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Full Length Research Paper

Evaluation of superoxide radical scavenging capacity and reducing power of areca flower extracts

En-Shyh Lin[1]* and Chia-Ching Li[2]

[1]Department of Beauty Science, National Taichung Nursing College, Taichung 40343, Taiwan.
[2]Department of Cosmetic Science, Vanung University, Taoyuan 32061, Taiwan.

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Areca is a traditional Chinese medicinal plant used to treat a variety of diseases. The antioxidant capacities of areca flower extracts were evaluated by using superoxide radical scavenging activity and reducing power, total phenolic content and total flavonoid content were also detected. In all the tested models, the areca flower extracts showed their ability to scavenge superoxide radical and reducing power in a does-dependent manner. The distilled water (AFW) extract had higher superoxide radical scavenging activity and reducing power than of distilled water/methanol (AFM) extract. These results indicated that areca flower extracts might be used as a potential source of natural antioxidant agent.

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

INTRODUCTION

Free radicals were the major interest for physiological and biochemical lesions. Our body generates free radicals as by-products of burning fuel for energy within the cells, exercising and vanishing off infections. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. Various environmental exposures such as pollution, smoke, the sun's ultraviolet light and radiation create free radicals (Hanson et al., 2006). Antioxidants inhibit or prevent oxidation of substrates and evolve to protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, hydroxy radical etc. An imbalance between antioxidants and ROS results in oxidative stress, which leads the cellular damage (Gülçin, 2010; Gülçin et al., 2010). 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. Interest in finding naturally occurring antioxidants in foods or medicines to replace synthetic antioxidants has increased considerably, given that synthetic antioxidants are being restricted due to their side effects (Zheng and Wang, 2001). Thereby, interest in finding natural antioxidants, 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, attention has been directed toward the development of natural antioxidants from plant sources (Chou et al., 2009; Lin et al., 2010a, b).

Areca (*Areca catechu*, Arecaceae) is one of the popular traditional herbal medicines used in Taiwan and widely distributed in East Africa, South Asia and Pacific islands. The areca flower is consumed as vegetables in Taiwan. Areca nut had been found to contain phenolics and alkaloids such as arecoline, arecaidine and guvacine (Zhang et al., 2008). Ingestion of large amount of areca therefore can cause various cholinergic effects, such as salivation, lacrimation, urinary incontinence, sweating, diarrhea and cardiac arrhythmia. However, several reports have indicated that areca constituents have beneficial effects on skin, suggesting the possible use in cosmeceuticals (Ashawat et al., 2007; Lee and Choi, 1999a, b; Lee et al., 2001; Padmaja et al., 1994). A very minor oral use of areca in Asia is as a dentifrice. The areca is burned to make a charcoal, which is pulverized and added to toothpaste (Small, 2004).

Although a lot of literature showed that areca nut or seed had a strong antioxidant activity (Lee et al., 2003; Wetwitayaklung et al., 2006; Zhang et al., 2010), little is known about the antioxidant potential of areca flower. In the present investigation, it was evaluated that the antioxidant activities by scavenging superoxide radical and reducing power of areca flower extracts using gallic acid

*Corresponding author. E-mail: eslin7620@hotmail.com. Tel: +886-4-22196816. Fax: +886-4-22259920.

as standard.

MATERIALS AND METHODS

Chemicals

Areca flower was picked from the western mountain area of Taiwan in April, 2008. The samples were cleaned, washed with distilled water, cut into small pieces. Gallic acid (GA), phenazine methosulfate (PMS), nicotinamide adenine dinucleotide (NADH) and nitroblue tetrazolium (NBT) were obtained from Sigma chemical (St. Louis, MO, U.S.A.). All other reagents were analytical grade.

Preparation of the extract

The areca flower extracts were prepared according to Köksal and Gülçin (2008) with modification (Chou et al., 2009). The areca flower was ground in a mortar and extracted twice using 50 ml of 100% distilled water (AFW) and 50% distilled water/methanol (AFM) under reflux for 5 h at 70°C, respectively. 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.

Scavenging activity on superoxide radical

Scavenging activity on superoxide radical was evaluated according to Gülçin et al. (2008) with modification (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 100 mM phosphate buffer (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 as scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100. Gallic acid was used for comparison.

Reducing power

The reducing power was determined according to Gülçin et al. (2008) with slight modification (Chou et al., 2009). The areca extract (0.25 ml) was mixed with 0.25 ml of 200 mM sodium phosphate buffer (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, allowed to stand for 10 min and the absorbance was measured at 700 nm. Higher absorbance indicated higher reducing power. Gallic acid was used for comparison.

Determination of total phenolic contents

Total phenolic contents were quantified according to the method of Slinkard and Singleton (1977) as modified by Chou et al. (2009). 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 it was 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. Gallic acid was used for constructing the standard curve (5 - 35 µg/ml; y = 0.0964x - 0.0519; R2 = 0.9856) and the results were expressed as µg of gallic acid equivalents/ml of areca extract.

Determination of total flavonoid contents

Total flavonoid contents were determined by a colorimetric method of Kim et al. (2003) as modified by Chou et al. (2009). 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 mixed well and the absorbance was read at 510 nm. Rutin was used for constructing the standard curve (10 -100 µg/ml; y = 0.0118x - 0.0108; R2 = 0.9989) and the results were expressed as µg of rutin equivalents/ml of areca extract.

Statistical analysis

All data were presented as the mean ± standard deviation (SD) of triplicate parallel measurements. Statistical analysis was performed using student's t test for paired values.

RESULTS AND DISCUSSION

Superoxide radical scavenging activity

Scavenging activity on superoxide radical in the fractions was determined by NBT-NADH-PMS system (Gülçin, 2007). Decrease of optical density values against control is the indication of the presence of bioactive compounds possessing superoxide radical scavenging activity. Superoxide anion, a reduced form of molecular oxygen, has been implicated in the initiating oxidation reactions associated with aging (Cotelle et al., 1996). Superoxide anions play an important role 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 et al., 2000). Also, superoxide anion is an oxygen-centered radical with selective reactivity.

These species are produced by a number of enzyme systems in auto-oxidation reactions and by non-enzymatic electron transfers that univalent reduce molecular oxygen. It can also reduce certain iron complex such as cytochrome c. As shown in Figure 1, both areca flower extracts effectively scavenged superoxide radical in a concentration-dependent manner. Both AFW and AFM extracts showed significant superoxide radical scavenging activities which were comparable with gallic acid ($p < 0.05$). Scavenging effect of areca flower extracts and gallic acid on the superoxide radical decreased in the following order: AFM > AFW > gallic acid, with 87.4 ± 0.1%, 81.9 ± 0.3% and 41.0 ± 0.1%, at the concentration of 20 µg/ml, respectively. This result was in agreement with other researchers, who demonstrated methanolic extract of areca nut has shown remarkable active-oxygen scavenging activity, especially indicated strong superoxide radical scavenging activity (Mizue et al., 1999; Patil et al.,

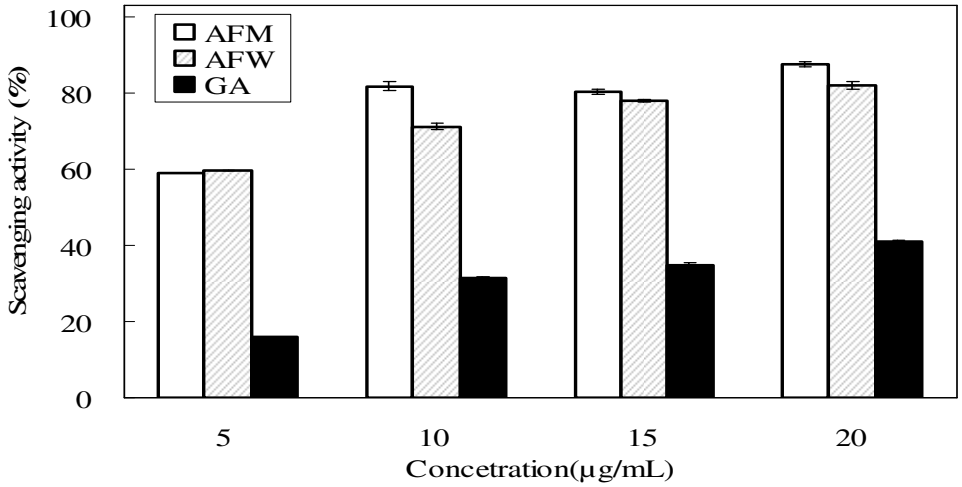

Figure 1. Scavenging activities on superoxide radical of AFW and AFM extracts of areca flower at different concentrations. GA was taken as the standard. Data expressed as mean ± SD (*n*=3).

2009).

Reducing power

The reducing capacity of the plant extract components may serve as a significant indicator of its potential antioxidant activity (Gülçin et al., 2003b; Meir et al., 1995). A higher absorbance indicates a higher ferric reducing power. Previous studies have reported that the electron donation capacity of bioactive compounds is associated with antioxidant activity (Siddhuraju et al., 2002; Yen et al., 1993). Figure 2 shows the reducing powder of both areca flower extracts compared with gallic acid. Reducing power increased with an increased in concentration. AFM extract displayed a higher reducing power compared to the AFW extract. Reducing powers of AFM extract was 1.48, whereas that of AFW extract was 0.83 at 25 µg/ml. However, gallic acid showed increase in reducing powers from 0.27 to 1.94 at 2.5-25 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). 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 generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Shimada et al., 1992; Shon et al., 2003). Hence, AFM extract may have the highest amounts of reductones and polyphenolics in areca flower extracts.

The results obtained in both antioxidant assays showed statistically significant difference between the AFW and AFM extracts. Furthermore, to check whether the total phenolic and flavonoid contents in areca flower extracts are responsible for these activities, correlation and regression analyses were performed, respectively. As indicated in Figures 3 and 4, total phenolic and flavonoid contents of both areca flower extracts showed significant and strong positive correlation with superoxide radical scavenging activity efficiency and reducing powder. This meant that % phenolic and flavonoid were not only factor of scavenging activity in AFM extract. However, this could be confirmed by the correlation between superoxide radical scavenging activity (y) and total phenolic or flavonoid content (x) of AFM extract which had a correlation coefficient of R2 = 0.752 (Figure 3). The R2 suggested that 75.2 % of the scavenging activity of AFM extract accessed from phenolic or flavonoid compounds. However, the result suggests a possible important role that the phenolic and flavonoid constituents of the AFW extracts might play in superoxide radical neutralization and reducing powder.

Figure 5 showed superoxide radical scavenging activity % significantly correlating with reducing powder for both areca flower extracts. This infers that both areca flower extracts differentially reducing powder by virtue of their varying degrees of superoxide radical quenching potential. These results clearly suggested that reducing power of areca flower extracts were also related to their ability to scavenge superoxide radical. Additionally, Robak and Gryglewski (1988) reported that antioxidant properties of some flavonoids are effective mainly via the scavenging of superoxide radical.

Amount of total phenolic and flavonoid contents

Most of the phenolic or polyphenolic compounds in nature

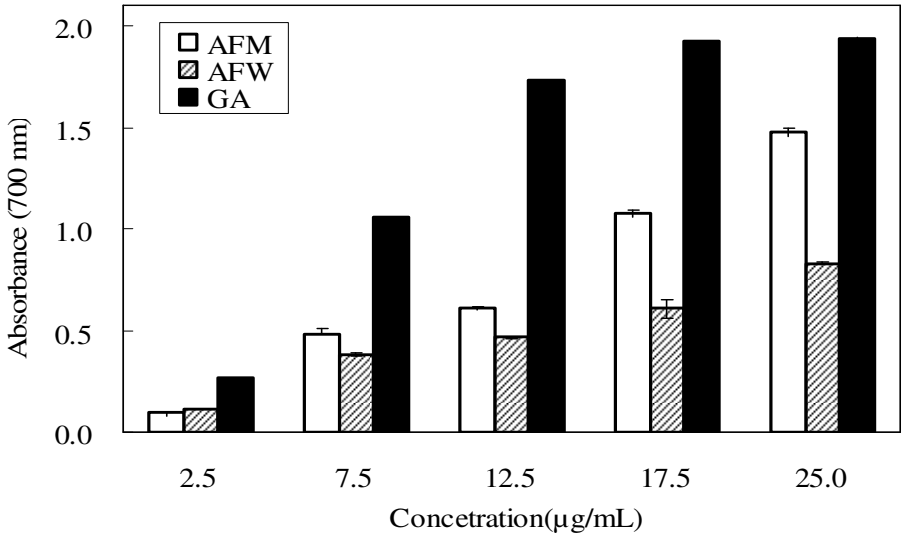

Figure 2. Reducing powers of AFW and AFM extracts of areca flower at different concentrations. GA was taken as the standard. Data expressed as mean ± SD (*n*=3).

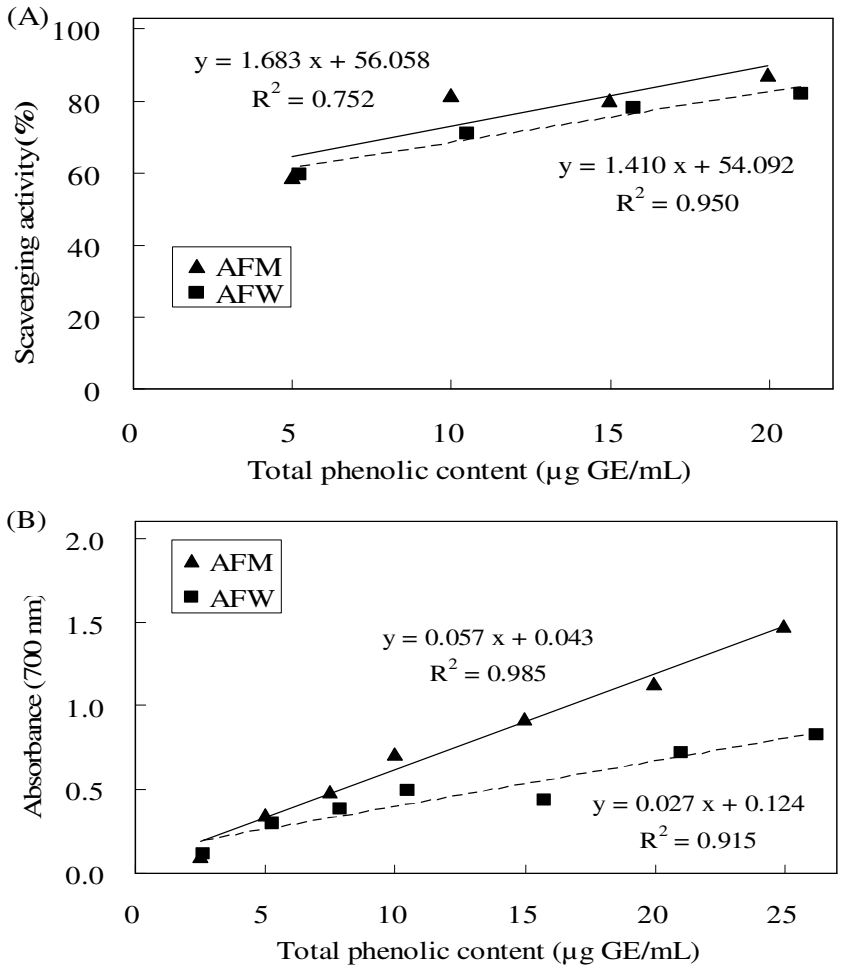

Figure 3. Linear correlation of total phenolic content in AFW and AFM extracts of areca flower. (A) Superoxide radical scavenging activity (%), (B) Reducing power.

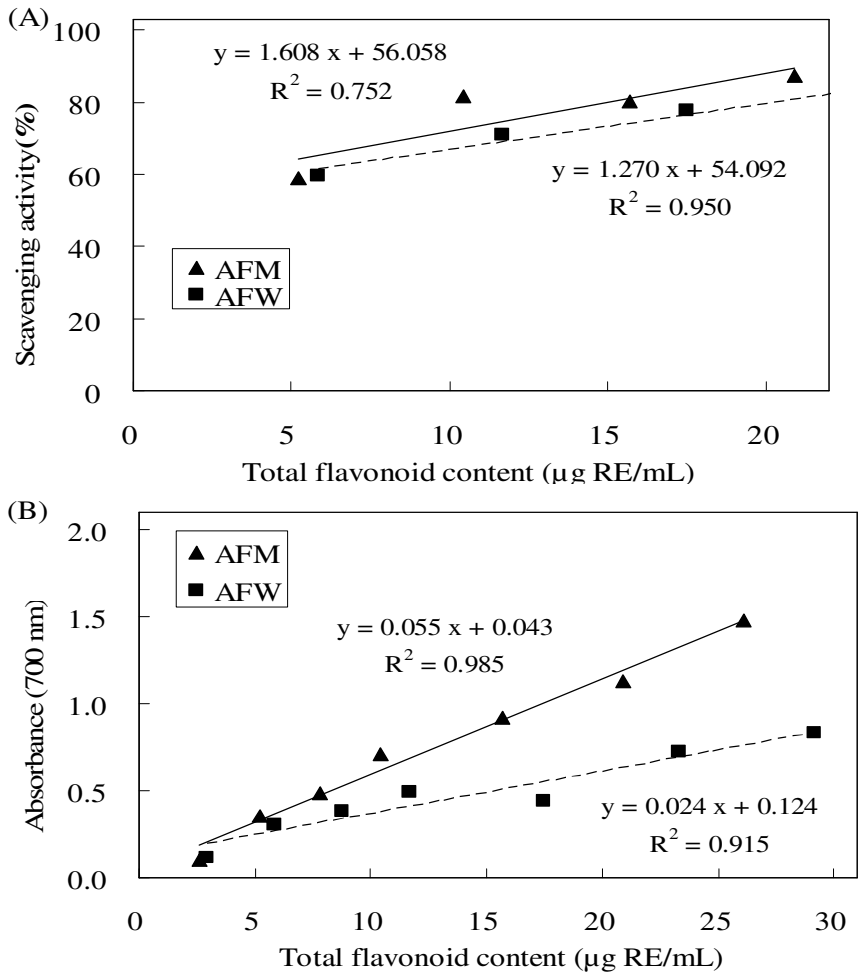

Figure 4. Linear correlation of total flavonoid content in AFW and AFM extracts of areca flower. (A) Superoxide radical scavenging activity (%), (B) Reducing power.

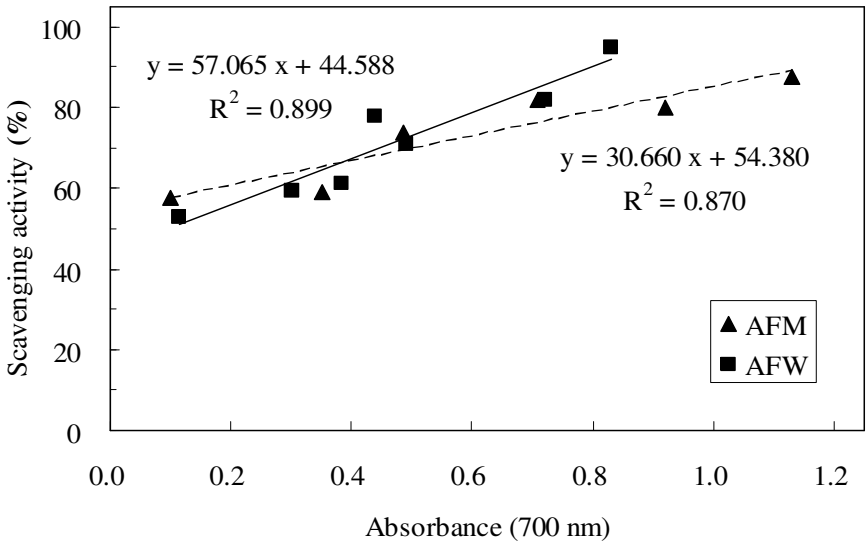

Figure 5. Relationship of superoxide radical scavenging activity (%) and reducing power between total phenolic content in AFW and AFM extracts of areca flower.

Table 1. Contents of phytochemicals in *A. catechu**.

Phytochemicals	Extracts	
	AFW	AFM
Total phenolics	406.43 ± 0.54†	840.73 ± 0.85†
Total flavonoids	451.47 ± 1.70‡	880.00 ± 2.79‡

* All data are expressed as mean ± SD 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.

have antioxidative activities, e.g. tocopherols, flavonoids and other organic acids. 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. Table 1 showed the flavonoids were the major components of both areca flower extracts. The significant differences were observed in the total phenolic content and flavonoid content among the areca flower extracts. AFM extract were found to have the highest total phenolic and total flavonoid content (840.73 ± 0.85 µg/ml and 880.00 ± 2.79 µg/ml, respectively) among the areca flower extracts. AFM extract contained the most total phenolics and flavonoids content, followed by AFW extract. The result suggests a possible important role that the extraction method was a major factor to determine the composition and their effective in the plant extract. Although total phenolics, tannic acid and gallic acid of areca nut have already been demonstrated (Lee and Choi, 1999b; Wang and Lee, 1996; Zhang et al., 2008), no exact component contributing the antioxidant activity to areca flower has been reported yet. Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities (Benavente-Garcia and Castillo, 2008; Hoensch and Kirch, 2005). Polyphenolics display important role in stabilizing lipid oxidation that associated with its antioxidant activity (Gülçin et al., 2003a).

In conclusion, the present study suggests that areca flower extracts are useful nutritional antioxidants for the nutraceutical industry. Total phenolics and flavonoids of areca flower extracts should be the most activity substances, but the components responsible for the antioxidative activity of areca flower extracts are currently unclear. Therefore, further research should be followed that required isolate and identify the bioactive compounds in areca flower extracts. After adequate treatment they can, for example, be included in foods with remarkable benefits for human or animal health.

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