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Quality analysis of *Areca catechu* L varieties from Mekong delta, in VietNam

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Abstract— *The areca nut palm (Areca catechu L., Arecaceae family) is an economically important palm species in the World providing livelihood options to millions of farmers. The objectives of this study were to extract from areca nut to determine the The phenolic content (TPC) , The flavonoid content (TFC) and Anthocyanin content (TAC) content of 8 samples areca nut in the Mekong Delta .TPC of different sample differed significantly ($p < 0.05$). The TPC measured in areca nut was significantly higher (135.78-162.27 mg). There were significant differences in the flavonoid content in areca nut ($p < 0.05$). The Anthocyanin contents in areca nut were in the range of (23.55-35.55mg). The contents of TFC of different arecanut palm sites were significantly different ($p < 0.05$) The TFC measured was significantly higher (365.71–495.12mg) . The trend of flavonoid content of different sites was similar to that of total phenolic content .Alkaloid levels in 8 Areca Nut at difference sites analysis .The content associated Alkaloid in eight varieties with different genotypes such as Guvacine ,arecoline , Arecaidine and arecoline in hoblies in different districts of Mekong were compared. The DPPH radical-scavenging rate significantly varied in different sites (34.6 to 66.9% $p < 0.05$). According to the differences of functional substances among varieties, it can provide guidance for consumers and theoretical basis for the production of healthy food.*

Keywords— ***extraction, phenolic content (TPC), flavonoid content (TFC), Anthocyanin content, Alkaloid***

I. INTRODUCTION

Although, areca nut palm is a tree that farmers in the Mekong Delta grow in a comnination with other fruit trees, it is just an interesting complementary occupation for farmers in that Mekong Delta region. The amount of areca palm produced is not enough to meet the demand of both domestic and foreign markets because areca nuts are an important raw material of many industries continuously today. Areca nut has been used in various kinds of traditional medications for the treatment of diseases such as, schizophrenia and glaucoma and it is also known to be a stimulant and aids in digestion (Rama et al., 2016). Extensive investigations demonstrated that AS possessed many pharmacological activities, such as antioxidant activity, anti-bacterial activity, anti-hypoglycemic activity, anti-inflammatory activity, anti-parasitic activity, and activity for promoting digestive functions, etc. (Shen et al., 2017; Peng et al., 2015). Particularly, these health benefits are related to the phyto-chemical constituents of the AS extracts, including phenolics, flavonoids and alkaloids, etc. (Sazwi et al., 2013).Polyphenols are very important plant constituents and they exert antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals(Maisuthisakul et al.,2012). However, polyphenols also possess pro-oxidant efect under certain conditions, such as high concentrations, high pH and the presence of redox-active transition metals(Antonio et al.,2015). The pro-oxidant activity of catechins at non-cytotoxic levels have been used as chemosensitizer for the treatment of cancer(Antonio et al.,2015). However, at high concentrations, certain polyphenols, such as catechins could exhibit cytotoxicity to normal cells (Elbling, et al .,2015). Thus, moderate amounts

of polyphenols could protect against diseases associated with oxidative stress such as cancer, coronary heart disease, infammation via mechanisms like antioxidant activity and neutralisation/modulation of human/bacterial/viral proteins/enzymes(Petti et al.,2009). In the recent years, the health benefits of the pigmented rice varieties have reported due to the presence of bioactive compounds. The phytochemical constituents (total phenolic, flavonoid and anthocyanin content) and individual phenolics and flavonoids of the extracts of sixteen genotypes of pigmented rice bran were evaluated using spectrophotometric and ultra-high performance liquid chromatography method. Antioxidative properties of the free and bound fractions were evaluated using nitric oxide and 1,1-diphenyl-2-picrylhydrazyl scavenging assays. Extracts were evaluated for antiproliferative activity against breast cancer cell lines (MCF-7 and MDA-MB-231) using the MTT assay(Ali et al .,2018). Phenolics, one of the most abundant groups of phytochemicals in whole grains, are considered natural antioxidants, which act as radical scavengers to decrease the incidence of oxidative stress-induced damage to large biological molecules, such as lipids, proteins, and DNA (Slavin .2004).

Therefore, the local agricultural sector needs to support farmers to improve varieties in agricultural production. Changing economic and social conditions, including the limited capabilities of farmers, have caused traditional agricultural production to change to more commercial production.

The predicting trends of agricultural product will allow to make right decision in economy nowadays. The right decision making about targeting and direction policies should be on the accurate information and current database and knowledge from inside and outside the country. This study would address suggestion for utilization of quantify flavonoids and polyphenols between 8 different sample *Areca catechu* L varieties from Mekong delta . Polyphenols and flavonoids and the mechanism of *Areca catechu* L growth and development from the limited description of previous works. Our studies have enriched the active compounds of areca nut and laid a solid foundation to improve the active compounds for the type of of Areca catechu L (areca nut)served as functional oral drugs for Vietnam.

II. METHODS AND MATERIALS

Sample Collection

Areca nut samples were collected from different districts of Mekong delta (Giong Trom, Ba Tri, Mo Cay(Ben Tre Province); Cai lay(Tien Giang) ; Tra Cu(Tra Vinh) ; Cai Rang, O Mon(CanTho city) and Binh Minh(Vinh Long)

Fresh seeds were taken from areca nuts and dried at 60 °C for 72 h to obtain a stable weight. They were chopped and ground by roller mill and then passed through a 14-mesh sieve before extracted.

Extraction of Polyphenol from Areca Seed

Maximum polyphenols (407.47 mg GAE g-1), total antioxidant activity with minimum arecoline (1.73 mg g-1 of sample) was achieved by using 80% acetone at pH 4 for 90 min with 10% w/v substrate under shaking conditions (chavan and singhal, 2013). To determine the antioxidant compounds, the optimum extraction conditions were used. 5 g of the finely powdered and dried areca seed sample was extracted using 55 ml of 70% ethanol at 70°C for 120 min by reflux. The extracts were filtered through Whatman No. 4 paper under reduced pressure, and then lyophilized by LGZ-10D Freezer Dryer. All the samples were redissolved

in 70% ethanol at a concentration of 5.0 mg/ml and analyzed for their content of polyphenols.

Determination of Polyphenol Content

Determination of total phenolic content (TPC) :TPC was determined by modified FC method.21 The extracts (0.5 ml; 1mg/ml stock solution) were mixed with 0.5 ml of distilled water and 1ml of FC reagent (pre-diluted, 10 times, with distilled water) and incubated for 5 min at room temperature (27± 2°C). After incubation, 2 ml of 700 mM sodium carbonate was added in the reaction mixtures, mixed and kept in dark for 45 min at room temperature. The absorbances of the samples were measured at 765 nm using a UV–Vis spectrophotometer (CECIL, CE 7200; Cambridge, UK). A calibration curve was prepared using standard solutions of gallic acid ranging from10 to 80 µg/ml (r2 =0.983). The amount of phenolics in different extracts was calculated from the calibration curve and was expressed as mg gallic acid equivalent (GAE) per gm of FL.

Estimation of total anthocyanin content (TAC)

Different areca nut samples (50 mg) were extracted with methanol/HCl (99:1 v/v) solute on at 4 °C for overnight. The observation of each sample were measured at 530 and 657 nm using a spectrophotometer. (UV-2120 Optizen, Mecasys, Korea), and relative anthocyanin levels were determined using the following formula:

$$\text{TAC= optical density (OD)530nm } -(0.25\times \text{ OD 657m})\times \text{ extraction volume (mL)}\times 1/\text{weight of sample (g)} \quad (1)$$

Cyanidin 3-glucoside was used as a standard and results were expressed as milligrams of cyanidin 3-glucoside equivalents (Cy3-GE)/100 gDM.

Determination of Flavonoid Content

Currently, the determination of TFC was depended on the aluminium chloride colorimetric method described by(Qiu, et al 2010). Briefly, a 50 µL supernatant was mixed with 100 µL distilled water. Then, 5% $NaNO_2$ was added into the mixture and incubated for 5 min. Subsequently, 10% $AlCl_3$ $6H_2O$ solution was drawn and added to the mixture for incubation for 3 min. Finally, 60 µL 4%NaOH was added to the termination reaction. The samples were read at 510 nm. Absolute methanol was used as the control, while a standard rutin curve was used to calculate the content of TFC. Results were recorded as mg of RE/100 g DW.

***Determination of DPPH* (1,1-Diphenyl-2-picrylhydrazyl) assay** Radical Scavenging Activity

The method by (Ghasemzadeh và ctv.,2015), was used with slight modifications to assess DPPH. The mixtures were shaken vigorously, and the sample was taken then incubated for 30 min in the dark. Mixture was measured at 517 nm.

$$\text{DPPH radical scavenging effect (\%)}=1-\text{Asample}-\text{Abackground}\text{Acontrol}\times 100\%\text{DPPH radical scavenging effect (\%)}=1-\text{Asample}-\text{Abackground}\text{Acontrol}\times 100\% \quad (2)$$

where A_{sample}, $A_{control}$, and $A_{background}$ refer to sample (sample and DPPH), control (without sample), and background (without sample), respectively.

Statistical Analyses

All measurements in this study were presented as means ± standard deviations. Each antioxidant activity assay was carried out three times from the same extracts in order to determine their reproducibility. Statistical differences and principal component analysis were analyzed with SPSS 25 (SPSS Inc., Chicago, IL, USA) (Li et al .2021). Canonical correspondence analysis and networks were conducted with Origin software.

III. RESULT AND DISCUSSION

3.1. The phenolic content (TPC) , The flavonoid content (TFC) and **Anthocyanin** (TAC) content of 8 samples areca nut in the Mekong Delta :TPC of different sample differed significantly ($p < 0.05$). The TPC measured in areca nut was significantly higher (135.78-162.27mg). The difference in the total phenolic content between varieties can be attributed to differences in genotype.

This may indicate that is shown in Table 1.

Polyphenolic content in areca nut components. Our study shows that phenolic acids were mostly detected in seed samples. The highest TPC content was observed in at Giong Trom and Tra Cu (162.27-161.45mg) respectively.

TAC content varied in different districts. High concentration of TAC content was observed in Tra Cu (35.25mg followed by Giong Trom (33.15mg) and less concentration of TAC was observed in Omon (23.55mg).

The contents of TFC hoblies in different districts of Giong Trom were compared (Table 1). There was a significant difference in TFC content between among hoblies of Mekong delta district. Giong Trom(Ben Tre) and Tra Cu(Tra Vinh) had higher concentration of TFC (495.12 and485.54 mg) compare to the other hoblies. Low

concentration of TFC was determined in the Binh Minh (Vinh Long) (411.85mg) and Omon (365.71mg) district.

Table 1: TPC, TAC, TFC content (mg/100) in Different at samples

Sites	TPC	TAC	TFC
Giong Trom(Ben Tre)	162.27a	33.15a	495.12a
MO Cay(Ben Tre)	142.5c	25.47b	444.74b
Ba Tri(Ben Tre)	154.5b	30.28a	412.26c
Cai Rang(Can Tho)	135.78d	30.27a	456.33b
O mon (Can Tho)	141.12c	23.55b	365.71d
Cai Lay(Tien Giang)	157.54b	32.22a	412.26c
Tra Cu(Tra Vinh)	161.45a	35.25a	485.54a
Binh Minh (Vinh Long)	158.44b	31.25a	411.85c

3.2. Alkaloid Levels **in 8** Areca Nut **varieties** :The content of free and associated Alkaloid in eight varieties with different genotypes of is presented in Table 2. The total Alkaloid .

Guvacine content varied in different Mekong districts. High concentration of **Guvacine** content was observed in Mo cay (2.60 ppm) followed by Giong Trom and (Tra Cu) Tra Vinh (2.48 ppm) and less concentration of **Guvacine** was observed in Ba Tri (1.79.ppm).

The contents of arecoline in hoblies in different districts of Giong Trom were compared (Table 2). There was a significant difference in arecoline content between among Mekong district. Giong trom (Ben Tre)had higher concentration of arecoline (2.2 ppm) compare to the other district . Low concentration of arecoline was determined in the BaTri (0.91ppm) and (1.2ppm) of O mon district.

The contents of **Arecaidine** in hoblies in different districts of Giong Trom were compared (Table 2). There was a significant difference in **Arecaidine** content between among Mekong district. Giong trom (Ben Tre)had higher concentration of arecoline (0.5 ppm) compare to the other district . Low concentration of arecoline was determined in the BaTri (0.18 ppm) and (0.18ppm) of Binh Minh districtof Vinh Long Province

The contents of arecoline in hoblies in different districts of Giong Trom were compared (Table 2). There was a significant difference in arecoline content between among Mekong district. Giong trom (Ben Tre)had higher concentration of arecoline (2.2 ppm) compare to the other district . Low concentration of arecoline was determined in the BaTri (0.91ppm) and (1.2ppm) of O mon district.

Table 2. Alkaloid Levels Measured in Areca Nut-Containing Products

lines	Guvacine (ppm)	Arecaidine (ppm)	Guvacoline (ppm)	Arecoline (ppm)	total alkaloids
Giong Trom(Ben Tre)	2.48b	0.50a	0.99a	2.22a	6.19a
MO Cay(Ben Tre)	2.61a	0.24c	0.87b	2.02a	5.74b
Ba Tri(Ben Tre)	1.79d	0.18d	0.32c	0.91c	3.19d
Cai Rang(Can Tho)	2.43b	0.28c	0.34c	1.36b	4.4c
O mon (Can Tho)	2.55b	0.41b	0.24d	1.2b	4.4c
Cai Lay(Tien Giang)	2.12c	0.15d	0.32c	1.25b	3.84d
Tra Cu(Tra Vinh)	2.48b	0.15d	0.98a	2.15a	5.84b
Binh Minh (Vinh Long)	1.89d	0.18d	0.32c	1.91b	4.3c

3.3./DPPH activity Free radicals are an intermediate metabolite of various biochemical reactions in human life activities. It has high chemical activity and is an effective defense system of the human body. However, the

excessive accumulation of free radicals that cannot be scavenged in time will attack life macromolecules and various organelles, and cause interhuman damage at the molecular, cellular and tissue level, which can further accelerate the human aging process and cause various chronic diseases (Akbari et al, 2022).Various mechanisms, such as free radical scavenging, capacity reduction, metal ions, and lipid peroxidation inhibition, have been studied to explain how areca nut extract can be used as an antioxidant (Ghasemzadeh et al.,2015). DPPH radical scavenging tests are based on the transfer of electrons from the molecule of the donor radical to the corresponding radical. The DPPH method is the simplest method for measuring the ability of antioxidants to block free radicals. DPPH thoroughly scavenged the effects of all extracts in areca nut increased with increasing concentration (Figure 2). The rate of DPPH significantly , areca nut (34.6 to 66.9%, $p < 0.05$). Areca nut of Cai Rang (Can tho) has the lowest antioxidant capacity for removing DPPH radicals and is significantly different from other sites ($p < 0.05$). The 8- sites extract demonstrated the highest DPPH activity, followed by areca nut extraction. DPPH activity of different sites of areca nut ranges from 34.6 to 66.9%, (figure 2)

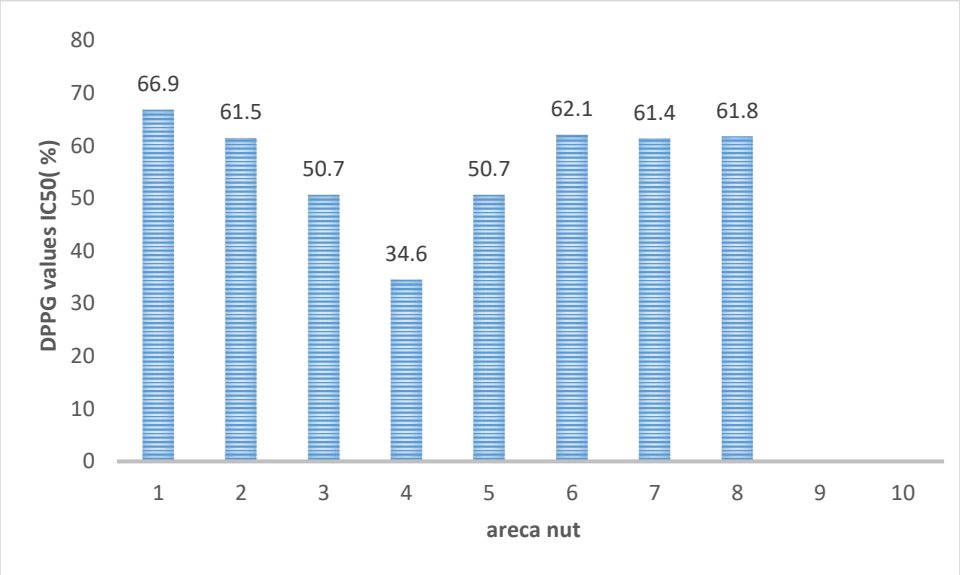

Fig.2: Antioxidant activity represented by $DPPH_{IC50}$ for different sites of areca nut. $DPPH_{IC50}$ value is an effective sample concentration at which DPPH radicals were scavenged by 50%.

IV. DISCUSSION

In our present investigation, we firstly studied TPC, TAC, TFC content (ppm) in Different at samples the from areca nut, and found that the three alkaloids including arecoline, guvacoline and homoarecoline, were predicted to possess good for properties, indicating that these alkaloids may possess good oral absorption and bioavailability. Nowadays, it is well known that an ideal candidate drug should possess some good characteristics of absorptive property and bioavailability besides pharmacological activity (Duchowicz et al .,2007).Guvacine is the most abundant of the four alkaloids measured, regardless of product type, contradicts the prevailing assertion in the literature that arecoline is the primary alkaloid in areca nuts te same with (Shih et al 2010). The results of the analyses revealed substantial variations in the levels of alkaloids across the tested products, with guvacine being the most abundant (1.39-8.16 mg/g), followed by arecoline (0.64-2.22 mg/g), arecaidine (0.14-1.70 mg/g) and guvacoline (0.17-0.99 mg/g). Substantial differences in the relative contribution of individual alkaloids to the total alkaloid content were also observed among the different products. (Vipin et al .,2017).In addition, Franke *et al*.2015 analyzed the aqueous extract of young and mature areca nuts and found significant differences in the total alkaloids and relative levels of individual alkaloids between them. Lower level of total alkaloids was observed in the young green nut compared to the mature nut, with arecoline being the major alkaloid. In the mature nut, however, guvacine was the major alkaloid with almost 3-fold higher concentration than arecoline, which is consistent with our data. For these observations suggest that the alkaloids contribution of differences sites .

V. CONCLUSIONS

Looking at a series of analytical results, the determination of polyphenols in areca nut extract aims to determine the quantitative profile of the quality of areca nut. Significant strides have been made in elucidating the

chemical structure of these bioactive compounds but while mass spectrometry based techniques certainly represent a powerful tool for defining areca nut phenolic profiles, We strongly believe that the research efforts undertaken to date constitute an excellent starting point towards the development of analytical tools aimed at investigating the phenolic, The flavonoid and Anthocyanin fraction of areca nut for demand in medicinal chemistry.

Studies related to antioxidant components may provide pharmacologic importance signifying ethnomedicinal uses of the plant species. The next need research for the geographic areas of raw plant material should also be analyzed and compared in the future research. Since the environmental factors e.g., nutrients and mineral in soil are also effect on the quality and quantity of phytochemical compounds in some species of medicinal plant and areca nut.

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