Arab Gulf J. scient. Res. 4 (2), pp. 495-507 (1986)

Phytochemical and Biological Investigations on Date Seeds (*Phoenix dactylifera* L.) Produced in Saudi Arabia.

Jaber S. Mossa, Mohamed S. Hifnawy and Abdel G. Mekkawi

Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh, Saudi Arabia.

ABSTRACT. Qualitative phytochemical similarly in date seeds of seven Saudi Arabian cultivars was observed, as the samples were found to contain sterols, sugars, flavonoids, minerals, and fixed oils. Microbiological and neuropharmacological screening, with the alcoholic extract of one cultivar, showed significant antimicrobial activity against certain microorganisms as well as a stimulant action on the motor activity of mice.

Date palms (*Phoneix dactylifera* L., Palmaceae) have been grown as a staple food for thousands of years in the desert areas of the world and dates are an economically important crop of Saudi Arabia. The number of these trees is estimated to be 9 million of which 7 million are fruiting trees classified under some 400 cultivars (Hussain and El-Zeid 1978). Production reaches up to 400,000 tons anually.

Most of the previously published work on dates has been concerned with the nutritional value of the fruits. However, date seeds have been subjected to some previous chemical investigations. The analytical characteristics of date stones, including moisture, ash, proteins, carbohydrates, fibers and fixed oil, were previously reported (Harvey 1936). A sterol, belonging to the ergosterol group was found in the seeds (Montignie 1942). The seeds were reported to contain 7.55% water soluble total sugars of which 3.21% are reducing (Mizuno and Miekami 1958); pentosan, fructose, glucose, sucrose, raffinose and stachyose were identified in the reducing sugar portion. A polysaccharide compound was located in the seeds and on hydrolysis gave galactose, mannose and xylose (Jindal and Mukherjee 1969). On the other hand, the hydrolyzed polysaccharide from the flesh and seed of khala dates grown in Saudi Arabia was shown to be composed of xylose, arabinose,

glucose and galactose (Hussain and El-Zeid 1975). Finally, a lignin compound and the steroid, estrone, were stated to be constituents of date seeds (Fernandez and Santaolla 1960 and Heftmann *et al.* 1965).

The present work was undertaken with the aim of chemical evaluation of the seeds of seven different cultivars of dates produced in the Kingdom. One of these cultivars was also screened microbiologically and neuropharmacologically.

Experimental

Seed Material

The seeds of seven cultivars obtained from the central region were isolated from their corresponding mature fruits. These cultivars were Khalas, Nabt-Saif, Nabt-Zamel, Negeeb, Sukkari, Sifree and Sugee. The seeds were washed with distilled water, air-dried and weighed, and their percentage relative to the weight of the fresh fruits were calculated. The seeds were then dried in a hot-air oven at about 60°C and powdered.

Methods

The powdered seeds were subjected to preliminary phytochemical screening for the main medicinal plant constituents *viz.* sterols and/or triterpenes, flavonoids, carbohydrates and/or glycosides, alkloids and proteins (Farnsworth 1966).

About 200 g portions of each of the seven cultivars under investigation were successively extracted using petroleum ether (60-80°C), ether, chloroform and ethanol. For the microbiological screening the investigated cultivar was further extraced with ethyl acetate just prior to the chloroform extraction. The solvent of each extract was evaporated under reduced pressure and the residue was phytochemically screened.

Lipid analysis

About 2 g portions of the petroleum ether extract of each cultivar were saponified by mixing with 100 ml of alcoholic KOH (5%) and refluxed on a boiling water bath for 3 hr. The saponified solution was diluted with distilled water and the unsaponifiable matter was separated with ether, concentated and analyzed by TLC and GLC. The mother liquor was acidified and the liberated fatty acids were similarly extracted with ether and methylated by refluxing the ether residue with absolute methanol containing a few drops of conc. H_2SO_4 for 3 hours. The methylated solution was diluted with water, and the methyl esters of the fatty acids were extracted with ether, concentrated, and analyzed by GLC.

Carbohydrate analysis

Ten mg portion of each of the alcohol extracts of the seven cultivars were separately silylated by dissolving in 1 ml of Tri-Sil-Z reagent (mixture of

trimethylsilylimidazole (TMSI) in dry pyridine) by warming at about 60-70°C. The silylated products were then analyzed by GLC.

Mineral analysis

Five g portions of the powder of each cultivar was ignited and ashed at about 750°C in a Muffle furnace for 24 hours. The total ash was weighed and the percentage was calculated. The ash was then dissolved in 5 ml of fuming HNO_3 and transferred quantitatively to a volumetric flask (100 ml) using deionized water. The solution was analyzed for its elemental composition using an atomic absorption spectrophotometer (the absorption mode was used) (Varian Publications 1978).

Antimicrobial Activity

Strains of *S. aureus* (No. 6571), *E. coli* (10418), *Pr. vulgaris* (4635), *Ps. aeruginosa* (10662) and *B. subtilis* (10400), from the National Collection of Type Cultures (NCTC), London, England and *C. albicans* (3153) from the Mycological Laboratory, London, School of Tropical Medicine and Hygiene, were used for this study. The ethanolic extract of one cultivar (Negeeb) was tested in concentrations of 1, 2 and 3 mg/ml of melted antibiotic assay agar medium. The overnight broth culture of the tested organism were diluted 1:100 with sterile water. The organisms were radially streaked over the agar containing the extract (Mitscher *et al.* 1972). A control plate was also streaked by the tested microorganisms. The microorganisms that responded to the extracts were further used for determination of the Minimum Inhibitory Concentration (MIC). This was measured using the tube dilution method (Kavanagh 1963). The extract was tested in 0.125, 0.5 and 1 mg/ml. All dilutions were prepared in antibiotic assay broth No. 1 with one drop of 10^{-2} dilution of an overnight broth culture of the test organism. All inocula were incubated overnight at 37°C before recording the inhibitory effect and the MIC. A standard streptomycin sulfate (Biochemie, MBH, Wien/Austria) was similarly tested and its MIC was recorded.

Neuropharmacological screening

Neuropharmacological studies were carried out in mice using five in each experiment, each weighing 30-40 g, according to the scheme of Irwin, 1961. An ethanolic extract of the Negeeb cultivar in the dose of 500 mg/kg of body weight was administered interaperitoneally (I.P.), the animals were observed for excitation, tremors, twitches, motor activity, motor co-ordination, pinna, coreneal reflexes and respiratory changes. Rectal temperature was recorded and mortality within 24 hr was noted.

Chromatographic methods

Thin layer chromatography (TLC) was performed on Silica Gel G layers using 10% ethyl acetate in n-hexane for development and anisaldehyde/H_2SO_4 for location.

For column chromatography, Silica gel S (70-230 mesh) was used as the stationary phase and for elution, n-hexane and n-hexane with increasing amounts of ether, and finally ether were used.

Gas liquid chromatography was performed on a Varian-Model 3700 Gas Chromatograph with dual FID using coiled glass columns (2 m × 2 mm I.D.) packed with 3% OV 1 on chromosorb W. The column temperature was 70°C increased by 10°C/min. to 220°C (for fatty acids) and 150°C to 250°C with 10°C/min (for sterols). The flame ionization detector (FID) was set at 250°C, the injector temperature was 200°C. The carrier gas was N_2 (40 ml/min.) with hydrogen and air for the detector (30 ml/min. and 300 ml/min. respectively). Sugar analysis by this technique was performed on a column of OV 17, and the column temperature was initially 150°C and increased to 320°C at 10°C/min with the injector temperature at 400°C.

Qualitative identifications were based on retention time comparison with authentic standards analyzed under the same conditions. This was confirmed by the spiking technique. Quantitative analysis was done using the area measurements derived by the automatic integrator. The percentages were determined relatively using the internal normalization method:

$$\text{Area } \% = \frac{\text{Peak area}}{\text{Total area}} \times 100$$

Results and Discussion

Date seeds as percentages of the fresh fruits (Table 1) reached up to 12.25% in the Sukkari cultivar and ranged from 6.37 to 10.18% in the other cultivars under investigation except in the Sugee cultivar which contained the smallest percentage (4.62%).

Preliminary phytochemical investigation showed qualitative similarity of the different cultivars under investigation. All contain sterols and/or triterpens, flavonoids, tannins, carbohydrates and proteins.

Table 1. Seed percentages based on wet weight of the fruit and the weight % of the dried seeds of different extracts.

Cultivar	Seed%	Pet. ether	Ether	$CHCl_3$	Alcohol
1. Khalas	7.28	5.4	0.52	0.39	6.08
2. Nabt-Saif	6.37	5.3	0.70	0.30	9.56
3. Nabt-Zamel	7.96	5.6	0.63	0.34	5.51
4. Negeeb	9.88	4.96	0.57	1.17	7.53
5. Sukkari	15.25	5.5	0.79	0.41	10.18
6. Sifree	10.18	5.6	0.74	0.51	8.51
7. Sugee	4.62	4.9	0.70	0.42	7.33

Successive solvent extraction (Table 1) resulted in the isolation of the fixed oil content in the petroleum ether extract (4.9% to 5.6%). Ether and chloroform extracted only small quantities of materials while most of the seed constituents were taken out with alcohol reaching a maximum amount (10.18%) in the Sukkari cultivar. Phytochemical and chromatographic screening showed qualitative similarities between the first three extracts (pet. ether, ether and chloroform). The alcohol extract, however, was rich in carbohydrates, proteins and possibly other more polar constituents. It acquired a red color which intensified on standing possibly due to the presence of some constituents which undergo oxidative changes by time or on exposure to light; further investigation of this extract is in progress.

TLC analysis of the first three extracts revealed the presence of a major spot, which colored dark violet with the spray reagent (R_f 0.45). It was much more prominent in the petroleum ether extract. On its column chromatographic fractionation this material was isolated. It was an oily liquid and spectral analysis revealed its aliphatic nature, IR (cm^{-1}) 2980, 1430, 1380, which are measures of aliphatic compounds. Its IR absorption characteristic for carbonyl groups (1720 cm^{-1}) and its R_f value changes by saponification of the pet. ether extract indicated its ester nature. It is likely a mixture of triglycerides. The unsaponifiable matter of the different cultivars was analyzed by GLC (Table 2) and showed the presence of estrone, one of the famous steroidal hormones, in the different samples except in the Negeeb cultivar which was also free of cholesterol, brassicasterol and compesterol. Cholesterol could be detected only in the Sukkari and Sugee cultivars (0.6 and 0.4%, respectively). Ergosterol was found only in the Negeeb cultivar in a moderate percentage (9%) a fact which needs further confirmation with other techniques *viz.* GC/MS; ergosterol is considered as a precursor for Vitamin D which is the end product of the controlled irradiation of ergosterol. β-sitosterol, on

Table 2. Steroidal contents of the seven cultivars under investigation (Relative % of total pet. ether extract as shown by GLC)*

Sterol / Cultivar	Estrone	Choles-terol	Brassica-sterol	Campe-sterol	β-sito-sterol	Ergo sterol
Khalas	+	–	0.6	2.0	12.2	–
Nabt-Saif	+	–	2.0	3.6	15.2	–
Nabt-Zamel	+	–	1.2	1.9	17.3	–
Negeeb	–	–	–	–	39	9
Sukkari	+	0.6	1.2	2.9	19.5	–
Sifree	+	–	3.1	3.1	24.5	–
Sugee	+	0.4	0.9	2.2	9.3	–

+ present in amounts less than 0.1%
– absent
* Data are the means of three determinations.

the other hand, was the most prominent in the unsaponifiable part of the investigated cultivars and reached a maximum amount (39%) in Negeeb and a minimum amount (9.3%) in Sugee cultivars. Figure 1 shows a GLC chromatogram of the unsap. matter of one cultivar (KHalas).

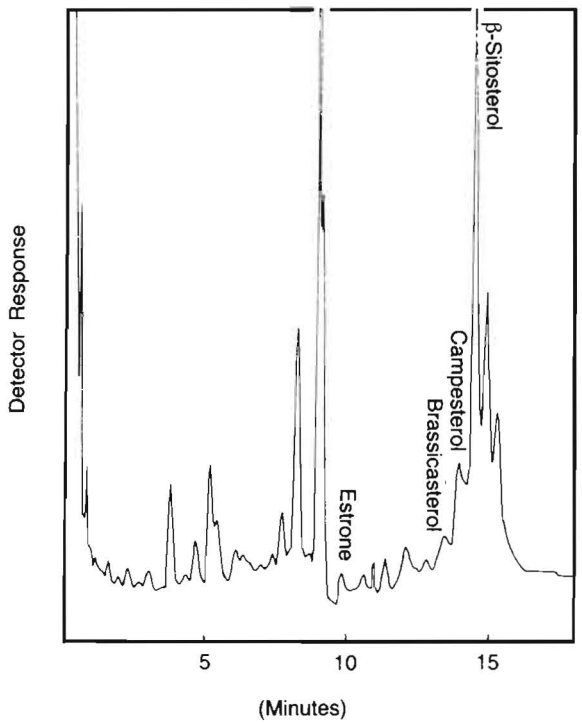

Fig. 1. GLC of Unsap. of Date Seeds (Khalas Cultivar)

The fatty acid analysis by GLC was performed on the saponifiable portion of the pet. ether extract after derivatization to the volatile methylesters (Table 3). Most of the investigated cultivars were found rich in unsaturated acids (linoleic and/or oleic which have the same retention times under the previously mentioned operating conditions). This combination ranged from 5.8% to 7.8% in the Negeeb and Sifree cultivars, respectively. Those cultivars which were relatively poor in their unsaturated acids were found rich in the saturated ones. Fig. 2 shows a GLC chromatogram of the fatty acid methylesters of the Khalas cultivar (C.F. Harvy, 1936 who reported only the physiocochemical characters of the oil).

Table 3. Fatty acid contents in the lipids of the investigated seeds. (Relative %).*

Fatty acid / Cultivar	1	2	3	4	5	6	7
Khalas	0.1	0.4	22.4	17.6	17.6	38.3	0.3
Nabt-Saif	0.2	0.4	24.7	14.2	11.9	48.0	0.5
Nabt-Zamel	0.2	0.4	21.0	18.3	15.7	42.3	0.4
Negeeb	–	–	18.0	11.0	9.0	58.0	–
Sukkari	0.2	0.4	22.2	19.3	15.9	39.0	0.5
Sifree	0.7	2.3	48.3	19.0	2.9	7.8	3.4
Sugee	0.1	1.6	54.4	23.5	3.0	9.4	1.7

1 = Octanoate; 2 = Decanoate; 3 = Dodecanoate;
4 = Tetradecanoate; 5 = Hexadecanoate; 6 = Linoleate/Oleate;
7 = Decosanoate.
* Each figure is the mean of three determinations.

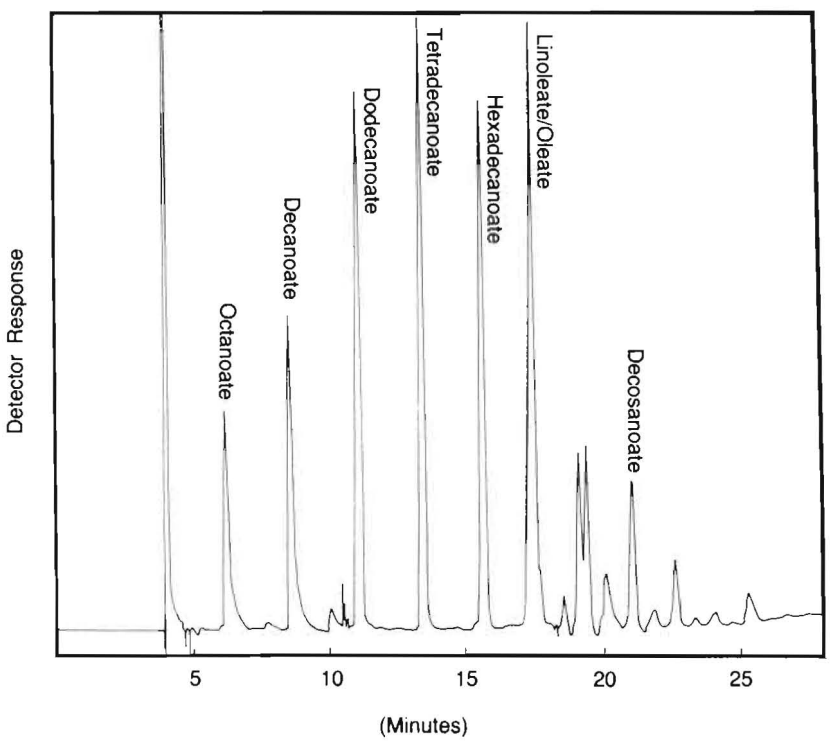

Fig. 2. GLC of Methyl Esters of Fatty Acids of Date Seeds (Khalas Cultivar)

The sugar content was found very prominent in the alcohol extract. A detailed study on the sugars of the seven cultivars is dealt with in another article. However, GLC analysis of the silylated extract, comparing with silylated authentic sugars, revealed the presence of different sugars (Table 4). Three of the investigated samples were found to contain mannose, glucose, sucrose, lactose, and maltose. Nabt-Zamel, contained only sucrose, lactose, and maltose, while Sugee was found nearly free of mannose. A GLC chromatogram of the silylated alcohol extract of the Khalas cultivar is reproduced in Fig. 3.

Table 4. Sugar content in the alcohol extract of the investigated seeds (Relative %)*

Sugar / Cultivar	Mannose	Glucose	Fructose	Sucrose		Maltose
Khalas	7	7	–	42	8	21
Nabt-Saif	19	21	–	24	12	4
Nabt-Zamel	–	–	–	37	28	17
Negeeb	13	9	–	32	4	16
Sukkari	12	7	–	30	22	12
Sifree	10	6	–	40	23	12
Sugee	–	16	–	27	23	15

* Each figure is the mean of three determinations.

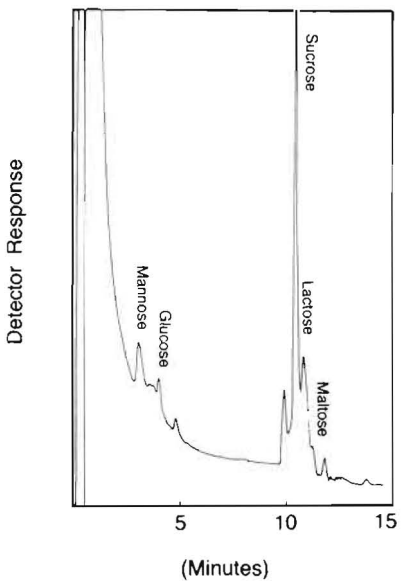

Fig. 3. GLC of Sugars of Date Seeds (Khalas Cultivar)

The mineral matter was analyzed by atomic absorption, and the results are recorded in μg/g amounts of dry powdered seeds (Table 5). It is apparent that date seeds are rich in macro and trace minerals essential for the normal health of mammals. Nutritionally, date seeds might be considered as sources for K^{+}, Ca^{++} and Fe^{+++} together with other elements.

Table 5. Ash % and mineral contents (μg/g) of the seeds under investigation.

Element \ Cultivar	1	2	3	4	5	6	7
Ash %	1.34	1.52	2.6	2.38	0.2	1.2	5.2
Fe^{+++}	4.95	8.55	8.55	4.9	16.6	9.03	5.33
Pb^{++}	0.17	0.23	0.48	1.3	4.0	0.28	0.1
Cd^{++}	1.1	0.9	2.25	0.002	9.0	1.75	0.0
Cu^{++}	0.93	1.2	1.8	0.326	2.0	1.0	0.63
Al^{+++}	3.17	3.13	4.4	4.4	23.9	12.75	6.15
Zn^{++}	1.0	1.3	2.03	0.663	2.4	10.0	2.39
Na^{++}	15.3	17.8	29.0	38.2	37.0	20.25	17.5
Mg^{++}	78.0	88.0	70.5	48.27	12.2	73.5	78.75
K^{+}	82.4	110.4	84.0	138.0	28.0	46.5	68.0
Ca^{++}	11.2	64.4	18.0	66.1	436.0	66.0	70.0

1 = Khalas; 2 = Nabt-Saif; 3 = Nabt-Zamel; 4 = Negeeb;
5 = Sukkari; 6 . Sifree; 7 = Sugee.

For the preliminary antimicrobial screen, the alcohol extract of one of the investigated cultivars (Negeeb) was tested against the listed microorganisms (Table 6), and the results are compared with those of a standard antibiotic (streptomycin sulfate). The data presented in the table reveal the activity of the extract against *S. aurus, Pr. vulgaris* and *B. subtilis* with an MIC of 0.5 mg/ml in case of *S. aurus* and *B. subtilis*. The antimicrobial effect, although comparatively weak when compared to streptomycin sulfare (MIC = 10 μg/ml), could be considered promising since the plant extracts are known to be very complex in their composition. Isolation of the different constituents, and their analysis for antimicrobial effect is in progress.

On the other hand, the neuropharmacological screen of the same extract (Table 7) showed that a stimulant action as it increased the motor activity when compared with control animals. In fact, the actual cause of this effect is not known and our progressing work on the alcohol extract of this cultivar and other cultivars deals with the full chemical and biological investigation of the active constituents.

Table 6. Antimicrobial activity Determination of alcohol extract of Negeeb cultivar as compared with streptomycin sulfare.

Extract	Method	Conc.	*S. Aureus*	*E. coli*	*Pr. vulgaris*	*Ps. aeruginosa*	*C. albicans*	*B. subtilis*
Ethanolic Extract	Streaking on agar.	1 mg	+	–	–	–	–	+
	mg extract/ml of agar.	2 mg	+	–	–	–	–	+
		3 mg	+	–	+	–	+	+
	MIC (broth dilution): extract, (mg/ml)		0.5	**	3	**	**	0.5
	Streptomycin sulfare (μg/ml)		10	10	10			10

+ Active. – Inactive, ** MIC was not determined due inactivity of the extract at this conc.

S. aureus = Staphylococcus aureus; E. coli = Escherichia coli; Pr. vulgaris = Proteus vulgaris; Ps. aeruginosa . Pseudomonas aeruginosa; C. albicans = Candida albicans; B. subtilis = Basillus subtilis;
MIC = Minimum Inhibitory Concentration.

Table 7. Neuropharmacological activity of alcohol extract of date seeds (Negeeb cultivar) in mice (500 mg/kg body weight, intraperitoneally)*

System/Observed Parameters		Ethanolic extract
AUTONOMIC RESPONSES	Respiration	Fast (Compared with control)
	Skin color	Normal
	Hypothermia	N.T (37.7)
	Piloerection	Negative to slight activity
	Salivation	Negative
	Urination	Negative
	Pupil Size	Normal
	Writhing	Negative
REFLEX	Pinna	Present
	Corneal	Present
MOTOR	Staggering	Negative
	Righting reflex	Not lost
C.N.S. EXCITATION	Straub tail	Positive
	Tremor	Positive
	Twitches	Positive (Head)
	Convulsions	Negative
	Agression	Negative
	Excitability	Positive

* This experiment was conducted on groups of 5 mice.

References

Farnsworth, N (1966) Biological and Phytochemical Screening of Plants, *J. Pharm. sci.* **55:** 225-276.

Fernandez, O. and **Santaolla, M.** (1960) Phytogenetic Chemistry of Lignins, Analysis of the seed covering of *Phoenix dactylifera. Rev. Real. Acad. Ciene. exact., Madrid* **54:** 201-207.

Harvey, R.G. (1936) Some Analytical Characteristics of Date Stone Oils, *Analyst* **61:** 403.

Heftmann, E., Tzeko, S. and **Bennett, R.D.** (1965) Identification of Estrone in Date Seeds by Thin Layer Chromatography. *Natur. Wissenchaften* **52:** 431-432.

Hussain, F. and **El-Zeid, A.** (1975) Chemical Composition of Khala Dates grown in Saudi Arabia, *Egypt. J. Hortic.* **2:** 209-214.

Hussain, F. and **El-Zeid, A.** (1978) *Studies on Physical and Chemical Characteristics of date varieties of Saudi Arabia,* Ministry of Agriculture and Water, Kingdom of Saudi Arabia.

Irwin S. (1964) in *Animal and Clinical Pharmacologic Techniques in Drug Evaluation,* Year Book Publishers, USA.

Jindal, V.K. and **Mukherjee, S.** (1969) Sugars in *Phoenix dactylifera* Seed Polysaccharides, *Curr. Sci.* **38:** 459-460.

Kavangh, F. (1963) *Analytical Microbiology,* Academic Press, New York, pp. 125-140.

Mitscher, L.A., Leu, R.P., Bathala, M.S., Wu-nan-Wu, Beal, J.L. and **White, R.** (1972) Antimicrobial agent from higher plants, I-introduction rationale and Methodology, *Lloydia* **35:** 157.

Mizuno, T. and **Miekami, Y.** (1958) Carbohydrates in the fruits of date palm, *Nippon Nagel Kazaku Kaishi* **32:** 829-832.

Montignie, E. (1942) Presence of Sterols in Dates, *Bull. Soc. Chim.* **8:** 545-546.

Varian Techtron Pty. Ltd. (1978) *Analytical Methods for Flame Spectroscopy* Springvale, Australia, *publication no.* 95/00009-00.

(Received 15/10/1985;
in revised form 12/02/1986)

Operating conditions

Fig. 1. *Column:* Coiled glass (2m × 2mm I.D.) packed with 3% OV 1 on chromosorb W; temperature 150°C increased by 10°C/min to 250°C; detector temperature, 250°C; injector temperature, 200°C; Carrier gas N_2 with 40 ml/min; H_2 flow, 30 ml/min; air flow, 300 ml/min.

Fig. 2. *Column:* Coiled glass (2m × 2mm I.D.) packed with 3% OV 1 on chromosorb W; temperature 70°C increased by 10°C/min to 220°C; detector temperature, 250°C; injector temperature, 200°C; Carrier gas N_2 with 40 ml/min; H_2 flow, 30 ml/min; air flow, 300 ml/min.

Fig. 3. *Column:* Coiled glass (2m × 2mm I.D.) packed with 3% OV 17 on chromosorb W; temperature 150°C increased by 10°C/min to 320°C; injector temperature, 400°C; Carrier gas N_2 with 40 ml/min; H_2 flow, 30 ml/min; air flow, 300 ml/min.

دراسات كيميائية وبيولوجية على بذور التمر المنتج في المملكة العربية السعودية

جابر سالم موسى، محمد سعيد حفناوي،
و عبد القادر مكاوي محمد

قسم العقاقير ـ كلية الصيدلة ـ جامعة الملك سعود
الرياض ـ المملكة العربية السعودية

يشمل هذا البحث نتائج الدراسة الكيميائية لسبعة أصناف من التمر منتجة في المنطقة الوسطى وهي: الخلاص، نبت سيف، نبت زامل، سكري، صفرى، نجيب والصقعي.

حيث تبين أن هناك تشابهاً نوعياً في محتوياتها الكيميائية ووجد بها مواد استيرولية و / أو ثلاثية التربينات، ومواد سكرية ومواد فلافونيدية ومعادن إلى جانب الزيوت الثابتة.

وكذلك فإن البحث يتضمن النتائج الأولية لفحص الخلاصة الكحولية لأحد الأصناف قيد الدراسة ميكروبيولوجياً إلى جانب دراسة تأثيراته الفارماكولوجية والعصبية حيث تبين أن للخلاصة الكحولية تأثير ملحوظ كمضاد لبعض الميكروبات المرضية. وكذلك تأثيراً منشطاً للجهاز الحركي عند الفئران.