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Applications of Date (*Phoenix dactylifera* L.) Fruits as Bioactive Ingredients in Functional Foods

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The demand for food bioactive ingredients from natural sources with low cost and broad range of applications is extremely increasing. In this study, five date varieties marketed in Malaysia were evaluated for their potential applications as functional food ingredients. The date fruits were extracted with 80% ethanol, and biological activities including antioxidant, antibacterial and anti-elastase activity were determined by referenced methods. Results of the study showed that the date variety Piyarom demonstrated the highest antioxidant activity (IC50 11.3 μg/ mL), strong antibacterial activity towards tested pathogens that was ranged in 62-76 %, and strong anti-elastase activities (61.2±4.9%). The varieties Ajwa and Anbar showed moderate antioxidant and antibacterial activity, while Deglet Nour and Rabbi exhibited low activities. The results revealed high potential of Piyarom extract to be used as ingredient for functional food applications and fulfilled the high demand for natural functional food ingredients.

Keywords: Antioxidant, Antibacterial activity, Anti-aging, anti-elastase, Functional food.

The date fruits (*Phoenix dactylifera* L.) are important economic commodity that is regularly consumed by Middle East populations for the health benefits and pleasant taste. The main food applications of date fruits are to be consumed as fresh and/or processed in many products such as date jam, date butter, date bars, date chutney, date relish, and date pickles, date oil and date coffee (Al-Mamary *et al*., 2014; Benmeddour *et al*., 2013). The date paste is widely used as filling for bakery products (Ghnimi *et al*., 2017), while date syrup is well known natural sweetener in dairy products (Kazemalilou and Alizadeh, 2017). Great number of research has been carried out on the date applications in different food formulas such as in meat, bakery, and dairy products. Date fruits were observed to enhance the nutritional value and the technological quality of the products (Manickavasagan *et al*., 2012). Nevertheless, date fruits have high potential to be used as functional food ingredient due to their properties including the high content of fiber, high content of bioactive compounds, abundant production during seasons, and broad range of applications (Ghnimi *et al*., 2017). Moreover, date fruits have many health benefits regardless of their varieties because of the nutrients content such as carbohydrates, minerals, proteins, lipids, moisture, and phenolic compounds (Al-Shahib and Marshall, 2003). In addition, date fruits phytochemicals are reported to have biological activities, such as antioxidant and anti-mutagenic (Vayalil, 2002), anti-carcinogenic (Rahmani *et al*., 2014), antimicrobial (Taleb *et al*., 2016), and anti-inflammatory (Tang *et al*., 2013).

On the other hand, a great number of researches demonstrated the correlation between the consumption of functional food rich with phytochemicals and the risk reduction of diet related diseases (Plaza *et al*., 2008). However, the extraction of the bioactive compounds and the

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good recovery of the compounds are crucial steps the preparation of the ingredients for functional food applications. The extraction solvent to be considered should facilitate high recovery of the bio-active compounds from the fruit. In the last two decades, modern consumer increased awareness regarding healthy diets and led to high demand for minimally processed functional foods. The high demand for functional foods resulted in increasing the demand for sustainable supply of functional food ingredients. Additionally, several chemically synthetic antioxidant and preservation agents are reported to have bad effects on consumers as observed with high exposure. Therefore, the aims of this study were to determine the nutritional value and proximate analysis of five date fruits varieties marketed in Malaysia. Moreover, the antioxidant, antibacterial and anti-elastase activities were evaluated for potential applications as natural ingredient in functional foods.

MATERIALS AND METHODS

Proximate analysis

Proximate analysis of the date fruits was determined by following the method of Association of Official Analytical Chemists (Flowers *et al*., 2006). The fresh fruits were subjected to the proper methods including the moisture (method 934.06), protein by Kjeldahl nitrogen (method 920.152), and ash (method 940.26). Total lipids were determined following the method as described by Bligh and Dyer procedure (Shahidi, 2001). Total carbohydrate was calculated by subtracting the total percentage value of other measurements from 100. Proximate analyses were expressed as grams per 100 g of fresh weight.

Sample extraction

Five date varieties marketed in Malaysia were purchased from local suppliers in Kuala Lumpur, Malaysia on May 2016. The varieties selected for this study were Piyarom and Rabbi imported from Iran, Anbar and Ajwa from Saudi Arabia and Deglet Nour from Tunisia. The date fruits were cleaned, the seeds removed and the edible parts were crushed into small pieces and then dried in oven at 40 °C until constant weight was reached. The dry dates sample (10 g) was dissolved in 80% ethanol and stirred at room temperature for 24 h. The extracts were filtered using filter paper (Whatman's No 1) and the solvent was evaporated using rotary evaporator at 40 °C and then dried at 40 °C for 72 h. The dried extract was then kept in the dark at 4 °C until further analysis.

Sugar content

Sugar content of date fruit extracts was determined using HPLC (Waters 486) equipped with the column Purospher® STAR NH2 (5 µm) (Merck, United States) by following the method described by Alasalvar *et al*., (2003). Dates sugars were extracted by acetonitrile/water in the ratio of 1:1, v/v for 2 min. The extract was placed in a water bath at 55-60 °C for 15 min. the extract further filtered using filter paper (Whatman's No 541) and the extraction was repeated three times. The mobile phase was acetonitrile and deionized water at the ratio of 75:25 v/v, and the flow rate was 1 mL·min^{-1}. The sugars were quantified from the peak areas compared to the calibration curve of the corresponding standards prepared in the range of 1 to 10 mg/100 mL of acetonitrile/water 1:1, v/v.

Mineral content

Minerals and trace elements of date fruits were digested in a HNO_3/H_2O at ratio of 5:1, and minerals were determined by mass spectrometry (ICP-MS, Perkin Elmer SCIEX, ELAN DRC-e, Scientific, Bremen, Germany). A standard mixture of Multi-Element Calibration Standard 3, No Hg was prepared in 1% nitric acid.

Antioxidant activity

The antioxidant activity of date fruit extract was determined following ABTS radical activity ($ABTS^+$) method described by Cai *et al*., (2004). The solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulphate, and incubated at room temperature for 16 h in darkness. The solution of $ABTS^+$ was diluted with 100% methanol and the absorbance was measured at 734 nm. The extract was dissolved in methanol to obtain concentration of 5 mg/mL and mixed with $ABTS^+$ solution at different concentrations. The mixture was kept at 23 °C for 6 min and the absorbance at 734 nm was immediately recorded. Antioxidant activity was calculated using the following equation:

% ABTS Inhibition = [(Abs control – Abs sample)] / Abs control X 100

Antibacterial activity

The antibacterial activity of the date extract that is dissolved in distilled water was

determined in liquid system following the antibacterial method described by Aween *et al*., (2012). The date fruits extract was tested against *Pseudomonas aeruginosa* ATCC10415, *Bacillus subtilis* ATCC11778, *Staphylococcus aureus* ATCC6538, *Escherichia coli* ATCC11229, *Salmonella typhimurium* ATCC14028. The freeze dry date fruits extracts were dissolved in distilled water (1 mg/mL) and 100 µL extract was mixed with 100 µL nutrient broth containing 10^6 of each selected pathogen separately in 96 wells micro-titer plates, while the negative control was nutrient broth and positive control was chlorexidin 1%. The plates were incubated at 37°C for 24 h then optical density was measured at 600 nm by using ELISA reader. The antibacterial activity was calculated by subtracting the 0 h readings from the 24 h readings, then using the following mathematical equation;

Antibacterial activity %=([negative control-LAB culture/negative control*100])

Anti-elastase activity

Anti-elastase activity of date extract was evaluated following the method described by Kraunsoe *et al*. (1996), with some modifications. Briefly, 25 µl of 0.1 M HEPES buffer (pH 7.5) was mixed with 1.4 mg/ml of date extract and 1 µg/ml elastase in 96-well plates. Negative control was 25 µL elastase and 50 µL HEPES buffer. The positive controls was mixture of 10 µg/ml lafin/N-methoxysuccinyl- Ala-Ala-Pro-Chloro, 25 µL elastase and 50 µL HEPES buffer. The solvent controls contained 25 µL each of elastase, HEPES buffer and either 10% MeOH, 10% DMSO or 30% DMSO. Extract controls (25 µL) were mixed with 150 µl HEPES buffer and served as colour controls. The plates were incubated for 20 min at room temperature, then 100 µL of the substrate N-Methoxysuccinyl- Ala-Ala-Pro-Val-p-nitroanilide (1 mM) was added and incubated for a 40 min at 25 °C. Absorbance was measured at 405 nm using spectrophotometer and the percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = [(A_{control} - A_{test\ sample}) / A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of buffer, elastase + solvent and A_{sample} is the absorbance of buffer, elastase + extract or elafin/ N-Methoxysuccinyl-Ala-Ala-Pro-Chloro.

Statistical analysis

All data were analysed by MINITAB16. One-way analysis of variance (ANOVA) employed to determine the statistical differences between the samples and the controls. The mean values difference was set at $p < 0.05$ which is considered significant. Additionally, multivariate data analysis was performed by the use of SIMCA-P software (Umetrics, Sweden).

RESULTS AND DISCUSSION

Proximate analysis

Proximate analysis carried out to determine the variation in the composition that can affect the nutrient content and biological activity of the sample. The results showed high variation among the studied samples (Table 1). Piyarom showed the highest protein and lipids content which was 3.3 g/100 g, and 0.67 g/100 g respectively. Deglet Nour had the highest content of ash as 2.1 g/100 g, and Ajwa had the highest content of carbohydrates (81.96 g/100 g). The moisture contents were 13.4, 14, 12.8, 17.7, and 15.1 for Ajwa, Rabbi, Piyarom, Deglet Nour, and Anbar, respectively. Previous studies on nutritional value of date fruits showed variation due to the different genetic origin, cultivation methods as well as environmental factors such as temperature, climate and soil. The results of this study are in agreement with the results reported by the previous studies (Al-Farsi *et al*., 2007) (Amira *et al*., 2011) (Baliga *et al*., 2011). The studies showed that date fruits contain sufficiently the amounts of most essential nutrients and could therefore be recommended for regular consumption to compensate the lost nutrients needed by the body (Baliga *et al*., 2011) (Al-Farsi *et al*., 2005). The low level of lipid content and high sugar content of is a good indicator of their nutritional value and health benefits. Furthermore, date fruits are known for their high content of carbohydrates, most of which are in the form of digestible simple sugars such as glucose, sucrose and fructose (Baliga *et al*., 2011). Sugars represent up to about 88% of the constituents of date fruits and high energy is derived from these sugars for the consumers. Nearly 100 g of the date fruit edible flesh provides about 314 kcal of energy (Al-Farsi *et al*., 2005). The high sugar content in the date fruits can used for many food applications.

Yield of extraction

The extracts yield is determined based

on the original sample weight and the final extract weight of the five date fruit varieties. The highest yield was obtained from Piyarom which is 23%, while the lowest yield was for Deglet Nour sample (16%). The other three samples extract yield was Ajwa 20%, Rabbi 21% and Anbara 19%. The extraction yield is very important for any plant extract to estimate the percentage of the total sample that can be used for further analysis. The yield percentage depends on the sample status for example how fresh the sample is, also depends on the solvent used for the extraction and the extraction method. These results are in agreement with the findings from previous studies by Al-Farsi *et al*., (2008) reported that high extraction efficiency is achieved by using ethanol for date fruits extraction, and this is in agreement with a preliminary study on the extraction optimization for date fruits (Unpublished data). Also, these results are in agreement with the findings from previous studies with another study by (Trabzuni *et al*., 2014) revealed that date fruits extracted by ethanol demonstrated significantly high yield ($P<0.05$) in comparison with other solvents.

Sugar content

The content of glucose and fructose

Table 1. Chemical composition of different date varieties

Date variety	Chemical composition (g/100 g) Moisture	Protein	Lipid	Ash	Carbohydrate
Ajwa	13.4 ± 0.55^{cd}	2.6 ±0.3 ^{c}	0.54 ±0.08 ^{b}	1.5 ±0.04 ^{c}	81.96 ±1.26 ^{a}
Rabbi	14.0 ±0.42 ^{c}	3.3 ±0.1 ^{a}	0.24 ±0.74 ^{d}	1.8±0.07 ^{b}	80.62±0.64 ^{b}
Piyarom	12.8 ±0.23^{d}	3.3 ±0.2 ^{a}	0.67±0.001 ^{a}	1.7 ±0.03 ^{b}	81.53 ±0.25 ^{a}
Deglet Nour	17.7±0.32a	2.4±0.1^{cd}	0.25±0.43 ^{d}	2.1 ±0.03 ^{a}	78.44 ±0.61 ^{c}
Anbar	15.1±0.17 ^{b}	2.9±0.1 ^{b}	0.46 ±0.09 ^{c}	1.6 ±0.05 ^{c}	79.94 ±0.66 ^{c}

Means within the same Colum having a common superscript are not significantly different (Pe”0.05)

Table 2. Sugars content (mg/100g DF) of the selected different varieties of date fruits

Date variety	Total sugar	Glucose	Fructose	Sucrose
Ajwa	81.96 ±1.26 ^{a}	47 ± 1.4 ^{b}	46 ± 2 ^{b}	ND
Rabbi	80.62±0.64 ^{b}	47 ± 0.87 ^{b}	48 ± 0.8 ^{b}	ND
Piyarom	81.53 ±0.25 ^{a}	45 ± 2.0 ^{a}	43 ± 0.95 ^{b}	7 ± 1.5 ^{d}
Deglet Nour	78.44 ±0.61 ^{c}	22 ± 1.2 ^{c}	23 ± 1.4^{c}	31±2.8^{c}
Anbar	79.94 ±0.66 ^{c}	47 ± 1.2 ^{b}	42 ± 1.2^{b}	9 ± 2.6 ^{d}

Means ± SD followed by the same letter, within a column are not significantly different ($P>0.05$)

Table 3. Mineral content (mg/100g DF) of the selected different varieties of date fruits

Mineral	Date varieties Ajwa	Rabbi	Piyarom	Deglet Nour	Anbara
Potassium (k)	282.9±1.07 ^{c}	378.59 ±0.36 ^{b}	471.7 ±0.86 ^{a}	192.85 ±0.03 ^{e}	235.08 ±0.69 ^{d}
Sodium (Na)	5.2 ±0.21 ^{c}	6.6 ±0.07 ^{b}	8.6 ±0.10 ^{a}	4.9 ±0.13 ^{c}	1.15 ±0.40 ^{d}
Magnesium (Mg)	36.01 ±0.77 ^{d}	45.17±0.31 ^{b}	49.7 ±0.23 ^{a}	38.4 ±0.94 ^{c}	34.9±0.70 ^{e}
Calcium (Ca)	0.483 ±0.08 ^{b}	0.516±0.040 ^{a}	0.311±0.01 ^{c}	0.14±0.06 ^{cb}	0. 207±0.01 ^{d}
Iron (Fe)	0.13^{d}±0.03	0.21 ±0.01 ^{c}	0.36 ±0.05 ^{b}	0.5 ±0.03 ^{a}	0.26 ±0.013 ^{c}
Selenium (Se)	0.520 ± 0.035^{ab}	0.4256 ± 0.056 ^{b}	0.5708 ± 0.038 ^{a}	0.5297 ± 0.024^{a}	0.4823 ± 0.033^{b}
Zinc (Zn)	1.696 ± 0.17^{c}	2.101 ± 0.26^{b}	2.684 ± 0.17^{a}	0.348 ± 0.019^{d}	1.009 ± 0.14 ^{c}

Means ± SD followed by the same letter, within a column are not significantly different ($P>0.05$).

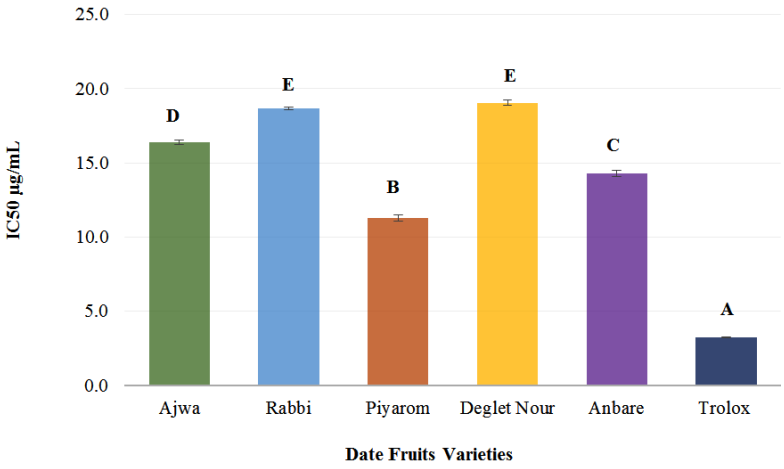

Fig. 1. ABTS antioxidant capacity of the selected different varieties of date extracts
Mean of different letters are significantly different at P< 0.05 Data are presented as mean ± SD of triplicates

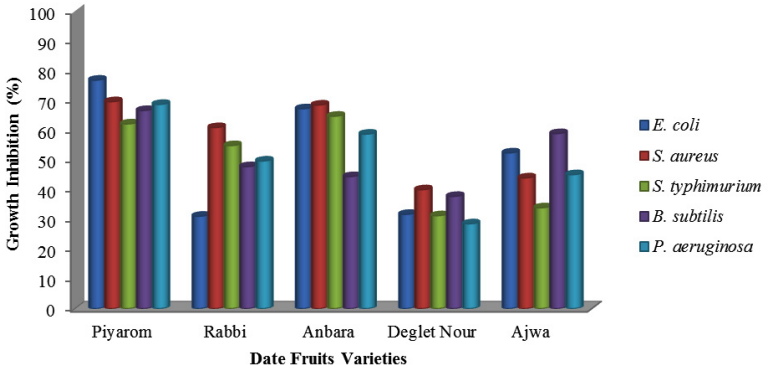

Fig. 2. Antibacterial activity of the selected date fruits varieties against 5 pathogens after the incubation for 24 h at 37 °C

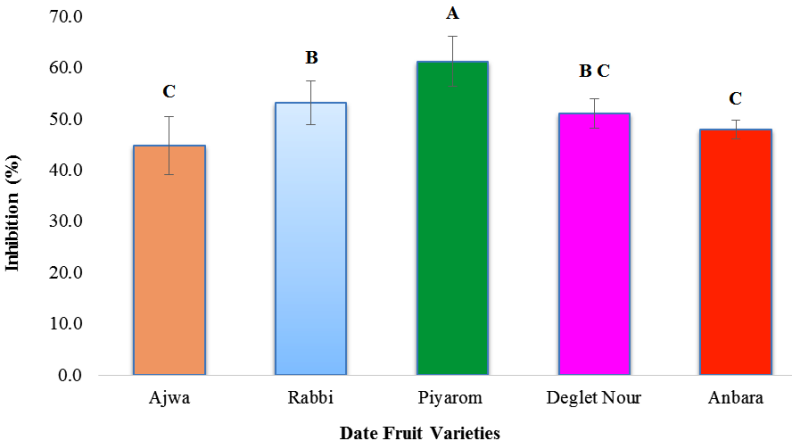

Fig. 3. The elastase inhibition activity of the selected varieties of date fruits

(monosaccharides) as well as sucrose (disaccharide) in the studied date varieties were determined and presented in Table 2. Results showed no significant difference in fructose and glucose content among all the tested date fruits. Deglet Nour dates had significantly (P d" 0.05) higher sucrose content than other cultivars and could be classified as sugar date. The other four cultivars (Ajwa, Piyarom, Rabbi, and Anbara) had significantly (P d" 0.05) higher levels of glucose and fructose, in almost equal amounts, and thus were classified as two-sugar dates. Results from this study are in agreement with those reported in a previous study (A. Em Mrabet, *et al*., 2008) in which Tunisian dates were also classified according to sugar type. Results of sugar contents were also in agreement with those reported in a previous study of some Saudi date cultivars (Al-Abdoulhadi *et al*., 2011).

Mineral Content

The date fruit varieties selected in this study was found to contain moderated amount of minerals (Table 3). The potassium content was the highest (192.85-471.7 mg/100 g DW), followed by magnesium (34.9-49.7 mg/100 g), sodium (1.15-8.6 mg/100 g), and selenium (0.4256-0.5708 mg/100 g). The minerals content significantly varied among the samples, Piyarom contained the highest amounts of potassium (471.7 mg/100 g), magnesium (49.7 mg/100 g), sodium (8.6 mg/100 g), and selenium (0.5708 mg/100 g), while Deglet Nour contain the highest iron and zinc contents 0.5 and 0.348 mg/100 g, respectively. In previous study mineral contents of date fruits were reported to be higher three to five times in comparison to the amounts found in grapes, apples, oranges, and bananas (Yu *et al*., 2008). These finding confirm that dates have high nutritional value. The high potassium and low sodium contents of dates is suitable for people with hypertension (Evans *et al*., 2002). (Shang *et al*., 2010) also reported that date fruits are a very good source of many minerals that are important for metabolism in human cells. For example, magnesium and calcium are essential for healthy bone development and for energy metabolism, and iron is essential for red blood cell production. Several studies demonstrated that date fruits contain suitable concentrations of calcium, potassium, and phosphorus, which are important for metabolism in human cells (Hassan and Bacha, 1982).

ABTS Antioxidant Capacity

The antioxidant activity of the dates was determined by ABTS assay as described by (Cheng *et al*., 2007). The ABTS is expressed as IC_{50}, the concentration required to decrease the initial concentration of ABTS by 50%. Results showed that different date varieties exhibited different antioxidant activity, and the range was between 11.3 and 19.1 mmol equivalents/100 g DW (Figure 1). The activity showed by Piyarom was significantly higher ($P < 0.05$), while the other samples extracts showed moderated antioxidant activity. The antioxidant activity for Piyarom in this study is in agreement with that of previous studies (Biglari *et al*., 2008). In addition, the Tunisia date (Deglet Nour) exhibited the lowest antioxidant activity that was similar to that of previous report by Souli *et al*., (2016).

Antibacterial activity

The antibacterial activity of the 5 date fruit samples was determined against five selected pathogens (Figure 2). Results showed that Piyarom has the highest antibacterial activity ranged between 60-70 % against all the tested pathogens. On the other hand, Deglet Nour demonstrated 30-40% antibacterial activity and is the lowest activity among the studied date fruit varieties. Anbar also exhibited high antibacterial activity while the other two samples had moderated antibacterial activity. The antibacterial activity of the date fruit samples depends on the presence of bioactive compounds such as phenolic compounds, flavonoids, and certain enzymes. Furthermore, the activity is affected by the extraction method and the solvents. In this study, the extraction was carried out using ethanol because of its polarity, low toxicity, and is well known for extracting bioactive compounds from different samples (Abarca-Vargas *et al*., 2016). Bounatirou *et al*., (2007) studied the antibacterial activity of Tunisian date fruits and the results showed moderate activity against gram negative and positive pathogens.

Anti-elastase activity

Elastin is a protein found in connective tissue which is responsible for the elasticity of the skin and lungs (Fulop *et al*., 2012; Kurtz and Oh, 2012; Debelle and Tamburro, 1999). This protein is catalysed by the enzyme elastase. Degradation of elastin by intracellular elastase increases with age and/or exposure to UV-radiation, leading to skin

aging (Labat-Robert *et al*., 2000). The five selected date fruit samples inhibited the elastase enzymes activity for up to 80% (Figure 3). Piyarom date showed the highest anti-elastase activity (61.2±4.9 %) which was significantly higher in comparison to the other samples. On the other hand, the lowest activity (44.8±5.7 %) was detected for Ajwa and it was approximately similar to the results for Anbara and Deglet Nour. To the best of the authors knowledge, the elastase inhibitory activity of the five-date varieties has not been reported before. Thus, it can be stipulated that Piyarom exhibit anti-aging property that may use in formulating functional food targeted for the elderly.

In conclusion Piyarom extracted by 80% ethanol demonstrated the highest phenolic compounds yield and the highest minerals content. No significant differences observed for the sugar content of the selected varieties. The antioxidant and antibacterial activity of Piyarom extract is significantly high in comparison to the other four samples. Moreover, Piyarom extract showed high potential for applications as anti-aging agent as the extract inhibited the elastase activity that is responsible for aging. The high yield of Piyarom extract (23%), the sugar and mineral content, and the biological activity demonstrated high potential for commercial applications in functional foods as bio-active ingredient to satisfy the high demand for bioactive ingredients. Further study is recommended to identify the bioactive compounds present in the Piyarom extract. Moreover, food applications should be carried out for selected food applications to determine the effects on nutrients and the technological properties.

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