antioxidant activity exhibited was 19.46 % to 80.77% inhibition with 68.27 -picromol Fe(II)/g dry extract. C. hystrix had high phenolic content, while, M. citrifolia showed high flavonoid content with ferric reducing potential whereas C. alata had high DPPH-scavenging activity.

KEYWORDS: herbal rice, antioxidant activity, phenolic content, aqueous extract, Siamese community, medicinal value

INTRODUCTION

Fresh herbs are largely consumed by many communities in Malaysia. Among them, Siamese community of Pasir Puteh, Kelantan widely consume herbal plants as a part of their dietary practice i.e., in the form of herbal rice. From various herbal plants used by the community, four have been selected for this study. Clitoria ternatea, Senna @ Cassia alata L., Citrus hystrix L. and Morinda citrifolia are commonly cooked with rice and consumed as ‘herbal rice’ within the community. These four plants were extensively investigated by various researches for their wide array of potential uses, mostly in medical field [46,44,28,47,50,51]. A number of traditional uses of these selected plants have been studied in many countries [27,26,9,1]. The need to study such benefits gained from natural food sources, is due to the existence of numerous diseases and illnesses which altered the mankind’s livelihood. At present, a large portion of the society is burdened due to ever-increasing needs for finding medication to cure different diseases encountered. In Malaysia, a large portion of the population is affected by various diseases and disorders such as diabetes, high blood pressure, high cholesterol,
obesity, cardiovascular disease, various forms of cancers, malnutrition etc. [16]. Amid multiple reasons for the mentioned diseases, improper nutrient consumption with insufficient antioxidants were identified as the major contributors to various diseases [35,32,36].

Antioxidants are molecules, either natural or synthetic, that helps in controlling oxidising molecules within the body and maintain the balance between Reactive Oxygen Species (ROS) and antioxidants [41,42,5]. The naturally-found antioxidants are non-enzymatic antioxidants such as Vitamin A, C & E, polyphenols, lycopene, ubiquitin, thiols, melatonin, etc. [35,6]. Although there are various synthetic antioxidants available, their effect within our biological systems, in a long run, has been the major concern for consumers [42,23]. Among the naturally-available antioxidant compounds, ascorbic acid, phenols and flavonoids of plant origin are of greater interest among numerous research groups. The focus into these set of compounds and further research shed a new light suggesting that phenols and flavonoids content are highly associated with the antioxidant capacity of a plant [7,33,12,19,15]. Many village communities consume herbal plants to stay healthy in which the antioxidants, phenolic and flavonoid compounds are found to be highly enriched. Thus, the present study focuses on finding the phenolic content and free radical scavenging potential of the selected four plants consumed by Siamese community of Pasir Puteh, Kelantan. This study will provide additional information to consumers in terms of antioxidant content in the selected plants and this information can be exploited in future to commercialise the nutritional benefits of these plants.

MATERIALS AND METHODS

Fresh samples were collected from Kampung Pasir Puteh, Kelantan, Malaysia. Samples include bunga telang (Clitoria ternatea), Gelenggang / Chum Hat in local dialect (Cassia glata L.), limau purut (Citrus hystrix L.) and noni (Morinda citrifolia). Samples were first washed with distilled water to remove debris and dirt. Approximately 50 g of the plant parts (leaf/ stem/ root/ rhizome) were cut into smaller pieces and extracted in distilled water using water bath at 70 °C for 2 hours. Extraction was done using method described elsewhere with slight modification [30]. The obtained extract were then filtered and concentrated. The concentrated extract was then placed in an oven at 40 °C until the extract dried. Upon drying the extract were stored in cool and dry place till further use.

Total phenolic contents of the extract were determined using FC assay with slight modification, using gallic acid as a standard (0.025 to 0.5 mg/ml) [45]. Reaction mixture was prepared by mixing 0.5 ml aqueous extract (100 µg/ml), 2.5 ml (10%) of Folin-Ciocalteu reagent and 2.5 ml (7.5%) of sodium carbonate. After incubating for 90 min at room temperature, the absorbance was determined spectrophotometrically at 765 nm. Gallic acid was used as standard and the TPC was expressed as mg GAE/g dry extract.

Total flavonoid content was determined according to the colorimetric assay [4]. One ml of aqueous plant extract (250 µg/ml) was mixed with 4 ml of distilled water. At zero time, 0.3 ml (5 % w/v) NaNO₂ was added. After 5 min, 0.3 ml (10 % w/v) AlCl₃ was added. At 6 min, 2 ml (1 M) NaOH solution was added. The volume was made up to 10 ml by adding distilled water and the mixture were shaken vigorously. The absorbance was then measured at 510 nm. Quercetin was used as a standard at various concentration (20, 40, 60, 80 and 100 mg/L, r² = 0.9816). The TFC was expressed as mg QE/g dry extract.

Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was carried out spectrophotometrically, described elsewhere with slight modification [45]. Briefly, 0.75 ml of 1 mM DPPH was reacted with 0.75 ml of 100 µg/ml extract. The sample mixture was then kept in dark for 30 min at room temperature. Ascorbic acid and butylated hydroxytoluene (BHT) were used as reference. The decrease in absorption was then measured at 517 nm. Radical scavenging activity was then calculated using the following equation:

\[
\text{% inhibition} = 100 - \left( \frac{(AB - AA)}{AB} \right) \times 100
\]

where, AB= Absorption of blank sample, AA= Absorption of tested extract solution at (t=30min).

FRAP assay was conducted as described by Benzie and Strain [8]. Briefly, 40 µl (100 µg/ml) plants extract was mixed with 3 ml of FRAP reagent. The mixture was incubated at 37 °C for 4 min and the absorbance was measured at 593 nm against blank prepared using distilled water and incubated for 1 hour instead of 4 min. FRAP reagent should be pre-warmed at 37 °C and should always be freshly prepared by mixing 2.5 ml of a 10 mM 2, 4, 6-tris(1-pyrindyl)-s-triazine (TPTZ) solution in 40 mM HCl with 2.5 ml of 20 mM FeCl₃, 6H₂O and 25 ml of 0.3 M acetate buffer, pH 3.6. Calibration curve was prepared, using an aqueous solution of ferrous sulphate FeSO₄. 7H₂O at various concentration (200, 400, 600, 800 and 1000 µM, r² = 0.9916). FRAP values were expressed as µmol Fe (II)/g dry extract.

All readings were measured in triplicate and were presented as mean ± standard deviation. Correlation analysis was done using Microsoft Excel software.
RESULTS AND DISCUSSION

Total Phenolic Content (TPC):

The Total Phenolic Content, in theory, represents all of its sub-components such as simple phenols, coumarins, tannins, phenolic acids, flavonoids, etc. [24]. When the plants were analysed for their phenolic content, a majority of the phenols present in the plant could be estimated through phenolic content assay. However, possibilities do exist that the estimation does not cover all the phenols present in it. This could be due to the fact that phenolic compounds exist either as free or bound phenols. Phenolic compounds, which are bound or exist as complexes with compounds such as proteins, carbohydrates, etc., require selective extraction method to hydrolyse it, before it could be estimated through phenolic content assay [24,17]. Thus, in the present study, the values observed during phenolic estimation are for free phenols present within the plant extract, because the extract was not subjected to either acid or alkaline hydrolysis, to hydrolyse the bound phenols. A large portion of this calculated phenolic content can possibly be contributed by the flavonoids present in the extract. This is because the TPC and TFC values were found to be almost similar to each other (Table 1).

Table 1: TPC and TFC of the four selected plant extracts

<table>
<thead>
<tr>
<th>Plant</th>
<th>TPC (mg GAE/g dry extract)</th>
<th>TFC (mg QE/g dry extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. alata L. (leaves)</td>
<td>71.01 ±12.10</td>
<td>94.00 ± 16.33</td>
</tr>
<tr>
<td>C. hystrix L. (leaves)</td>
<td>130.04 ± 12.10</td>
<td>120.67 ± 24.94</td>
</tr>
<tr>
<td>C. ternatea (flowers)</td>
<td>72.40 ± 4.74</td>
<td>60.67 ± 24.94</td>
</tr>
<tr>
<td>M. citrifolia (leaves)</td>
<td>61.63 ± 5.47</td>
<td>187.33 ± 9.43</td>
</tr>
</tbody>
</table>

The table 1 shows the Total Phenolic Content (TPC) of four plant extracts analysed via Folin-Ciocalteu colorimetry. The TPC of the four plant extracts ranged from 61.63 ± 5.47 to 130.04 ± 12.10 mg GAE/g dry extract. The highest TPC was observed in C. hystrix extract (130.04 ± 12.10 mg GAE/g dry extract). C. ternatea, C. alata and M. citrifolia had moderate level of TPC compared to C. hystrix, with values of 72.40 ± 4.74, 71.01 ± 12.10 and 61.63 ± 5.47 mg GAE/g dry extract, respectively. Overall, M. citrifolia had the least TPC (i.e., 61.63 ± 5.47 mg GAE/g) among the plant extracts analysed. C. hystrix showed highest TPC and almost similar TPC was also observed by Jamilah et al., [22]. In contrast to C. hystrix, M. citrifolia was found to contain least amount of TPC among the four samples tested. It was three times less compared to the findings of Serafini et al., [41] in which the results reported 198.8 mg GAE/g of extract. The low value of TPC found in M. citrifolia in the present study may be attributed to different extraction and drying method [41]. The longer extraction time and oven drying process might have resulted in low TPC values in M. citrifolia [21]. C. ternatea showed higher TPC (72.40 ± 4.74 mg GAE/g) when extracted at 70°C when compared with extraction at room temperature (20.7 ± 0.1 mg GAE/g) as observed by Rabeta and An Nabil [34]. This could be again explained due to the difference in extraction procedure, extraction parameters or geographical location etc [3,40]. Further, Ruenroengklin et al., [39] reported that TPC increases in litchi fruit pericarp with increase in extraction temperature from 30°C to 80°C.

Total Flavonoid Content (TFC):

Flavonoids are one of the major classes of phenolic group in plants. Flavonoids exist both as free-moving and bound flavonoids. As a common lab practice, bound flavonoids are subjected to hydrolysis to convert flavonoid glycoside into aglycone [24]. However, in the present study, the estimated TFC was also based on free flavonoids present in the plant extract. The total Flavonoid Content was determined for the selected four herbal plants and the content ranged from 60.67 to 187.33 mg QE/g dry extract (Table 1).

The highest TFC was observed in M. citrifolia followed by C. hystrix, C. alata and C. ternatea with values, 187.33 ± 9.43, 120.67 ± 24.94, 94.00 ± 16.33 and 60.67 ± 24.94 mg QE/g dry extract, respectively. The aqueous extract of C. ternatea showed similar TFC value (60.67 mg QE/g) with methanolic flower extract (67.2 mg QE/g dry flower extract) as reported by Laksmi et al., [25]. This shows that the aqueous and methanolic extract possess similar TFC [11]. The TFC of C. alata was comparatively very high (94.00 mg QE/g), while Devendra et al., [11] obtained only 4.25 mg QE/100 g of extract. This could be justified by the geographical differences, climate and soil composition of both study sites. Both locations would have different effects upon the growth and nutritional content level of plant [3,40]. Aqueous C. hystrix extract exhibited considerably a large amount of TFC (120.67 mg GE/g). However, Fidianny et al., [14] recorded a mere 3.0 g QE/100 g of ethanolic extract. Rohman et al., [38] studied ethyl acetate extract of M. citrifolia fruit and obtained 1.19 g QE/100 g dry material. It was exceptionally low with respect to the aqueous M. citrifolia leaves extract containing 187.33 mg QE/g of TFC. As per the trend analysis of TFC present in aqueous extracts of four selected plants and in comparison with previous studies, it can be inferred that aqueous extract provide better TFC. Thus, it can be concluded that common water extraction provide generous amount of flavonoid compounds to herbal plant consumers.
DPPH free radical-scavenging assay:

The antioxidant activities of the four plant extracts were determined using DPPH radical, a stable organic nitrogen radical. The extracts were tested to measure its ability to scavenge DPPH free radical in the specified time period. The assay was conducted via UV-Vis Spectrophotometer to detect the colour change which indicates the amount of free radical scavenged by the extract. In the assay conducted, C. alata showed highest percentage of inhibition i.e., 80.77 %, followed by C. ternatea and C. hystrix with 38.56 % and 30.47% of inhibition respectively. M. citrifolia recorded the lowest amount of DPPH free radical-scavenging activity i.e., 19.46 % inhibition as given in table 2. C. alata has been reported to contain rich antioxidant compounds resulting in high free radical inhibition capacity [9].

The scavenging potential of each extract was compared with reference to the scavenging capacity of standard Ascorbic Acid (AA) and Butylated Hydroxyl Toluene (BHT). C. alata revealed highest antioxidant capacity with 250.877 mg AAE/g and 3518.72 mg BHTE/g dry extract (Figure 2). Subsequently, C. ternatea, C. hystrix and M. citrifolia recorded 140.035, 101.844 and 68.266 mg AAE/g dry extract, respectively. With respect to BHT, C. ternatea, C. hystrix and M. citrifolia showed 1540.95, 1167.46 and 632.70 mg BHTE/g dry extract, respectively.

<table>
<thead>
<tr>
<th>Plant</th>
<th>(% DPPH inhibition)</th>
<th>AA Equivalent (mg AAE/g dry extract)</th>
<th>BHT Equivalent (mg BHTE/g dry extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. alata (leaves)</td>
<td>80.77 ± 0.43</td>
<td>1881.58 ± 9.50</td>
<td>3518.72 ± 19.91</td>
</tr>
<tr>
<td>C. hystrix (leaves)</td>
<td>30.74 ± 0.46</td>
<td>763.83 ± 10.26</td>
<td>1167.46 ± 21.52</td>
</tr>
<tr>
<td>C. ternatea (flowers)</td>
<td>38.56 ± 2.18</td>
<td>938.74 ± 48.70</td>
<td>1540.95 ± 102.06</td>
</tr>
<tr>
<td>M. citrifolia (leaves)</td>
<td>19.46 ± 0.08</td>
<td>512.00 ± 1.75</td>
<td>632.70 ± 3.67</td>
</tr>
</tbody>
</table>

The scavenging potential of each extract was compared with reference to the scavenging capacity of standard Ascorbic Acid (AA) and Butylated Hydroxyl Toluene (BHT). C. alata revealed highest antioxidant capacity with 250.877 mg AAE/g and 3518.72 mg BHTE/g dry extract (Figure 2). Subsequently, C. ternatea, C. hystrix and M. citrifolia recorded 140.035, 101.844 and 68.266 mg AAE/g dry extract, respectively. With respect to BHT, C. ternatea, C. hystrix and M. citrifolia showed 1540.95, 1167.46 and 632.70 mg BHTE/g dry extract, respectively.

Fig. 1: Ascorbic Acid Equivalent (AAE) and Butylated Hydroxyl Toluene Equivalent (BHTE) of four selected plant extracts.

Ferric Reducing/Antioxidant Power (FRAP) assay:

The table 3 shows Ferric Reducing/Antioxidant Power (FRAP) of four plant extracts ranging from 363.00 to 389.67 µmol Fe (II)/g dry extract. The FRAP values of all the four plants are closely similar indicating equal level of ferric reducing potential. The highest ferric reducing potential was observed in M. citrifolia (389.67 µmol Fe (II)/g) followed by C. ternatea extract (368.56 µmol Fe (II)/g). Meanwhile, C. alata and C. hystrix exhibited equal ferric reducing potential of 363.00 µmol Fe (II)/g dry extract.

<table>
<thead>
<tr>
<th>Plant</th>
<th>FRAP (µmol Fe (II)/g dry extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. alata (leaves)</td>
<td>363.00 ± 5.44</td>
</tr>
<tr>
<td>C. hystrix (leaves)</td>
<td>363.00 ± 7.20</td>
</tr>
<tr>
<td>C. ternatea (flowers)</td>
<td>368.56 ± 4.16</td>
</tr>
<tr>
<td>M. citrifolia (leaves)</td>
<td>389.67 ± 38.59</td>
</tr>
</tbody>
</table>
The current FRAP values of the four selected plants are very high from those reported by previous researchers [29,2,49,21]. The high ferric reducing potential observed in the current study could be the result of a healthy and pristine environment along with uninterrupted temperate environmental condition from where the present samples were collected. In a suitable geographical and environmental condition, plants can produce rich bio-active compounds in their system with high antioxidant potential [18].

![Graph showing FRAP assay of four selected plants](image)

**Fig. 2:** FRAP assay of the four selected plants

Correlation analysis was carried out to observe the relationship among TPC, TFC and antioxidant capacities of the four extracts. It was observed that there exists a negative correlation between TPC-DPPH (R = -0.214) and TPC-FRAP (R = -0.550) whereas it is positive correlation between TFC-FRAP (R = 0.786) followed by negative correlation between TFC-DPPH (R = -0.605). The phenolic content, in general, is regarded as the compounds that promote a plant’s antioxidant capacity. However the observation from the present study indicates that the antioxidant capacity shown is not only dependent on the phenolic content of plants, but in some cases, it also exists due to other bio-active compounds too [19,48,33,13]. In line with the current findings, the TFC present in the plant extracts significantly contribute to the antioxidant capacity especially the FRAP antioxidant mechanism. The antioxidant activity can also be executed through ‘mutually stimulating effect’ among the various bioactive elements and compounds present in the plant other than phenolics [31,37,10,29].

**Conclusion:**

The present findings inferred that the four plants, being consumed as a part of daily dietary intake by Siamese community of Pasir Puteh, Kelantan, contain substantial amount of antioxidant and phenolic compounds. *C. alata* has exhibited the highest antioxidant potential whereas *C. hystrix* possess the highest TPC and *M. citrifolia* is rich in TFC which further showed highest Ferric Reducing potential/Antioxidant Power among the four plant extracts analysed. It is also to be noted that the temperature used, extraction time, mechanism of extraction and drying have unintended effects on antioxidant and phenolic content. The antioxidant capacity has been found to be not significantly correlated with phenolic content and it indicates that antioxidant could be the result of a mutually-stimulating effect of numerous bioactive compounds found in the plant extract. The findings suggest that the plants used in this present study could be exploited to provide inexpensive means for rural community to acquire sufficient antioxidant and phenolic compounds from natural resources.

**ACKNOWLEDGMENTS**

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