MATHEWS Open Access Journals

ISSN: 2572-6447

Research Article | Mathews Journal of Diabetes & Obesity

Microwave-Assisted Extraction of Phenolic Compounds from Date Palm Saps (Phoenix Dactylifera L.) and Their Antioxidant, Antidiabetic and Antibacterial Activities Evaluation

Raed Abdennabi[1,2]*, Nicolas Gaboriaud[2], Vivek Ahluwalia[2], Job Tchoumtchoua[2], Amine Elgheryeni[3], Alexios Leandros Skaltsounis[2], Neji Gharsallah[1]

[1]Laboratory of Plant Biotechnology, Faculty of Science, B.P. 1171, 3000, University of Sfax, Tunisia.

[2]Laboratory of Pharmacognosy and Natural Products Chemistry Athens 15771, Greece.

[3]Uiversity of Toulouse II Jean Jaurès, France

Corresponding Author: Raed Abdennabi, Laboratory of Plant Biotechnology, Faculty of Science, B.P. 1171, 3000, University of Sfax, Tunisia, **Tel:** +216 98 69 43 98; **Email:** raedabdennabi@gmail.com

Received Date: 24 Oct 2017

Accepted Date: 16 Nov 2017

Published Date: 20 Nov 2017

Citation: Abdennabi R, Gaboriaud N, Ahluwali V, Tchoumtchoua J, et all. (2017). Microwave-Assisted Extraction of Phenolic Compounds from Date Palm Saps (Phoenix Dactylifera L.) and Their Antioxidant, Antidiabetic and Antibacterial Activities Evaluation. M J Diab. 2(2): 010.

ABSTRACT

Date palm sap (DPS) of Phoenix dactylifera L. is a popular juice consumed as a fresh drink called “Lagmi” and listed in folk remedies for diabetes. This study was aimed to at developping optimal microwave assisted extraction conditions for recovery of phenolic compounds from date palm saps and to evaluate their antioxidant, antidiabetic and antibacterial. Extraction process was optimized for the first the time with with inexpensive solvent and optimization was done for first time extact polyphenolic compounds from Phoenix dactylifera. The extraction of phenolic compounds was achieved by Microwave Hydro-distillation (MWHD) with some modifications. The results showed that the extracts from saps of four date palm varieties are rich in polyphenols and flavonoids (Thokar variety: 26.630 ± 0.007 mg GAE/ g, Khalet variety: 13.520 ± 0.056 mg QEd/ g. The polyphenols content helps to have an antibacterial activity with the most of the extracts such as Khalet variety 16 mm against Staphylococcus aureus. The highest antioxidant capacity was obtained with Thokar variety 220.000 ±1.414 (mg alpha tocopherol/g). The extracts exhibited an interesting antidiabetic activity which is more important for Thokar variety with IC50 of 91.024 µg/ml. The results obtained herein indicate that DPS may contain interesting compounds which can be used as additives in food, cosmetic and medical preparations.

KEYWORDS

Date Palm Sap; Antioxidant Capacity; Antidiabetic; Antibacterial; Bioactive Phenolic Compounds.

INTRODUCTION

Date palm (Phoenix dactylifera) is one of the major crops in the south of Tunisia and it is also a source of income for local population [1]. It is also known as “Bread of Desert” as every part of the date palm is useful and it provides nutrition, used as a staple food and used by food industry as raw material. The palm tree sap, called “lagmi” is a white syrupy liquid having nutritional composition rich in sugars, minerals and phytochemicals [1]. Palm fruit is rich source of phenolic compoundsand has been used for centuries for its pharmaceutical properties [2]. There is a renewed interest to identify and quantify phytochemicals with antioxidant properties from plants. Antioxidant constituents from fruits and vegetables are known for their potential health benefits [3-6]. Among phytochemicals, phenolic compounds are known to be effective in prevention of chronic and acute diseases such as cancer, cardiovascular disorders, and inflammations [7].

Kchaou et al, [8] analyzed effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties and demonstrated that these have potential antioxidant activity.

Previous phytochemical studies on date palm sap have suggested that it contains sugars, and phenolic compounds such as flavonoids and bi-flavonoids, phenolic glycosides, and gallic acid derivatives [9] and its storage is very difficult in front of the fast fermentation. Extraction is a very important step in natural product chemistry as extraction process play a very important role in the quality and quantity of bioactive molecules. Recently, microwave extraction has been successfully applied to numerous biologically active compounds from a wide variety of natural resources [10, 11, 12]. In general, the compounds are extracted more selectively and more quickly by this technique with similar or better yields compared with conventional extraction processes. Moreover, this technique uses less energy and solvent and is therefore more environmentally friendly [13, 14, 15]. We applied this technique for the first time for extraction process in date palm sap.

Therefore, the aim of this study was to develop the optimal microwave assisted extraction conditions (Green extraction) for recovery of phenolic compounds and antioxidant properties from the Date Palm Sap using water.

MATERIALS AND METHODS

Plant Material and Procedure Preparation

The sap from the cultivar (Beser, Ameri, Khalet, Thokar,) was collected by a traditional tapping method from palms in Tozeur region (Tunisia). Sap samples were rapidly stored at a freezing temperature (-20°C) to protect them from fermentation.

Extraction of phenolic compounds by Microwave Hydro-distillation (MWHD) with some modifications

In microwave hydro-distillation (MWHD), the hydrodistillation (HD) apparatus is placed inside a microwave oven with a side orifice through which an external cooler joins the vessel containing the Date Palm Sap (500 ml) material and water (500 ml) inside the oven. The oven is operated at full power, which causes water to boil vigorously and reflux [16]. 500 ml of Sap distillated are poured into a separatory funnel, 500 ml of Diethylether are added to the bulb and the mixture is stirred for 10 min. After stirring, the two phases are obtained: organic phase that essentially contains fat and other material. The aqueous phase was filtered through a Wattman filter to remove solid residues and then dried under vacuum at 40°C.

Total flavonoid content

The total flavonoid was determined according to the method of Djeridane et al. [17] where complex flavonoid aluminium was formed with maximum absorbance at 430 nm. Quercetin was used to make the calibration curve. About 1mL of diluted sample was mixed with 1mL of 2% aluminium trichloride ($AlCl_3$) methanolic solution. After incubation at room temperature for 15min, the absorbance of the reaction mixture was measured at 430nm, and the total flavonoid content was expressed in mg quercetin equivalents per gram of extract (mg QE/g extract).

Total antioxidant capacity by phosphomolybdenum method

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method [18]. 0.3 mL of extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then, the absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer (UVmini-1240) against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic (1000, 500, 250, 125, 62.5 and 31.25 µg/mL) with methanol.

Total polyphenols content

The total phenolic content in the extracts was determined with Folin-Ciocalteau reagent using the method of [19]. A standard curve was plotted using gallic acid as a standard. The gallic acid was mixed with methanol, and their absorbances were recorded at 750 nm. 100 μL of diluted sample was added to 2mL of 2% Na_2CO_3 aqueous solution. After 2 min, 100 μL of 50% Folin-Ciocalteau reagent was added. The final mixture was shaken and then incubated at room for 30 min in the dark at room temperature. The absorbance of all samples was measured at 750 nm, and the results are expressed in mg gallic acid equivalents per gram extract (mg GAE/g extract).

Antibacterial assay

The extracts isolated from Date palm sap of the four verities were tested against five microrganisms, Pseudomonas aeruginosa, S. aureus, E. faecalis, Bacillus cereusand, Bacillus subtitilus. All strains are the right of microbial biotechnology laboratory, Faculty of Sciences of Sfax, Tunisia. The bacterial cultures were first grown on Muller Hinton agar (MH) plates at 37°C for 18 to 24 h prior to seeding onto the nutrient agar. One or several colonies of the respective bacteria were transferred into API suspension medium (bioMerieux) and adjusted to 0.5 McFarland turbidity standards with a Densimat (bioMerieux) [20, 21]. The inocula of the respective bacteria were streaked into MH agar plates using a sterile swab and were then dried at 37 °C during 15 min. A sterile wells of 6 mm of diameter was founded at the surface of MH agar and 50µl of extract (1mg/mL) was dropped onto each well [22]. The treated Petri dishes were incubated at 37°C for 18 to 24 h. The antibacterial

activity was evaluated by measuring the clear zone surrounding the wells. Standard discs of the antibiotic penicillin were applied as a positive antibacterial controls.

Determination of α-Amylase activity

The in vitro α-amylase inhibition activity of all samples was determined based on the spectrophotometric assay using acarbose as the reference compound [23]. The sample of date palm sap extract was dissolved in DMSO to give concentrations from 50, 100 and 200 mg/ml. The enzyme α-amylase solution (0.5 mg/mL) was prepared by mixing of 50 mg of α-amylase in 100 ml of 40mM phosphate buffer, pH 6.9. Positive control, acarbose was obtained by dissolving in phosphate buffer. The assay was conducted by mixing 80 ml of sample, 20 ml of a-amylase solution and 1 ml of 2-chloro-4- nitrophenol-a-D-maltotrioside (CNPG3) (2.25 mM). The mixture was incubated at 37°C for 5 min. The absorbance was measured at 405 nm spectrophotometrically (Jenway 6405 UV/Visible, Great Britain). Similarly, a control reaction was carried out without the sample/acarbose. Percentage inhibition (PI) was calculated by the expression:

$$PI = \frac{(\text{Absorbance control} - \text{Absorbance test})}{(\text{Absorbance control})} \times 100$$

RESULTS AND DISCUSSION

Microwave-assisted extraction

Table 1 shows the weight of the date palm sap extracts in solvent diethyl ether. Range of extracts weight was 0.70-2.89 g. It has been established that the extraction weight increases with the time of microwave hydro-distillation (MWHD) 30 mn ± 10. In this study, highest weight was obtained in diethyl ether with Thokar variety (2.89g) and lowest with Beser variety (0.7g). Those results were more important than the amount obtained from tomato [24].

Table 1: The weight of product obtained.

Sap sample	weight (g)
Thokkar	2.89
Beser	0.7
Ameri	0.772
Khalet	0.88

Antimicrobial assays

Date Plam sap displayed varied antibacterial activities across the studied pathogens (Table2). DSP inhibited the growth of bacterial strains producing zone inhibition diameters from 6 mm to 15mm with Gram (-) and Gram (+) bacteria, respectively. Among Gram (+) bacteria, the strongest activity of Date palm sap was observed for Khalet variety against Staphylococcus aureus (16 mm). This value is highest than positive control which it is sensitive to the bacteria, followed by Enterococcus faecalis (15 mm) with Ameri variety. However, there is no activity with Beser variety. Generally, this higher resistance among Gram (-) bacteria could be ascribed to the presence of their outer membrane, surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering. The absence of this barrier in Gram (+) bacteria allows the direct contact of the extract's hydrophobic constituents with the phospholipids bilayer of and

Table 2: Antibacterial activity of the Date palm sap microwave extract.

Strains	*Bacillus cereus*	*Bacillus subtitilus*	*Staphylococcus aureus*	*Pseudomonas aeuginosa*	*Enterococcusfaecalis*
Thokar	-	9	10	9	-
Khalet	-	16	16		10
Ameri	13	13	13	6	15
Beser	-	-	-	-	-
Penicillin	R	14	15	R	14

leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems. The higher resistance of Gram positive bacteria and the less inhibition of the extracts against Pseudomonas aeuginosa (9 mm with Thokar variety and 6 mm with Ameri variety) would be due to the presence of 5-Epipentenomycin I in date palm sap [9].

Amylase activity assays

This assay evaluated the ability of date palm sap extracts to inhibit the activity of α-amylase, a digestive enzyme secreted from the pancreas and salivary gland. Alpha-amylase is involved in important biological processes such as digestion of carbohydrates. Many crude drugs inhibit α-amylase activity [25]. Natural α-amylase inhibitors are beneficial in reducing post-prandial hyperglycemia by delaying the digestion of carbohydrates and consequently the absorption of glucose. Table 3 indicated that only 3(DPS) extracts showed a potent inhibition of α-amylase enzyme. The IC_{50} values related to Thokar and Khalet varieties against α-amylase were 91.024 and 145.45 µg/ml, respectively. Moreover, α-amylase activity underwent a strong inhibition via Acarbose (IC_{50} = 14.88 µg/ml).

It should be mentioned that Acarbose has been used for management of post-prandial hyperglycemia but it was reported that this agent was associated with several health side effects [26]. The potent α-amylase inhibitory activity of date palm sap depended on their total phenolics and flavonoids contents. In fact, many phenolic compounds and specially flavonoids have been reported as potential antidiabetic agents because they exert a good inhibiting action of α-amylase and could have potential prevention in diabetes mellitus as part of a dietary strategy [27].

Table 3: Alpha-Amylase inhibition assays of date palm sap.

Sap	%Inhibition	IC_{50} µg/ml
Thokar	54.93	91.024
Khalet	68.75	145.45
Ameri	35.47	281.92
Beser	-	-
Acarbose	88.72	14.88

Table 4: Total phenolic and flavonoid content and antioxidant capacity.

Sap	TPC[a] (mgGAEb/ g)	TFC[c](mg QEd/ g)	TAC (mg alpha tocopherol/g)
Thokar	26.63 ±0,007	9.64±0,042	220 ±1,414
Khalet	14.47±0,021	13.52±0,056	160±2,828
Besr	24.7±0,282	11.64±0,021	140±0,707
Ameri	20.45±0,070	10.92±0,056	130±1,414

Evaluation of polyphenol and flavonoid contents.

Polyphenols have received considerable attention because of their physiological function, including antioxidant, antidiabetic, antimutagenic and antitumour activities [28]. Phenolic compounds such as flavonoids, and phenolic acids are widely distributed in plants, which have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, with beneficial implications for human health [29]. Table 4 summarizes the results from the quantitative determination of the flavonoids and total phenol contents of the different Product isolated from different varieties of date palm sap. All extracts were found to be rich in flavonoids and polyphenols. The extract from Thokar variety had a high level of phenolic (26.630 ± 0,007 mg GAE/g extract) and a less one of flavonoid (9.640 mg ± 0,042 QE /g extract) contents. The extract from Khalet variety contained less amounts of polyphenols (14.470 ± 0,021 mg GAE/g extract) and flavonoids (13 ± 0,056 mg QE /g extract).

Total antioxidant capacity evaluation

The antioxidant capacity of the DPS extracts was measured spectrophotometrically through phosphomolybdenum method, which was based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate /Mo (V) compounds with a maximum absorption at 695 nm. Table 4 shows that the antioxidant capacity of the different extracts from date palm sap was found to increase in this order:

Ameri < Beser < Khalt < Thokar

The high content of polyphenol and flavonoide in the Thokkar variety allows it to have the highest antioxidant capacity

CONCLUSION

The microwave assisted extraction was shown to be a good technology used at the first time in date palm saps ”Lagmi”. DPS extracts were shown to be rich in polyphenols and flavonoids as well as to possess antioxidant, antibacterial, and antidiabetic activities. Their yields varied with the date palm varieties. The results showed that the extraction efficiency of polyphenols, flavonoids, antioxidant properties and biological activities with the microwave assisted extraction Is necessary in view of the problem of conservation of the sap and its fresh use.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors have not been involved at any stage of the study. They participated neither in the design and the conduction of the experiment, nor, in the analysis of the data or the preparation of the manuscript for publication.

ACKNOWLEDGEMENTS

Authors would like to thank the Laboratory of Pharmacognosy and Natural Products Chemistry Athens 15771, Greece.

ABBREVIATIONS

DSP, date palm sap; GA, gallic acid; QE, quercetin; $AlCl_3$, aluminiumtrichloride; FRAP, Ferric Reducing Antioxidant Power; Na_2CO_3, Sodium carbonate; DMSO, Dimethyl sulfoxide; $CNPG_3$, 2-chloro-4- nitrophenol-a-D-maltotrioside; Ringer's phosphate solution.

REFERENCES

1. Thabet IB, Besbes S, Attia H, Deroanne C, et al. (2009). Physicochemical characteristics of Date Sap ''Lagmi'' from Deglet Nour palm (Phoenix dactylifera L.). Int J Food Prop. 12: 659-670.
2. Gruca M, van Andel TR and Balslev H. (2014). Ritual uses of palms in traditional medicine in sub-Saharan Africa: a review. J ethnobiol ethnomed. 10: 60.
3. Kaur C, Walia S, Nagal S, Walia S, et al. (2013). Functional quality and antioxidant composition of selected tomato (Solanum lycopersicon L) cultivars grown in Northern India. LWT - Food Sci Technol. 50:139-145.
4. Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, et al. (2010). Antioxidants: its medicinal and pharmacological applications. Afr J Pure Appl Chem. 4:142-151.
5. Pandey KB and Rizvi SI. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2(5). 270-278.
6. Vladi Vladimir-Knežević S, Blažeković B, Bival Štefan M and Babac M. (2012). Plant polyphenols as antioxidants influencing the human health. Phytochemicals as Nutraceuticals– Global Approaches to Their Role in Nutrition and Health. 155-180.
7. Linseisen J and Rohrmann S. (2008). Biomarkers of dietary intake of flavonoids and phenolic acids for studying diet–cancer relationship in humans. Eur J Clin Nutr. 47:60-68.
8. Kchaoua W, Abbèsa F, Bleckerb C, Attiaa H, et al. (2013). Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (Phoenix dactylifera L.). Ind Crops Prod. 45:262-269.
9. Abdennabi R, Bardaa S, Mehdi M, Rateb ME, et al. (2016). Phoenix dactylifera L. date palm sap (Beser variety) enhances wound healing in Wistar rats: phytochemical and histological assessment. Int J Biol Macromolec. 88: 443-450.
10. Li Y, Fabiano-Tixier AS, Vian MA and Chemat F. (2013). Solvent-free microwave extraction of bioactive compounds provides a tool for green analytical chemistry. Trends Anal. Chem. 47:1-11.
11. Martins S, Aguilar CN, Garza-Rodriguez I, Mussatto SI, et al. (2010). Kinetic study of nordihydroguaiaretic acid recovery from Larrea tridentata by microwave-assisted extraction. J. Chem. Tech. 85:1142 -1147.
12. Wang J, Zhang J, Zhao B, Wang X, et al. (2010). A comparison study on microwave-assisted extraction of Potentilla anserina L. polysaccharides with conventional method: molecule weight and antioxidant activities evaluation. Carbohydr Polym. 80(1): 84-93.
13. Eskilsson CS and Bjoklund EJ. (2010). Analytical-scale microwave-assisted extraction. J. Chromatogr. A. 902(1): 227-250.
14. Belanger JM and Pare JR. (2006). Applications of microwave-assisted processes (MAP™) to environmental analysis. Anal Bioanal Chem. 386(4): 1049-1058.
15. Srogi K. (2006). A review: application of microwave techniques for environmental analytical Chemistry. Anal Lett. 39(7):1261-1288.
16. Golmakani M-T and Rezaei K. (2008). Comparison of microwave-assisted hydrodistillation with the traditional hydrodistillation method in the extraction of essential oils from Thymus vulgaris L. Food Chem. 109(4): 925-930.
17. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, et al. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 97(4): 654-660.
18. Prieto P, Pineda M and Aguilar M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex, specific application to the determination of vitamin E. Anal. Biochem 269(2):337-341.
19. Chen HY, Lin YC and Hsieh CL. (2007). Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. Food Chem. 104 (4):1418-1424.
20. Saïdana D, Mahjoub S, Boussaada O, Chriaa J. (2008). Antibacterial and Antifungal Activities of the Essential Oils of two Saltcedar Species from Tunisia. J Am Oil Chem. Soc. 85: 817-826.
21. Hichri F, Ben Jannet H, Cheriaa J, Jegham S, et al. (2003). Antibacterial activities of a few prepared derivatives of oleanolic acid and of other natural triterpenic compounds. C R Chim. 6(4): 473-483.
22. Bel Haj Khether F, Ammar S, Saidana D, Daami-Remadi M, et al. (2008). Chemical composition, antibacterial and antifungal activities of Trichoderma sp. growing in Tunisia. Ann Microbiol. 58(2):303-308.

23. Gella FJ, Gubern G, Vidal R and Canalias F. (1997). Determination of total and pancreatic alpha-amylase in human serum with 2-chloro-4-nitrophenyl-alpha-D-maltotrioside as substrate. Clin Chim Acta. 259(1-2):147-160.

24. José Pinela , MA Prieto , Ana Maria Carvalho , Maria Filomena Barreiro , et al. (2016). Microwave-assisted extraction of phenolic acids and flavonoids and production of antioxidant ingredients from tomato: A nutraceutical-oriented optimization study. Separation and Purification Technology 164:114-124.

25. Kobayashi K, Saito Y, Nakazawa I and Yoshizaki F. (2000). Screening of crude drugs for influence on amylase activity and postprandial blood glucose in mouse plasma. Biol Pharm Bull. 23(10):1250-1253.

26. Laborda R, Manzano I, Gamón M, Isabel G, et al. (2013). Effects of Rosmarinus officinalis and Salvia officinalis essential oils on Tetranychus urticae Koch (Acari: Tetranychidae). Ind Crop Prod. 48:106-110.

27. Orhan I, Aslan S, Kartal M, Bilge S, et al. (2008). Inhibitory effect of Turkish Rosmarinus officinalis L. on acetylcholinesterase and butyrylcholinesterase enzymes. Food Chem. 108: 663-668.

28. Othman A, Ismail A, Ghani NA and Adenan I. (2007). Antioxidant capacity and phenolic content of cocoa beans. Food Chem. 100(4):1523-1530.

29. Govindarajan R, Singh DP and Rawat AKS. (2007). High-performance liquid chromatographic method for the quantification of phenolics in “Chyavanprash‟ a potent Ayurvedic drug. J Pharm Biomed Anal. 43(2): 527-532.