Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs

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Abstract:

Ten water extracts from herbs were prepared by boiling in hot water for 10 min. The herbal extracts were studied for their antioxidant activity and phenolic compound contents. In this study, the extracts of Carthamus tinctorius L., Hibiscus sabdariffa L., Chrysanthemum indicum L., Aegle marmelos L. and Jubliang exhibited strong scavenging activity against DPPH radicals (> 90%), and their ferric reducing antioxidant power (FRAP) value ranged from 1140.5 to 2295.5 µmol/g. The content of phenolic compound in the extracts was determined using the Folin-Ciocalteu reagent and calculated as gallic acid equivalents (GAE). The herbal extracts that gave high phenolic compound contents (GAE > 130 mg/g) were Jubliang, A. marmelos L., C. indicum L., H. sabdariffa L., C. tinctorius L. They are related with ferric ion reduction (FRAP value) and the percentages of scavenging activity from DPPH assay. Afterwards, the seven herbal extracts (i.e. A. marmelos L., Andrographis paniculata Nees, C. indicum L., Cymbopogon citratus Stapf., H. sabdariffa L., Jubliang and Zingiber officinale Rosc.) of different phenolic compound contents and antioxidant activity were selected for antimutagenicity assay using Ames test. At the concentrations of 8.13 - 9.26 mg/plate, the antimutagenicity of Jubliang and A. paniculata Nees was strong (> 60% inhibition) while that of A. marmelos L. and C. indicum L. was moderate (40 - 60% inhibition). The present study has revealed that phenolic contents of herbal drinks, except that of A. paniculata Nees, were correlated with antimutagenic activity. Thus, herbal extracts exhibited good sources of water soluble antioxidants, phenolic compounds and antimutagens.

Key words: Antimutagenic activity, Antioxidant activity, Herbal drinks, Phenolic compounds
Introduction

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to radiation, ozone, cigarette smoking, air pollutants and industrial chemicals [1]. The free radicals are major cause of human cancer and other diseases. That the risk of diseases can be reduced by increased consumption of antioxidants which are abundant in food [2]. Herbal drinks are becoming popular especially among health-conscious consumers since these beverages are prepared from natural ingredients. Indigenous people have been using herbs, such as Hibiscus sabdariffa L., Chrysanthemum indicum L., Aegle marmelos L. for a long time as panacea drinks. Many investigators reported that some herbal drinks contain many compounds such as polyphenols, flavonoids, isoflavones, glucosinolates. Polyphenols belong to the category of natural antioxidants [3]. A wide variety of phenolic substances derived from edible plants have been reported to retain marked antioxidant and anti-inflammatory activities, which contribute to their chemopreventive potential [4,5]. Tseng et al. [6] reported that Thai herbs inhibited the mutagenesis induced by various chemicals in animal experiment. Hence, it is of importance to investigate the phenolic contents, the antioxidant activity and antimutagenicity of some water extract of herbs often consumed by Thai people, and to determine the relationships between antioxidant activity, phenolic contents and the antimutagenic activity.

Materials and Methods

Plant material

Ten commercial grinded herbs: fruits of Aegle marmelos L. and Garcinia atroviridis Griff. ex T. Anderson., dried leaves of Andrographis paniculata Nees and Schefflera leucaantha R. Vig. (Hanumaanprasankhai), dried flowers of Carthamus tinctorius L., Chrysanthemum indicum L. and Hibiscus sabdariffa L., stems of Cymbopogon citratus Stapf., rhizome of Zingiber officinale Rosc. and Jubliang (a mixture of eight herbs namely, Bombax ceiba L., Chrysanthemum morifolium, Imperata cylindrical (L) P. Beauv., Lopatherum gracile Brogn., Nelumbo nucifera Gaertn., Oroxylum indicum L., Pragmites communis Trin and Prunella vulgaris) were selected for the study. These were purchased from a health market in Bangkok.

Preparation of water extract of herbs

Each sample of 1 g was extracted with 150 ml of boiling water on a hot plate for 10 min. The extract was then filtered through a cotton mesh and filter paper Whatman no. 1. The filtrate was used directly for antioxidant assay without storage.

Antioxidant activity assay

Free radical scavenging activity (DPPH assay):

The antioxidant activity of the water extract from each herb was tested using a stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as described by Fukumoto and Mazza [7] with some modifications. The extract was allowed to react with DPPH in order to evaluate the free radical scavenging activity. The activity was monitored by a decrease in an absorbance at 520 nm. An aliquot of 22 µl of the extract or blank reagent (dimethyl sulfoxide, DMSO) or standard Trolox (0.04-1.28 mM in 80% methanol) was added to 200 µl of DPPH in 80% methanol (150 µM) in a 96-well flat-bottom microplate (Bibby Sterilin Ltd, UK). After incubation at 37 °C for 30 min, the absorbance of the solution was read in a microplate reader (Sunrise, Tecan Co., Austria) using a 520 mm filter. The radical scavenging activity was calculated as a percentage of DPPH scavenging activity using the equation: % scavenging activity = 100 × [1 - (AE/AD)], where AE is the absorbance of the DPPH solution with an extract added, and AD is the absorbance of the DPPH solution with nothing added [8].

Ferric reducing antioxidant power (FRAP) assay:

Each of 20 µl of extract or standard (ferrous sulfate) or appropriate blank reagent (DMSO) was added to each well in a 96-well microtiter plate and was
run in triplicate. FRAP reagent (150 µl), freshly prepared and warmed at 37 °C according to the procedure described by Griffin and Bhagooli [9], was added to each well. The change in absorbance at 600 nm from the initial blank was read after 8 min using a microplate reader and compared to that of a standard solution. The FRAP values of the extracts were determined from a calibration curve of ferrous sulfate solutions at concentrations of 62.5, 125, 250, 500 and 1000 µM, expressed as µM of ferric iron reduced per gram of dried sample. The antioxidant activity was measured by its ability to reduce the Fe^{3+}/ferricyanide complex by forming ferrous products. Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 600 nm. Increased absorbance at 600 nm indicates a stronger reducing power [10].

**Determination of total phenolic contents**

The total phenolic contents of water extracts were determined according to the method described by Amarowicz et al. [8], Swain and Hillis [11], Naczk and Shahidi [12] and modified the procedures of measurement by using a microplate reader. Briefly, 10 µl of each extract was transferred into a 96-well microplate containing 160 µl of distilled water. After mixing the contents, 10 µl of Folin-Ciocalteu reagent and 20 µl of a saturated sodium carbonate solution were added. The plate was mixed well and the absorbance of blue mixtures was recorded at 750 nm with microplate reader after 30 min incubation. The readings of sample and reagent blanks were subtracted from the reading of reagent with extract. The total phenolic contents were calculated as a gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions (ranging from 25 to 800 mg/ml), and expressed as mg of gallic acid per gram of dry sample. All measurements were done in triplicate.

**Antimutagenicity assay**

Seven herbal extracts (Aegle marmelos L., Andrographis paniculata Nees, Chrysanthemum indicum L., Cymbopogon citratus Stapt., Hibiscus sabdariffa L., Jubliang and Zingiber officinale Rosc.) were concentrated and used for evaluating the antimutagenic activity. The pre-incubation method of Ames test described by Yahagi et al. [13] was used to determine the antimutagenicity. One hundred microliters of the Salmonella typhimurium strain TA100 were added to the test tube containing 500 µl of 0.5 M trisodium phosphate-potassium chloride buffer and 100 µl of the selected concentrations of each extract. One hundred microliters of a direct mutagen, nitrite-treated aminopyrene that was prepared by the method described by Kangsadalampai et al. [14], was added to the test tube. The mixture was incubated at 37 °C for 20 min in a shaking water bath. Then, 2 ml of top agar with 0.5 mM L-histidine and 0.5 mM D-biotin was added to the mixture. The sample tube was mixed and quickly poured onto an agar plate. The plate was inverted and incubated at 37 °C for 48 h. Colonies of the His+ revertant were counted. The inhibitory effect of each herbal extracts on mutagenicity of the standard direct mutagen was determined as percentage of inhibition, which is calculated as follows:

\[
\text{Percentage of inhibition} = \frac{(A-B)}{(A-C)} \times 100
\]

Where A is the number of revertants per plate induced by the mutagen; B is the number of revertants per plate induced by the mutagen in the presence of the extracts; and C is the number of spontaneous revertants per plate. The inhibition of herbal extracts was considered as being strong, moderate or weak when the value is higher than 60%, 40-60% or 20-40%, respectively, and negligible effect when the value is less than 20% [15].

**Results**

The antioxidant activity and total phenolic contents vary considerably among herbs. The antioxidant activity of herbal extracts was investigated using DPPH and FRAP assays. The reduction of DPPH by antioxidants in the herbal extracts expressed as the percentage of radical scavenging activity was ranked (from highest to lowest) as follows: Carthamus tinctorius L., Jubliang, Aegle marmelos L., Chrysanthemum indicum L., Hibiscus sabdariffa L., Zingiber officinale Rosc.
Rosc., Cymbopogon citratus Stapf., Schefflera leucantha R.Vig., Andrographis paniculata Nees, Garcinia atroviridis Griff. ex T. Anderson. (Table 1). For the reducing (FRAP value) power, the extracts obtained from Jubliang, Hibiscus sabdariffa L., Chrysanthemum indicum L., Aegle marmelos L., Carthamus tinctorius L., Zingiber officinale Rosc., Cymbopogon citratus Stapf., Schefflera leucantha R. Vig., Andrographis paniculata Nees and Garcinia atroviridis Griff. ex T. Anderson had the FRAP values of 2295.5 ± 26.22, 2220 ± 39.18, 1626 ± 153.54, 1444.43 ± 61.11, 1140.5 ± 113.49, 794.31 ± 18.37, 523.36 ± 9.09, 486.92 ± 7.14 and 376.93 ± 15.79 µmol/g, respectively.

Total phenolic contents were determined using the Folin-Ciocalteu reagent and expressed as gallic acid equivalents (GAE) per gram. The total phenolic contents, calculated using the standard curve of gallic acid ($R^2 = 0.9988$), were varied from 36.76 to 283 mg of GAE per gram. The total phenolic contents among different herbal extracts were as follows: Jubliang > Aegle marmelos L. > Chrysanthemum indicum L. > Hibiscus sabdariffa L. > Carthamus tinctorius L. > Schefflera leucantha R. Vig. > Cymbopogon citratus Stapf. > Zingiber officinale Rosc. > Andrographis paniculata Nees > Garcinia atroviridis Griff. ex T. Anderson (Table 1). Overall, the highest and the lowest phenolic content were found in Jubliang and Garcinia atroviridis Griff. ex T. Anderson, respectively. Similar observation was found for their antioxidant activity.

Almost all of the extracts showed that the total phenolic contents were related to the ferric ion reduction (FRAP value) and the scavenging activity (DPPH assay). The herbal extracts with high antioxidant activity exhibited relatively high total phenolic contents. From the data, the herbal extracts can be classified into three antioxidant activity groups, as shown in Table 2.

Seven extracts from Aegle marmelos L., Andrographis paniculata Nees, Chrysanthemum indicum L., Cymbopogon citratus Stapf., Hibiscus sabdariffa L., Jubliang and Zingiber officinale Rosc. containing different phenolic contents or antioxidant activity were used for antimutagenic assay. The results showed that almost all of the extracts were not mutagenic on S. typhimurium strain TA100 (data not shown). The effect of herbal extracts on the mutagenic activity of nitrite treated 1-aminopyrene in the absence of metabolic activation is shown in Figure 1. The results revealed that the mutagenicity of nitrite treated 1-aminopyrene was inhibited to a greater or lesser extent by an addition of herbal extracts on S. typhimurium strain TA100. At the high concentrations of the extracts (8.13-9.26 mg/plate), Jubliang and Andrographis paniculata Nees showed strong antimutagenicity (> 60% inhibition) while Aegle marmelos L. showed moderate activity (40-60% inhibition). At low and moderate concentrations, almost all of the extracts had weak antimutagenicity (20-40% inhibition) except for the extract of Hibiscus sabdariffa L. (at moderate concentration) showing moderate activity. Interestingly, the lowest concentration of the extract from Hibiscus sabdariffa L. showed nearly moderate antimutagenicity (39% inhibition). Although, Andrographis paniculata Nees had low total phenolic contents and antioxidant properties, it showed high antimutagenicity. From the data, the phenolic contents did not always correspond with the antimutagenicity activity.

**Discussion**

In this study, ten herbs were extracted with hot water, suitable for the evaluation of antioxidant activity and total phenolic contents of actual condition of herbal drinks. Jubliang, Aegle marmelos L., Chrysanthemum indicum L., Hibiscus sabdariffa L. and Carthamus tinctorius L. which contained high phenolic compounds exhibited high antioxidant activity when determined by DPPH and FRAP assays. It, thus, confirms that phenolic compounds have an important role in antioxidant activities [16]. A good correspond between antioxidant activity and phenolic compounds was found in Bulgarian medicinal plants [17], Chinese medicinal plants [18], some fruits, vegetables and grain products [19]. The phenolic hydroxyl groups present in plant antioxidants have redox properties [20,21] allowing them to act as a reducing agent and a hydrogen donator in the two assays. Thus, phenolic compounds
### Table 1. Antioxidant activity and total phenolic contents of tested herbal extracts.

<table>
<thead>
<tr>
<th>Herbal extracts</th>
<th>Total phenolic contents (mg of GAE/g)</th>
<th>FRAP value (µmol/g)</th>
<th>DPPH (% Scavenging effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Garcinia atroviridis</em> Griff. ex T. Anderson.</td>
<td>36.76 ± 0.49</td>
<td>376.93 ± 15.79</td>
<td>46.52</td>
</tr>
<tr>
<td><em>Andrographis paniculata</em> Nees (พิชปะ랗ใน)</td>
<td>38.65 ± 4.26</td>
<td>486.92 ± 7.14</td>
<td>58.02</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> Rosc. (ขิง)</td>
<td>59.86 ± 9.02</td>
<td>1030.5 ± 11.49</td>
<td>84.88</td>
</tr>
<tr>
<td><em>Cymbopogon citratus</em> Stapf. (ตะไคร่)</td>
<td>83.83 ± 18.53</td>
<td>794.31 ± 18.37</td>
<td>81.69</td>
</tr>
<tr>
<td><em>Schefflera leucantha</em> R.Vig. (หัวสามประจักษ์)</td>
<td>129.86 ± 1.95</td>
<td>523.36 ± 9.09</td>
<td>64.45</td>
</tr>
<tr>
<td><em>Carthamus tinctorius</em> L. (ทองคำ)</td>
<td>139.98 ± 18.02</td>
<td>1140.5 ± 5.05</td>
<td>96.65</td>
</tr>
<tr>
<td><em>Chrysanthemum indicum</em> L. (เกียงยา)</td>
<td>214.34 ± 17.09</td>
<td>1626 ± 153.54</td>
<td>93.33</td>
</tr>
<tr>
<td><em>Aegle marmelos</em> L. (มะขาม)</td>
<td>267.22 ± 17.79</td>
<td>1444.43 ± 61.11</td>
<td>94.07</td>
</tr>
<tr>
<td>Jubliang (จุบที่)</td>
<td>283 ± 14.23</td>
<td>2295.5 ± 26.22</td>
<td>95.59</td>
</tr>
</tbody>
</table>

Values expressed are means ± S.D. of three replicate experiments

### Table 2. Classification of antioxidant activity of water extracts from herbs.

<table>
<thead>
<tr>
<th>Herbal extracts</th>
<th>Phenolic contents (mg of GAE/g)</th>
<th>FRAP value (µmol/g)</th>
<th>DPPH (% Scavenging effect)</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jubliang</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aegle marmelos</em> L.</td>
<td></td>
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<tr>
<td><em>Chrysanthemum indicum</em> L.</td>
<td>&gt;130</td>
<td>&gt;1000</td>
<td>&gt;90%</td>
<td>High</td>
</tr>
<tr>
<td><em>Hibiscus sabdariffa</em> L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Carthamus tinctorius</em> L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Schefflera leucantha</em> R.Vig.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cymbopogon citratus</em> Stapf.</td>
<td>50-130</td>
<td>500-1000</td>
<td>60-90%</td>
<td>Moderate</td>
</tr>
<tr>
<td><em>Zingiber officinale</em> Rosc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Andrographis paniculata</em> Nees</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Garcinia atroviridis</em> Griff. Ex T. Anderson.</td>
<td>&lt;50</td>
<td>&lt;500</td>
<td>&lt;60%</td>
<td>Low</td>
</tr>
</tbody>
</table>
could be the major antioxidant in these herbal drinks. An exception was found on Schefflera leucantha R. Vig. which had high phenolic contents but it showed moderate antioxidant activity. It implied that the phenolic contents might not correspond to the antioxidant activity. The finding was in agreement with the study of Kahkonen et al. [22], who found no correspond between antioxidant activity and phenolic contents on some plant extracts. Ivanova [17] proposed that not all phenolic compounds possessed radical quenching activity. The method of extracting the herbs with hot water might give the different results in antioxidant activity from other published reports. The difference would be due to organic solvents used in isolation that extracted only some selective components. Tiwari et al. [23] suggested that individual components may lose their synergistic characteristics probably present in their natural mixture form.

Among the herbal drinks Jubliang showed the highest FRAP value, highest phenolic contents and highest antimutagenic activity. Jubliang is the only herbal drink that consists of eight herbs. Some of the components of Jubliang, namely Pragmites communis [24] and Lophatherum gracile [25] had been reported to have high antioxidant activity in DPPH assay. Possible synergistic interactions among herbal components that had high antioxidant and antimutagenic activity in Jubliang are warrant for further study.

The antioxidant properties of herbs may be attributed to the plant pigments that are the main components of each herbal extract. The red pigment presented in the flowers of Hibiscus species are anthocyanins (cyanidin-3-glucoside and delphinidin-3-glucoside) which may act as an antioxidant [26, 27].

Stich and Rosin [28] proposed that phenolics had inhibitory effects on genotoxicity of several mutagens. It was found that such compounds were associated with low incidence of various cancers [29-31]. The present study has revealed that phenolic contents of herbal extracts were correlated with antimutagenic activity. An exception was found on the extract from leaves of Andrographis paniculata Nees. It had strong antimitagenic effect but had less phenolic contents. It is proposed that the antimitogenic effect was due to the high chlorophyll content extracted with hot water. Several researchers [32-34] found that chlorophylls had antimitogenic activity in in vitro against aromatic nitro compounds, which is in agreement with the present results. Some investigators found that chlorophylls exhibited antimitogenic activity (in Ames test) by complex formation [35]. The purified chlorophyll protects against the adduct formation of DNA in isolated hepatocytes by interfering with carcinogen activation [36].

Although the extract from Cymbopogon citratus Stapf. has moderate antioxidant activity, it showed low antimitogenic activity. Although Vinitketkumnuen et al. [37] found that mutagenicity of aflatoxin B1, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, IQ, MNNG and AF-2 was inhibited by the extract of lemon grass in a dose dependent manner, but no effect was found on the mutagenic activity of benzo[a]pyrene in Salmonella typhimurium strains TA98 and TA100. Benzo[a]pyrene is a polycyclic aromatic hydrocarbon which has a structure related to nitropyrene, a standard mutagen in this experiment.

The non-enzymatic formation of direct mutagens such as products from treating 1-aminopyrene with nitrite in acidic solution (pH 3.0-3.4) is physiologically important, especially with regard to the etiology of gastric cancer. The reduction of His+ revertant of TA100 induced by nitrite-treated aminopyrene suggests that the herbal extracts may contain some compounds that interfere with the mutagenic metabolism of ultimate mutagen/carcinogen, or form a complex of phenolic compounds with 1-nitropyrene, or scavenge the electrophilic metabolites [38].

**Conclusion**

The study indicates the presence of antioxidants, phenolic compounds and antimutagens in hot water extract of herbs consumed in Thailand. The high antioxidant and antimutagenic activity found in some herbal extracts may help to protect against free radical and reduce mutagen formed in the pH of stomach digestion.
Figure 1. Effect of herbal extracts on the mutagenic inhibition of nitrite treated 1-aminopyrene to Salmonella typhimurium strain TA100.

References


