Identification and Quantification of Phenolic Compounds in Mangifera Indica Waterlily Kernel and Their Free Radical Scavenging Activity

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***Abstract*—The aim of this study was to determine phenolic compounds and antioxidant capacity of extract prepared from *Mangifera indica* waterlily kernel. Acidified methanolic extract prepared from *M. indica* waterlily kernel was analyzed. The total phenolic content was determined by the Folin_Ciocalteu method. Antioxidant activity was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric-reducing antioxidant power (FRAP) assays. Characterization of phenolic compounds was conducted using HPLCDAD and TSQ-ESI-MS and Peak identities were confirmed by comparing their retention times, UV_vis absorption spectra, and mass spectra with authentic standards. The total phenolic content of *M. indica* waterlily kernel was 8.6 mg GAE/g DW. The seeds extract showed a strong potency of antioxidant activity as confirmed by the DPPH radical scavenging activity, …. and FRAP assays. Epigallocatechin (EGC), chlorogenic acid, apiginin, and epicatechin were the major compounds among the 12 phenolics that have been identified and quantified in *M. indica* waterlily kernel with 21.8, 13.7, 3.2 and 2.7 mg/g *M. indica* waterlily kernel, respectively. The 12 phenolic compounds identified in *M. indica* waterlily kernel using HPLCDAD and TSQ-ESI-MS had high antioxidant activity and should be considered as a prospective antioxidant.**

***Index Terms*—mangifera indica, phenolic compounds, antioxidants, bioactive analysis**

I. Introduction

Oxidative stress, due to reactive oxygen species (ROS), is implicated in the development of many chronic diseases including diabetes mellitus and cancers [1]. Antioxidants have been demonstrated to play important roles in ROS scavenging via several mechanisms [2]. In particular, epidemiological studies have demonstrated that increased consumption of fruits and vegetables is associated with reduced risks of chronic diseases like cancers, likely due to their antioxidant-rich contents including phenolic compounds [3], [4]. There are over 500 mango varieties, mostly restricted to tropical Asia [5], which are commonly used as fruits and for other purposes. Peels and seeds are the major by- products generated during the processing of mango amounting from 35 to 60% of the total fruit weight [6]. In addition to the nutritional content of the fleshy parts of mangoes, [7] reported that mango peels contain valuable compounds, such as polyphenols, carotenoids and vitamins that are known to be antioxidant-rich. These antioxidants could scavenge radicals in biological systems leading to reduced risk of oxidative stress-related diseases. Furthermore, several studies have reported on the phenolics of Mangifera indica [8], [9]. However, to date there is still little information on phytochemical components of Mangifera indica waterlily kernel and their functional effects especially related to radical scavenging activity and antioxidant properties. Thus the aim of this study was to evaluate the phenolic compounds from the extract of M. indica waterlily kernel and their antioxidant potentials.

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II. Materials and Method

A. Chemicals

All standards (gallic acid, protocatechuic acid, p-coumaric acid, chlorogenic acid, ellagic acid, vanillic acid, 4-hydroxybenzoic acid, ferulic acid, methyl gallate, ethyl gallate, (+)-catechin, mangiferin, morin, rutin, daidzein and kaempferol) used for identification and quantification were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). Also, 2,4,6-Tripyridyl-S- triazine (TPTZ), Butylatedhydroxytoluene (BHT) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Fisher Scientific (Loughborough, UK) while the Folin-Ciocalteu reagent was obtained from Merck (Darmstadt, Germany). HPLC grade solvents were obtained from Fisher Scientific (Loughborough, UK).

B. Preparation of Standard Curves

The standard stock solutions were prepared by dissolving standards in methanol to a concentration of

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100 µg/mL. For the calibration curves, four additional concentrations (20, 40, 60 and 80 µg/mL methanol) were prepared by the dilution of the stock solutions with methanol.

C. Sample Preparation

Mango fruits were procured from a local market at Kuala Lumpur in between the month of June to July, 2012. Extraction of samples- M. indica waterlily kernel was soaked in water, and washed to remove adhering flesh. They were then air-dried, and subsequently kept in an oven at 45 ℃ for 2 d. The dried kernels were finely ground with a Waring blender 7011HS (Osaka Chemical Co. Ltd., Kita-Ku, Osaka, Japan) and stored at 4 ℃ until analysis. Ethanol (95 %) was added to the kernel powder at 10:1 (v/w) and the mixture shaken continuously at 200 rpm and 37 ℃ for 24 h in an incubator shaker (INNOVA 4000, New Jersey, USA). Insoluble materials were then removed by filtration and the filtrates centrifuged for 10 min at 4000 rpm using Benchtop Centrifuge Z200A (Labnet International, Inc., Woodbridge, NJ, USA). The residues were discarded and the supernatant dried using 1L Rotary Evaporator N1001S-WD (Tokyo Rikakikai Co., Ltd., Koishikawa Bunkyo-ku, Tokyo, Japan) until the extract was fully concentrated. Total polyphenols were extracted by adding 40 mL of methanol (50%, v/v) to 500 mg of M. indica waterlily kernel with constant shaking in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) at 200 rpm for 1 h at room temperature. Extracts were centrifuged at 1500g for 10 min at 23 ℃ in a Hettichi centrifuge (Zentrifugen, Germany); 40 mL of acetone (70% v/v) was then added to the residue with constant shaking for 1 h at room temperature and centrifuged at 1500g for 10 min at 23 ℃. Supernatant extracts were combined and adjusted to 100 mL with distilled water [10]. Polyphenols were determined spectrophotometrically by the Folin-Ciocalteau method as described previously [11]. Briefly, 0.1 ml of the sample extract was added to 0.75 ml of Folin–Ciocalteu reagent followed by addition of 0.75 ml of 6% sodium carbonate. The mixture was stirred and allowed to stand for 90 min. The absorbance was read at 725 nm using a UV/Vis 1601 spectrophotometry (Shimadzu, Kyoto, Japan). A blank consisting of combined methanol 50% and acetone 70% (v/ v) and reagents was used as a control. The results were expressed as mg GAE/g of dry matter utilising a calibration curve of gallic acid at a concentration ranging from 0.02 mg/ml to 0.1 mg/ml.

D. Ferric-Reducing Antioxidant Power (FRAP) Assay

The FRAP reagent was prepared freshly by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6 with 1 volume of 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mmol/L HCl and with 1 volume of 20 mmol/L FeCl3.6H2O. The reagent was warmed to 37 ℃ and absorbance was read at 593 nm (A1) against deionized water as blank. Mangifera indica waterlily kernel extract and vitamin C as a positive control were dissolved in 10 mL of distilled water at 0 (control), 50,100, 200, 300 and 400 µg/mL, then 50 µL of sample and positive control and 150 µL of deionized water were added to1550 µL of FRAP reagent. After adding the sample to the FRAP reagent, a second absorbance at 593 nm was read after 8 min (A2). Finally A1 was deducted from A2 to determine the FRAP value of the sample. A standard curve was plotted using different concentrations (100 -1000 µmol/L) of $FeSO_4.7H_2O$. The final results were expressed as the concentrations of antioxidants having a ferric-reducing capability equivalent to that of 1 µmol/L $FeSO_4.7H_2O$ [12].

E. 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) Assay

The DPPH free radical-scavenging activity was evaluated by the method of Yang *et al.* [13]. M. indica waterlily kernel extract and BHT as a positive control were dissolved in 10 mL of distilled water at 0 (control), 50, 100, 150, 200 and 250 µg/mL. Then, 1 mL of M. indica waterlily kernel extract and BHT solutions was mixed with 2 mL of 0.2 mmol/L DPPH (in ethanol) separately, after which each solution was mixed thoroughly and incubated at 25 ℃ for 20 min then absorbance was read spectrophotometrically at 517 nm. The inhibition of DPPH radical by M. indica waterlily kernel extract was calculated according to the following equation: Scavenging activity (%) = 100 – [As / A0 X 100] As is the absorbance of the sample and A0 is the absorbance of the blank control.

Chromatographic analysis- To obtain higher concentrations of the phenolic compounds, hydrolysis reaction for the samples was done as reported by Nuutila *et al.* [14]. Briefly, 500 mg of M. indica waterlily kernel extract were placed in a test tube and extracted with 40 mL of acidified methanol (4 mL of HCl was added to obtain a final concentration of 1.2 M) and 2 mg ascorbic acid as an antioxidant. Samples were shaken at 240 rpm on an orbital shaker at 35°C for 16 h. Then, the mixture was centrifuged at 1500 x g for 10 min, the supernatants were collected and brought to a final volume of 50 mL with deionized water. The hydrolyzed sample was stored at -80°C. Before use, the extract was filtered through a 0.45 µm nylon membrane filter (SRP 15, Machery Nagel, Düren, Germany) and then analyzed by HPLC.

The HPLC method was validated in terms of the linearity of calibration curves that have been previously determined for the standards and the components in M. indica waterlily kernel extract. Linearity of detector responses was determined on five concentration levels with three injections for each concentration. A linear relationship between peak area and concentration (20-100 g/mL) was observed for each standard with a correlation /coefficient ranging from $r = 0.9912$ to $r = 0.9993$.

The reproducibility of the injection integration procedure was determined for standards and for the twelve M. indica waterlily kernel extract components 1-12 (Fig. 2). The solutions of standards and samples were prepared 3 times and each solution was injected 3 times. The relative standard deviations of peak area for each compound with replicate injection were calculated.

Quantitative analysis of M. indica waterlily kernel extract were performed on an Agilent series 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA)

equipped with a diode array detector (DAD) following the procedure described by He and Xia (2007) [3]. Briefly, chromatographic analysis were performed on a Lichrospher C18, 5μm (250 mm x 4 mm, i.d.) column from Merck (Darmstadt, Germany) utilizing a mobile phase consisting of 0.5% (v/v) acetic acid (mobile phase A) and 100% methanol (mobile phase B) at a flow rate of 0.8 mL/min with a gradient elution program and 30 min run time. The gradient elution started at 100% phase A with a linear decrease to 10 % at 20 min to 25 min, the next 5 min phase A increased back to 100%. The injection volume was 20 μL at 25˚C oven temperature. The majority of the phenolic compounds in M. indica waterlily kernel extract showed a UV-Vis spectra at 280 nm, rather than 254, 320, and 365 nm. When there were variations in retention times, peak identifications were supported by spiking with authentic standards or confirmed by LC-ESI-MS analysis.

F. Mass Spectrometry

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. It is used for determining masses of particles, for determining the molecule and for elucidating the chemical structures of molecules, such as peptides, polyphenols and other chemical compounds. Electrospray ionization mass spectrometric (ESI–MS) analysis of phenolic compounds in M. indica waterlily kernel extract was performed to support phenolics identification using an applied TSQ Quantum Ultra- LCMS (Thermo Fisher, San Diego, CA, USA). The mass spectra were operated in both negative and positive electrospray ionization ESI modes and high resolution with extended resolution up to 3000 Daltons was used for the best resolution. The spray voltage used was 3500 V. The sheath/ auxiliary /sweep gas was 99% pure nitrogen and sheath gas pressure was 30 psi with 5 psi for auxiliary gas pressure. The capillary temperature was 270 ℃. The injection volume was 10 μL and the flush speed was 100 μL/s.

A preliminary study of the UV spectrum of the peaks gave a first indication of the family of phenolic compounds. Thus, the surveyor method of PDA-LCMS was set as following: run time was 30 min with rise time 1 sec, scan wavelength was performed at 200-600 nm; the scan bandwidth was 1 nm with scan rate of 5 (Hz) and scan step of 1 nm. The channel sample rate was performed in 10 (Hz) with the three following channels: channel A, the wavelength was 214 nm and bandwidth was 9 nm; channel B wavelength was 254 nm and 9 nm for bandwidth; channel C, 280 nm, 9 nm for wavelength and bandwidth, respectively. A gradient elution was performed with 0.2% (v/v) acetic acid in deionized water as solvent A and 100% methanol as solvent B with the flow rate of 0.1 mL/min according to the following program: first 20 min, the linear gradient of solvent A was from 95% to 10% and 5% to 90% of solvent B, the running was constant for 5 min and then linearly increasing from 10% to 95% for solvent A in contrast of solvent 90% to 5% for solvent B. In the MS analysis (full scan), data were collected over a mass of 100 to 800 m/z. Identification of the phenolic compounds of M. indica waterlily kernel extract was achieved by comparison of their retention times, UV–Vis absorption spectra and mass spectra with authentic standards.

Statistical analysis- Each analysis was done in triplicate. Results were expressed as mean values ± standard deviations. Data were statistically analysed by One-Way ANOVA procedure with SPSS software Version 19.0 (Chicago, IL USA), followed by the Duncan test. The confidence limits used in this study were based on 95% confidence ($p < 0.05$).

III. Results and Discussions

A. Total Extractable Polyphenols (TEP)

The content of TEP in the Mangifera indica waterlily kernel was 8.57 ± 0.3 mg gallic acid equivalents (GAE)/100 mg dry sample. The present results are in agreement with those reported previously. Ajila *et al.* [15] had reported 96 mg GAE/g of Mangifera indica ripe peels, while other have reported higher (with amount at 102 mg GAE/g) or slightly lower values (70 mg GAE/g) [16], [17]. This suggests that M. indica waterlily kernel is rich in polyphenol compounds. Interestingly, the total polyphenols in M. indica waterlily kernel extract estimated using Folin–Ciocalteau method showed different result compared to that calculated from the sum of the individual phenolic compounds using the HPLC method (53.6 mg/g d.w.). The higher value observed due to Folin-Ciocalteu method could be related to interference of sugars and protein [18]. As can be recalled, polyphenols have been associated with high antioxidant potentials and increased capacity for scavenging free radicals. The implication of such an effect is that fewer radicals due to the scavenging ability of polyphenols from foods like Mango kernel could protect against development of oxidative stress-related diseases caused by excess free radicals.

B. FRAP

The FRAP assay is traditionally used to assess the antioxidant potentials of extrascts from food or plants. In this study, the antioxidant capacities of M. indica waterlily kernel extract and ascorbic acid as control were determined and are shown in Fig. 1. The M. indica waterlily kernel exhibited a high antioxidant power similar to what ascorbic acid produced. The highest concentration of M. indica waterlily kernel used (400 μg/mL), showed an antioxidant power (1289 μM/L) that was comparable to what a similar concentration of ascorbic acid produced (1324 μmol/L). The current findings are higher than what was reported for the same concentration of Mangifera pajang Kort. pulp (770 μmol/L) [19]. In addition, a study by Abu Bakar *et al.* [20] demonstrated that the antioxidant activity of unripe peel of Mangifera pajang Kort. using FRAP assay was 343.17 μmol/g sample, also lower than what was found in the current study. This is likely due to complete ripening of the fruit studied in the current study, which may have enhanced the antioxidants higher than in unripe fruits. Moreover, studies have demonstrated that polyphenolic

content of ripe mango peel is normally higher than the unripe peel [21], [22].

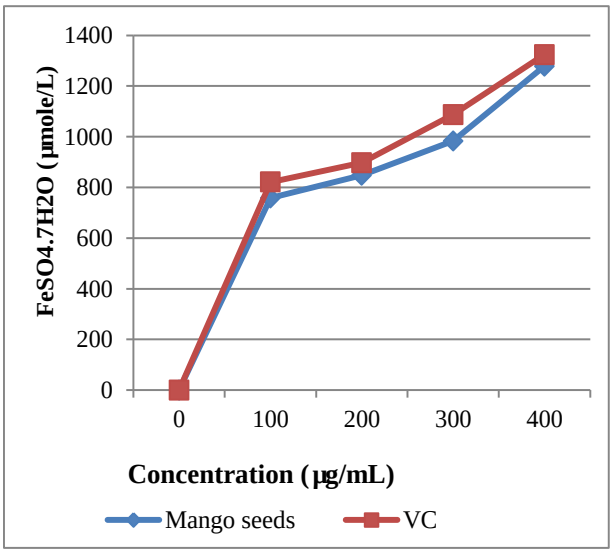

Figure 1. Ferric-reducing antioxidant power (FRAP) of M. indica waterlily kernel extract against that of Ascorbic Acid; treatment with different concentrations of M. indica extract and Ascorbic Acid showed a dose-dependent effect that was similar, with no significant differences ($p > 0.05$).

TABLE I. RADICAL-SCAVENGING ACTIVITIES OF *MANGIFERA INDICA* WATERLILY KERNEL AND BHT

Sample	Amount ((µg)	DPPH scavenging activity %
BHT	50	57.53 ±0.6
	100	85.50 ±0.9
	150	89.07 ±0.7
	200	92.33 ±0.7
	250	98.17 ±0.8
M. indica waterlily	50	50.20 ±0.3
	100	78.83±0.6
	150	82.23±0.6
	200	87.73 ±0.6
	250	95.37±0.7

C. DPPH Activity

Table II shows the DPPH radical- scavenging activities of different concentrations of M. indica waterlily kernel extract compared to control (Butylatedhydroxytoluene, BHT). At a concentration of 250 µg/mL, the extract scavenged more than 95% of the DPPH radical, which was comparable to that of BHT (98%). Its capacity for high scavenging activity may be attributed to the high amounts of TEP in M. indica waterlily kernel extract. This is in agreement with what Maciel *et al.* reported [16]; 200 mg/mL of M. indica extract produced scavenging activity of over 79.6 %. The antioxidant effects of M. indica may be as a result of its electron-donating ability. It is expected that higher levels of polyphenols would possess an increased ability to donate electrons thus quenching free radicals, and our results are in agreement; high phenolic content of Mangifera indica waterlily kernel produced an equally higher radical-scavenging capacity in a dose-dependent manner.

There were significant increases in scavenging activities of both BHT and M. indica dose-dependently ($p < 0.05$), and BHT showed slightly higher values at every concentration ($p < 0.05$).

In the current study, a hydrolysis method was used to prepare the extract, which may have improved the antioxidant capacity of the extract as a result of increased release of the phenolic compounds. Already, Soong and Barlow [8] demonstrated that gallic acid and ellagic acid in their free forms produced higher antioxidant activities compared to their conjugated forms. They demonstrated that hydrolysis was a good method for releasing these compounds and thus increasing their antioxidant capacities. Furthermore, Nuutila *et al.* [14] showed that hydrolysis increased the release of phenolic acid and flavonoides.

D. Identification of Phenolic Compounds of Mangifera Indica Waterlily Kernel

Mango seeds are known to contain a mixture of polyphenols as phenolic acid, flavonoids and xanthones. To determine which of these were more abundant in the extract used in this study, chromatographic techniques were used as shown in Fig. 2. Reverse-phase HPLC was used to analyse the extract, which showed the presence of gallic acid (1), chlorogenic acid (2), epicatechin (3), 4-hydroxybenzoic acid (4), mangiferin (5), syringic acid (6), EGC (7), p- coumaric acid (8), rutin (9), myricetin (10), quercetin (11) and apigenin (12) (Fig. 2). The compounds were confirmed by comparing those from the extract to the retention times of their standards, as shown in Table II.

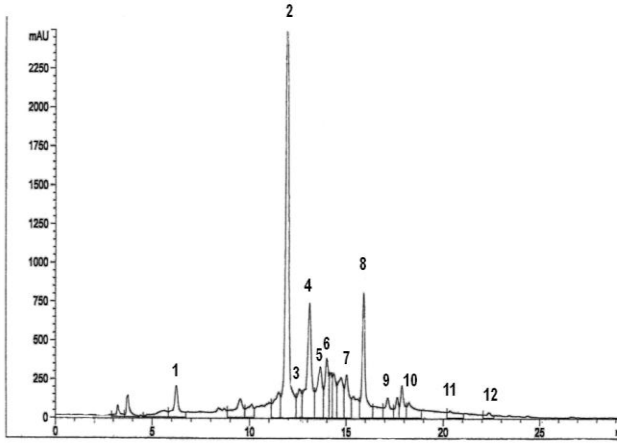

Figure 2. HPLC profile of phenolic compounds in *M.indica* waterlily kernel peak identification: 1, gallic acid; 2, chlorogenic acid; 3, epicatechin; 4, 4-hydroxybenzoic acid; 5, mangiferin; 6, syringic acid; 7, EGC; 8, *P*-coumaric acid; 9, rutin; 10, myricetin; 11, quercetin; 12, apigenin.

Masibo and He [23] have reported that major phenolic acid identified in mango parts (pulp, peel, seed, leaf, and stem bark extracts) include gallic acid, methyl gallate,

digallic acid, ellagic acid, β-glucogallin, and α-gallotannin. In addition, Kim *et al.* [24] reported the presence of gallic acid, p- coumaric acid, coumaric acid, ferulic acid and p-OH-benzoic acid, while Ribeiro and Schieber [25] reported that gallic acid, ferulic, protocatechuic, caffeic, coumaric, ellagic and 4-caffeoylquinic acids were the major compounds present in Mangifera indica peel, flesh and kernel. Following HPLC analyses, more structural information using LCMS (Liquid Chromatography Mass Spectrometry) was obtained for positive identification and to make up for shifts in retention times on HPLC. To confirm the result of HPLC identification, the structures and molecular formulas of the 12 phenolic compounds were determined from their electrospray ionization tandem mass spectrometry (ESI-MS) spectra (Table II).

TABLE II. PHENOLIC COMPOUNDS IDENTIFIED BY HPLC-ESI-MS AND THEIR QUANTITIES IN *M. INDICA* WATERLILY KERNEL

t_R (mn)	Compound	MW	Relative abundance
6.2	Gallic acid	170.12	4.1 E4
11.9	Chlorogenic acid	354.31	1.1 E7
12.6	Epicatechin	290	3.6 E4
13.0	4-hydroxybenzoic acid	138.12	7.5 E7
13.6	Mangiferin	422.33	6.7 E4
13.9	Syringic acid	198	2.6 E5
15.0	EGC	306.27	7.4 E5
15.9	*P*-coumaric acid	164.16	1.7 E5
17.1	Rutin	610.16	5.1 E4
18.2	Myricetin	318	2.4 E5
20.3	Quercetin	302	1.7 E6
22.3	Apigenin	270	3.6 E5

n.d. not detected in signed mode; [a] Highest compound intensity in signed mode.

Following identification of the compounds using full-scan conditions, the extracted-ion at m/z values corresponding to the [M – H]-, [M+H]+ ions of the studied compounds were analyzed. Already, Selles et al. [26] has demonstrated the use of structure characterization for HPLC quantitative determination of phenolic constituents from Mangifera indica stem bark including gallic acid, methyl gallate, mangiferin, 4-hydroxy benzoic acid, catechin, epicatechin and propyl benzoate. Identification and quantification of Xanthone and flavonols including mangiferin, kaempferol and their related glycosides have also been reported in peels of Brazilian mango cultivars, which were detected in the negative mode [M – H] - [9]. In the current study, the identities of all compounds were confirmed by comparison of the data with those of standard compounds. The corresponding UV–Vis and MS data for the 12 compounds identified were obtained and used in the structural characterization.

A study by Charrouf *et al.* [27] reported that phenolic compounds including gallic acid, protocatechuic acid, catechin and rutin were detected in negative mode at masses of 169, 153, 289 and 609 m/z, respectively using ions full scan of [M – H] - which agreed with the masses in the current study. Whereas gallic acid, p-coumaric acid, ferulic acid, ellagic acid, kaempferol were detected in strawberry fruit at the same wavelength as in this study, 272, 326, 238, 368 and 266 nm, respectively [28].

E. Quantitative Analysis of Phenolic Compounds

The quantitative analyses of the phenolic compounds in M.indica waterlily kernel extract was performed by HPLC. The concentration of each compound in the studied extract was determined by from the calibration equation of the corresponding standard and showed that EGC, chlorogenic acid, apiginin, epicatechin, rutin, p-coumaric acid, myricetin, mangiferin, 4-hydroxybenzoic acid, gallic acid, quercetin and syringic acid were present at concentrations of 21.8, 13.7, 3.2, 2.7, 2.5, 2.4, 2.0, 1.8, 1.2,1.1, 0.8 and 0.5 mg/g dry weight, respectively (Table III).

TABLE III. QUANTIFICATION OF PHENOLIC COMPOUNDS IN *M.INDICA* WATERLILY KERNEL USING HPLC-DAD

Compound	Regression equation [a]	Correlation coefficient	Amount (mg/g)
Gallic acid	y = 69.833x + 197.52	0.9993	1.11 ±0.04
Epicatechin	y = 25.138x + 53.55	0.993	2.67 ±0.16
P-coumaric acid	y = 87.467x + 299.64	0.9959	2.43 ±0.13
Mangiferin	y = 83.324x - 36.381	0.9982	1.77 ±0.05
Rutin	y = 23.167x + 52.143	0.9947	2.49 ±0.08
Chlorogenic acid	y = 55.228x +197.36	0.9959	13.66 ± 0.5
EGC	y = 4.514x +13.672	0.9984	21.79 ±1.2
4-hydroxybenzoic	y = 222.13x + 775.25	0.9985	1.21 ±0.03
Syringic acid	y = 90.205x + 626.65	0.9985	0.52 ±0.01
Myricetin	y = 35.17x + 77.259	0.9985	1.98 ±0.06
Quercetin	y = 45.627x + 327.55	0.9996	0.75 ±0.01
Apigenin	y = 5.7729x -5.72	0.9978	3.21 ±0.05
Total			53.58±0.81

a y expresses the detection response (peak area mAU*s) and x the concentration for phenolic compounds (in µg/mL); amount (mean value ±standard deviation; n = 3)

Berardini *et al.* [29] demonstrated the amounts of phenolic compounds like mangiferin and kaempferol in peels of different mango (Mangifera indica L) cultivars to be 1.3 and 0.7 mg/g, respectively. These are lower than those reported in the current study. The hydrolysis used in the current study could have increased the release and thus amounts of phenolic compounds detected [14], [30], [31]. Furthermore, the results obtained in the current study are higher than those reported for Mangifera indica stem bark by NúñezSellés *et al.* [32]. They demonstrated the presence of several phenolic compounds such as gallic acid, benzoic acid, methyl gallate, propyl gallate, mangiferin and catechin at concentrations of 208 mg/100g, 198mg, 445mg, 476mg, and 7140 mg, respectively. The high amount reported in the current study could be due to the higher proportion of phenolic compounds found in peel compared to the stem bark.

This is supported by the report of Masibo and He [23] that mango peel is richer in polyphenols compared to that of pulp, leave, seed and steam bark.

Some studies have reported that mango peels are a rich source of polypenols [33], phenolic acid and flavonols [23], flavonol and xanthone, mangiferin [34]. A number of phenolic compounds have been reported in mango (Mangifera indica) peel including ellagic acid, mangiferin, mangiferin gallate, isomangiferin, isomangiferin gallate, quercetin, kaempferol, rhamnetin and their related conjugates [23]. Berardini *et al.* [21] established that the antioxidative capacity of the mango peel extract was higher than that of standard mangiferin and quercetin 3-O-glucoside, thus suggesting that the antioxidative capacity of the peel extract cannot be attributed to a single component but to the synergistic effect of all the compounds present. In addition to their effects as antioxidant and anticancer agent, a study reported that phenolic acids such as gallic acid have antimicrobial activity for controlling dental caries and periodontal disease [35]. Another phenolic, Mangiferin, has a wide range of pharmacological effects including hypolipidimic, antidiabetic, anti-HIV, antitumor, immunomodulatory and antioxidant activities [36]. The functional effects of the M. indica could be attributable to its high antioxidant activity as shown in the present study, and therefore its potentials in neutralizing free radicals could have profound implications on oxidative stress.

VI. Conclusions

The present study demonstrated that the extract obtained from M.indica waterlily kernel is a rich source of polyphenols (phenolic acid, flvonoides and xanthones). The antioxidant capacity of the M.indica waterlily kernel as determined by different antioxidant assays (DPPH and FRAP assays) exhibited a strong potency due to the presence of associated polyphenols with considerable amounts of phenols (86 mg/g M. indica waterlily kernel). EGC, chlorogenic acid, apiginin, epicatechin and rutin were found to be the major phenolic compounds among 12 phenolics identified and quantified. The study was able to show that hydrolysis produced higher concentrations of phenolic compounds likely due to increased release of the phenolics from the food matrix and finally their resultant good separation on chromatographic analyses.

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