2020

Evaluation of the level of biomolecules isolated from date palm seeds (Phoenix dactylifera L) and in vitro Antioxidant property

Najla BENTRAD Dr
USTHB, bentrad.najla@gmail.com

Rabéa Gaceb-Terrak
gacebTerrak@yahoo.fr

Follow this and additional works at: https://www.biomedicinej.com/biomedicine

Part of the Life Sciences Commons, and the Medical Sciences Commons

This work is licensed under a Creative Commons Attribution 4.0 License.

Recommended Citation
BENTRAD, Najla Dr and Gaceb-Terrak, Rabéa (2020) "Evaluation of the level of biomolecules isolated from date palm seeds (Phoenix dactylifera L) and in vitro Antioxidant property," BioMedicine: Vol. 10 : Iss. 2 , Article 4.
DOI: 10.37796/2211-8039.1017

This Original Articles is brought to you for free and open access by BioMedicine. It has been accepted for inclusion in BioMedicine by an authorized editor of BioMedicine.
Evaluation of the level of biomolecules isolated from date palm seeds (Phoenix dactylifera L) and in vitro Antioxidant property

Cover Page Footnote
Dear editor Before publication process, please take into consideration this last version of the article after review by the seconds co-authors 'Gaceb-Terrak Rabéa'. Cordially Bentrad Najla

This original articles is available in BioMedicine: https://www.biomedicinej.com/biomedicine/vol10/iss2/4
Evaluation of the level of biomolecules isolated from date palm seeds (*Phoenix dactylifera*) and *in vitro* Antioxidant property

Najla Bentrad*, Rabéa Gaceb-Terrak

Laboratory Research on Arid Zones, Faculty of Biological Sciences, Department of Biology and Physiology of Organisms, University of Sciences and Technology Houari Boumediene (USTHB), BP 32, 16111 El-Alia, Bab Ezzouar, Algiers Algeria

Abstract

Date palm fruits and by-products such as seeds are a source of various elements with significant nutritional values like fibres, minerals, essential fatty acids, amino acids and phenolic compounds. The experimental part was carried out on date palm seeds from Bent Kbala cultivar, the chemical composition of the organic fraction was determined using the method of UV-visible spectrophotometer, thin layer chromatography (TLC) and high-performance liquid chromatography-diode array detection (HPLC-DAD).

The results revealed the presence of catechin tannins and approximately 17 phenolic compounds, including two compounds, which were identified for the first time in the date palm sub-product, especially in seeds such as naringenin and rutine. The assessment of the antioxidant potential shows that date palm seeds have a significant potential compared to standard antioxidants commonly used in cosmetics and neutraceuticals industries.

**Keywords:** Date palm seeds, polyphenols, TLC, HPLC-DAD, antioxidant activity

Introduction

Plants have been extensively investigated for its antioxidant properties, since phenolic compounds are widely used and considered to be potential sources of antioxidants [1]. The interest in natural antioxidants, especially polyphenolic compounds, continues to increase, these secondary metabolites are consumed daily by dietary intakes. There are many industrial requirements for use of natural sources of antioxidants:

- High concentration of active molecules, adequate food supply, regular and, if possible, non-seasonal, good raw material conservation, reasonable cost, easy use, process of extraction, low aromatic character of the preparations, absence of toxicity in the extract, authorization and legal use in the food, pharmaceutical and cosmetic industries. In the process of oxidative stress regulation, flavonoids can act in various ways, it has been shown that flavonoids act *in vitro* to reduce dehydroascorbic acid via glutathione, against which they act as donors of hydrogen [2--3].

The selection of a convenient antioxidant is based on the properties required such as efficiency, solubility, heat stability and the nature of the food to be protected. In general, their antioxidant efficacy is due to their high flavonol and flavanol content, in particular epigallocatechin [4]. The search for new sources of natural antioxidants from by-products is also aimed at agricultural and food industries [5] for example cereal bran [6], fruit pips and citrus fruits [7] and grape berries in particular [8].

The beneficial nutritional values of the date palm fruit (*Phoenix dactylifera*) have long been claimed for consumption and human health [9]. Date palm seeds were considered as date fruit waste and it is now used as animal feed [10]. On the other side,
date fruit seeds are used as food supplements [11] and have been roasted and milled and taken as coffee drink. The seed coffee powder meal has actually been widely marketed [11–12]. In traditional medicines, powdered seeds, is used as eyeshadow [11].

Also, it has been shown that the natural date seeds extract has a strong ability to inhibit *Pseudomonas ATCC 14209* B1 phage infectivity (infectivity), which is known to be resistant to disinfection [13]. According to another research, extracts isolated from date palm sub-products have shown significant anti-microbial potential [14–16].

The aim of the present study is firstly, to identified phenolic compounds from date palm seeds (by-products) using Spectrophotometer UV-Visible, TLC and HPLC-DAD. Secondly, to determine in vitro antioxidant properties by evaluation of the anti-radical activity of organic extracts of seeds and antioxidants standards by the method using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) considered relatively as a stable free radical.

**Materials & methods**

**Plant material**

Date palm fruit of Bent Kbala (BK) cultivars were collected from the region of Metlili, Ghardaia in Algeria (32° 16’ North, 32° 16’ East). The seeds were obtained by removing the endocarp, while the teguments around the seeds were preserved. The seeds (BK) were reduced to very fine powder by an electric grinder (type KSW 445 CB) and stored in a hermetically sealed bottle, protected from moisture and light.

**Total polyphenols**

A maceration of 5 g of fine seeds powder in 100 mL pure methanol (99% MeOH) was performed for 24 hours under constant stirring on an agitator type “Heidolph Promax 2020” at 140 stirring min⁻¹. The obtained methanol extracts are filtered using a Buchner funnel and stored for 48 hours in the refrigerator at 6 °C. The quantitative determination of total polyphenols was carried out in accordance with the colorimetric method using Folin-Ciocalteu reagent after the addition of sodium bicarbonate at 20% [17]. The absorption of phenolic acid standards and seeds extract at 765 nm was read using the spectrophotometer “JENWAY 7305 UV/VIS”. The concentration of phenolic compounds and tannins is determined by the equation of reference molecules such as gallic acid and catechin tannin calibration curve. The results are expressed in µg gallic acid equivalent per gram of dry wight (GAE·g⁻¹ DW) for phenolic acids and catechin equivalents (CE·g⁻¹ DW) for condensed tannins. Antioxidant tests were also used in this fraction.

**Catechin tannins**

Under these conditions, tannins are condensed into red precipitation, which confirms their presence in the medium. In order to distinguish between the two types of tannins (Gallic or catechin tannins), we used the Stiasny reagent, which is based on the Diallo method [18]: For 10 mL of extract, we added 5 mL of the Stiasny reagent, followed by 15 minutes of heating in a water bath at 90 °C. A revelation of greenish or blackish blue coloration of the extract results from the presence catechin or gallic tannins.

**Extraction of flavonic aglycons**

The seeds extract is obtained according the protocol optimized by Lebreton and co-workers [19]: Approximately 1g of dry vegetable powder is hydrolyzed with 80 mL of hydrochloric acid (2N), the mixture was heated in a bath of boiling water for 40 min. After cooling, the hydrolyzed powder is filtered and then transferred to a separating glass funnel, the separation is carried out twice (20-20 mL) with diethyl ether.

Following the separation of organic phase containing active compounds, it is evaporated under a ventilated host and then taken into pure methanol. This fraction is used for phytochemical investigation to recognize bioactive compounds by Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC-DAD). In addition, antioxidant tests were carried out using the bioactive compounds of this fraction (flavonic aglycons). In our previous work, the determination of the chemical content of aglycones was already carried out using Gas chromatography—mass spectrometry [15–16].

**Thin layer chromatography (TLC) analysis**

TLC is a semi-qualitative analytical method that allows chemical compounds to be separated and partially identified in a complex mixture. The separation of the flavonoids was carried out by a single-dimensional TLC on silica gel F254 with a thickness of 0.2 mm and an aluminum support of 20 × 20 cm, which was developed in a mobile phase using the solvent system (eluent): Acetic acid/Chloroform (9-1: v/v).
Following separation, the partial identification of the flavonoids is carried out by comparing the calculated frontal ratio (FR) of each substance to the standards or molecules [20] under the ultraviolet light of the UV lamp at two wavelengths of 365 and 254 nm.

The disclosure was performed with ammonia vapor (NH₃), the color reaction allows an unknown substance to be partially connected to a class of phenolic compounds. The flavonoids fluorescence that gives us information about the various phenolic classes. Each substance is characterized by FR that corresponds to the ratio between the molecule displacement (d) and the solvent front displacement (D).

High performance liquid chromatography (HPLC-DAD) analysis

The chromatograph is equipped with a brand system the Agilent 1100 series, a quaternary pump and an automatic injector. The column is of the type Hypersil™ BDS C₁₈, 5 µm, 250 × 4.6 mm at 30 °C. The mobile phase consists of water acidified with acetic acid (0.2%) at a pH of 3.1 and acetonitrile with a linear elution gradient for 30 minutes at 1 mL/min. The detection of separable substances is based on a diode array detector (DAD Diode Array Detector), which enables both to measure absorbance directly on a plurality of wavelengths: 200, 254, 280, 330 and 355 nm selected according to the molecules maximum absorbance.

Evaluation of the antioxidant activity

The study of the anti-radical activity of seeds extracts from the date palm and natural antioxidants of reference was carried out in accordance with the method described by Blois [21] using 2,2-diphenyl-1-picryl-hydrazyl (DPPH), which it is a relatively stable free radical. Antioxidants reduce the violet color of DPPH to a yellow color, diphenyl-picryl-hydrazine, whose color intensity is inversely proportional to the antioxidant's ability to give protons in the mixture. The DPPH radical is an oxidant that the antioxidant potential can be measured at 517 nm.

A 0.3 mM solution of DPPH is prepared in methanol according to the protocol established by Gulcin et al. [22] and adapted by Bouhlali et al. [23]; 800 µL of this solution are added to 2 mL of reference antioxidant. Our choice has been made with known natural antioxidants: Vitamin E (10 µg mL⁻¹), ferulic, gallic and L-ascorbic acids (5 µg mL⁻¹). The organic seeds extract was assessed for its antioxidant potential. As before, 800 µL of DPPH solution (0.3 mM) is added to 2 mL of seeds extract. The following formula is used to estimate the anti-radical potential of crude extract from plant or reference substances as follows:

\[ (I \%) = \left\{ \frac{A_0 - A_1}{A_0} \times 100 \right\} \]

\( I \%: \) Percent inhibition of DPPH

\( A_0: \) Absorbance of white (methanol)

\( A_1: \) Absorbance of the tested sample (organic extract or reference molecule)

50% inhibition concentration: IC₅₀

After spectrophotometric determination at a wavelength equal to 517 nm, the concentration that trapped 50% of the radical DPPH or the 50% inhibition concentration designated IC₅₀ is graphically determined.

Statistical analysis

The experimental data are expressed in standard mean error (m ± SEM) from n separate experiments and the significant differences between the experiments were recorded at p < 0.05.

Results & discussion

Detection and identification of phenolic compounds

Quantification of phenolic compounds and catechin tannins

According to this work, the seeds containing a low amount of phenolic acids, it is approximately estimated at 2.5 ± 0.453 mg.g⁻¹ gallic acid equivalent (GAE), but it contains a significant amount of catechic tannins and it has been recorded at approximately 31.3 ± 0.01 mg.g⁻¹ catechin equivalents (CE). The condensed tannins (non-hydrolyzable tannins) derive from the condensation of flavan-3-ol monomers such as catechin and/or epicatechin. The identification of these phenolic polymers is carried out by a chemical reaction which is added a few drops of ferric chloride solution (FeCl₃³⁻) to the crude phenol extract. We note that the seed extracts from date palm show a positive response. The presence of catechin tannins is revealed by a black to intense greenish-black precipitate. However, this tests conducted to demonstrate the presence of hydrolysable tannins showed a negative reaction, leading to the conclusion, date palm seeds of Bent Kbala cultivars do not contain hydrolysable tannins. Few studies have been done on the composition of catechin tannins in the date palm seeds, a major by-product of the date.
Furthermore, cytohistological techniques have already revealed them in seeds teguments of some other date cultivars such as Deglet Nour and Takberucht [24]. We have shown that anthocyanidins from date seeds inhibit the proliferation of some endophyte fungi from five special fungi from *Fusarium oxysporum* spice [15], which causes significant economic damage to cultivated plants and the environment.

Tannins are generally used in the case of venous insufficiency, hemorrhoidal symptoms, oral hygiene, as antihemorrhagic, anti-dysenteric, cardial erethic disorders and sleep disorders. Tannins are widely used in the skin tanning industry in industrial applications, but we must note their great importance in food industries such as cider and fruit juice.

**Flavonic aglycones and phenolic acids identified by TLC**

In our previous work [15], the determination of the quantitative content of flavonic aglycones was carried out. The TLC of organic extracts (ether phase) provided a good separation of the molecules and an acceptable visibility of the spots under UV (wood light). By calculating the frontal ratios (FR) and taking into account the fluorescence of each revealed compound, we detected the presence of five phenolic compounds (Table 1). The substances are numbered in ascending order of their revealed frontal ratios (FR) at 365 nm (Band I) and 254 nm (Band II). All these results show that the organic extract shows brownish yellow spots observed below 245 nm after spraying with NH₃⁻⁻⁻ corre-sponding to phenolic acids; the UV fluorescence of which is dark blue below 365 nm. Also, aglycone flavonol emit a yellow fluorescence of less than 365 nm.

**Phenolic compounds profiled by HPLC-DAD**

The biochemical investigation of natural extract from date seeds by chromatography by diode cluster discovery (HPLC-DAD) at distinctive wavelengths shows the proximity of seventeen (17) phenolic compounds, of which thirteen (13) are identified at 200 nm, ten (10) at 280 nm, nine (9) at 250 and/or 355 nm, and five (5) at 254 nm. The following families and classes belong to these phenolic compounds (Table 2):

The chromatographic analysis by HPLC-DAD in organic seed extracts is revealed: Quercetol and Isorhamnetin, these molecules detected and identified in seed samples are already found in palm leaves of date cultivars. In the seeds organic extract, a flavone was found, it is Apigenin. The date palm leaves, have already shown this flavone but in their C- glycosylated form, Vitexin [25].

For the first time in the date palm, a new phenolic compound is highlighted, we note that this compound is detected at all wavelengths used. It corresponds to O-glycosylated flavanone, commonly referred to as Prunin or Naringenin 7-O-glucoside. A Quercetol 3-O-rutinoside is a glycosylated flavonol compound, is showed for the first time in the date palm seed.

HPLC-DAD analysis has revealed phenolic acids whose basic skeleton is C₆-C₁ or C₆-C₆ of these compounds are: Six phenolic acids of the benzoic series with a C₆-C₁ basic skeleton, derived from benzoic acid, were identified in the analyzed seed samples, namely hydroxybenzoic acids:

Five phenolic acids derived from coumaric acid (C₆-C₃) was identified such as trans- cinnamic,

| Compound                  | Retention time (RT) | IUPAC nomenclature                      | Wavelength | \(\lambda_{max} (nm)\) |
|---------------------------|---------------------|----------------------------------------|-------------|------------------------|
| N'                        |                     |                                       |             |                        |
| 1                         | 3.736               | Hydroxyquinate                         | 200         |                        |
| 2                         | 6.468               | Acid para -hydroxybenzoic              | 200, 280    |                        |
| 3                         | 6.657               | Resorcylic acid                        | 200, 230, 254 |                        |
| 4                         | 7.070               | Caffeic acid                           | 254         |                        |
| 5                         | 7.251               | Dihydroxycinnamic acid                 | 200, 230, 254, 280 |          |
| 6                         | 8.647               | Rutin                                  | 200, 230, 280 |                        |
| 7                         | 8.781               | Hydroxyccinnamic acid                  | 200, 230, 280, 355 |          |
| 8                         | 9.273               | Ferulic acid                           | 200, 230, 280, 355 |          |
| 9                         | 9.627               | Syringic acid                          | 200         |                        |
| 10                        | 9.897               | Salicylic acid                         | 355         |                        |
| 11                        | 10.377              | Naringenin 7- O - glucoside            | 200, 230, 254, 280, 355 |          |
| 12                        | 10.899              | Acid 3,4,5 trimethoxybenzoic m- anisic acid | 200, 230 |          |
| 13                        | 11.828              | Quercetin                              | 280, 355    |                        |
| 14                        | 12.873              | Apigenin                               | 200, 230, 280, 355 |          |
| 15                        | 13.840              | Trans-cinnamic acid                    | 254, 280, 355 |          |
| 16                        | 14.458              | Apigenin                               | 200, 230, 280, 355 |          |
| 17                        | 15.080              | Isorhamnetin                           | 200, 355    |                        |

**Table 2. Main phenolic compounds detected at different wavelengths and identified by HPLC-DAD in the organic extract of date palm seeds.**
endothelial tissues [38]. Rutin inhibits the growth of very high concentrations of vascular tissues.

Rutin acts as an angiogenesis inhibitor and inhibits the growth of these compounds have been found for the flavonoids. Flavonoids generally have the ability to inhibit the effects [31].

Quercetin, for example, is a powerful antioxidant, better than ascorbic acid (vitamin C), as it inhibits linoleic acid oxidation [32]. Quercetin also carries out biological activities in the prevention and treatment of hypertension and endothelial dysfunction in rats with spontaneous hypertension [33], as well as in rats with hypertension caused by chronic inhibition of nitric oxide synthase [34], or in rats with renal hypertension [35]. The antioxidant and chelating ability of flavonoids is attributed to most of the biological activities. Epidemiological studies have shown, among other things, that flavonol-rich foods reduce the risk of coronary heart disease mortality [36]. Several studies have shown that a flavonoid-rich diet can have beneficial health effects. Rutin, also known as rutoside, quercetin-3-O- rutinoside or sophorin, is a glycoside that combines Quercetin (flavonol) and rutinose (disaccharide).

Rutin is also an antioxidant that acts on the inhibition of LDL oxidation compared to Quercetin, Apigenin, Kaempferol and Luteolin [37]. In vitro, Rutin acts as an angiogenesis inhibitor and inhibits the growth of very high concentrations of vascular endothelial tissues [38]. Rutin inhibits the aggregation of platelets and reduces capillary permeability, thinning the blood and improving circulation [39]. In most plants, isorhamnetin is a neuroprotective compound used to treat brain disorders, neurosensory syndrome, peripheral blood flow disorders and brain failure [40]. In phenylpropanoids derived from cinnamic acid, numerous cis forms have been found, such as cis-para-coumaric, cis-ferulic and cis-caffeic acids in plants. In mammals, such as acids, hydroxycinnamic acid (para-4-hydroxycinnamic acid) has antioxidant properties and may reduce the risk of abdominal cancer [41].

Anti-radical activity (scavenger effect) of DPPH

After the addition of DPPH solution, we noticed a dissipation of the initial purple color, in the organic extract seeds to yellow color. Also a positive response to the DPPH test was observed with antioxidants molecules (ferulic, gallic, citric, L-ascorbic acids and alpha- tocopherol), there is a significant variation in this antioxidant activity (p > 0.05). However, there is no antioxidant activity was registered in macerate seed (BK). The antioxidant results are expressed as a percentage (Table 3).

Date palm seed extract showed the highest inhibition rate (89.89 ± 0.02%) and alpha- tocopherol (84.4 ± 0.04%) a powerful antioxidant vitamin. The anti-radical power of gallic acids (70.5 ± 0.156%), L-ascorbic acids (61.5 ± 0.04%) and citric acids (62.6 ± 0.15%) is less than those observed previously in seeds extract and vitamin E. Ferulic acid, a reference phenol acid with the lowest anti-radical power (48.73 ± 0.173%). From these data (Table 3), each extract was determined graphically at a concentration of 50% (IC50) of the radical DPPH.

This concentration is 0.031 μg mL⁻¹ for organic seed extract, so the organic extract BK requires the lowest concentration complex with 50 percent of the free radical DPPH (IC50 = 0.031 μg mL⁻¹), the antioxidant activity is in fact closely related to the extract concentrations because the lowest IC50 shows the most important antioxidant activity compared to other standard antioxidants.

Table 3. Results of the free radical inhibition test and extracts studied.

| Compounds | Antioxidant activity (%) | IC50 (μg.mL⁻1) |
|-----------|-------------------------|---------------|
| Seeds     | 84.97 ± 0.024           | 0.031 ± 0.033 |
| α-Tocopherol | 84.40 ± 0.040         | 1.50 ± 0.43   |
| Gallic acid | 70.50 ± 0.156         | 3.21 ± 0.71   |
| Citric acid | 62.60 ± 0.150         | 1.79 ± 0.12   |
| Acid L-ascorbic | 61.53 ± 0.040     | 2.50 ± 0.53   |
| Ferulic acid | 48.73 ± 0.173         | 4.96 ± 0.17   |
| Macerat   | -                       | -             |

Table 3. Results of the free radical inhibition test and extracts studied.
The synergistic interactions between antioxidants in the mixture depend not only on the concentration of antioxidants, but also on the structure and nature of the biological activity involved [42]. Polyphenols appear to be effective suppliers of hydrogen to the DPPH radical due to their chemical structure [43].

They act primarily as primary antioxidants to stabilize the peroxide radicals, but they can also deactivate reactive oxygen species such as superoxide ions \((\text{O}_2^-)\), hydroxyl radicals \(\text{OH}\) or singlet oxygen [44]. In fact, in particular, phenolic compounds and flavonoids are recognized as potentially antioxidant, the scavenger effect of flavonoids (FLOH) is attributed to their low potential redox, which allows the transfer of hydrogen atoms through hydroxyl groups and stable radical molecules (RH) to reduce free radicals (R*) [45]. Middleton and co-workers [46] showed that free radical scavenging depend on several structural factors, such as the most effective flavonoids containing 3',4' dihydroxyl groups on ring B and/or a 3-OH group on cycle C.

The presence of free OH in position 3 on the C ring is favorable for antioxidant activity and, for the same molecule, its glycosylation leads to a decrease in activity, which is more pronounced when sugar is a diholoside [44, 46].

Conclusion

This work initiates the investigation of active compounds from date palm seeds (sub-product of Phoenix dactylifera) with antioxidant interest. The extraction of organic fractions and biochemical characterization using spectrophotometers UV-Visible, TLC and HPLC-DAD allowed the identification of a very diverse range of phenolic compounds, such as flavonoids, three flavonols (quercetin, isorhamnetin and kaempferol), four flavones (luteolin, chrysoeriol, tricin and apigenin), However, a flavanone (prunin or naringenin 7-O-glucoside) and an O- glycoside (rutin) are identified for the first time in date palms seeds. Phenolic acids have also been identified, such as \(p\)-hydroxybenzoic, salicylic, resorcylic, \(m\)-anisic, syringic and 3,4,5- trimethoxybenzoic (C\(_6\)-C\(_1\)) and five acids listed below, caffeic, dihydroxycinnamic and ferulic (C\(_6\)-C\(_3\)), and a single phenol: Benzoquinone (hydroxyquinone).

The organic seed extract from Bent Kbala cultivar has the highest antioxidant activity \((\text{IC}_{50} = 0.031 \, \mu\text{g.mL}^{-1})\) compared to other antioxidants in relation to the antioxidant activity. In the future, it would also be interesting to consider the valuation of the date product (pulp) and sub-product (seed), which enables different antioxidant molecules to be produced. This would enable concentrated food (powder or granules) to be produced in accordance with scientific standards.

Funding

The General Direction of Scientific Research and Technological Development (DGRSDT) in Algeria and the General Direction of National Security (DGSN), central laboratory of the scientific police.

Conflicts of interest statement

The authors wish to disclose no conflicts of interest.

References

1. Shahidi F. Natural Antioxidants: Chemistry, Health Effects, and Application. Champaign, Illinois: AOCS Press; 1997. p. 64–75.
2. Peterson J, Dwyer J. Flavonoids: dietary occurrence and biochemical activity. Nutr Res 1998;18:1995–2018.
3. Bruneton J. Pharmacognosie, phytochimie, plantes medicinales. 4ème Ed. Tec & Doc; 2009. p. 245. Lavoisier.
4. Gramza A, Korczak. Tea constituents as antioxidants in lipid systems. Trends Food Sci Technol 2005;16:351–8.
5. Fki I, Allouche N, Sayadi S. The use of polyphenolic extract, purified hydroxytyrosol and 3,4-dihydroxyphenyl acetic acid from olive mill wastewater for the symbolization of refined oils: a potential alternative to synthetic antioxidants. Food Chem 2005;93:197–204.
6. Duh PD, Yen GC. Antioxidant efficacy of methanolic extracts of peanut hulls in soybean and peanut oils. J Am Oil Chem Soc 1997;74:745–8.
7. Anagnostopoulou M, Kefalas P, Papageorgiou V, Assimopoulou A, Boskou D. Radical scavenging activity of various extracts and fractions of sweet orange peel. Food Chem 2006;94:19–25.
8. Guendez R, Kallithraka S, Makris D, Kefalas P. Determination of low molecular weight polyphenolic constituents in grape seed extracts: correlation with antiradical activity. Food Chem 2005;89:1–9.
9. Chaira N, Smaali MI, Martinez-Tome M, Mrabet A, Murcia MA, Ferchichi A. Simple phenolic composition, flavonoid contents and antioxidant capacities in water-methanol extracts of Tunisian common date cultivars (Phoenix dactylifera L.). Int J Food Sci Nutr 2009;60(57):316–29.
10. Habib HM, Ibrahim WH. Nutritional quality evaluation of eighteen date pits varieties. Int J Food Sci Nutr 2009;60:99–111.
11. Benchelah AC, Maka M. Les dattes de la préhistoire à nos jours. Phytothérapie 2006;1:43–7.
12. Ali-Mohamed AY, Khamis ASH. Mineral ion content of the seeds of six cultivars of Bahraini date palm (Phoenix dactylifera). J Agric Food Chem 2004;52:6522–5.
13. Jassim SAA, Naji MA. In vitro evaluation of the antiviral activity of an extract of date palm (Phoenix dactylifera L.) pitis on a pseudomonas phage. Evid Based Compl Alter Med 2007;15:1–6.
14. Azouaoui-Ait Kettout T, Gaceb-Terrak R, Rahmania F. The effect in vitro of flavonoid aglycones extracts from roots of date palm cultivars on Fusarium oxysporum f. sp. albedinis. Int J Agric Eng 2013;7(9):739–41.
15. Bentrad N, Gaceb-Terrak R, Bennmalek Y, Rahmania F. Studies on chemical composition and antimicrobial activities of bioactive molecules from date palm (Phoenix dactylifera L.) pollens and seeds. Afr J Tradit Compl Altern Med 2017;14:242–56.
16 Bentrad N, Gaceb-Terrak R, Rahmania F. Identification and evaluation of antibacterial agents present in lipophilic fractions isolated from sub-products of Phoenix dactylifera. Nat Prod Res 2017;31:2544–8.
17 Ribéreau-Gayon P. In: Dunod, editor. Les composés phénoliques des végétaux; 1968. p. 254. Paris.
18 Singleton VL, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999;299: 152–72.
19 Diallo AM. Niafunke. Thesis. Algiers-Algeria: University of Science and Technology Houari Boumediene; 2010.
20 Lebreton PH, Jay M, Voirin B, Bouchez MP. L’identification des flavonoïdes de la maïs d’Arménie; 1987.
21 Blois MS. Antioxidant determination by the use of a stable free radical. Nature 1958;181:1199–200.
22 Gulcin I, Alici AH, Cesur M. Determination of In Vitro Antioxidant and Radical Scavenging Activities of Propofol. Chem Pharm Bull 2005;53:281–5.
23 Bouhlali ET, Alem C, Ennassir J, Benlyas M, Nait-Mbark A, Zegzouti YF. Phytochemical compositions and antioxidant capacity of three date (Phoenix dactylifera L.) seeds varieties grown in the South East Morocco. J Saudi Soc Agric Sci 2015;3:63–7. https://doi.org/10.1016/j.jssas.2015.11.002.
24 Gaceb-Terrak R. Contribution to the knowledge of interactions between date palm (Phoenix dactylifera L.) and causal agent of bayoud (Fusarium oxysporum L ap albedinis) by phytochemical analyses of lipids and phenylpropanoids. PhD Thesis. Algiers-Algeria: University of Science and Technology Houari Boumediene; 2010.
25 Gaceb-Terrak R. Contribution to the study of Fusarium wilt of the date palm Phoenix dactylifera: Identification of flavonoids. Thesis of magister. Algiers-Algeria: University of Sciences and Technology Houari Boumediene; 1987.
26 Psotova J, Kolár M, Sousek J, Svagera Z, Vicar J, Ulrichová J. Biological activities of Prunella vulgaris extract. Phytotherapy Res 2003;17:1082–7.
27 Al-Farsi MA, Lee CY. Optimization of phenolic and dietary fibre extraction from date seeds. Food Chem 2008;108: 977–85.
28 Mansouri A, Embarek G, Kokkalou E, Kefalas P. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix dactylifera). Food Chem 2005;89:411–20.
29 Larson RA. The antioxidants of higher plants. Phytochem 1988;27:969–78.
30 Ouaflà S. Chemotaxonomic study by flavonoids of date palm cultivars (Phoenix dactylifera L.). Thesis of magister. Algiers-Algeria: University of Sciences and Technology Houari Boumediene; 1987.
31 Hooper L, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, Ryder JJ, Hall WL, Cassidy A. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2008;88:58–50.
32 Courret C, Collin S. Effect of the number of flavonol units on the antioxidative activity of prolycanidin fractions isolated from chocolate. J Agric Food Chem 2003;51:6816–22.
33 Duarte J, Pérez-Palencia R, Vargas F, Ocete MA, Pérez-Vizcaíno F, Zarzuelo A, Tamargo J. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. Br J Pharmacol 2001;133(1):117–24.
34 Duarte J, Jiménez R, O’Valle F, Galisteo M, Pérez-Palencia R, Vargas F, Perez-Vizcaíno F, Zarzuelo A, Tamargo J. Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. J Hypertens 2002;20:1843–54.
35 García-Saura MF, Galisteo M, Villar IC, Bermejo A, Zarzuelo A, Vargas F, Duarte J. Effects of chronic quercetin treatment in experimental renovascular hypertension. Mol Cell Biochem 2005;270:147–55.
36 Huxley RR, Neil HA. The relation between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. Eur J Clin Nutr 2003;57:904–8.
37 Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. J Nutr Sci Vitaminol 2001; 47(5):357–62.
38 Luo H, Jiang BH, King SM, Chen YC. Inhibition of cell growth and VEGF expression in ovarian cancer cells by flavonoids. Nutr Cancer 2008;60(6):800–9.
39 Navarro-Núñez L, Lozano ML, Palomo M, Martínez C, Vicente V, Castillo J, Benavente- García O, Diaz-Ricart M, Escobar G, Rivera J. Apigenin inhibits platelet adhesion and thrombus formation and synergizes with aspirin in the suppression of the arachidonic acid pathway. J Agric Food Chem 2008;56(9):2970–6.
40 Kleijnen J, Knipschild P. Ginkgo biloba for cerebral insufficiency. Br J Clin Pharmacol 1992;34:352–8.
41 Ferguson LR, Shuo-tun Z, Harris PJ. Antioxidant and anti-inflammatory effects of plant cell wall hydroxycinnamic acids in cultured HT-29. Mol Nutr Food Res 2005;49(6):585–93.
42 Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H, Bourgou S, Ksouri R, Bellila A, Skandrani I, Falleh H. Flavonoid composition and biological activities of Tunisian Nigella sativa L. shoots and roots. Comptes Rendus Biologies 2008;331:48–55.
43 Turkmen N, Velioglu YS, Sari F, Polat G. Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. Molecules 2007;12:484–96.
44 Wiseman SA, Balentine DA, Balz F. Antioxidants in tea. Criti Rev Food Sci Nutr 1997;37(6):693–704.
45 Javanovic SV, Steenken S, Tomic M, Marjanovic B, Simic MJ. Flavonoids as antioxidants. J Am Chem Soc 1994;116:4846–51.
46 Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Pharmacological Rev 2000;52:673–751.