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QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITIES OF *ARECA CATECHU* NUT HUSK

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Abstract

Areca cathechu nut also locally known as 'pinang' contains countless nutritional and functional components with different bioactivities. The areca nut is covered by fruit shell of husk that contains a lot of fibre. However, its husk was often thrown away and discarded as a waste. Thus, this study attempts to analyse antioxidant capacity of areca nut husk. The areca nut husk was extracted using three types of solvent of different polarity including *n*-hexane, ethyl acetate and ethanol. Qualitative phytochemical screening was carried out to determine the phytochemical constituents present in the extracts. The antioxidant capacity of areca nut husk was then evaluated quantitatively by determining total phenolic content and DPPH assay, respectively. Ethanolic extract revealed the presence of alkaloid, steroids, terpenoids, tannins, saponins, glycoside, flavonoid and phenol. Folin-Ciocalteu method was used to quantify the total phenolic content in the extracts using a calibration curve of gallic acid. Total phenolic content of ethanolic extract (558.33 mg GAE/g) was found higher than that of ethyl acetate and *n*-hexane extracts which were 436.48 mg GAE/g and 290 mg GAE/g, respectively. The ethanolic extract exhibited higher DPPH radical scavenging activity compared to ethyl acetate and *n*-hexane extracts with 72.14% ± 0.17 percentage of inhibition and IC_{50} value of 118.33 ± 0.51. The results obtained from this study revealed the potential of this underutilized areca nut husk as promising natural antioxidant.

Keywords: *Areca catechu*; areca nut husk; phytochemical; antioxidant

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Introduction

Antioxidant is a substance that can prohibit or delay the oxidation of an oxidizable substrate in a chain reaction. It has stabilizing effect that appear to be very crucial in the prevention of many diseases (Zhang *et al*., 2009). According to Farhat *et al*. (2013), antioxidants are significantly used to reduce oxidative stress; the stress that may affect and damage biological molecules. Antioxidant can be divided into two types which are synthetic and natural antioxidants. Synthetic antioxidants are widely used in the food industry to extend shelf life. However, the use of synthetic antioxidant was restricted due their toxicity and carcinogenic effects (Zhang *et al*., 2009). Nowadays, interest in finding natural antioxidants that have less or no negative side effects has greatly increased (Diem *et al*., 2013). The number of antioxidant compounds synthesized by plant as secondary products, mainly phenolic, serve as plant defence against reactive oxygen species (ROS) (Zhang *et al*., 2009).

Areca catechu Linn. also known as betel nut, Palm or Pinang is a species of slender palm which grows and widely distributed in various part of Southern and Southeast Asia including China, India, Indonesia, Malaysia and Africa, etc. (Wang *et al*., 2021). It comes from the genus of *Areca* and family of Arecaceae. Areca nut is the dark red seeds (kernels) of *A. catechu* and it is one of the famous chewable items with the purpose of dispersing accumulated fluid in the abdominal cavity and killing worms. It were frequently used in traditional herbal medicine. The areca nut contains countless nutritional and functional components with different bioactivities (Hu *et al*., 2022). Figure 1 shows the areca nut that commonly grown in Malaysia. Many researchers used nuts, young shoots, buds, leaves, or roots of areca in various medicinal field (Zhang *et al*., 2014). Each part has different benefit and usage. Areca nut has been used as therapeutic agent to treat many diseases such as leucoderma, anaemia and obesity. These pharmacology activities are contributed to abundant phenolic compounds in the areca nuts.

Extensive investigations have verified that the extract of areca nut comprises many bioactive compounds, such as phenolics, flavonoids, and polysaccharide, etc. These compounds have contributed to multiple pharmacological activities, such as antimicrobial, antioxidant, anti-osteoporosis, anti-inflammatory, and anti-parasitic effects, etc (Hu *et al*., 2022). In previous study, hydroalcoholic extract of areca nut was reported to possess as significant analgesic, anti-inflammatory and *in vitro* antioxidant activities (Bhandare *et al*., 2010; Wang *et al*., 2021). It was also reported that the phenolic content and antioxidant activities of methanolic extract of areca nut was higher than that of its root (Hamsar *et al*., 2011). Areca nut are covered with fruit shell of husk that contains a lot of fibre. About 60 - 80% of total volume and weight of areca nut is the husk. However, the husk always thrown away as a waste and the underutilized areca nut husk often left to decay on the field after harvesting which lead to landfill problem (Subramani *et al*., 2019).

Figure 1. Fruit of areca nut (Tile *et al*. 2017).

The phenolic compounds or also known as polyphenols are phytochemicals that exhibit antioxidant activity and consequently give a beneficial physiological effect. Polyphenols are the most prevalent antioxidant phytochemicals in the plant kingdom and reportedly possess both singlet oxygen quenching and radical scavenging activities. Many studies have been conducted on the areca nut which prove to contains many phenolics and tannins compounds (Zhang *et al*., 2014). However, to date, the study on the areca nut husk is still too little and not well established. In this study, phytochemical screening and determination of antioxidant activities were carried out on areca nut husk extract. Three types of solvent of different polarity were used to extract areca nut husk using maceration extraction method. Futhermore, the phytochemical screening and antioxidant activities including total phenolic content and DPPH assay of areca nut husk extract were also determined.

Methods

Materials

Areca nut husk was collected locally from Parit Raja district, Batu Pahat, in the state of Johor, Malaysia. The areca husk was manually separated from their fresh fruit and cleaned. The husk then was dried, ground into smaller particles and was kept in a desiccator until further analysis. The solvents such as ethanol, ethyl acetate and *n*-hexane were purchased from Merck (Darmstadt, Germany), respectively.

The standard ascorbic acid and gallic acid were purchased from Sigma-Aldrich (M) Sdn. Bhd. All other chemicals used in this study were of analytical grade.

Extraction of areca nut husk

The areca nut husk was extracted using ethanol (polar), ethyl acetate (semi polar) and *n*-hexane (non-polar) solvents. The maceration method was used to extract phytochemicals compounds from the areca nut husk. Approximately 40 g of the husk particles was soaked in solvent 1:10 (w/v) and left for three days. After 72 hours, the solution was filtered and each filtrate was concentrated using rotary evaporator to remove solvents (Yeo *et al*., 2014).

Preliminary qualitative phytochemical analysis

Phytochemical screening was carried out on the crude extracts to detect the presence of numerous bioactive constituents including alkaloids, flavanoids, steriods, terpenoids, saponins, tannins, glycosides and phenolics. The phytochemical screening test was conducted according to Riaz *et al*. (2012) with slight modifications. The alkaloid was tested by treating small quantity of the crude extracts with few drops of HCl and was filtered. The filtrates then was tested with Wagner's reagent. The formation of alkaloid in the crude extracts was indicated by the presence of reddish brown precipitate. As for the steroids test, the steroid content in crude extract was determined by adding 2 mL of acetic anhydride and 2 mL of concentrated H_2SO_4 to 2 mL of crude extracts. The presence of steroids was confirmed by the color change from violet to green. For terpenoids test, chloroform (2 mL) was mixed together with 1 mL of crude extracts. After that, 3 mL of concentrated H_2SO_4 was added. The formation of layer, a reddish brown coloration at the interface is indicated the presence of terpenoids. In the test for tannins, crude extracts (2 mL) was treated with 5% of $FeCl_3$ and the presence of tannins was confirmed with deep blue-black or blue green color reactions. As for saponins test, about 3 mL of distilled water was shaken with 2 mL of crude extract. The presence of saponin was shown by production of foam that had persisted for 10 minutes. In the test for glycosides, crude extract was mixed with 2 mL of glacial acetic acid containing 2 drops of 2% solution of $FeCl_3$. The mixture was poured into another test tube containing 2 mL of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of glycosides (Rajamani *et al*., 2016). For flavonoid test, about 2 mL of crude extract was treated with few drops of concentrated H_2SO_4 and 5 mL of ammonia solution. The appearance of yellow colouration was confirmed the presence of flavonoid. Finally for phenols test, the phenolic content was investigated by adding 3 or 4 drops of 5% $FeCl_3$ solution to 2 mL of crude extract. The presence of phenols was shown by the formation of dark green color.

Total phenolic content

Total phenolic content of the ethanolic, ethyl acetate and *n*-hexane areca nut husk extracts were determined according to Rao *et al*. (2016) with minor modifications. About 10 mg of the extract was liquefied in 10 mL of the solvent to yield a concentration of 1 mg/mL. About 500 uL of sample was added to 2.5 mL of 10% Folin-Ciocalteu's reagent. After 5 min, 2.5 mL of 7.5% Na_2CO_3 was added. Blank was prepared by mixing 500 uL of distilled water, 2.5 mL of 10% Folin-ciocalteu's reagent and 2.5 mL of 7.5% Na_2CO_3. The reaction mixture was placed in the dark for 2 h, and the absorbance was recorded at 725 nm (UV-Vis spectrophotometer, PG Instrument, T80/T80+ spectrophotometer). The same procedure was repeated for the standard solution of gallic acid with different concentration (100, 50, 25, 12.5, 6.25 ug/mL) and the calibration line was constructed (Shen *et al*, 2017). Based on the measured absorbance, the concentration of phenolics (ug/mL) was read from the calibration line and the content of phenolic in the extract was expressed in terms of gallic acid equivalent (ug of gallic acid/ug of extract).

DPPH radical scavenging assay

DPPH is the most easy, simple and reasonably costly method and hence it might have been used mostly for the antioxidant evaluation of a sample. DPPH radical scavenging assay of areca nut husk extract was determined according to the method adapted from Shen *et al*. (2017) with some modifications. Briefly, 200 µL of sample with different concentration (1000, 500, 250, 125, 62.5, 31.3, 15.63, 7.8

ug/mL) was mixed with 3.8 mL of DPPH in methanol. After, 30 min incubation in darkness, the absorbance at 517 nm was measured against methanol blank. Ascorbic acid with different concentration (1000, 500, 250, 125, 62.5, 31.3, 15.63, 7.8 ug/mL) was used as standard. The blank was methanol, and DPPH in methanol was used as the negative control. Ascorbic acid, synthetic antioxidant was used as a positive control. The percentage DPPH inhibition was calculated using Eq.1:

$$\text{Inhibition (\%)} = \frac{(A0-A1)}{A0} \times 100\% \qquad \text{(Eq. 1)}$$

where A_0 is absorbance of negative control, A_1 is absorbance of the extract or standard. The experiment was performed in triplicate. The percentage radical scavenging activity versus extract concentration curve was then plotted and the concentration of the sample that was required for 50% radical scavenging activity was determined and expressed as the IC_{50} value. Lower IC_{50} values indicated high antioxidant capacity.

Result and Discussion

Preliminary qualitative phytochemical analysis

In analysing the group of compounds present in the areca nut husk, the extract from three different types of solvent of different polarity (ethanol, ethyl acetate and *n*-hexane) were used for comparison in determining the presence of secondary metabolites of alkaloids, steroids, terpenoids, tannins, saponins, glycosides, flavonoids and phenols. The results were recorded and tabulated in Table 1. It was found that the crude extract from ethanol gave the positive result for all tests. All the bioactive constituents present in ethanolic areca nut husk were found almost similar to those present in the aqueous extract of areca nut. The aqueous extract areca nut also proved to contain alkaloids, saponins, phenols and glycosides (Ramajani *et al*., 2016). Sarikurkcu *et al*. (2020) have investigated the effects of different solvents (ethyl acetate, methanol and water) on the extraction of phenolic compounds and verified that methanol was the best solvent. In general, solubility of secondary metabolites in plant matrix increased with polarity of the solvents (Boulekbache-Makhlouf *et al*., 2013). Water with good polarity was not a much more efficient extraction solvent than organic solvent. The extraction yields of bioactive compounds are related to solvent types and the solubility of those components in these solvents (Suchinina *et al*., 2011). In this study, ethanol is a higher polarity type of solvent followed by ethyl acetate and *n*-hexane. Solubility of secondary metabolites compounds of areca nut husk extract in ethanol is higher than that of ethyl acetate and *n*-hexane, hence, showed the presence of more group of compounds than that of ethyl acetate and *n*-hexane solvents. Ethanol being organic and nontoxic might have higher frequency of use for extraction purpose compared to methanol as toxicity of methanol limits its use in some extraction, while the use of water in the extraction needs a different step of freeze drying to remove it from extract after extraction (Alam *et al*., 2013).

Total phenolic content

Total phenolic contents recorded from three different extracts are given in Table 2. The values varied from 290 to 558.33 mg GAE/mL, with reference to gallic acid standard curve ($y = 0.00676 + 0.00957x$, $r^2 = 0.9982$) as shown in Figure 2. Total phenolic content in ethanolic extract is the highest compared to ethyl acetate and *n*-hexane. Phenolic compounds that contained in the plant have redox properties that allow them to act as antioxidant. The results in Table 2 show that the ethanolic extract gave total phenolic content of 558.33 ± 2.88 mg GAE/g which is higher than ethyl acetate and *n*-hexane extract which approximately 436.48 ± 3.00 mg GAE/g and 290 ± 3.00 mg GAE/g, respectively. Higher phenolic content in ethanol extract is responsible for bioactivity and expected to exhibit good result in antioxidant activities. This is because plant extracts with higher levels of total phenolics exhibit greater free radical scavenging (Ghasemzadeh *et al*., 2011).

Wang *et al.* (2021) have conducted the study on *in vitro* antioxidant activities of the areca nut extract extracted by 50% ethanol/acetone. The total phenolic content obtained was 57.92 mg GAE/g with high amount of bioactive compounds including catechin, quercetin, qrecoline, guvacoline and guvacine.

Broad polarity of solvent are increasingly used to recover many important bioactive compounds from medicinal plants (Ruegas-Ramón *et al*., 2017). The ethyl acetate and *n*-hexane solvents, due to their lower polarity will resulted lower solubility of secondary metabolites, thus, reduced the total phenolic contents, respectively. The result showed that ethanolic extract of areca nut husk form this study could be considered as better potential antioxidant agent due to its high total phenolic content recorded compared to the ethyl acetate and *n*-hexane extracts.

Table 1. Phytochemical analysis of areca nut husk extracts.

Extract	Compounds	Results	Observation
n-hexane	Alkaloids	(+)	Reddish brown precipitate formed
	Steroids	(+)	Color change to green
	Terpenoids	(+)	Two layers formed, reddish brown coloration at interface
	Tannins	(-)	Yellow color formed, light green precipitate
	Saponins	(-)	No foam formed
	Glycosides	(+)	Light green color, brown ring at interphase
	Flavonoids	(+)	Yellow coloration formed
	Phenols	(-)	Color change to light green
ethyl acetate	Alkaloids	(+)	Reddish brown precipitate formed
	Steroids	(-)	No change
	Terpenoids	(+)	Two layers formed, reddish brown coloration at interface
	Tannins	(+)	Color change to green
	Saponins	(+)	Foam formed
	Glycosides	(-)	No change
	Flavonoids	(-)	No change
	Phenols	(+)	Dark green color formed
Ethanol	Alkaloids	(+)	Reddish brown precipitate formed
	Steroids	(+)	Color change to dark green
	Terpenoids	(+)	Two layers formed, reddish brown coloration at interface
	Tannins	(+)	Color change to greenish brown
	Saponins	(+)	Foam formed
	Glycosides	(+)	Two layers formed, reddish brown coloration at interface
	Flavonoids	(+)	Yellow coloration formed
	Phenols	(+)	Dark green color, brown precipitate formed

*Note: (+): presence; (-): absence

Table 2. Total phenolic content of areca nut husk extracted by ethanol, ethyl acetate and *n*-hexane.

Type of solvent used	Total phenolics content (mg GAE/g)
Ethanol	558.33 ± 2.88
Ethyl acetate	436.48 ± 3.00
n-hexane	290.00 ± 3.00

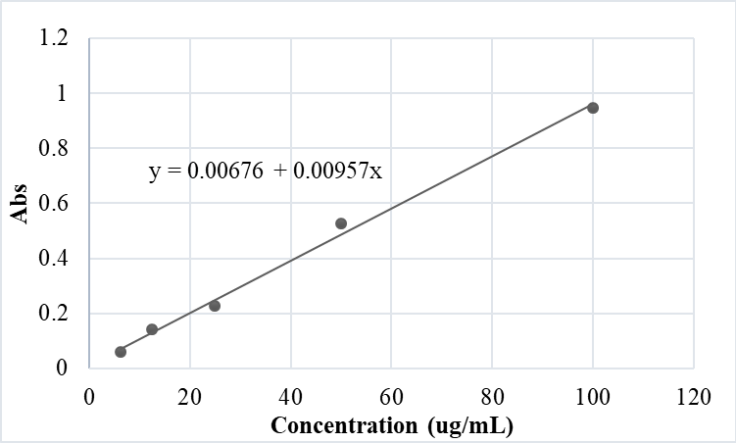

Figure 2. Calibration curve of gallic acid

DPPH radical scavenging assay

Before DPPH radical scavenging assay was done, DPPH screening method was carried out to confirm if the extract has an active compound or not. Methanol was used as a blank while ascorbic acid was used as a standard. All samples were run in triplicate. The percentage inhibition were calculated against blank based on the absorbance and the results are tabulated in Table 3.

Table 3. Percentage inhibition of DPPH screening of ascorbic acid, ethanol, ethyl acetate, *n*-hexane extracts.

Extract	Absorbance at 1000 ppm	Percent inhibition (%)
Ascorbic acid	0.082	96.57
Ethanol	0.241	70.64
Ethyl acetate	0.323	57.26
n-hexane	0.538	22.16

Based on the results, ethanol and ethyl acetate extracts are considered to have active compound for antioxidant scavenging activities due to the percentage inhibition which is more than 50% at concentration of 1000 ppm. While *n*-hexane was found inactive due to lower percentage inhibition that is less than 50%. Extract from ethanol and ethyl acetate were then proceed for DPPH radical scavening assay.

DPPH radical scavenging assay was used to evaluate the antioxidant properties, as the method is much more simpler and convenient. Stable free radical can be found in DPPH molecule. The antioxidant properties that presence allow the electron to be donated to DPPH which led the change in color of the solution from purple to yellowish. In this study, the reduction potential of areca nut husk extract can be confirmed. Scavenging activity of DPPH is based on one-electron reduction which represents the free radical reducing activity of antioxidants. Ascorbic acid, a synthetic antioxidant were used as positive control. All samples were run in triplicate. Different concentration (1000, 500, 250, 125, 62.5, 31.3, 15.63 and 7.8 ppm) of sample were mixed with 3.8 mL DPPH solution and then incubated for 30 minutes before analysis using UV-Vis. The IC_{50} value was calculated and lowest IC_{50} was detected in the ethanolic extract followed by ethyl acetate extract as tabulated in Table 4. As the lower IC_{50} value possesses a higher antioxidant activity, the ethanolic extract has a higher ability to scavenging free radical compared to ethyl acetate and *n*-hexane extracts. It can be concluded that the extracts from high polarity solvents (ethanol) were much more effective radical scavengers than those that using less polarity solvents (ethyl acetate and *n*-hexane) (Ghasemzadeh *et al*., 2011). High antioxidant activity

showed by the ethanolic extract has a positive relationship with total phenolic content, where high total phenolic content gives a high antioxidant capacity due to the linear correlation between the two parameters.

The plots of absorbance in different concentrations of extracts are shown in Figure 3. Significant reductions in absorbance were observed when the concentration extracts and standard ascorbic acid were increased indicating significant reduction of the concentration of the DPPH radical in the reaction mixture due to the free radical scavenging activity (Hartwig *et al*., 2012). DPPH is able to accept an electron or hydrogen radical to form a stable diamagnetic molecule. Changes in color, from purple to yellow indicates a decrease in absorbance of DPPH radical (Figure 4). This demonstrated that the antioxidant found in a mixture solution interact with free radicals (Jadid *et al*., 2017). Figure 5 shows the percentage inhibition was increased in a concentration dependent manner. The 1000 ppm extract showed the best antioxidant activity, where the ascorbic acid (positive standard) was the highest (99.41 ± 0.20%), followed by ethanol and ethyl acetate (72.14 ± 0.17% and 59.56 ± 0.20%), respectively (Table 1). The antioxidant measurement has revealed that ethanolic extract of areca nut husk has greater activity than ethyl acetate extract.

Table 4. IC_{50} of ethanolic and ethyl acetate extracts.

Extract	**Percentage inhibition at 1000 ppm (%)**	**IC_{50}**
Ascorbic acid	99.41 ± 0.20	30.90 ± 0.24
Ethanol	72.14 ± 0.17	118.33 ± 0.51
Ethyl acetate	59.56 ± 0.20	992.53 ± 0.64

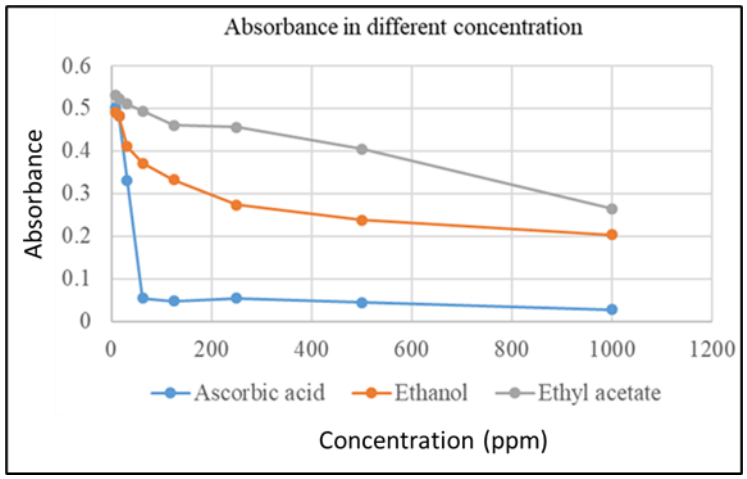

Figure 3. Absorbance of ascorbic acid (positive standard), ethanol and ethyl acetate extracts in different concentrations.

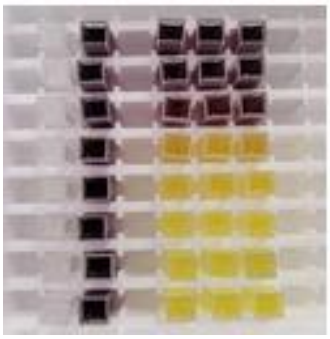

Figure 4. Color of mixture solution changes from purple to yellow indicates decrease in absorbance of DPPH radical.

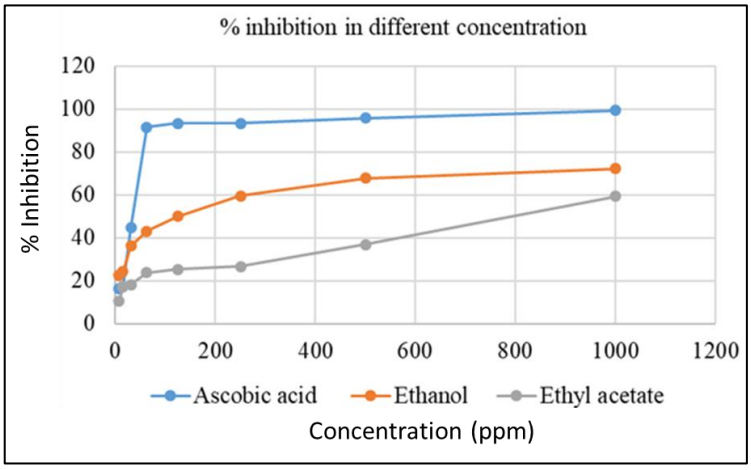

Figure 5. Percentage inhibition of ascorbic acid (positive standard), ethanol and ethyl acetate extracts in different concentrations

Conclusion

In this study, ethanolic extract of areca nut husk showed the most number of biologically active constituents present as compared to *n*-hexane and ethyl acetate extracts. The detected bioactive contituents present are including alkaloids, steroids, terpenoids, tannins, saponins, glycosides, flavonoids and phenols. Moreover, among all the three type of solvents, ethanolic extract of areca nut husk was also found to exhibit the best antioxidant activities with total phenolic content of 558.33 ± 2.88 mg GAE/g, percentage inhibition of 72.14%± 0.17 and IC_{50} value of 118.33 ± 0.51. Thus, the finding from this study revealed the high potential of areca nut husk to be utilized as natural antioxidant and a detailed study of its biological function will further substantiate its medicinal values.

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Author Contribution

All authors contributed significantly to this work and are in agreement with the content of the manuscript. NA Sapaat – Writing–original draft, data curation; A Abdullah – Supervision; writing–review & editing; SAISM Ghazali – writing–review & editing; NM Nor – Validation, writing–review & editing.

Conflict of Interest

The authors declare no conflict of interest.

References

Alam, M. N., Bristi, N. J. and Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal* 21: 143–152.

Bhandare, A. M., Kshirsagar, A. D., Vyawahare, N. S., Hadambar, A. A. and Thorve, V. S. (2010). Potential analgesic, anti-inflammatory and antioxidant activities of hydroalcoholic extract of *Areca catechu* L. nut. *Food and Chemical Toxicology* 48: 3412–3417.

Boulekbache-Makhlouf, L., Slimani, S., and Madani, K. (2013). Total phenolic content, antioxidant and antibacterial activities of fruits of Eucalyptus globulus cultivated in Algeria. *Industrial Crops and Products* 41: 85–89.

Diem, Q., Elisa, A., and Tran-nguyen, P. L. (2013). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, *22*(3), 296–302.

Farhat, M. B., Landoulsi, A., and Chaouch-Hamada, R., (2013). Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats. *Industrial Crops and Products*, 904–914.

Ghasemzadeh, A., Jaafar, H. Z. and Rahmat, A. (2011). Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale Roscoe*) extracts. *Journal of Medicinal Plants Research*, *5*(7), 1147–1154.

Hamsar, M. N., Ismail, S., Mordi, M. N., Ramanathan, S., and Mansor, S. M. (2011). Antioxidant activity and the effect of different parts of areca catechu extracts on Glutathione-S-Transferase activity in vitro. *Free Radicals and Antioxidants* 1 (1): 28–33.

Hartwig, V. G., Brumovsky, L. A., Fretes, R. M., and Boado, L. S. (2012). A novel procedure to measure the antioxidant capacity of *yerba maté* extracts. *Ciência e Tecnologia de Aliment, Campinas* 32 (1): 126–133.

Hu, X., Wu, L., Du, M., and Wang, L., (2022). Eco-friendly synthesis of size-controlled silver nanoparticles by using Areca catechu nut aqueous extract and investigation of their potent antioxidant and anti-bacterial activities. *Arabian Journal of Chemistry* 15 (103763): 1–15.

Jadid, N., Hidayati, D., Hartanti, S. R., Arraniry, B. A., Rachman, R. Y., and Wikanta, W. (2017) Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay. Proceeding of International Biology Conference, AIP Conference Proceeding 1854, 020019.

Rajamani, R., Arts, K., and Kuppusamy, K. S., (2016). Preliminary phytochemical screening of aqueous extract of betel nut and betel leaves. *International Journal of Biosciences and Nanosciences*, *3*(1), 14–18.

Rao M.U., Razak, A.M. and Khamsah, S. P., (2016). Phytochemical screening, total flavonoid and phenolic content assays of various solvent extracts of tepal of *musa paradisiaca. Malaysian Journal of Analytical Sciences*, *20*(5), 1181–1190.

Riaz, T., Abbasi, M. A., Aziz-Ur-Rehman, Shahzadi, T., Ajaib, M., and Khan, K. M., (2012). Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscosa*. *Journal of the Serbian Chemical Society*, *77(1)*, 1–17.

Ruegas-Ramón M., Figueroa-Espinoza, M. C. and Durand, E. (2017). Application of deep eutectic solvent (DES) for phenolic compounds extraction: Overview, challenges, and opportunities. *Journal of Agricultural and Food Chemistry* 65 (18): 3591–3601.

Sarikurkcu, C., Sahinler, S.S., Ceylan, O. and Tepe, B. (2020). *Onosma pulchra*: phytochemical composition, antioxidant, skin-whitening and anti-diabetic activity. *Industrial Crops and Products* 154, 112632.

Shen, X., Chen, W., Zheng, Y., Lei, X., Tang, M., Wang, H., and Song, F., (2017). Chemical composition, antibacterial and antioxidant activities of hydrosols from different parts of *Areca catechu L* . and *Cocos nucifera* L . *Industrial Crops and Products*, *96*, 110–119.

Suchinina, T. V., Shestakova, T. S., Petrichenko, V. M. and Novikova, V. V. (2011). Solvent polarity effect on the composition of biologically active substances, UV spectral characteristics, and antibacterial activity of *Euphrasia brevipila* herb extracts. *Pharmaceutical Chemistry Journal* 44: 683–686.

Subramani, B. S., Shihari, S., Manu, B., Babunarayan, K. S., (2019). Evaluation of pyrolyzed areca husk as a potential adsorbent for the removal. *Journal of Environmental Management*, *246*, 345–354.

Wang, R., Pan, F., He, R., Kuang, F., Wang, L., and Lin, X., (2021). Arecanut (*Areca catechu* L.) seed extracts extracted by conventional and eco-friendly solvents: Relation between phytochemical compositions and

biological activities by multivariate analysis. *Journal of Applied Research on Medicinal and Aromatic Plants* 25 (100336): 1–12.

Yeo, Y. L., Chia, Y. Y., Lee, C. H., Sow, H. S., and Yap, W. S., (2014). Effectiveness of maceration periods with different extraction solvents on in-vitro antimicrobial activity from fruit of *Momordica*. *Journal of Applied Pharmaceutical Science*, *4*(10), 16–23.

Zhang, W., Huang, W., Chen, W., Han, L., and Zhang, H., (2014). Optimization of extraction conditions of areca seed polyphenols and evaluation of their antioxidant activities. *Molecules*, 16416–16427.

Zhang, W., Li, B., Han, L., and Zhang, H., (2009). Antioxidant activities of extracts from areca (*Areca catecthu* L .) flower , husk and seed. *African Journal of Biotechnology*, *8*(16), 3887–3892.