Bentrad et al., Afr J Tradit Complement Altern Med., (2017) 14 (3) : 242-256
doi:10.21010/ajtcam.v14i3.26

STUDIES ON CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF BIOACTIVE MOLECULES FROM DATE PALM (*PHOENIX DACTYLIFERA* L.) POLLENS AND SEEDS

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Abstract

Background: Natural resources have been the crucial origin of chemical elements. They have been used in many traditions as alternative medicines. The chemical profiling of some plant extracts and essential oils related to different plants were followed to unveil their most active components. In this paper, *Phoenix dactilyfera* L was selected as a host plant to investigate the composition of different organs with different cultivars.
Materials and method: The antibacterial and antifungal activities of the extracts have been tested using different techniques, including optical density and GC/MS analyses of the natural extracts.
Results: GC/MS analysis revealed the presence of abundant oleic (36.69%) and lauric (20.49%) acids in date seeds. However,the pollen contains a high amount of palmitic (22.27 %), linoleic (33.4%) and linolenic (17.055%) acids.
Moreover, the largest inhibition zone is obtained with the organic extract of Deglet Nour which showed a strong antibacterial activity against *Escherichia coli* and pollen extract showed also a strong inhibition against *Escherichia coli, Staphylococcus aureus, S. aureus* MRSA and *Enterococcus faecalis*. Aqueous extracts of date palm seeds of and pollen seem to have a fongitoxique activity from a concentration of 6 $mg.mL^{-1}$ and $12mg.mL^{-1}$ of cyanidine; as well as the organic extracts of pollen with a concentration of 90 $\mu g.mL^{-1}$ induce an inhibition to the growth of five special forms of *Fusarium oxysporum*.
Conclusion: The bioactive compounds of date palm can be used for drug development and in the food industry.

Key words: Pollen, dates seeds, fatty acids, flavonoids, pathogenic bacteria, fungitoxicity.

Introduction

The search for new, bioactive chemical compounds have received a great interest, either in drug development or in the food industry. In fact, production of natural drugs or fungicides from plant sources has become an essential element in developing countries, bordering on high rate of health and environmental problems, and related infectious diseases (Sashi and al., 2003). Therefore, the therapeutic properties of plants have become an essential element of healthcare all over the world. Consequently, new bioactive molecules merit consideration for their best therapeutic uses, such as antibiotics and antifungal medication, as well as to preserve the environment and people's health. Based on the World Health Organization's (WHO) investigation, almost 80% of African populations rely exclusively on plants for their primary healthcare needs (Sujatha, 2005), while about 61% of herbal medicines are commercialized all over the world (Patel and Kumar, 2008). Furthermore, essential constituents of plants are not only used as plant extracts for therapy, but they are also used in the search for precursors of potential active chemical compounds and in drug development (Cragg and al., 1997).

Due to the alarming increase in the number of pathogenic microorganisms which are resistant to drugs and, therefore, compromise the existing antibiotic and antifungal agents, updating of antibiotic and antifungal formula or addition of a new active agent has become a challenging research field (Boulenouar and al., 2009).

Phoenix dactylifera L. (date palm) is specie belonging to Arecaceae family represents a source of food interest for populations of desert regions. It is distributed in arid areas, particularly in South Asia, North Africa and parts of Central America (Zaid and De Wet, 1999).

In Algeria, various parts of date palm as well as the date fruits and seeds are used for prophylaxis and treatment of many human diseases. According to an ethnobotanical study, parts of date palm are traditionally used to treat anemia and demineralization, in infusion for cold, as a gargle for sore throat, crushed in water to treat hemorrhoids, constipation and jaundice. Green dates are toning, aphrodisiac and can treat intestinal disorders such as diarrhea (Benchelah and Maka, 2006). Date palm syrup is useful in the treatment of broncho-pulmonary infections and is also used as a sedative. The powdered seeds are used as food supplements, and as coffee substitute (Bellakhdar, 1997).

The date palm pulp is used as a sweetener in the preparation of beer as well as in other industrial processes, especially in the production of syrup and confectionery (Rahman and al., 2007). Date palm seed meal has been

doi:10.21010/ajtcam.v14i3.26
marketed to replace coffee powder (Martin-Sanchez and al., 2013). Some researchers have described the importance of date pollen in traditional medicine. It is widely used to treat male infertility. Indeed, the aphrodisiac effect of date palm pollen extract may be attributed to the presence of alkaloids, flavonoids and saponins in the extract (Abedi and al., 2014).

Baliga and co-workers (Baliga and al., 2011) reported the beneficial effects of phenolic compounds from date fruits. They are considered as antioxidants, anti-carcinogenic, anti-microbial, anti-mutagenic, anti-inflammatory agents, and they reduce the risk of cardiovascular disease. The *Pseudomonas* phage ATCC 14209-B1 is known as a resistant germ to disinfection. However, it is known that the natural extract of date's seeds have a strong ability to inhibit the infectivity of this germ [Jassim and Naji, 2007).

Moreover, extraction and purification of organic molecules from many natural sources have become essential. Some of these compounds are used by the pharmaceutical and food industries as safe additives and functional foods (Shahidi and Naczk, 2004), and to prevent serious systemic infections of plants (Boulenouar and al., 2009).

The current study was conducted in order to identify some bioactive compounds extracted from date palm (*Phoenix dactylifera* L.) seeds and pollen, and to evaluate their antibacterial and antifungal properties.

Material and methods

Plant material

The plant material is made of pollen (AP) from herbalist market and three cultivars of date seeds of *Phoenix dactilyfera* L. collected from different areas of Algeria. All samples were harvested at the same annual period. All cultivars were identified in our laboratory (LRZA): Deglet Nour (DN) from Tolga, region of Biskra (34°40′ North, 5°30′East), Takerbucht (TK) from the Experimental Station INRA Adrar (27°54′ North, 0°17′1" West), Bent Kbala (BK) from Metlili, region of Ghardaia (32°16′North, 32°16′ East).

Dates were depulped by removing the endocarp surrounding the seeds, while the teguments were preserved. Seeds were reduced completely in powder using an electric grinder (type KSW 445 CB). The plant powder is stored separately in sealed glass bottles.

The different extracts from date seeds and pollen were obtained according to protocol optimized by Lebreton and co-workers (1967):

Acid hydrolysis

First order, an acid hydrolysis was performed on 20 g dry plant material blinded with 80 mL of hydrochloric acid (2N HCl). The mixture prepared into Erlenmeyer flasks was boiled in water bath at 100 °C for 40 minutes.

Extraction of bioactive compounds

At the end, the acid mixture is separated twice into two fractions with diethyl ether (60-60 mL). The colors of fractions obtained are light yellow corresponding to the flavonic aglycons (organic fraction) and orange-red rich in anthocyanins and C-glycosides (aqueous fraction).

Quantitative determination of flavonoids content

The organic fraction is evaporated and the dry residue is taken up in unmixed ethanol (95°). The content of flavonic aglycons is determined using a differential assay between flavones and flavonols using the chelating properties of $AlCl_3$ (1%) dissolved in ethanol.

The optical density is measured by spectrophotometer UV-visible (type JUNWAY 7300) at 420 nm after 10 min of incubation. The height differential peak (Δ OD) is proportional to the concentration of quercetin in the sample analyzed. The content of flavonic aglycons is expressed and calculated using the following formula:

$$T \text{ flavonic aglycones} = (\Delta OD / \varepsilon). M. V. d / p$$

With:

T: flavonic aglycons content (in $\mu g.g^{-1}$); Δ OD: Optical Density differential peak at 420 nm; ε: molar absorption coefficient of quercetin (= 23000); M: molar mass of quercetin equal to 302; V: volume of the ethanol solution of aglycon; d: dilution factor; p: dry weight of hydrolyzed plant powder in g. The aqueous fraction contains anthocyanidins which have an absorption maximum at 520 nm; the proanthocyanins content is expressed by the following formula:

$$T \text{ anthocyanins} = (OD / \varepsilon). M. V. d / p$$

With:

doi:10.21010/ajtcam.v14i3.26

T: anthocyanins content (in $mg.g^{-1}$); OD: optical density at the maximum absorption of 520 nm; ε: molar absorption coefficient of cyanidin (= 34700); M: molar mass of procyanidin equal to 306; V: volume of aqueous solution; d: dilution factor; p: dry weight of hydrolyzed plant powder in g. In the same aqueous fraction contains also C-glycosides and it were concentrated by 15 mL of n-butanol and dosed at 340 nm and content is expressed by the following formula:

$$T\ C\text{-glycosides} = (OD / \varepsilon).\ M.\ V.\ d / p$$

With:

T: C-glycosides content (in $mg.g^{-1}$); OD: optical density at the maximum absorption of 340 nm; ε: molar absorption coefficient of orientin (luteolin-8-C-glucoside) equal to 18850; M: molar mass of orientin equal to 448; V: volume of n-butanol solution; d: dilution factor; p: dry weight of hydrolyzed plant powder in g.

Chromatographic analysis of organic fraction by GC/MS

The GC-MS analysis of organic extracts were performed using a HP 6800 chromatograph (Agilent Technologies) coupled to HP 5973 MSD mass spectrometer (Agilent Technologies). The instrument is equipped with capillary HP-5MS column (5% phenyl and 95% dimethyl polysiloxane) with 30 m x 0.25 mm x 0.25 µm dimensions.

The temperature in the column was programmed at 60 °C, ramp up 6 $°C.min^{-1}$ to 290 °C and then held isothermal for 5 min. The helium is used as a vector gas at a flow rate of 0.8 $mL.min^{-1}$. The injector temperature was 250 °C; the volume of samples injected is 1 µL in split mode (20:1), with methanol as a solvent (time limit at 4 min).

The electron ionization is effected by electron impact (EI) with ionization energy of 70 eV. The interface temperature set at 280 °C and the ionization source was 230 °C. The analyzer is quadrupole type (150 °C). The mass spectrum was recorded using a mass detector scan mode (rang 34-550 amu).

The identification of chemical compounds was performed according to their elution order and by comparison of their mass spectra and retention Time (RT) with those registered in *Wiley 7* and *02 NIST* databases. The proportions of chemicals constituents were obtained by percentages peak-area (Area %).

Assay for antimicrobial activities

Bacterial strains

In this part, five virulent bacteria have been used: *Escherichia coli* (ATCC 25992), *Pseudomonas aeruginosa* (ATCC 27852), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* resistant to methicillin (MRSA) (ATCC 43300) and *Enterococcus faecalis* (ATCC 29212).

Yeast strain

*Candida albicans (*ATCC 02091) a dermatophyte fungi.

Special forms of filamentous fungus *Fusarium oxysporum*

The five special forms (f. sp.) of *Fusarium oxysporum* used during our experiment are *F. oxysporum* f. sp. *canariensis* (NRRL 38338), *F. oxysporum* f. sp. *lycopersici* (NRRL 38554), *F. oxysporum* f. sp. *albedinis* isolated from a collected palm tree, *F. oxysporum* f. sp. *melonis* (NRRL 38535) and *F. oxysporum* f. sp. *phaseoli*.

Agar-well diffusion method and preparation of extracts and microbial cultures

The antibacterial activity was determined using the well diffusion method (2002); the microbial strains were grown in nutrient broth and incubated at 37 °C during 24 hours. Then, dilutions at 1/10 were prepared individually for each bacterial strain and inoculated by swabbing on the surface of Muller-Hinton agar, however, the yeast were inoculated on the surface of Sabouraud agar. After that, all microbial stains were incubated for 20 min at 37 °C. The agar was cut into discs of 5 mm diameter and wells were filled with 100 µL containing 30 $µg.mL^{-1}$ of bioactive compounds. The negative control was performed using dimethylsulfoxide (DMSO).

Determination of inhibitory potential of organic extracts and pures substances

Antimicrobial activity was determined as reported Paudel and co-workers (2014) by measuring the inhibition zone after incubation of 24 hours at 37 °C for bacterial stain and after 48 h the yeast. The experimentation was performed in five replicate and presented by average value.

doi:10.21010/ajtcam.v14i3.26

Preparation of range's concentrations of date palm seeds and pollen extracts

In this part, the choice was focused on date palm seeds of Deglet Nour, cultivar known to be sensitive to fungal infection caused by *Fusarium oxysporum* f. sp *albedinis* and Takerbucht cultivar highly resistant to this pathogen. However, pollen has never been valued in this context. Preparation of concentration range was achieved after extraction carried out on 20 g of seeds powder and 10 g of pollen powder according to the method cited above. The two fractions were analyzed with a spectrophotometer UV-visible, and then tested on *Fusarium oxysporum*. The antifungal effect of organic extracts of seeds (DN, TK and BK) were realized with two concentrations 15 µg and 30 $\mu g.mL^{-1}$, while the pollen extract (AP) was tested with concentration 90 $\mu g.mL^{-1}$. The aqueous extracts were prepared as follows; the initial concentration of aqueous extract tested is 12 $mg.mL^{-1}$. Then the extract was diluted to lower concentration of 6 $mg.mL^{-1}$.

Fungitoxic effect of extracts on the virulence of *Fusarium oxysporum*

The incorporation of flavonic extracts of seeds and pollen of *Phoenix dactilyfera* L. was made according to the method of solid dilution in Czapeck-Dox (Rapilly, 1968).
The bioactive extracts are directly added in various series of concentrations, control boxes contain the DMSO instead of the extract. The cultures of *Fusarium* obtained from PDA (potato dextrose agar) medium grew during 7 days after that, disks (5 mm in diameters) are taken from colonies and putted down in the bottom of the boxes. After one day of incubation in darkness and sterile conditions in 27 °C, the mycelia growth of *F. oxysporum* was measured in mm in all cultures of each concentration during one month; the growth of *F. oxysporum* was measured daily. The rate of inhibition (I %) was calculated according to the following equation (Idris and al., 2007):

$$I\ (\%) = 100 \times (dC - dE) / dC$$

Where: I (%): rate of inhibition expressed in percentage; dC: diameter of colonies in boxes containing DMSO (negative control); dE: diameter of colonies in boxes containing the plant extract.

Statistical analysis

The collected data and experimental results were statistically evaluated by Kruskal-Wallis and Friedman tests using XLstat program (Microsoft Corporation, USA) and the significance of differences among experiments was recorded at $P < 0.05$.

Results and Discussion

Contents of total flavonoids

The phytochemical analysis of organic extracts of date palm showed the presence of three classes of flavonoids (flavonic aglycons: flavonols and flavones, anthocyanidins and C-glycosylflavones). During the acid hydrolysis, the flavonic aglycons comprise the fragile C-O-C glycosyl bonds which were easily broken and it allow a selective separation of three classes of flavonoids and the average contents is shown in Figure 1. The results of total contents flavonoids revealed the same content between all date seeds cultivars however the difference is observed only in case of pollen, the differences were statistically significant ($P < 0.05$).

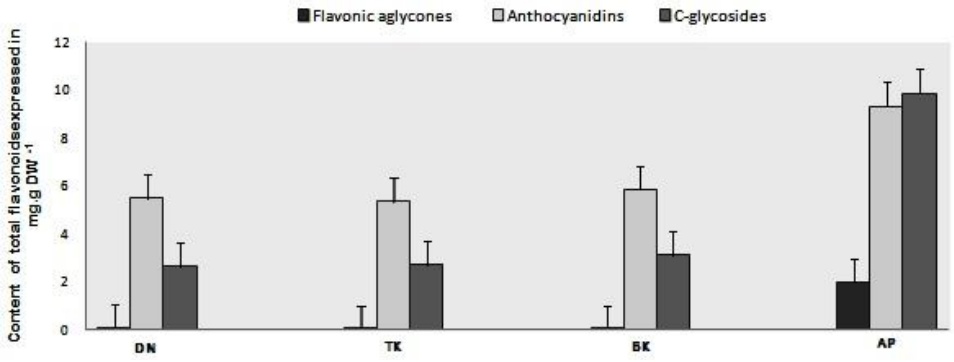

Figure 1: Contents of total flavonoids in date seeds and pollen of *Phoenix dactylifera* L. DN: Deglet Nour,

doi:10.21010/ajtcam.v14i3.26

TK:Takerbucht, BK: Bent Kbala, AP: Pollen, DW: dry weight.

Table 1: Chemical composition (area in %) organic extracts of date palm seeds (*Phoenix dactylifera* L.) detected and identified by GC-MS.

RT	Compound	IUPAC name	Area %		
			DN	TK	BK
7.19	Phenol	Hydroxybenzene		0.11	0.18
7.32	Pentanoic acid, 4-oxo-, methyl ester	Levulinic acid, methyl ester	2.42	1.34	0.89
9.12	Pentanoic acid, 4-oxo-	Levulinic acid / Laevulinic acid			0.37
10.61	Octanoic acid, methyl ester	Caprylic acid, methyl ester	1.39	0.69	0.65
14.68	Phenol, 3-(1,1-dimethylethyl)-	3-tert-butyl-phenol	0.32	0.22	0.42
15.31	Decanoic acid, methyl ester	Capric acid, methyl ester	0.93	0.59	0.46
16.12	Phenol, 2-methoxy-4-(2-propenyl)-	Eugenol		0.26	0.20
16.95	Tetradecane	n-Tetradecane			0.13
19.42	Phenol, 2,6-bis (1,1-dimethylethyl)-4-methyl-	Butylated hydroxytoluene / Ionol	3.16	2.08	3.73
19.59	Dodecanoic acid, methyl ester	Lauric acid, methyl ester	22.58	19.93	15.77
20.04	Nonanedioic acid, dimethyl ester	Azelaic acid, dimethyl ester		0.21	
20.40	Dodecanoic acid	Lauric acid			2.80
20.78	Benzenepropanoic acid, 4-hydroxy-, methyl ester	p-Hydroxy-hydrocinnamic acid, methyl ester	0.18	0.21	0.19
23.40	Tetradecanoic acid, methyl ester	Myristic acid, methyl ester	10.72	12.10	10.26
24.04	Tetradecanoic acid	Myristic acid			0.79
24.46	n-Butyl laurate	Lauric acid, butyl ester	0.22		0.16
25.15	Methyl 13-methyltetradecanoate	Methyl 13-methylmyristate		0.08	
25.96	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Di-iso-butyl phthalate			0.19
26.51	9-Hexadecenoic acid, methyl ester, (Z)-	Palmitoleic acid methyl ester		0.08	
26.86	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	9.24	11.26	10.55
27.43	Hexadecanoic acid	Palmitic acid / Cetylic acid			1.06
27.82	NI				0.57
28.10	Phosphorothioic acid, O,O-diethyl O-(4-nitrophenyl) ester	Parathion	0.19	0.21	0.32
28.46	Hexadecanoic acid, 14-methyl-, methyl ester	Methyl 14-methylhexadecanoate		0.11	
29.56	9,12-Octadecadienoic acid, methyl ester	Linoleic acid, methyl ester	8.33	8.42	6.54
29.69	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid, methyl ester	34.40	35.59	32.64
30.02	Octadecanoic acid, methyl ester	Stearic acid, methyl ester	3.52	2.97	2.89
30.25	9-Octadecenoic acid, (Z)-	Oleic acid / cis-Oleic acid			5.20
30.55	9-Octadecenoic acid (E)-	Elaidic acid / trans-Oleic acid			0.46
30.63	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid, methyl ester	0.16		0.40
31.61	NI			0.15	
32.56	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid, methyl ester	0.61	0.60	0.48
32.91	Eicosanoic acid, methyl ester	Arachidic acid, methyl ester	0.39	0.31	0.35
33.36	1-Nonadecene	Nonadec-1-ene	0.34		0.34

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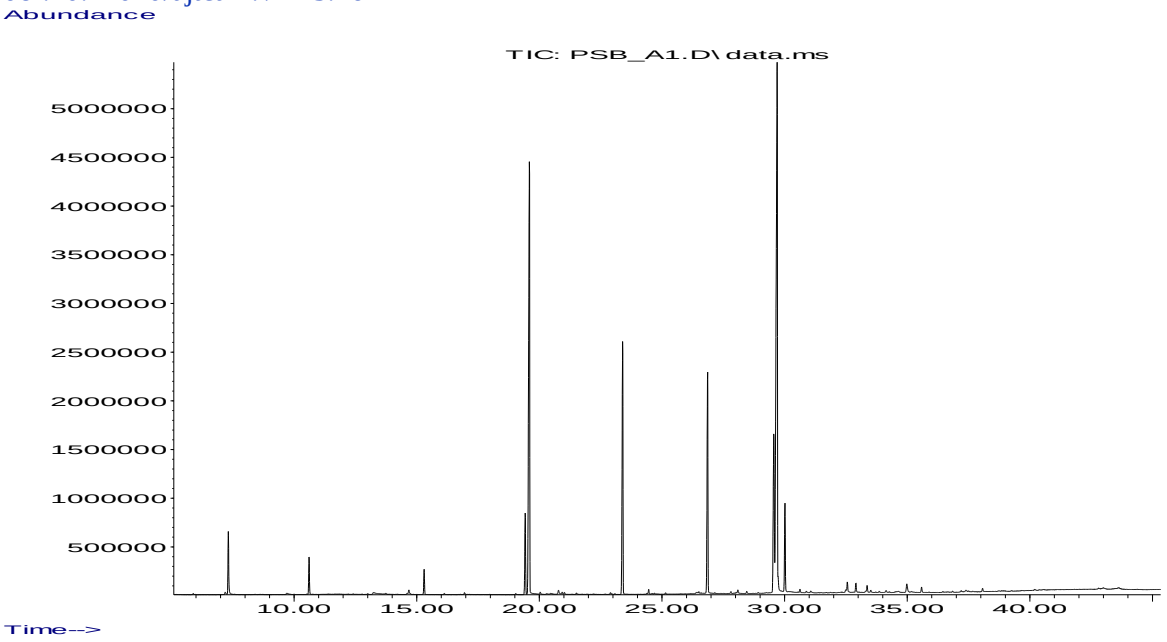
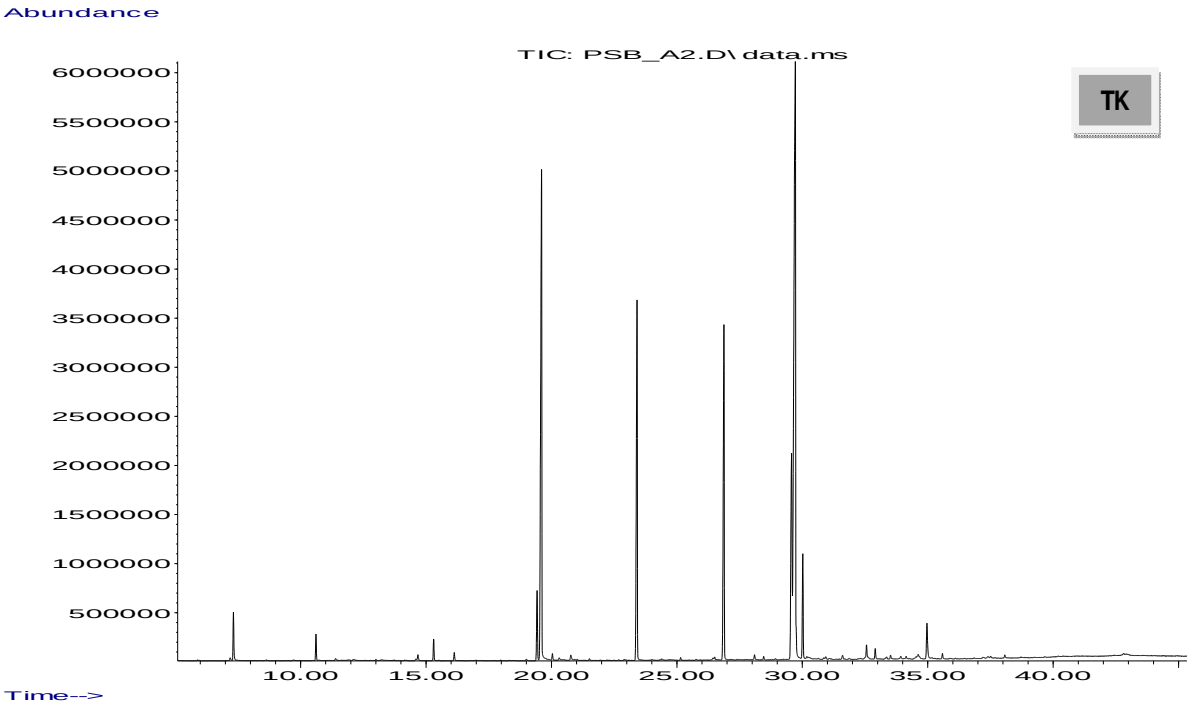
Table 2: Chemical composition (area in %) organic extracts of pollen (*Phoenix dactylifera* L.) detected and identified by GC-MS.

RT	Compound	IUPAC name	Area %
			AP
10.31	NI		0.06
15.23	NI		0.04
15.66	Phénol, 2,6-bis (1,1-dimethylethyl)	2,6-bis (1,1-dimethylethyl) phenol	0.83
15.70	Octanoic acid, methyl ester	Caprylic acid, methyl ester	1.13
16.52	NI		0.01
17.26	NI		0.03
17.63	Heneicosanoic acid, methyl ester	Methyl heneicosanoate	0.04
17.90	NI		0.03
18.05	Tetradecanoic acid, methyl ester	Myristic acid, methyl ester	1.2
18.74	methyl 9-methyltetradecanoate	9-Methylmyristic acid	0.07
18.83	Pentadecanoic acid, methyl ester	n-Pentadecanoic acid methyl ester	0.20
18.86	NI		0.18
19.14	Pentadecanoic acid, methyl ester	n-Pentadecanoic acid methyl ester	0.17
19.80	NI		0.03
20.02	9-Hexadecenoic acid, methyl ester	Palmitoleic acid, methyl ester	9.59
20.24	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	14.4
20.30	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	7.88
20,87	13,16-octadecadiynoic, methyl ester	Methyl 13,16-octadecadiynoate	0.05
20.91	NI		0.03
20.98	NI		0.05
21.04	Éthyle linoléate	Linoleic acid methyl ester	0.13
21.33	Heptadecanoic acid methyl ester	Margaric acid methyl ester	0.48
21.47	NI		0.03
22.40	9,12-Octadecadienoic acid (Z-Z)-	Linoleic acid	12.89
22.47	9,12-Octadecadienoic acid (Z-Z)-	Linoleic acid	20.51
22.57	9,12,15-Octadecatrienoic acid, methyl ester	Linolenic acid	16.61
22.64	11-Octadecanoic acid, methyl ester	trans-Vaccenic acid, methyl ester	3.79
22.68	9,12,15-Octadecatrienoic acid, methyl ester	Linolenic acid	0.45
22.83	Octadecanoic acid, methyl ester	Stearic acid, methyl ester	4.15
23.13	NI		0.1
23.80	NI		0.1
25.83	4-butenylcyclohexane	Cyclohexane, 1-buten-1-yl-	0.13
25.98	Tricyclo [5.1.0.0(2,8)] octane		0.19
26.22	NI		0.07
26.26	NI		0.03

doi:10.21010/ajtcam.v14i3.26





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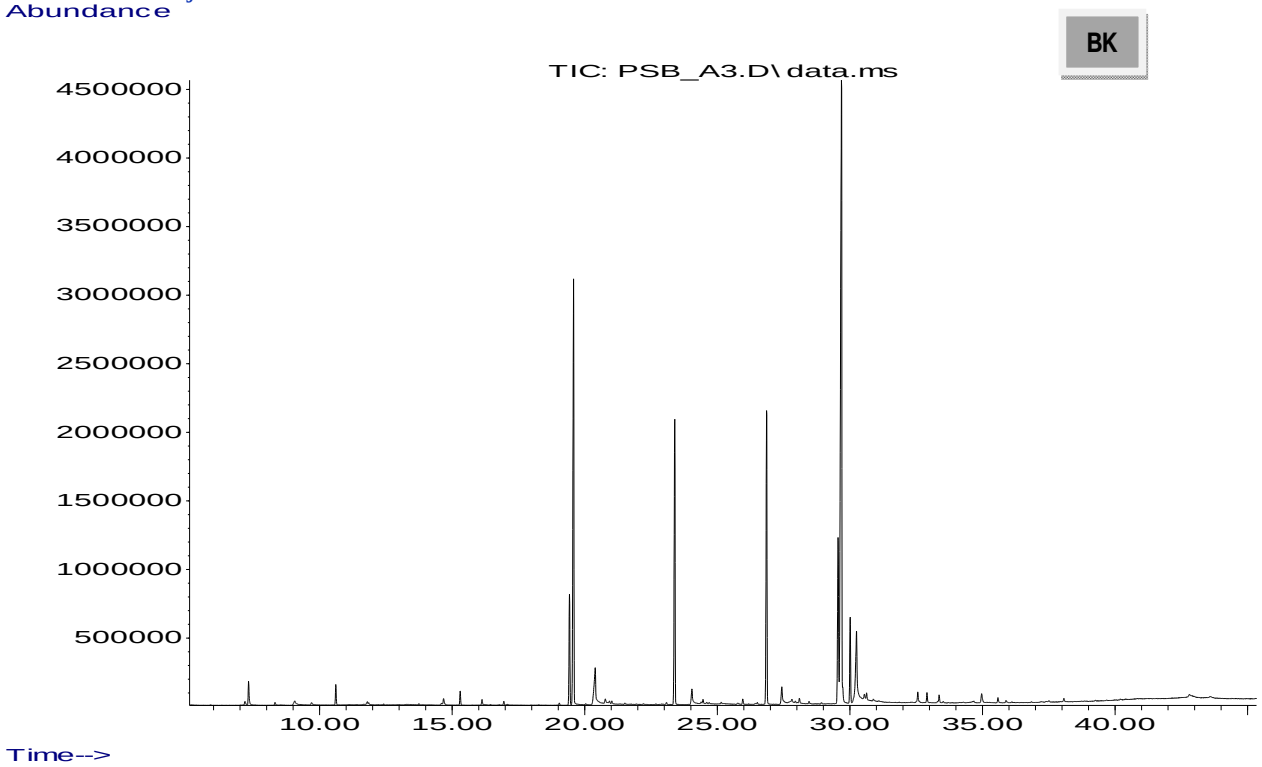


Figure 1: The GC/MS chromatograms of organic extracts of three cultivars (DN, TK and BK)

Flavonic aglycones

Two forms of aglycons are obtained during acid hydrolysis, flavonols and flavones deriving respectively from the O-glycosylflavonols and O-glycosylflavones. The content of flavonic aglycons is expressed in mg.g DW^{-1} milligrams of quercetin (flavonol control) by gram of dry weight (DW) and their values were signifcantly different ($P < 0.05$). The most important amounts of are present in date palm pollen (AP) about 2 ± 0.19 $mg.g^{-1}$ DW. However date seeds of Deglet Nour (DN), Takerbucht (TK) and Bent Kbala (BK) have low contents of flavonic aglycons with approximately 0.03 ± 0.02 $mg.g^{-1}$ DW.

Anthocyanidins

Proanthocyanidins are red pigment, obtained after oxidation of polymers's condensed tannins in acid solvent. The instability of these molecules in aqueous or alkaline solutions explains the choice of aqueous acidified solvents (2N HCl) which favor the distribution of stable forms of flavylium cations.

The anthocyanidins contents were significantly different ($P < 0.05$) and are expressed in mg of cyanidin by g of DW. Date palm pollen contains 9.33 ± 0.3 $mg.g^{-1}$ DW, while seeds of the three cultivars contain approximately 5.57 $g.g^{-1}$ DW.

C-Glycosides

The C-glycosides or C-glycosylflavones are partially resistant to acid hydrolysis regarding their stable glycosidic bond. The differences between seeds cultivars and pollen were statistically significant ($P \leq 0.05$). Indeed, pollen contains the highest amount of C-glycosides (9.88 ± 1 $mg.g^{-1}$ DW) compared to seeds. Even so, the C-glycosides content of Deglet Nour, Takerbucht and Bent Kbala seeds are almost identical, they contain respectively 2.67 ± 0.34 ; 2.72 ± 0.46 and 3.12 ± 0.69 $mg.g^{-1}$ DW.

As a rule, the molecular content vary with plant organs, many elements such as genetic factors, maturity, climate, exposure to sun light contribute to these differences, but plants taxonomically related tend to produce similar flavonoids (Havsteen, 2002). *In situ*, plants produce flavonoids in response to environmental factors such as harmful ultraviolet radiation or as a deterrent against pests to survive in microbial invasions (Grayer and Harborne, 1994).

doi:10.21010/ajtcam.v14i3.26

Fatty acids and volatile compounds profiled by GC/MS

All Tables and chromatogrammes of chemical screening

The chemical composition of three date seeds cultivars and pollen are expressed by average (area in %) and is shown in Table 1 and Table 2. There is no significant difference ($P > 0.05$) between all analyzed samples. The most abundant elements are represented by free fatty acids. These fatty acid methyl ester (FAME) and ester bonds of some fatty acids were formed under transesterification into methanol.

The analysis performed by GC/MS of seeds extracts revealed that the highest amounts of fatty acids compounds are oleic C18:1 (36.84%) and lauric acids C12:0 (20.36%), so the presence of an additional C12 in our list mentions the richness of the local cultivars in lauric acid. These results reveal that date seeds oil is oleic-lauric type. Other major fatty acids elements were found in seeds extracts, such as linoleic acid C18:2 (33.4%), linolenic C18:3 (17.06%) and palmitic C16 (22.27%).

The GC-MS analysis shows an abundant amount of saturated and unsaturated fatty acids (Table 1) in pollen extract (AP), it contains oleic acid at 37%, palmitic acid at 22.27 %, then 17 % for linolenic and 33 % for linoleic. Also, some minor volatils compounds (about 1.35%) were identified in AP extract.

Many researches are in agreement with the composition revealed for the tested samples and it mention that oleic acid is the most abundant compound of seeds oil into some Arecaceae as *Phoenix dactylifera* L. (Besbes and al., 2004). The oil of seeds of *Phoenix dactylifera* L. (Deglet Nour cultivar) is characterized by oleic-lauric type whose respective rates about 39.17%-24.34%. The seeds of *Washingtonia filifera* and *Phoenix theophrasti* Gr (Liolios and al., 2009) are oleic-lauric type (40.60%-17.87 and 88%-17.02%).

In general, oils containing oleic acid confer beneficial health effects. It has been proven that this fatty acid represses the intense expression of the oncogene HER2 (erbB-2), induces apoptosis in carcinoma cells, this antitumor effect is also tested with linoleic acid (Carrillo and al., 2012). It was revealed that linoleic acid is an essential fatty acid for humans and animals providing many physiological benefits such as reducing the incidence and severity of cancer, boosting the immune system, modulate stearyl CoA desaturase, known also for his ability to reduce body fat (Aydin, 2005). In fact, linolenic acid is considered as an essential fatty acid, Brouwer and co-workers (2004) have shown the correlation between the large consumption of linolenic acid, and their positive effects to human health such as the reduce of cardiovascular disease and increase prostate cancer risks.

Other studies have proved that palmitic, stearic, oleic, linoleic and linolenic acids have antibacterial activities (McGaw and al., 2002). Lauric acid present also a bactericidal proprieties and could be used as a natural treatment against *Propionibacterium acnes* (Nakatsujil and al., 2009). Furthermore, Doering and co-workers (Doering and al., 1994) demonstrated that myristic acid has a trypanocide effect which could be incorporated into anti-trypanosome drug.

Some phenolic compounds (about 3.75%) was also found in seeds and among them butylated hydroxy toluene BHT at 3% considered as a powerful antioxidant which is used as a food additive. In fact, eugenol was detected in few amounts (0.23%) only in date seeds of Takerbucht and Bent Kbala. Eugenol is an element which has many pharmacological proprieties such as antibacrobial, antioxidant, anti-inflammatory, anticancer, antipyretic, analgesic, anesthetic and insecticide activities and for this reason it is widely incorporated in cosmetic and pharmaceutical products (Kong and al., 2014).

The profiling using GC-MS represents some disadvantage especially when samples contain complex molecules. For example, in stationary phase of capillary column a significant interfacial adsorption of some solutes can occur and causes important deviation of retention index data. This is attributed to temperature programming rate or speed carrier gas operations. As results the retention index (IR) can be modified, consequently, the standardization of GC-MS analysis still in progress (Acampora-Zellner and al., 2008).

Determination of antimicrobial activities

Depending to diameter of inhibition zone, the antibacterial activity can be divided into four interval, results are expressed in mm as follow: diameter of inhibition zone less or equal to 10 mm the antibacterial activity is low, inhibition zone is between 10 and 15 mm the antibacterial activity is moderate, inhibition zone between 15 and 20 mm, the antibacterial activity is strong and inhibition zone upper or equal to 20 mm, the inhibition obtained is extremely strong (Paudel and al., 2014):

The results shown in Table 2 present antimicrobial activities against Gram negative, Gram positive bacteria species, and yeast strain (*C. albicans*), the diameter of inhibition zone from 10 to 26 mm. Consequently, there is a significant difference ($P \leq 0.05$) between all antimicrobial treatments.

The largest inhibition zone tested with organics extracts was obtained with DN extract against *Escherichia coli* (20.3 ± 0.5 mm); also, the pollen extract is qualified by a strong antimicrobial effect against Gram-positive bacteria, *Enterococcus faecalis* (20.5 ± 0.71 mm), *Staphylococcus aureus* (19.50 ± 0.71 mm), *S. aureus* MRSA (20 ± 0 mm) and yeast stain *Candida albicans* (19.38 ± 1.11). Compared to other naturals extracts, TK extract present a moderate antibacterial activity against all pathogenic bacteria previously tested. Despite the fact that natural's extracts reduce

doi:10.21010/ajtcam.v14i3.26
microorganism growth but is not effective enough to inhibit completely all microbial development, it is clear to mention that organic extracts of date seeds and pollen represent an important source of phenolic compounds and free fatty acids involved as antimicrobial agents. On other side, the antibacterial potential was also tested for the pure substances found in the tegument of date seeds such as catechol, vanillin, benzoic acid, resorcylic acid, syringic acid, cinnamic acid and ferulic acid (Gaceb-Terrak, 2010). Despite the similar structure of some flavonoids, their capacity as antimicrobial agents is different. A highly antibacterial activity is obtained with catechol against *Escherichia coli* (25 ± 0 mm), hydroxyquinone showed also a highly antibacterial activity against *Pseudomonas aeruginosa* (26 ± 0.58 mm), *Staphylococcus aureus* (21.00 ± 1.00 mm) and *S.aureus* MRSA (21.25 ± 1.00 mm). In addition, the vanillin shows a strong antibacterial activity only against *Enterococcus faecalis* (17.5 ± 0.71 mm) and *Candida albicans* (16.63± 0.75 mm). The benzoic acid presents a very strong antibacterial activity especially against *Enterococcus faecalis* (20.5 ± 0.71 mm); however, their hydroxybenzoic derivatives (gallic, resorcylic and syringic acids) showed a moderate activity against some bacteria (Table 3). However, the cinnamic acid and its hydroxycinnamic derivative (ferulic acid) have also a moderate antimicrobial effect.

doi:10.21010/ajtcam.v14i3.26

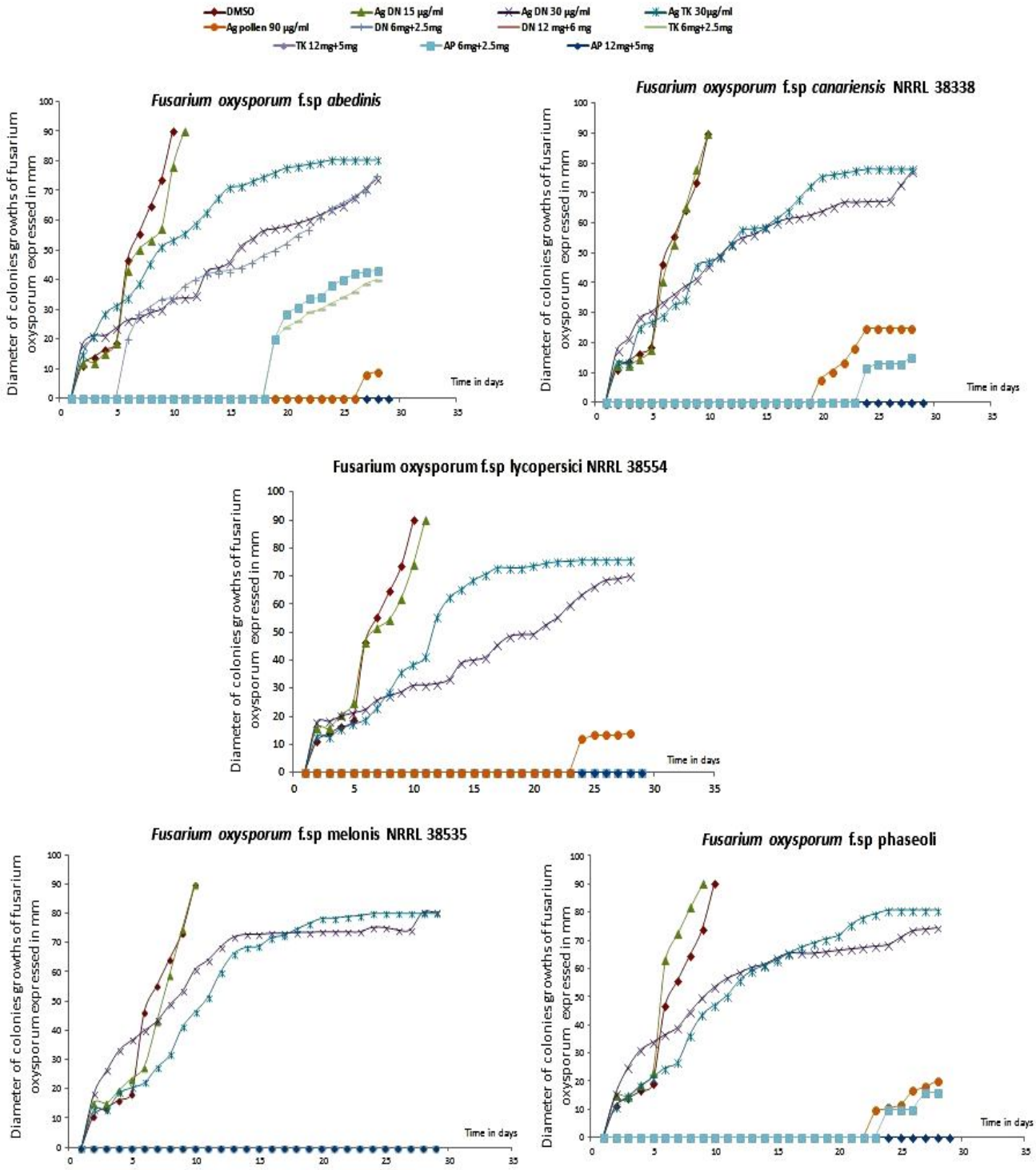
Table 3: Inhibition zones of bioactive compounds of *Phoenix dactylifera* L. against pathogenic bacteria and fungi at concentration of 30 µg.mL^{-1} (Mean ± SEM, n =5)

	Diameter of inhibition zone (mm)					
	Gram-negative bacteria		Gram-positive bacteria			Fungi
Extract	*Escherichiacoli* ATCC 25992	*Pseudomonas aeruginosa* ATCC 27852	*Staphylococcus aureus* ATCC 25923	*Staphylococcus aureus* (SARM) ATCC 43300	*Enterococcus faecalis* ATCC 29212	*Candida albicans* ATCC 02091
DN	20.30 ± 0.50	14.18 ± 0.29	15.50 ± 0.77	17 ± 1.5	13.67 ± 1.23	11.67 ± 0.58
TK	15.33 ± 0.58	11.75 ± 0.50	11.00 ± 1.00	14.75 ± 0.00	11.36 ± 0.80	11.33 ± 0.58
AP	17.00 ± 0.00	-	19.50 ± 0.71	20.00 ± 0.00	20.50 ± 0.71	19.38 ± 1.11
Catechol	25.00 ± 0.00	17.00 ± 1.00	15.00 ± 0.00	14.50 ± 0.71	17.00 ± 0.00	-
Hydroxyquinone	22.33 ± 1.52	26.00 ± 0.50	21.00 ± 1.00	21.25 ± 1.00	17.00 ± 1.00	-
Vanillin	-	-	-	13.33 ± 0.58	17.50 ± 0.71	16.63 ± 0.75
Benzoic acid	-	12.00 ± 0.00	-	13.00 ± 0.00	19.50 ± 0.71	-
Gallic acid	-	12.75 ± 0.29	-	15.00 ± 0.00	16.13 ± 0.85	-
Resorcylic acid	-	11.50 ± 0.70	15.83 ± 0.76	-	13.33 ± 0.58	-
Syringic acid	-	12.25 ± 0.29	11.60 ± 1.00	-	11.33 ± 0.58	-
Cinnamic acid	-	13.83 ± 0.76	-	10.00 ± 0.00	13.50 ± 0.71	-
Ferulic acid	-	11.00 ± 0.00	10.67 ± 0.58	10.33 ± 0.58	12.30 ± 1.44	11.25± 0.50
Control solution	-	-	-	-	-	-

doi:10.21010/ajtcam.v14i3.26

Determination of antifungal activities of *Fusarium oxysporum*

The curves of kinetics growth obtained of *Fusarium oxysporum* present a regular development (Figure 2), the rate of inhibition of organic and aqueous extracts are graphically determined. The evaluations of inhibitory potential of natural extracts were statistically significant ($P < 0.05$).



doi:10.21010/ajtcam.v14i3.26

Figure 2: *In vitro* growth of five special form of *Fusarium oxysporum* in the presence of aqueous and organics extract of pollen and date palm seeds of Deglet Nour and Takerbucht.

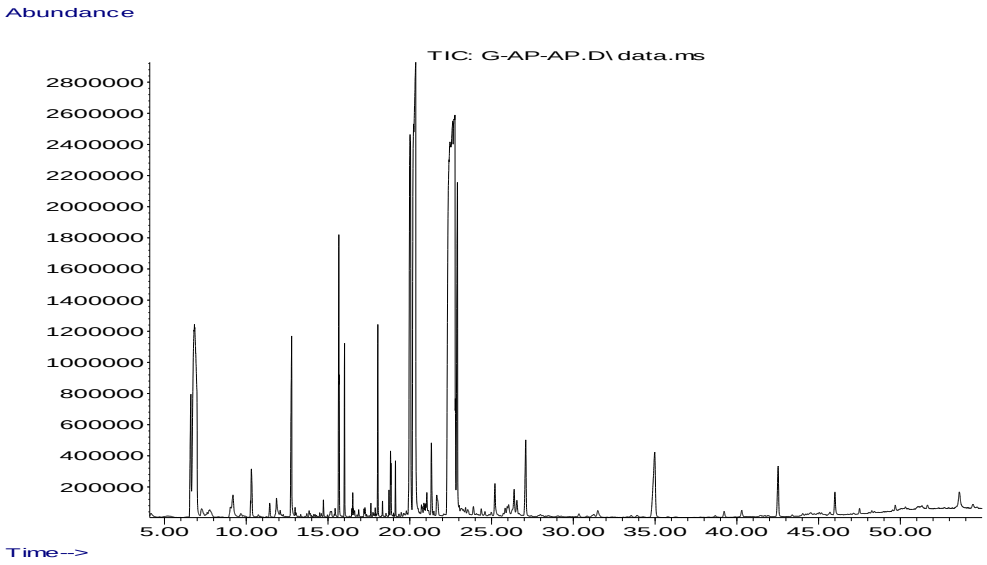


Figure 2: The GC/MS chromatograms of organic extract of pollen (AP).

After several weeks of incubation, only the fraction containing anthocyanins and C-glycosides presents the best antifungal activity when it is tested *in vitro*, the important inhibition was obtained with the aqueous fraction (anthocyanins and C-glycosides) of the seeds and pollen (100% inhibition), at 12 $mg.mL^{-1}$ of cyanidin as well as for 6 $mg.mL^{-1}$ of cyanidin present from 82 to 100% of inhibition potential.

The filamentous fungus are sensitive to the aqueous extracts containing the polymers of anthocyanidins (condensed tannins) and there is a progressive decrease in the growth as the concentration of extracts increases, just for *Fusarium oxysporum* f. sp. *albedinis*, *F. oxysporum* f. sp. *canariensis* and *F. oxysporum* f. sp. *phaseoli* as consequence, the fungitoxic effect of aqueous extracts were significantly different ($P \leq 0.05$). Regardless the concentration of the natural treatments the results of inhibitory effect obtained with *F. oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *lycopersici* were not statistically significant ($P > 0.05$).

The organic extracts of Deglet Nour and Takerbucht do not induce the inhibition of *Fusarium oxysporum* growth, whereas the organic extract of the pollen leads to an important inhibition (from 72 to 100%) for the most part of fungi at 90 $\mu g.mL^{-1}$. So, the differences between all organic extracts tested were statistically significant ($P \leq 0.05$).

Few fungal species of *Fusarium* genus present a pathogenic behavior and among them *F. solani*, *F. oxysporum*, *F. verticilloides* and *F. moniliforme* (Sifuentes-Osornio and Corzo-Leon, 2012). Their fatal effects are induced by mycotoxins which present a cutaneous toxicity, also it is considered as a hematotoxic, immunotoxic element (Brochard and Le Bâcle, 2010). So, mycotoxins produce an acute and/or a chronic poisonings to animals and human after consumption of contaminated food.

Some similar works have shown that secondary metabolites isolated from date seeds have an inhibition potential against the growth of pathogenic microbes (Al-daihan and Shafi-Bhat, 2012).

The obtained bioactives extracts from different parts of date palms may present a source of healthy food for animals and human nutrition and an alternative agent to reduce the cost of food disinfection and processing related to fungi and bacteria contaminations.

Conclusion

The phytochemical analysis of organic extracts of date palm showed the presence of three classes of flavonoids (flavonic aglycones: flavonols and flavones, anthocyanidins and C-glycosylflavones). a significant number of saturated and unsaturated fatty acids are abundant in the extract, it contains oleic at 37%, lauric acids at 20% in date seeds and palmitic acid at 22,27 %, then 17, % for linolenic and 33 % for linoleic acids in pollen extracts. Organic extracts of date

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seeds and pollen, as well as phenolic substances, some of them are identified in tegument of date seeds and qualified by an antibacterial behavior against gram positive and gram negative bacteria. The antifungal properties of anthocyanidins polymers have been screened which give an important value of these molecules for many applications related to pharmaceutical engineering and food industries using natural additives.

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