PHYTOCHEMICAL SCREENING OF SOME *INVIVO* AND *INVITRO* DATE PALM TISSUES

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ABSTRACT

The preliminary photochemical screening of the different date palm tissues, in vivo and in vitro tissues, namely, shoot tip, pollen grain, leaves, fruits, callus, embryogenesis and in vitro leaf tissue revealed the presence of carbohydrates, alkaloids, steroids, flavonoids and tannins. The separation and identification of steroids by Thin Layer Chromatography (TLC) in the *in vivo* and in vitro tissues of both Zaghloul and Sewi cultivars revealed the presence at 5-7 spots from which two steroids namely cholesterol's and ß-sitosterol were identified in both tissues, in addition to stigma sterol which was detected in pollen grains. The spectrophotometric determination of total steroids in both tissues of the tow cultivars showed higher values in pollen grain and shoot tip in case of *Invivo* tissues. Also in leaf and roots of the *invitro* tissues.

INTRODUCTION

Natural substances are employed, either directly or indirectly, by a large number of industries. Natural plant products (Phytochemicals) figure prominently in several of these. For example, Phytochemicals are utilized to a large extent by the pharmaceutical, cosmetics, food, agrochemical, and chemurgic industries. Economically important plants serve as irreplaceable sources of industrial oils (both volatile and fixed), flavors and fragrances, resins (e.g. rosin and tall oil), gums, natural rubber, waxes, saponins and other surfactants, dyes, pharmaceuticals, pesticides (e.g., insecticides and rodenticides), and many specialty products (***Balandrin and Klocke, 1988).***

The steroids form a group of structurally related compounds, which are widely distributed in animals and plants. Steroids belong to a large group of compounds known as terpenoids or isopernoids. Terpenes are

formed by the polymerization of isoprene units, and steroids are triterpenes or triterpenoids. Steroid family includes the sterols, vitamin D, bile acids, a number of sex hormones and adrenal cortex hormones, some hydrocarbons and other compounds are also included with steroids***. (Abd El-Rahaman, 1991).***

MATERIALS AND METHODS

I. Preliminary photochemical screening:

Some explants of date palm (*Phoenix dactylifera* L.) were collected from Zaghloul and Sewi cv to be used as explant source in this study. These explants were taken from the following parts:

In vivo **explant:** Gommar (shoot tips), pollen grains, Leaves, fruits.

In vitro **explant:** Callus, embryos, germinated embryos and leaves.

*** Preparation of extracts:**

1. 100 GM of Gommar (shoot tip) was extracted with 300 ml of ethyl alcohol 70%.
2. 5 GM of pollen grains was extracted with 100 ml of ethyl alcohol 70%.
3. 50 GM of leaves were extracted with 100 ml of ethyl alcohol 70%.
4. 50 GM of fruits were extracted with 100 ml of ethyl alcohol 70%.
5. 25 gm of callus was extracted with 50 ml of ethyl alcohol 70%.
6. 25 GM of Embryos were extracted with 50ml of ethyl alcohol 70%.
7. 25 gm of parts of Germinated embryos was extracted with 50 ml of ethyl alcohol 70%.
8. 25 gm of leaves were extracted with 50 ml of alcohol 70%.

The obtained extracts were subjected to the following photochemical screening tests according to ***(Ateya, 1975).***

1. Test for carbohydrates and glycoside
2. Test of cardenolides:
3. Test of alkaloids:
4. Tests for sterols and/or triterpenes:
a. Liebermann-Burchard test:
b. Salkowski test:
5. Tests of flavonoids:
6. Test of tannins:

II. Isolation and identification of steroids by TLC technique: according to (**Ateya, 1975).**

III. Quantitative Determination of Total Steroids in the unsaponifiable fraction by Spectrophotometer: The total steroids were determined in the unsaponifiable fraction by the reaction with Denigee reagent according to (Pharco 1993).

RESULTS AND DISCUSSION

1. Preliminary photochemical screening of some *In vivo* and *in vitro* date palm tissues:

The screened results to detect the presence of Carbohydrates, alkaloids, triterpenes (sterols and steroids), cardenolides, flavonoids, Tannins were recorded in Table (1).

Carbohydrates:

All samples contained carbohydrate in the different grades. The samples of shoot tip, pollen grain, and leaves had more carbohydrates than callus and embryogenesis. The germinated embryos and leaves (*in vitro*) were of lower values.

Alkaloids: Alkaloids were present in all samples but in minute values as indicated by Dragen'dorf test.

Sterols: All the samples of both cv gave positive reaction to both Liberman-Burchard and Salkowski tests indicating the presence of sterols. The tests on shoot tip and pollen grains of both cultivars indicated

Table (1): The results of phytochemical screening for some explants of date palm, Zaghloul and Sewi cultivars.

Constituents			In vivo								In vitro							
			Gommar		Pollen grains		Leaves		Fruits		Callus		Embryos		Germinated embryos		Leaves	
			Z.	S.	Z.	S.	Z.	S.	Z.	S.	Z.	S.	Z.	S.	Z.	S.	Z.	S.
Carbohydrates			+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	+	+	+	+
Alkaloids			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sterols and Steroids	A		+++	+++	+++	+++	++	++	++	++	++	++	+	+	+	+	+	+
	B		+++	+++	+++	+++	++	++	++	++	++	++	+	+	++	+	++	++
Cardenolides			-	-	-	-	-	-	+	++	-	-	-	-	-	-	-	-
Flavonoids	A		++	++	+++	+++	-	-	+	+	+	+	+	+	-	-	-	-
	B		++	++	+	+	-	-	-	-	+	+	+	+	-	-	-	-
	C	1	-	-	++	++	-	-	-	-	-	-	-	-	-	-	-	-
		2	++	+	++	+	-	-	-	-	++	+	+	+	-	-	-	-
Tannins	A		+++	++	+++	+++	++	++	+++	+++	+	+	+	+	+	++	++	++
	B		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Z = ZAGHLOUL ***S = SEWI***

\+ = Small value ++ = Moderate value +++ = High value

the presence of steroid in high value followed by the callus and embryogenesis of Sewi cultivar. The low values were detected in germinated embryos and leaves of Zaghloul cv, also the leaves in vitro of Sewi cultivar contains lower value.

Cardenolides: Cardenolides were detected in leaves, callus and fruit samples, while the other samples gave negative test for cardenolides.

Flavonoids: Three tests (A, B and C) in Table (1) were carried out to detect the flavonoids. The first was for flavonoids in general (A) the second was for free flavonoids (B) and the third was for combined C_1 as well as to detect flavonal and/or flavonone C_2. With regard to the results obtained from the falconoid test (A) reagent, it can be said that flavonoids are present in all samples except these of fruit, callus, leaves (in-vivo), germinated embryos and leaves (*in vitro*). Free flavonoids are present in all samples (B) except leaves, fruits, germinated embryos and leaves (*in vitro*). The combined flavonoids (C_1) are present in the pollen grain only. C2 flavonoids are present in the shoot tip, pollen grain, callus and embryos.

Tannins: Two tests were carried out, the first was the ferric chloride test, which was used to detect the catechol tannins. The second was used to detect the free gallic acid. The ferric chloride test gave positive results indicating the presence of catechol tannins in all samples. While, the 2nd test gave negative result.

The previously obtained results are in agreement with that obtained by **(Mahran et al (1976). Mossa et al (1986)** it could be concluded from the previous results that carbohydrates steroids, flavonoids and tannins are mostly found in all tested samples, either *in vivo* or *in vitro* tissues. Accordingly, although such compounds tested qualitatively using color tests (i.e. first step). They were markedly present in the *in vivo* tissues more than the *in vitro* ones. It is of interest to do trials to increase such compounds of biological values, especially the steroids, which is a principal target in out work.

2. Quantitative determination of total steroids by spectrophotometer

In vivo tissues

From the obtained results (Table 2) it appears that Zaghloul CV. tissues contains more total steroids than Sewi CV. regardless of tested organ. The highest value was formed in the pollen grains of Zaghloul CV. While, the lowest value was formed in root tissues of both CV. It could be observed that the steroid content in the *in vivo* tissue of Zaghloul was in the highest value in pollen grains, followed by shoot tip tissue fruit and male flowers. The lowest value was that of roots. On the other hand the highest value in Sewi cultivar was that of pollen grain, followed by shoot tip and fruit tissues, respectively.

Table (2): Quantitative determination of total steroids by spectrophotometer in the untreated in vivo and in vitro of two date palm tissues, Zaghloul and Sewi cultivars.

Type of explant	Zaghloul (mg/g)	Sewi (mg/g)
1- In vivo		
Shoot tip (Gommar)	0.524	0.212
Pollen grains.	0.166	0.127
Leaf	0.249	0.234
Root	0.069	0.500
Female flowers	0.249	0.104
Male flowers	0.256	0.120
Fruit	0.393	0.198
2. In vitro		
Callus	0.099	0.085
Emberyogenic callus	0.075	0.060
Embryos	0.079	0.075
Germinated embryo	0.050	0.031
Leaf	0.339	0.308
Roots	0.135	0.126

***In vitro* tissues:**

Data of Table (2) of show that leaf tissues of Zaghloul CV. contain the highest total steroid, regardless of the tested organ. The lowest value was formed in the germinated embryo of both CV. These results show clearly that the steroid content in the *in vitro* tissues of Zaghloul cv exhibit high value in leaf tissues followed by roots and callus. The germinated embryo tissues exhibited lower value in this concern. On the other hand the Sewi tissues had different trend where the highest value was that of leaf followed by roots. The lowest value was that of germinated embryo tissues. The decrement of steroid content in Sewi cv than Zaghloul cv may be attributed to the genetic make up of both cultivars.

3. Separation and identification of steroids by thin layer chromatography in the *in vivo* and *in vitro* tissues obtained from two date palm cultivars (Zaghloul and Sewi).

The results in table (3) show the thin layer chromatograms (TLC) of steroids separated from the unsaponifiable fraction in the lipid extracts of both in vitro and *in vivo* tissues of Zaghloul and Sewi cultivars. The identification was carried out using values obtained from authentic compounds.

A. *In vivo* tissues:

The separation was carried out on shoot tip (Gommar) and pollen grains in both cultivars shoot tip:

1. Shoot tip (Gommar)

The separation of steroid in shoot tip tissues revealed the presence of 5 spots having R.f. values as 0.1, 0.19, 0.13, 0.39 and 0.95 in Zaghloul cv and . Two spots were identified as cholesterol (Rf 0.30) and B-sitosterol (Rf 0.39). The rest of spots could not be identified in this respect. With regard to the Sewi cultivar, spots were separated having Rf values as 0.07, 0.29, 0.42, 0.51 and 0.92. The same steroids were also identified (i.e. cholesterol and B-sitosterol R.f 0.29 and 0.42 respectively)

2. Pollen grains:

The chromatogram of the separated steroids in pollen grains tissue of Zaghloul cultivar show the presence of 7 spots having R.f values as 0.08, 0.15, 0.29, 0.39, 0.53, 0.62 and 0.96. Two spots were identified cholesterol (R.f. 0.29) and B-sitosterol (R.f 0.39). With regard to the Sewi cultivars 6 spots were separated having the following R.f values, 0.10, 0.28, 0.35, 0.40, 0.51, 0.92. The identified compounds were the same as Zaghloul cultivar in addition to stigmasterol (R.f 0.35). The obtained results agreed with those obtained by Bennett and Heftman (1966), and Das and Banerjee (1980).

Table (3): Separation of components of unsaponifiable matter of data palm (*Phoenix dactylifera* L.) by TLC

Type of Explant	Cultivar	No. of Fractions	Rf. Values	
Gommar "Shoot tip"	Zaghloul	1	0.10	Unidentified
		2	0.19	Unidentified
		3	0.30	Cholesterol
		4	0.39	B-sitosterol
		5	0.95	Non of sterols and triterpenes
Gommar "Shoot tip"	Sewi	1	0.07	Unidentified
		2	0.29	Cholesterol
		3	0.42	B-sitosterol
		4	0.51	Unidentified
		5	0.92	Non of sterols and triterpenes
Pollen grains	Zaghloul	1	0.08	Unidentified
		2	0.15	Unidentified
		3	0.28	Cholesterol
		4	0.39	B-sitosterol
		5	0.53	Unidentified
		6	0.62	Unidentified
		7	0.96	Non of sterols and triterpenes
Pollen grains	Sewi	1	0.10	Unidentified
		2	0.28	Cholesterol
		3	0.33	Stigmasterol
		4	0.40	B-sitosterol
		5	0.51	Unidentified
		6	0.98	Non of sterols and triterpenes
Callus "in vitro"	Zaghloul	1	0.13	Unidentified
		2	0.27	Cholesterol
		3	0.41	B-sitosterol
		4	0.96	Non of sterols and triterpenes
Callus "in vitro"	Sewi	1	0.14	Unidentified
		2	0.29	Cholesterol
		3	0.40	B-sitosterol
		4	0.57	Unidentified
		5	0.99	Non of sterols and triterpenes.
Embryos In vitro	Zaghloul	1	0.09	Unidentified
		2	0.30	Cholesterol
		3	0.39	B-sitosterol
		4	0.97	Non of sterols and triterpenes.
Embryos In vitro	Sewi	1	0.10	Unidentified
		2	0.28	Cholesterol
		3	0.39	B-sitosterol
		4	0.55	Unidentified
		5	0.97	Non of sterols and triterpenes

B. In vitro Tissues:

1. Callus

The TLC chromatogram of steroids in callus tissue of Zaghloul cultivar revealed the presence of 4 spots having R.f values as 0.13, 0.27, 0.41, 0.96. Two spots were identified as cholesterol (R.f. 0.27) and β-sitosterol (R.f. 0.41). With regard to the steroids in callus tissue of Sewi

cultivar, it could be said that the results previously obtained for Zaghloul cultivar can be applied on Sewi cultivar except of one more spot having R.f value of 0.57. Consequently, two steroid compounds were identified as cholesterol (R.f 0.29) and B-sitosterol (R.f 0.40).

2. Embryos tissues:

The separation of steroids by TLC in the *in vitro* tissue of embryo in Zaghloul cultivar revealed the presence of 4 spots having R.f. values as 0.09, 0.30, 0.39 and 0.97, cholesterol (R.f 0.30) and B.sitosterol were identified. A similar TLC chromatogram was mostly obtained except of one more spot of R.f value (0.55).

It could be concluded from the previous result that two sterol compounds were identified in all samples cholesterol and B-sitosterol in addition to stigmasterol, which was detected in the pollen grains of Sewi cultivar. It may be mentioned that the entire identified compound may be used by the plant as precursors for synthesis of the different steroids of medicinal importance in this concern.

Butenandt and Jacobi (1993) isolated oestrone from the press cake of palm Kernel and so far the first time from the plant origin, but the botanical source of which was not specific. ***Zaki et al. (1993)*** declared that pollen grains of palm trees, known to contain the steroid hormone estrone, are used in Egyptian folk medicine to treat male and female infertility. Several steroids, including the brassinosteroid, 24-epi-costasterone, were isolated from pollen grains of *Phoenix dactylifera* and identified by GC-MS. ***Amer and Zahran (1999)*** mentioned that the pollen grains contained estrogen, guercetin, β- amyrin, β- sitosterol, steroid, cholesterol's, and estrone. Also, date kermels contained estrogen, cholesterol's, campestral, stigmasterol, and β-Sitosterol.

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