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# Fatty Acids Derivatives and Steroidal Saponins: Abundance in the Resistant Date Palm to *Fusarium oxysporum* f. sp. *albedinis*, Causal Agent of Bayoud Disease

R. Gaceb-Terrak and F. Rahmania

***Abstract*****—**Takerbucht is the only cultivar of date palm known as being resistant to the bayoud disease, caused by *Fusarium oxysporum* f. sp. *albedinis* (*F.o.a.*). In the aim to understand more about the defense mechanisms implied, we realized phytochemical analyses of this cultivar leaflets and roots and this, for the first time, using gas chromatography-mass spectrometry (GC-MS).The examination of our results shows that fifty-four molecules have been detected, fourteen of which are common to leaflets and roots. This study revealed also the organs' richness in derivatives fatty acids: both saturated and unsaturated are represented mainly by methyl esters of Hexadecanoic and 9,12,15-Octadecatrienoic acids. 1-Dodecanethiol, derivative Dodecanoic acid is only present in roots. It’s of great interest to note that the screening revealed the steroidal saponins abundance, among which Yamogenin acetate and Diosgenin, exclusively detected in Takerbucht. They may play an essential role, in the date palm resistance to the bayoud disease.

***Keywords*****—**Analysis by GC-MS, leaflets and roots of resistant date palm to *F.o.a.*, derivatives fatty acids, steroidal saponins.

## I. Introduction

THE date palm, *Phoenix dactylifera* L. is of many and various interests. Its ecological role is essential to oasis development and life in the desert [1]; its socio-economic importance is related to dates which occupy a prominent place as a source of foreign currency and in human and animal food; moreover, this fruit has significant agro-food opportunities [2].

Many studies have been done on diseases caused by fungi and cause significant damage in plants [3]. Several affections of the date palm are caused by *Fusarium* species, the most dangerous being vascular wilt or bayoud caused by *Fusarium oxysporum* f. sp. *albedinis*. This disease destructed many palm groves of Morocco and Algeria. Today, it is progressing towards north-western Mauritania [4]. In Algeria, Takerbucht is the only resistant cultivar to this disease.

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The chemical investigations occurring in our laboratory could contribute to a better appreciation of the molecular profile of the bioactive resistant cultivar, including the identification of new antifungal compounds. The date palm has many interesting biological activities that have been revealed, in recent years, by numerous authors. Thus, *in vitro* tests showed that leaf extracts present both antifungal activity against *F.o.a.*, causal agent of vascular fusariosis of date palm [5] and antibacterial against pathogenic germs Gram$^-$ and Gram$^+$ [6].

The studies we undertake aim at searching for natural bioactive molecules able to contribute at this plant Saharan valorization.

## II. Materials and Methods

### A. Plant Material

Our experimentation was carried out on leaflets and roots collected in march 2010 from five date palm trees belonging to Takerbucht cultivar, growing in the experimental station of the National Institute of Agronomic Research (INRA) of Adrar, in the South-west (27°54’ Northern, 0°17’1’’ West) of the Algerian Sahara.

### B. Extraction and Separation of Organic Phase

The dry material (5 g) is hydrolyzed during 40 min by hydrochloric acid (2N) in boiling “bain-marie”. Three successive extractions with diethyl ether permitted the organic phase separation. The aqueous phase containing orange-red colored anthocyanidins is eliminated. The organic phase is evaporated; the dry residues are taken again by absolute methanol.

### C. Analysis by GC-MS

The analysis of methanolic leaflets and roots extracts was performed using a gas chromatograph Hewlett Packard 6890 coupled to a mass spectrometer, Electron Impact HP 5973 type. Detection and identification of compounds were done by comparing the mass spectra we obtained with those of the National Institute of Standards and Technology Library (Nist98). Each compound detected is characterized by its retention time (RT), its distribution area and its recognition %

defined by Nist98. The chromatograms are traced with OriginPro 8.0 software.

## III. Results and Discussion

The analytical process applied allowed the separation of complex mixtures. Fifty-four molecules are detected by GC-MS, they represent a total area of 99,98 %; fourteen from them are common to both plant organs. The chromatographic profiles of date palm leaflets are little diversified than what observed in roots. Twenty substances were detected in leaflets; nineteen among these compounds were identified (Table I and Fig. 1 (a)). Forty-eight substances are detected in roots among which forty-three are identified (Table I and Fig. 1(b)).

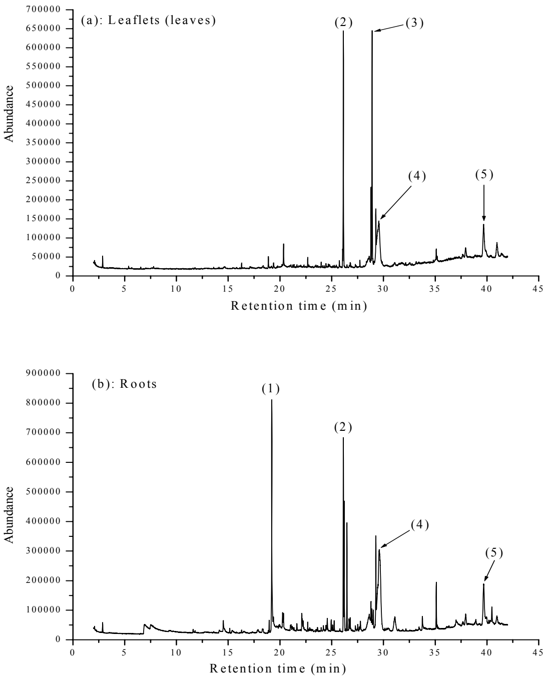


Fig. 1 GC-MS chromatograms of leaflets (a) and roots (b) extracts of Takerbucht, resistant date palm cultivar (1): 1-Dodecanethiol; (2): Hexadecanoic acid, methyl ester; (3): 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; (4): Yamogenin acetate and (5): Diosgenin

This study showed the extracts' richness in fatty acids derivatives and steroidal saponins. Among these molecules, five are abundant; they represent an area of 68,98 % in the leaves and 53,32 % in the roots (Table I). The comparison of their mass spectra and their chromatographic profiles, obtained under our experimental conditions, with those of the Nist98 library, allowed the identification of these substances (Fig. 1 (a), 1 (b) and 2).

Saturated and unsaturated fatty acids derivatives are mainly represented by 1-Dodecanethiol only present in the roots and methyl esters of Hexadecanoic and 9,12,15-Octadecatrienoic

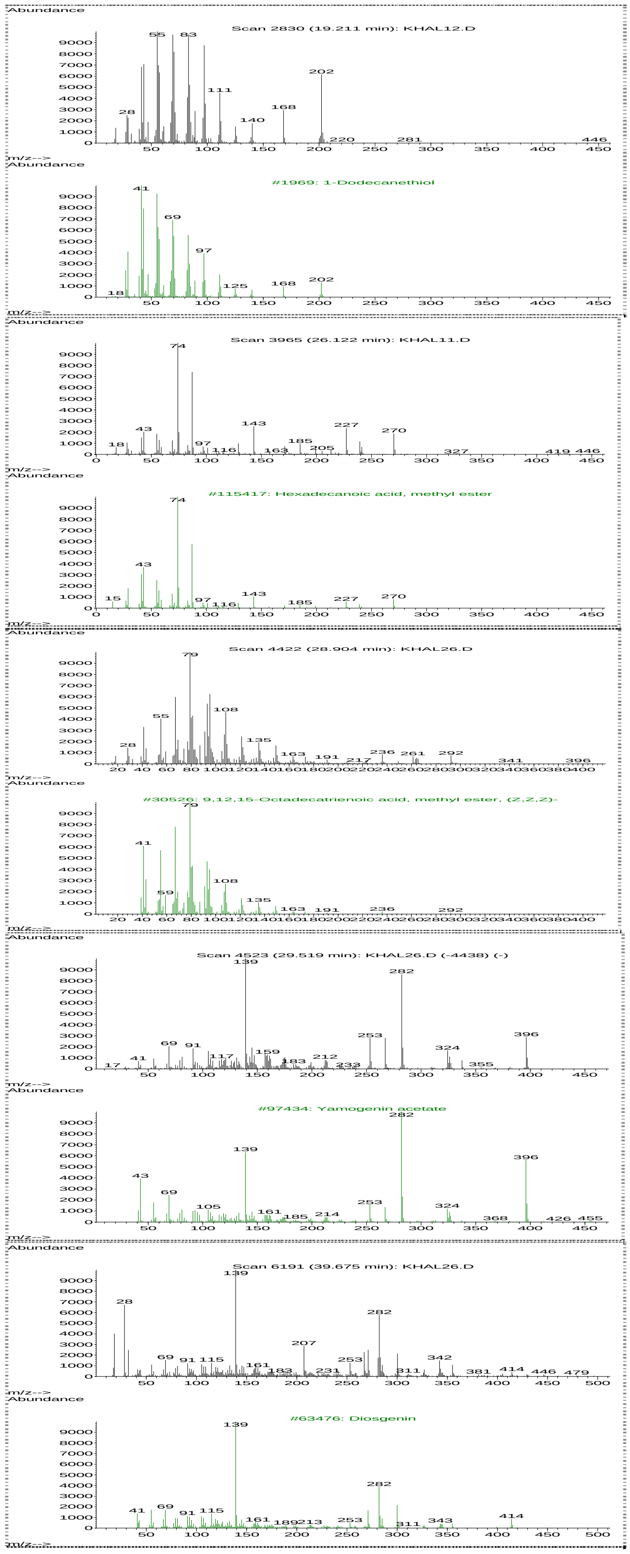


Fig. 2 Mass spectra of derivatives fatty acids (1-Dodecanethiol; Hexadecanoic acid, methyl ester and and 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-) and steroidal saponins (Yamogenin acetate and Diosgenin) identified from leaflets and roots of resistant cultivar

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acids present as well in leaves as in roots. The steroidal saponins of spirostane type ($C_{27}$), Yamogenin acetate and Diosgenin are very abundant in the two considered organs (Tables I and II and Fig. 3).

TABLE I
CHEMICAL COMPOSITION OF LEAFLETS AND ROOTS EXTRACTS OF TAKERBUCHT, RESISTANT DATE PALM CULTIVAR

| Compound (IUPAC nomenclature) | RT | Area (%) | |
|---|---|---|---|
| | (min) | Leaves | Roots |
| Toluene | 2.86 | 0,80 | 0,41 |
| Phenol | 6.90 | | 0,18 |
| 2-Methoxy-4-vinylphenol | 14.52 | | 1,11 |
| 9-Borabicyclo[3.3.1]nonane, 9-(3-methyl-2-butyl)- | 15.16 | | 0,20 |
| Tetradecane | 16.29 | 0,48 | |
| Dodecanoic acid, methyl ester | 18.88 | 0,93 | |
| Octanoic acid | 18.96 | | 0,49 |
| Benzoic acid, 4-hydroxy- | 19.05 | | 1,44 |
| 1-Dodecanethiol | 19.21 | | 10,29 |
| Nonanedioic acid, dimethyl ester | 19.38 | 0,69 | |
| 4-(Methylthio) thiobenzoic acid, S-methyl ester | 20.26 | | 0,93 |
| Hexadecane | 20.33 | | 0,90 |
| 2-Octanol, 8,8-dimethoxy- | 20.35 | 1,92 | |
| p-Octylacetophenone | 21.01 | | 0,16 |
| Benzaldehyde, 4-hydroxy-3,5-dimethoxy- | 21.61 | | 0,41 |
| Cinnarizine | 22.11 | | 1,29 |
| NI | 22.24 | | 0,60 |
| Methyl tetradecanoate | 22.68 | 0,57 | 0,28 |
| Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide | 23.62 | | 0,24 |
| Octadecane | 23.98 | 0,50 | 0,25 |
| 5,6,7,8-Tetrahydro-1-phenylnaphthalene | 24.20 | | 0,45 |
| Pentadecanoic acid, methyl ester | 24.44 | | 0,24 |
| Aspidinol | 24.57 | | 0,73 |
| 4-O-Nitrophenylhydrazono-3-methyl-2-pyrazolin-5-one | 24.95 | | 0,61 |
| Dodecane, 1-iodo- | 25.08 | | 0,31 |
| 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester | 25.24 | | 0,44 |
| 9-Hexadecenoic acid, methyl ester, (Z)- | 25.94 | 0,73 | |
| Hexadecanoic acid, methyl ester | 26.14 | 16,93 | 7,21 |
| 1,3,5-Benzenetricarboxylic acid, trimethyl ester | 26.20 | | 5,16 |
| Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate | 26.47 | | 4,00 |
| 1,3-Dioxolane, 4,4,5-trimethyl-2-pentadecyl- | 26.68 | | 0,88 |
| Dibutyl phthalate | 26.78 | | 0,79 |
| 3,5-Di-tert-butyl-4-hydroxybenzoic acid | 27.29 | | 0,49 |
| NI | 27.52 | | 0,43 |
| Ethanone, 1-(9-anthracenyl)- | 27.66 | | 0,18 |
| Heptadecanoic acid, methyl ester | 27.72 | 0,67 | |
| Fenchol, exo- | 27.77 | | 0,81 |
| NI | 28.61 | 4,70 | 4,33 |
| 8,11-Octadecadienoic acid, methyl ester | 28.80 | 5,40 | 1,81 |
| 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 28.92 | 18,30 | 1,08 |
| Benzo[b]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl- | 28.96 | | 0,51 |
| 6-Methoxy-8-[6-aminohexylamino]lepidine diphosphate | 29.02 | | 0,83 |
| Octadecanoic acid, methyl ester | 29.27 | 5,15 | 4,81 |
| Yamogenin acetate | 29.55 | 22,05 | 28,42 |
| Undecanoic acid, 2,6,10-trimethyl-, methyl ester | 33.76 | | 0,91 |
| Bis(2-ethylhexyl) phthalate | 35.10 | 1,65 | 1,91 |
| Sulazepam | 35.19 | 0,67 | 0,15 |
| NI | 36.99 | | 1,10 |
| 6-(2-Formylhydrazino)-N,N'-bis(isopropyl)-1,3,5-triazine-2,4-diamine | 37.95 | 2,23 | 1,24 |
| NI | 38.93 | | 0,69 |
| Diosgenin (5) | 39.68 | 11,70 | 6,32 |
| Benzoic acid, 3-methyl-2-trimethylsilyloxy-, trimethylsilyl ester | 39.76 | | 1,16 |
| Disulfide, didodecyl | 40.47 | | 1,50 |
| 2-Ethylacridine | 40.95 | 3,91 | 1,30 |
| Total area (%) | | 99,98 | 99,98 |
| Number of compound | | 20 | 48 |
| Unidentified compounds (NI) | | 1 | 5 |

These results show that leaves and roots of the resistant date palm cultivar contain high quantity of saturated and polyunsaturated fatty acids. In previous work, it had been shown that the susceptible Algerian cultivars, Deglet Nour and Tgaza, are less rich in α-linolenic acid, a trienoic fatty acid [7].

TABLE II
CHEMICAL CHARACTERISTICS OF FIVE ABUNDANT MOLECULES IN LEAVES AND ROOTS EXTRACTS OF TAKERBUCHT, RESISTANT DATE PALM

| IUPAC Nomenclature | Synonyms | Abundance | |
|---|---|---|---|
| | | Leaves | Roots |
| 1-Dodecanethiol | Lauryl mercaptan | - | ++ |
| Hexadecanoic acid, methyl ester | Palmitic acid, methyl ester | ++ | + |
| 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | α-Linolenic acid, methyl ester | ++ | + |
| Yamogenin acetate | (3β,25*R*)-spirost-5-en-3-ol, acetate | +++ | +++ |
| Diosgenin | (3β,25*R*)-spirost-5-en-3-ol | ++ | + |

- : absence (area = 0%); +: area < 10%; ++: 10% < area < 20%; +++: area > 20%.

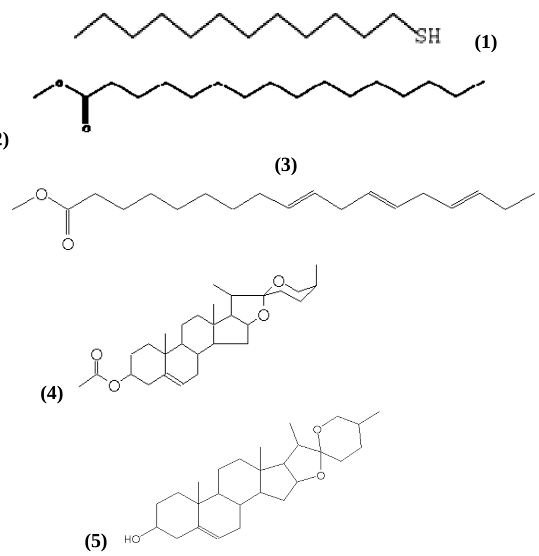


Fig. 3 Chemical structure of 1-Dodecanethiol (1); Hexadecanoic acid, methyl ester (2); 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (3); Yamogenin acetate (4) and Diosgenin (5)

Derivatives of linoleic and linolenic acids accumulate in tomato (*Lycopersicum esculentum* Mill.) resistant to *Phytophtora* sp. and to *Verticillium albo-atrum* [8]-[9]. It had also been clearly shown that in oil palm (*Elaeis guineensis* Jacq.), young plants inoculated with *F. oxysporum* f. sp. *elaeidis* accumulate dienic alcohols from linoleic acid, methyl ester; these derivatives are toxic *in vitro* for pathogens [10]. Linolenic acid, methyl ester isolated from leaves of *Arachis* *hypogaea* L. causes a fungicidal activity towards *Puccinia arachidis*, a rust pathogen of groundnut [11].

Linolenic acid is a main component of thylakoid membranes of chloroplasts; it is known for his role in the defense signals in plants and contributes as a first messenger in activation of defense proteins (PR proteins). This key molecule involved in signal propagation in the plant cell, it probably induces the expression of a secondary messenger (jasmonic acid) implied in a series of the plant response to a stress [12]. Linoleic and linolenic acids are involved in the mechanisms of defense against fungal pathogen by producing hydroxylated and peroxidised fatty acids [13].

In addition, assays realized *in vitro* showed that steroidal saponins extracted from *Allium sativum* L. are toxic against *Tricoderma viride* [14]. The same molecules extracted from seeds of *Trigonella foenum-graecum* L. and tested on several fungi, pathogenic for plants, man, or saprophytes had no toxicity if they possess a furostane skeleton, however, spirostane form reveals a significant antifungal activity [15]. Similarly, saponins with spirostane skeleton extracted from monocotyledons and tested *in vitro* against some pathogenic fungi such as *Candida albicans*, *C. glabrata*, *C. krusei*, *Cryptococcus neoformans* and *Aspergillus fumigatus*, induce significant fungicidal activity [16]. In addition these molecules may present antibacterial and antiparasitic actions [17]-[18].

Thanks to their various biological and useful activities, as low toxicity, these steroidal saponins are more and more used in the medical field. Their possible valorization and use in the industrial field could be considered. Studies of antifungal activities of the major substances identified in leaves and roots of Takerbucht, a resistant date palm cultivar to *Fusarium oxysporum* f. sp. *albedinis* is underway in our laboratory.

## IV. Conclusion

Relying upon the results obtained from this analysis, it is interesting to test *in vitro* antifungal activity of bioactive molecules isolated from leaves and roots of resistant date palm cultivar (work in progress in our laboratory). However, a detailed study would be necessary to extrapolate from the laboratory to the natural environment (oasis), in order to eradicate the bayoud disease.

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