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Antiradical capacity and reducing power of different extraction method of Areca catechu seed

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Areca catechu is a common traditional Chinese medicinal plant used to treat dyspepsia, constipation, beriberi and oedema. The antiradical capacities of different extraction method of A. catechu extracts were evaluated by scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radicals. Reducing power, total phenolic and flavonoid contents were also detected. Based on DPPH radical scavenging activity, the 100% methanolic extract (AME of 38.77%) is the most effective followed by 50% water/methanol (AWM) and 100% water (AWE) extracts (22.92 and 21.47%, respectively). In addition, the extracts of AME (78.04%), AWM (75.77%) and AWE (68.42%) were effective in superoxide radical scavenging activity in comparison with the control (ascorbic acid and gallic acid). The reducing power, total phenolic and flavonoid contents of the AME extract was found to be superior to other extracts. The results indicated that different extraction method of A. catechu extracts were related to their antiradical activities and reducing power. The present study suggests that A. catechu extracts are useful nutritional antioxidants for the nutraceutical industry.

Key words: Areca catechu, antioxidant activity, radical scavenging, reducing power.

INTRODUCTION

Areca catechu (Areaceae) is popularly used in traditional herbal medicines in Taiwan; it is called “bin lang”. It is widely distributed in Southeast Asia, East Africa, and Pacific islands. In traditional Chinese medicine, A. catechu seed is commonly known in folklore medicine for treatment of various diseases such as dyspepsia, constipation, beriberi and oedema. They had been found to contain phenolics and alkaloids such as arecoline, arecaidine and guvacine (Zhang et al., 2008). Ingestion of large amount of A. catechu therefore can cause various cholinergic effects, such as salivation, lacrimation, urinary incontinence, sweating, diarrhea and cardiac arrhythmia; however, A. catechu constituents are known through several experiments to have beneficial effects on skin, suggesting the possible use in cosmetics industries (Ashawat et al., 2007; Lee and Choi, 1999a, b; Lee et al., 2001; Padmaja et al., 1994). A very minor oral use of A. catechu in Asia is as a dentifrice. The A. catechu is burned to make a charcoal, which is pulverized and added to toothpaste (Small, 2004).

Free radicals are a major interest for physiological and biochemical lesions. Various environmental exposures such as pollution, smoke, ultraviolet light and radiation generate free radicals (Hanson et al., 2006). Antioxidant inhibits the generation and activity of free radicals, and evolves to protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydroxy radical etc. An imbalance between antioxidant and ROS results in oxidative stress, which leads to cellular damage. This long-term damage occurs as a result of skin irritations or allergic reactions such as hives and itchy rashes as well as continuous aging of skin. Many synthetic chemicals such as phenolic compounds are found to be strong radical scavengers, but they usually have side effects. Thus, demands for natural antioxidants from plant sources with more stability and better

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Abbreviations: ROS, Reactive oxygen species; PMS, phenazine methosulfate; NADH, nicotinamide adenine dinucleotide; NBT, nitroblue tetrazolium; AWE, 100% water; AWM, 50% water/methanol; AME, 100% methanol; DPPH, 1,1-diphenyl-2-picrylhydrazyl.
anti-oxidant effects are increasing due to the rejection of synthetically produced one by the consumers (Zheng and Wang, 2001). Thereby, interest in finding natural anti-oxidants, without undesirable side effects, has increased greatly. The numbers of antioxidant compound by plants play important roles in preventing diseases induced by free radicals (Hirose et al., 1994). Therefore, researches are on-going for the development of natural-originated antioxidants in recent years. Research have focused on natural antioxidants and numerous crude extracts to possess antioxidant properties (Chou et al., 2009; Kim et al., 1995; Lee et al., 2003; Lim et al., 1996; Lin et al., 2010a, b).

Although previous studies focused mainly on some antioxidant activity of A. catechu extracts (Pithayanukul et al., 2009; Wettitayaklung et al., 2006; Zhang et al., 2010), no data is available in the literature on the antioxidant activity of various extraction methods of A. catechu. In general, the extraction solvent is another major factor to determine the composition of effective components and their contents in the resulting extract from the plant (Hamida et al., 2002; Lin and Lee, 2010). Therefore, the present work is aimed at investigating different extraction methods of A. catechu of antiradical capacities by scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radicals and reducing power using ascorbic acid and gallic acid as standard; total phenolic content and total flavonoid content were also detected.

MATERIALS AND METHODS

Chemicals

The dried seeds of A. catechu were purchased locally (Goangder Tarng Ginseng Co., Taoyuan, Taiwan). The samples were cleaned, washed with distilled water and cut into small pieces. Ascorbic acid was obtained from Fluka (Buchs, Switzerland), Gallic acid, DPPH, phenazine methosulfate (PMS), nicotinamide adenine dinucleotide (NADH) and nitroblue tetrazolium (NBT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade and obtained from either Sigma-Aldrich or Merck Chemical Co. (Darmstadt, Germany).

Preparation of A. catechu extract

The dried A. catechu were ground in a mortar, and extracted twice using 50 ml of either 100% water (AWE) or 50% water/methanol (AWM) or 100% methanol (AME) under reflux for 5 h at 70°C. The supernatants were separated from the solid residue by paper filtration (No. 1, Advantec, Tokyo, Japan). The extracts were combined and evaporated at 60°C under reduced pressure. All dried extracts were stored at 4°C until use.

Determination of total phenolic and flavonoid contents

Total phenolic contents were determined applying the Folin-Ciocalteu colorimetric method by Lin and Lee (2010). Basically, areca extract solution (0.05 ml) was mixed with 0.05 ml of Folin-Ciocalteu’s phenol reagent. Then, 0.5 ml of a 15% sodium carbonate solution was added to the mixture and then adjusted to 1 ml with 0.4 ml of distilled water. The reaction was allowed to stand for 10 min with intermittent shaking, after which the absorbance was read at 725 nm using a spectrophotometer (Ultrospec 2100 pro, Amersham, Hong Kong). Gallic acid was used for constructing the standard curve and the results were expressed as μg of gallic acid equivalents/ml of areca extract.

Flavonoid contents were determined by a colorimetric method described by Lin and Lee (2010). The areca extract (0.05 ml) was mixed with 0.4 ml of distilled water and 0.02 ml of a 7.5% sodium nitrite solution, followed by 15% aluminum chloride solution (0.02 ml). After 6 min, 0.2 ml of 1 M sodium hydroxide and 1 ml of distilled water were added to prepare the mixture. The solution was properly mixed and the absorbance was read at 510 nm. Rutin was used for constructing the standard curve and the results were expressed as μg of rutin equivalents/ml of areca extract.

Assay of antioxidant activities

DPPH radical assay

Scavenging effect on DPPH radical was measured by the method of Chou et al. (2009). Briefly, 0.1 ml of a 1 mM methanol solution of DPPH was incubated with varying concentrations of areca extract. After a 30 min incubation period at room temperature, absorbance of the resulting solution was read at 517 nm. DPPH radical scavenging activity was expressed as the inhibition percentage and was calculated as (1 - absorbance of sample/absorbance of control) × 100. Ascorbic acid and gallic acid were used for comparison.

Superoxide radical scavenging activity

Measurement of superoxide radical scavenging activity was evaluated according to the method of Chou et al. (2009). The reaction mixture contained the same volume of 120 μM PMS, 936 μM NADH, areca extract and 300 μM NBT in a total volume of 1 ml of phosphate buffer (100 mM, pH 7.4). After 5 min of incubation at ambient temperature, absorbance of the resulting solution was measured at 560 nm. The superoxide radical activity was calculated using the following formula:

\[
\text{Scavenging effect (\%)} = \left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \right) \times 100
\]

Ascorbic acid and gallic acid were used for comparison.

Reducing power

The reducing power was estimated by the method of Chou et al. (2009). The areca extract (0.25 ml) was mixed with 0.25 ml of sodium phosphate buffer (200 mM, pH 6.6) and 0.25 ml of 1% potassium ferricyanide. Then the mixture was incubated at 50°C for 20 min. After 0.25 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (0.5 ml) was mixed with 0.4 ml of deionized water and 0.1 ml of 0.1% ferric chloride solution to allow for standing for 10 min, and the absorbance was measured at 700 nm. A higher absorbance indicated a higher reducing power. Ascorbic acid and gallic acid were used for comparison.

Statistical analysis

All data were presented as the mean ± standard deviation of triplicate parallel measurements. Statistical analysis was performed using Student’s t test for paired values.
RESULTS AND DISCUSSION

DPPH radical scavenging activity

Numerous antioxidant methods and modifications have been proposed to investigate antioxidant activity and to explain how antioxidants function. The DPPH radical scavenging activity in a relatively short time, compared with other methods of various natural compounds or crude extracts of plants (Ahn, 2009; Lee et al., 2003). The method widely used to predict the ability of flavonoids to transfer H atoms to radicals is based on the free radical, 1,1-diphenyl-2-picrylhydrazyl in the DPPH assay. The antioxidants were able to reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydrazine. Figure 1 depicts the DPPH radical scavenging ability of different extraction method of A. catechu extracts and standards (ascorbic acid and gallic acid). Obviously, the AME extract of A. catechu showed moderate activities of 24.15 ± 0.52% to 38.77 ± 0.75% at a concentration of 2.5 to 25 µg/ml tested. The AWM extract showed slight DPPH radical scavenging abilities of 18.53 ± 0.61 to 22.92 ± 1.79% at 2.5 to 25 µg/ml, whereas those of AWE extract scavenged DPPH radicals by 16.31 ± 1.04 to 21.47 ± 1.46%. Ascorbic acid and gallic acid showed excellent scavenging abilities of 32.21 ± 0.37 and 52.26 ± 0.58% at 25 µg/ml, respectively. Upon comparison, methanolic extract of A. catechu showed higher scavenging activities than ascorbic acid, water and water/methanolic extracts.

In vitro studies clearly indicated that the methanolic extract of A. catechu has significant antioxidant activity. This result is in agreement with Ahn (2009). In addition, Zhang et al. (2010) showed that the ethyl acetate extract exhibited considerable antioxidant activity by DPPH of areca nut. However, other investigators (Wetwitayaklung et al., 2006) have reported that the change of the content of the phenols and tannin in A. catechu at different growth stages were determined.

Superoxide radical scavenging activity

Superoxide anion, a reduced form of molecular oxygen, has been implicated in initiating oxidation reactions associated with aging (Cotelle et al., 1996). Superoxide anions play important roles in formation of other reactive oxygen species such as singlet oxygen, hydrogen peroxide and hydroxyl radical, which induce oxidative damage in DNA, lipids, and proteins (Aurand et al., 1977; Pietta, 2000). Also, superoxide anion is an oxygen-centered radical with selective reactivity. In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the presence of bioactive compounds possessing superoxide radical scavenging activity. Figure 2 shows the comparison of the superoxide radical elimination abilities of all A. catechu extracts with different
Figure 2. Scavenging activities on superoxide radical of 100% methanol (AME), 100% water (AWE) and 50% water/methanol (AWM) extracts of A. catechu at different concentrations. Ascorbic acid (Vitamin C) and gallic acid (GA) were taken as the standards. Data are expressed as mean ± standard deviation (n = 3).

standards such as ascorbic acid and gallic acid. The finding shows that A. catechu extracts were more effective at radical elimination than that of ascorbic and gallic acid (P < 0.05). Scavenging effect of different extraction method of A. catechu extracts and standards on superoxide radical decreased in the following order: AME > AWM > AWE > gallic acid > ascorbic acid, with 78.04 ± 0.20, 75.77 ± 0.87, 68.42 ± 0.36, 33.89 ± 0.25 and 14.57 ± 0.20% at 10 µg/ml, respectively. These results clearly suggested that different extraction method of A. catechu extracts were also related to their ability to scavenge superoxide radical. This result is in agreement with other researchers (Mizue et al., 1999; Patil et al., 2009), who demonstrated that methanolic extract of areca nut showed remarkable active-oxygen scavenging activity and especially, indicated strong superoxide radical scavenging activity.

Reducing power

The reducing power of the plant extract components might serve as a significant indicator of its potential antioxidant activity (Gülçin et al., 2003b; Meir et al., 1995). As indicated in Figure 3, the reducing powers of different extraction method of A. catechu extracts were enhanced by increasing concentration of samples. The reducing powers of different extraction method of A. catechu extracts were in the following order: AME (1.59 ± 0.04) > AWM (1.48 ± 0.04) > AWE (1.10 ± 0.01) at 25 µg/ml. With regards to reducing capacity, higher reducing powers might be attributed to higher amounts of total phenolic and flavonoid and the reducing power of a compound may reflect its antioxidant potential (Lee et al., 2007). Zhang et al. (2009) mentioned that ethanolic extract of areca seed may have the highest amounts of reductones and polyphenolics. Reducing power indicates compounds that are electron donors, which can act as primary and secondary antioxidants (Yen and Chen, 1995). Different studies have been indicated that the reducing properties are gene- rally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain through the donation of a hydrogen atom (Shimada et al., 1992; Shon et al., 2003).

Amount of total phenolic and flavonoid contents

Most of the phenolic or polyphenolic compounds in nature have antioxidative activities, example include tocopherol, flavonoid and other organic acid (Kim et al., 1995). Water with alcohol was selected as the extraction solvent since both are commonly used in the food industry in a variety of ways and are more highly stable in the human body than any other solvents. As shown in Table 1, the flavonoids were the major components of all A. catechu extracts. Significant differences were observed in the total phenolic content and flavonoid content among the A. catechu extracts. The extract of AME was found to have the highest total phenolic and flavonoid contents (466.70...
Figure 3. Reducing powers of 100% methanol (AME), 100% water (AWE) and 50% water/methanol (AWM) extracts of *A. catechu* at different concentrations. Ascorbic acid (Vitamin C) and gallic acid (GA) were taken as the standards. Data are expressed as mean ± standard deviation (*n* = 3).

Table 1. Contents of phytochemicals of different extraction method of *A. catechu* extracts.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Phytochemical</th>
<th>Total phenolic (g/ml)</th>
<th>Total flavonoid (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AME</td>
<td></td>
<td>466.70 ± 0.79</td>
<td>933.33 ± 1.31</td>
</tr>
<tr>
<td>AWE</td>
<td></td>
<td>278.25 ± 0.54</td>
<td>286.36 ± 1.17</td>
</tr>
<tr>
<td>AWM</td>
<td></td>
<td>461.17 ± 0.55</td>
<td>731.98 ± 2.15</td>
</tr>
</tbody>
</table>

AME, 100% methanol extract; AWE, 100% water extract; AWM, 50% water/methanol extract. All data are expressed as mean ± standard deviation of triplicate tests. Total phenolic content was expressed as µg gallic acid equivalents/ml extract. Total flavonoid content was expressed as µg rutin equivalent/ml extract.

± 0.79 µg/ml and 933.33 ± 1.31 µg/ml, respectively) among all *A. catechu* extracts evaluated, followed by AWM and AWE extracts. Based on the study, it seemed that the potent antiradical activity and reducing power of *A. catechu* might result from its high contents of phenolic and flavonoid type compounds. This result is in agreement with Ahn (2009), who demonstrated that, the higher the contents of phenol and tannin in *A. catechu* L. methanol extract, the higher the antioxidant ability; the active compounds are presumed to be phenolic and tannic with hydroxyl groups.

Polyphenolics display important role in stabilizing lipid oxidation associated with its antioxidant activity (Gülçin et al., 2003a). Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimitogenic and anti-carcinogenic activities (Benavente-Garcia and Castillo, 2008; Hoensch and Kirch, 2005). In general, medicinal plants of the same scientific name differ in composition of effective components and their contents, depending on origin and growth conditions (Wetwitayaklung et al., 2006). In view of previous studies, the major compounds of *A. catechu* are polyphenolic compounds, gallic acid, alkaloids, tannin, arecoline and arecaidine (Lee and Choi, 1999b; Patil et al., 2009; Wang and Lee, 1996; Zhang et al., 2008).

Conclusion

In the present study, the methanolic extract of *A. catechu* exhibited strong antiradical activities and reducing power. In addition, the AME extract contains significant amount of phenols and flavonoids, which play a major role in controlling oxidation. The results imply that *A. catechu* extracts may be used as an antioxidant, leading to the possibility of developing natural antioxidant material. It was of our paramount interest to further identify the specific antioxidant components in methanolic extract of *A. catechu* which may be a new health-care food supplement or functional cosmetic for special use in the future.

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REFERENCES


