Total phenolic, flavonoid content and metabolite profiling of methanol extract of date (*Phoenix dactylifera*) seeds by LC-QTOF-MS

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Total Phenolic, Flavonoid Content and Metabolite Profiling of Methanol Extract of Date (*Phoenix dactylifera*) Seeds by LC-QTOF-MS

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Abstract. *Phoenix dactylifera* L. (date palm) has been used in traditional medicine to treat illnesses. Extracts of *P. dactylifera* are reported to possess valuable pharmacological attributes. Date seeds are part of the palm trees that have not been widely used, based on previous studies that date seeds contain secondary metabolites such as phenolic acids compounds, flavonoids, carotenoids, sterols, terpenoids and anthocyanin. The aim of this study was to identify the total phenolic and flavonoids content from methanol extract of date seeds and characterize their metabolite profiles using the LC-QTOF-MS instrument. Date seeds were extracted using methanol 96% by maceration method so that thick extracts were obtained which were then analysed for the total phenolic and flavonoids content using UV-Vis spectrophotometer instruments, while the profiles of secondary metabolites used LC-QTOF-MS instruments. The results of the analysis of total phenolic and flavonoid of methanol extract of date seeds were 11.11 ± 0.71 g GAE/100 g and 2.15 ± 0.28 g QE/100 g, LC-QTOF-MS, it was concluded that the majority of date seed methanol extract contained phenolic acids compounds, flavonoids, carotenoids and sterols.

INTRODUCTION

Phoenix dactylifera L. (date palm), commonly known as the date palm is a primeval plant and has been cultivated for its edible fruit in the desert oasis of the Arab world for centuries. The fruits are a rich source of carbohydrates, dietary fibers, certain essential vitamins and minerals. The date pits are also an excellent source of dietary fiber and contain considerable amounts of minerals, lipids and protein. In addition to its dietary use the dates are of medicinal use and are used to treat a variety of ailments in the various traditional systems of medicine [5]. Date palm has been used in traditional medicine to treat illnesses, date fruits is related to the therapeutic implications in the control of diseases, through antioxidant, anti-inflammatory, anti-tumor, anti-diabetic, antifungal, antiviral, antibacterial, immunomodulatory, antiparasitic, hepatoprotective, antiinflammatory and anticoccidial activities effects [16]

Dietary antioxidants, including phenolic compounds, in dates may help to protect the body from various degenerative disorders by minimizing oxidative stress [17]. Studies with seven Algerian varieties such as Tazizaout, Ougherouss, Akerbouche, Tazerzait, Tafiziouine, Deglet-Nour and Tantbouchte contain ferulic, coumaric and sinapic acid are present in all the varieties as the major compounds. Coumaric acid is present in almost all varieties as *p*-coumaric acid, excepting the varieties Tazerzait, Tafiziouine and Tazizaout, where its derivatives were found. The presence of the characteristic compound of the family of Palmae was also detected, which is 5-o-

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caffeoylshikimic acid in all varieties, except for the variety Tazizaout [15]. Conducted a study of three Omani date varieties that have shown the presence of both free (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) and bound phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and *o*-coumaric acid) [2].

Flesh of fruit palm also contain flavonoids, carotenoids and sterols. Assessed the flavonoid content in the Deglet Noor variety during the Khalal stage of maturity and identified thirteen flavonoid glycosides of luteolin, quercetin, and apigenin [10]. It was also observed that both methylated and sulfated forms of luteolin and quercetin are present as mono-, di-, and triglycosylated conjugates while apigenin is present only as the diglycoside. Quercetin and luteolin formed primarily O-glycosidic linkages whereas apigenin was present as the C-glycoside. Kikuchi and Miki, 1978 have examined cholesterol, campesterol, stigmasterol, *β*-sitosterol and isofucosterol contained in dates. Conducted research that dates contain carotenoids such as lutein, *β*-carotene and neoxanthin [6].

Studies of the different parts of the date palm tree are needed, to get more comparative information and to discover the bioactive compounds present in both edible and non-edible parts (by-products). Although the previous published research have studied the phenolic composition profiling of different edible parts and by-products of date palm (*Phoenix dactylifera* L.) by using HPLC-DAD-ESI/MS^n but seed have not studied [1]. Seed of date palm, agricultural by-products are considered undervalued materials due to their removal from foods and the problems that arise from their treatment and disposal in the environment. In this context, the present work investigates seed of date trees of the variety Shiva (Egypt), with regard to their bioactive phenolic compounds (flavonoids and phenolic acids) extracted with methanol and analyzed using LC-QTOF-MS as a powerful analytical technique. Taking into account the considerable economic importance of the disposal of by-products of the date industry, it is important to identify the potential applications of the new sources of phytochemicals.

MATERIALS AND METHODS

Plant material

Date seeds of Shiva variety (Egypt) were obtained from a date palm juice factory, CV. Amal Mulia Sejahtera, Bogor, West Java, Indonesia. Date seeds were cleaned, washed with tap water, and dried in the oven at 50 °C overnight, then grounded into 40 mesh powder using a mill machine. Then it put in polyethylene plastic, and stored at 4 °C until extraction.

Extraction of Date Seeds Powder

1 Kg of date seeds powder were macerated with methanol (1:2) at room temperature until colourless filtrates. The filtrates were filtered and concentrated with a vacuum evaporator at 40 °C. The extracts were stored in a freezer at -20 °C until analysis.

Determination of Total Phenolic and Flavonoid Contents

Total phenolic content was determined using Folin-Ciocalteu reagent at 10%. The calibration curve was prepared using Gallic acid. The absorbance was measured at 760 nm. The total phenolic contents were expressed as g of gallic acid equivalent (GAE) /100 g fresh weight (FW) with modification [11]. Flavonoid contents were quantified using 10% $AlCl_3$ reagent. The method based on the formation of the complex flavonoids□aluminum, having an absorption maximum at 430 nm. Total flavonoid contents were expressed as mg of quercetin equivalent (QE)/g dry weight (DW) with modification [7].

Establishment of the Secondary Metabolites Profile Using LC-QTOF-MS

This analysis was performed using an LC-QTOF-MS system with a Finnigan MAT Spectra System P4000 pump coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer. The separation was performed on a 125 × 2 mm Superspher 100-4 RP-18 column at a flow rate of 0.33 mL/min and an injection volume of 1 μL. The detection was monitored at 254 and 365 nm and also by MS-ESI(+) spectroscopy at a probe temperature of 450 ^oC, probe voltage of 4.9 kV and at 20 and 100 eV in the mass analyser. The following gradient

programme was used: (A) AcOH (2.5%) and (B) MeOH/AcOH (2.5%) (3:2), 70% A at 0 min, 60% A at 12 min, 40% A at 32 min and 20% A at 34 min. The data were processed using the Xcalibur 1.2 software.

RESULTS AND DISCUSSION

Total Phenolic and Flavonoid Content

The result of the analysis of total phenolic of methanol extract of date seeds were 11.11 ± 0.71 g GAE/100 g FW. That result was higher than previous studies, total phenolics in date seeds from three varieties (Mabseeli, Um-sellah, and Shahal) were 4.430 ± 0.297, 4.293 ± 0.180, and 3.102 ± 0.58 g GAE/100 g FW, respectively [3]. Various factors such as variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, storage conditions, and amount of sunlight received, cultural methods, process and stabilization conditions, climatic conditions, use of different analytical methods and use of different phenolic acid standards and extraction solvents, might be responsible for the observed differences. On the other hand, that total phenolic content in methanolic extract is higher than that measured in ethyl acetate extract, this result is in agreement that polar solvents are among the most employed for polyphenols extraction [4]. Therefore, dates and their by-products (particularly seeds) may be considered as rich sources of total phenolics.

Flavonoid content in samples were 21.50 ± 2.80 mg QE/g DW, that result was lower than previous studies, flavonoids in methanol extracts of date palm seeds were obtained depending on the variety, 1211 ± 81 mg QE /g DW in Deglet Nour variety, 1210 ± 63 mg QE/g DW in Ruchdi variety, 1270 ± 112 mg QE/g DW in the Ftimi variety, and 1450 ± 153 mg QE/g DW in Kentichi varieties [4]. It is well known that phenolic compounds, especially flavonoids are endowed with several biological activities such as antioxidant and antibacterial properties [12]

General Profile the Secondary Metabolite Profile Using LC-QTOF-MS (ESI^+)

Under the conditions used, most of the compounds detected had intensive signals corresponding to the pseudo-molecular ion $[M+H]^-$ and $[M+H]^+$. Formation of $[M+H_2O]^+$, $[M+Na^+]$ and $[M+CH3OH]^+$: was observed as well. Adducts are expected in positive electrospray ionisation (ESI^+) [15] . The identification of the individual secondary metabolite profile was achieved by comparison of their MS data with the literature [8];[9];[14];[18]. Figure 1 shows the HPLC chromatogram of the *P. dactylifera* seed extract at the wave lengths 254 and 365 nm.

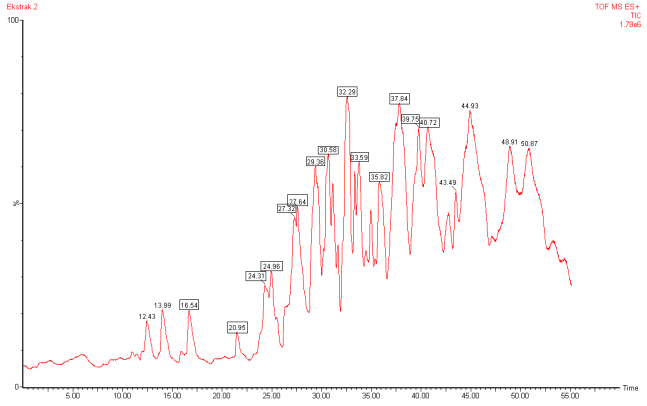

FIGURE 1. HPLC chromatogram of the *P. dactylifera* seed extract

Phenolic Acids

The most apparent compounds are the cinnamic acids (**1**) and their derivatives (Figure 2). Ferulic (**2**), coumaric, and sinapic acids (**3**) are present as the major compounds and caffeic acid (**4**) are present as the minor compounds. Coumaric acid is present as *p*-coumaric (**5**) and *o*-coumaric acid (**6**). The presence of a compound 5-*o*-caffeoylshikimic acid (**7**) was also detected. The molecular weight of cinnamic acid and its derivatives are shown in Table 1.

Flavonoids

Compared with the cinnamic acids detected, the concentration of flavonoids was very low. Some flavonoid compounds that appear are groups of catechins namely catechin (**8**) and epicatechin (**9**), besides quercetin (**10**), luteolin (**11**) and apigenin (**12**) (Figure 2). The molecular weight of flavonoids is shown in Table 1.

Carotenoids

Carotenoids appear the least when compared to other secondary metabolites. At least there are two carotenoin compounds that appear, namely *β*-carotene (**13**) and Lutein (**14**) (Figure 2). The molecular weight of carotenoids is shown in Table 1.

Sterols

Sterol groups also appear among several compounds Cholesterol (**15**), Campesterol (**16**), *β*-sitosterol (**17**), Stigmasterol (**18**), Fucosterol (**19**) and 7*α*-hydroxy *β*-sitosterol (**20**) (Figure 2). The molecular weight of sterols is shown in Table 1.

TABLE 1. Analysis of the LC-QTOF-MS chromatograms

R.T (min)	$[M+H]^+$ (m/z)	λ_{max} (nm)	Identification (Compounds)	Group of Secondary Metabolites
29.66	149.0246	242, 286, 322	Cinnamic acid (1)	Phenolic acids
31.61	194.9787	242, 318	Ferulic acid (2)	
32.29	225.0344	242, 318	Sinapic acid (3)	
31.07	181.0679	262, 294	Caffeic acid (4)	
30.53	165.0939	238, 310	p-coumaric acid (5)	
30.58	165.0942	238, 310	o-coumaric acid (6)	
20.95	335.2598	242, 326	5-o-caffeoylshikimic acid (7)	
27.32	290.2783	242, 256, 311	Catechin (8)	Flavonoids
27.32	290.2783	242, 256, 311	Epicatechin (9)	
24.31	302.2344	242, 286, 322	Quercetin (10)	
16.54	286.2379	262, 322	Luteolin (11)	
24.96	270.2498	242, 286, 322	Apigenin (12)	
37.84	536.2783	256, 311	*β*-carotene (13)	Carotenoids
39.75	568.9342	242, 322	Lutein (14)	
29.36	387.1093	-	Cholesterol (15)	Sterols
27.32	400.7095	-	Campesterol (16)	
27.64	413.2679	-	*β*-sitosterol (17)	
30.53	411.3847	-	Stigmasterol (18)	
31.07	411.3666	-	Fucosterol (19)	
30.53	429.3767	-	7*α*-hydroxy *β*-sitosterol (20)	

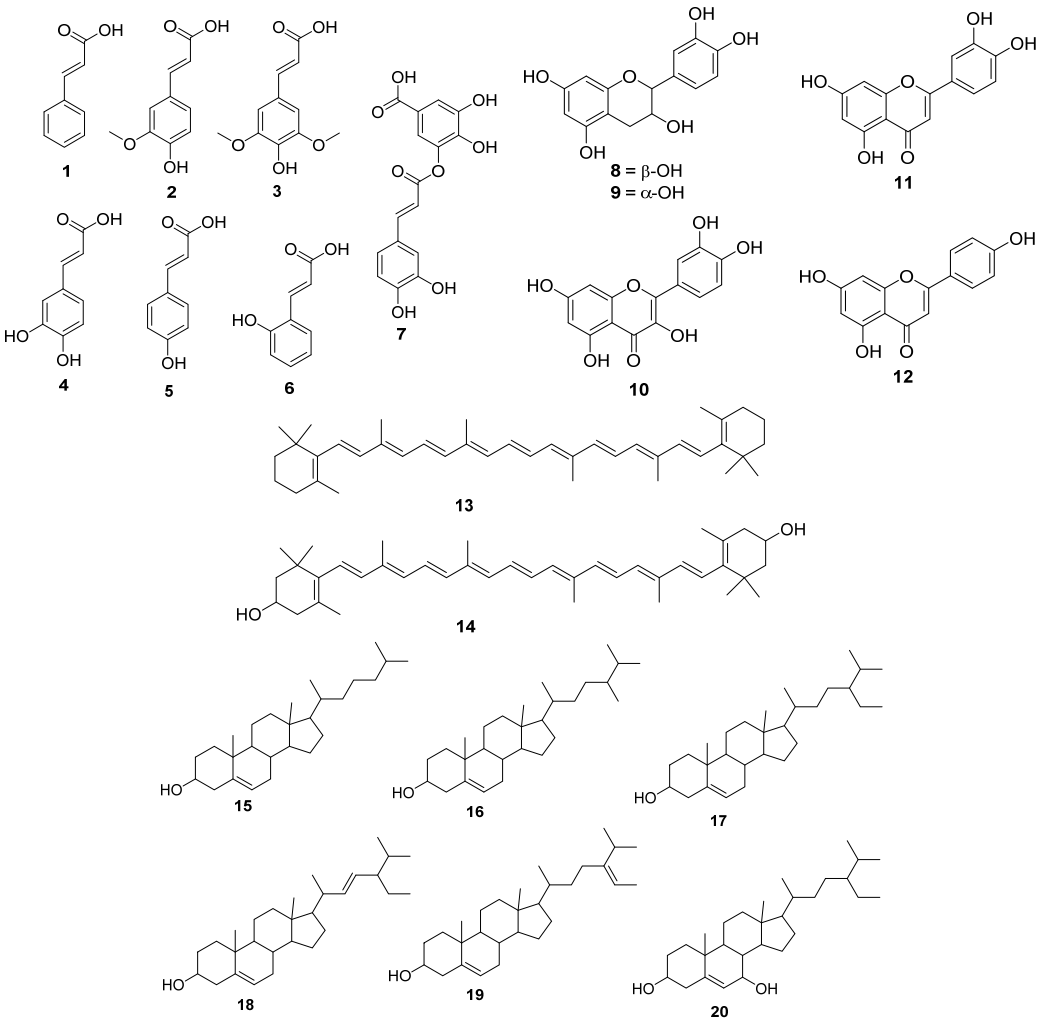

FIGURE 2. Structures of secondary metabolites present in date seed extract

Pharmacological Activities of Dates

Animal studies have shown that oral feeding of p-coumaric acid present in date increases the expression of antioxidant enzyme genes in rat cardiac tissue [19]. The date components phenolic acids are reported to possess antimutagenic effects [20]. The investigations have shown that the anthocyanins, carotenoids, procyanidins and flavonoids present in dates are known to possess membrane protective effects [21]. In vitro studies have shown that flavonoids possess antifungal activities against *C. albicans* and *C. krusei*, and that their presence in the extract may have been responsible for the observed antifungal effects [22-23]. The studies have shown that the date constituents polyphenols [24], and βcarotene [25], possess anti-inflammatory effects in different models of study. The date constituents flavonoids [26], and β-sitosterol [27] possess gastroprotective effects against different ulcerogens.

Preclinical studies have shown that the phytochemicals caffeic acid [28], β-sitosterol [29], catechin [30], and quercetin [30-31] present possess cardioprotective and antihyperlipidemic effects in various animal models of study [32-34]. Studies have confirmed that ferulic acid [35], caffeic acid and quercetin [36], β-carotene [37], apigenin [38] and luteolin [39], the date constituents have all been reported to possess hepatoprotective effects against the ccl4-induced hepatic damage in rodents. Previous studies have shown that quercetin [40] possess protective effects against the gentamicin-induced nephrotoxicity in rats. Experimental studies have shown that carotenoids [41] and quercetin [42] possess immunostimulatory effects.

CONCLUSION

The date seed contain some secondary metabolites are incorporated into four major groups namely phenolic acids compounds, flavonoids, carotenoids and sterols. Secondary metabolite compounds on dates have different pharmacological effects. However, further studies are needed so that it is easy to characterize flavonoid glycosides, using LC-QTOF-MS with other isolation methods such as partitioning first.

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