

**PHYTOCHEMICAL STUDIES ON *Asphodelus fistulosus* SYN. *A. tenuifolius* Cav.**

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**ABSTRACT**

*Asphodelus fistulosus* Syn. *A. tenuifolius* Cav. belongs to the family Liliaceae. It is distributed in Sinai desert (North & South Sinai) as an annual herb, flowering in spring after heavy rains.

The preliminary phytochemical screening showed that the plant contains mainly anthraquinones, flavonoids, alkaloids, sterols and terpens.

The phytochemical investigation of the plant revealed the presence of six sugars (raffinose, maltose galactose, glucose, sucrose and rammnose), fifteen amino acids that were in free form or enclosed in protein structure with different percentages, nine fatty acids, eleven hydrocarbons, mainly octacozane, squalene and hexacosane, besides one type of sterol ( $\beta$ -sitosterol). Alkaloidal investigation supported by spectral methods, revealed the presence of choline and anabsine.

**Key words :** *Asphodelus fistulosus*, *A. tenuifolius*, *phytochemistry*.

**1. INTRODUCTION**

*Asphodelus fistulosus* belongs to the family *Liliaceae* which includes several genera reported as remedies in folk medicine (Walt and Breyer-Brandwijk, 1962). Their value in this respect has been attributed to various constituents, which may be either alkaloids as colchicine in *Colchicum* spp. (Boit, 1961) and cardiac glycosides as in *Urginea* spp. (Stoll, 1937 & 1956).

*A. fistulosus* belongs to the genus *Asphodelus* which is commonly used as remedial plants in folk medicine as mentioned by Hammouda *et al.*, (1972), Boulos (1983), Chiej (1984), Iwu (1993) and Ghazanfar (1994).

*A. fistulosus* L. var *tenuifolius* (Arabic name : Burway) is one of the wild annual herbs which are widely distributed in Sinai desert. It is used as laxative, diuretic and as a cure for external ulcer (Ghazanfar 1994). Accordingly; *A. fistulosus* was the target of this study.

Fell *et al.*, (1968) recorded four free sugars (stachyose, raffinose, melibiose and sucrose) in *Asphodelus microcarpus* seeds and three sugars (stachyose, raffinose and sucrose) in *A. fistulosus* seeds. Rizk and Hammouda (1970) found that the tuber of *Asphodelus microcarpus* contains mucilage (2.7 D.Wp) that composed of glucose, galactose and arabinose.

Mohammed *et al.*, (1961) recorded that the seed of *A. fistulosus* contains 16% protein, from which 13 amino acids were identified. This protein source is suggested as a possible human food source.

Fell *et al.*, (1968) obtained from the fixed oil of *A. microcarpus* and *A. fistulosus* seeds:  $\beta$ -sitosterol,  $\beta$ -amyrin, campesterol and stigmasterol. The yield of the unsaponifiable matter (sterols) was 1.03 and 0.98% from *A. microcarpus* and *A. fistulosus*, respectively. Also five fatty acids were identified in seed oil of both species; they were myristic, palmitic, stearic, oleic and linoleic acids. Rizk and Hammouda (1970) found that the unsaponifiable matter (sterol) in the tubers of *A. microcarpus* amounted to about 17%, fucosterol was isolated from the unsaponifiable fraction. By thin layer and gas liquid chromatographic (GLC) analysis of fatty acids, they detected: myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, behenic and lignoceric acids.

Hammouda *et al.*, (1972) found  $\beta$ -sitosterol- $\beta$ -D-glucoside and two unidentified components from the tubers of *A. microcarpus*. Singh and Saxene (1976) recorded in *Asphodelus albus* nine fatty acids besides  $\beta$ -amyrin,  $\beta$ -sitosterol, stigmasterol, campesterol and fucosterol.

Tackholm and Drar (1954) declared that Bedouins at Sidi-Barrani in Egypt were using the tubers of *A. microcarpus* as remedy for withering and paralysis. Gum that can be obtained from cuts in the unripe capsules of *A. microcarpus* was used for local application in North Africa. Adding, the juice obtained from the fruits was employed

for earache. Gomes (1992) reported that the tubers of *A. lusitanicus* were used in Portuguese traditional medicine to treat several skin diseases.

## 2. MATERIALS AND METHODS

*Asphodelus fistulosus* plants were collected during 1999 season (winter and spring) from its natural locality, at Wadi Om-Sura and El-Arish; North Sinai. The collected whole plant was dried in an oven at 60°C for 48 hours and ground to fine powder, then used in the following investigation:

### 2.1. Preliminary phytochemical screening

#### 2.1.1. Steam distillation

About 50 g of fresh plants were subjected to steam distillation to extract volatile oil, according to Balbaa *et al.* (1981).

#### 2.1.2. Preparation of the extract for further screening

About 20 g of air-dried plant powder were refluxed with 150 ml of 80% ethyl alcohol for 6 hours, then filtered. The residue was then washed several times with hot alcohol. The combined filtrates were collected, concentrated under reduced pressure at 50 °C, and used for the following tests:

Test for tannins according to Balbaa (1986), test for sterols and terpenes (Libermann - Burchard's test) according to Fieser and Fieser (1959) and Salkowski reaction's according to Brieskorn and Klinger - Hand (1961), test for flavonoids according to Wall *et al.* (1954), test for alkaloids according to (Woo *et al.*, 1977), test for carbohydrates and/or glycosides using Molish's and Fehling's reagent according to Balbaa (1986), test for saponins according to (Wall *et al.*, 1954 and Balbaa 1986), and test for resins according to Balbaa (1986), test for anthraquinones according to Balbaa (1986).

### 2.2. Investigation of carbohydrates

Investigations of the free sugars were determined according to Chapline and Kennedy (1994) and chromatographic separations and combined sugars according to Abou-Zeid *et al.* (1995).



### **2.3. Investigation of amino acids**

The free amino acids and protein were determined using amino acid analyzer according to Pellet and Young (1980).

### **2.4. Investigation of lipids**

The lipids were extracted from the powdered plant with petroleum ether ( B.p. 40 - 60°C ) : ether (1:1 v/v ) for 24 hours using Soxhelt apparatus. The lipids were obtained by distilling off the solvent and the last traces of the solvents were removed by heating the liquid sample in a vacuum oven at 50°C to a constant weight.

#### **2.4.1. The fundamental chemical properties**

Acids, iodine, saponification and ester values were determined according to Farag (1995).

#### **2.4.2. Chromatographic investigation of lipid contents**

The lipids were extracted with petroleum ether: diethyl ether (1:1) using Soxhelt apparatus, then filtered.

##### **2.4.2.1. Identification of fatty acids by GLC**

Methylation of fatty acids was carried out by Trimethyl silylation reaction. The fatty acid methyl ester was then subjected to gas-liquid chromatographic analysis (GLC). The relative properties of each individual compound were estimated as the ratio of the partial areas to the total areas as mentioned by Farag *et al.* (1980).

##### **2.4.2.2. Identification of unsaponifiable matter by GLC**

The hydrocarbons and sterols compounds were identified by using a Hewlett Packard gas chromatography, model 5890, equipped with flame ionization detector.

The relative percentage of each unsaponifiable compound was determined using traingluatation method according to Nelson *et al.* (1969). The results of Itoh *et al.* (1973) and Farag *et al.* (1986) were used as a guide to characterize some of the unknown compounds.

### **2.5. Investigation of alkaloids**

Alkaloids were extracted from the dried plant powder of *A. fistulosus* according to Woo *et al.* (1977), investigated according to Stahl (1969), Hammouda *et al.* (1971) and Awaad (1995) and identified by using MS and NMR measurements.

### 3. RESULTS AND DISCUSSION

#### 3.1. Preliminary phytochemical screening of *A. fistulosus*

The preliminary phytochemical screening on *A. fistulosus*, collected from Wadi Om-Sura revealed the presence of alkaloids, anthraquinones and tannins. No saponins, resins or volatile oils were present in *A. fistulosus* as shown in Table (1).

Table. (1): Preliminary phytochemical screening of *A. fistulosus*.

Test	Results
Alkaloids	(+)
Flavonoids	(+)
Saponins	(-)
Resins	(-)
Carbohydrates and/or glycosides	(+)
Terpens	(+)
Anthraquinones	(+)
Tannins	(+)
Sterols	(+)
Volatile oils	(-)

#### 3.2. Investigation of carbohydrates

##### 3.2.1. Free sugars

Table (2) shows the result of investigating the free sugars of *A. fistulosus* using Whatmann No. 1 comparative paper chromatography, solvent system n-butanol : acetic acid : water (4:1.5) and aniline hydrogen phosphate as spraying reagent and revealed the presence of (raffinose, glucose, galactose, maltose and sucrose) as compared by pure authentic samples of sugars.

Table (2): Free sugars of *A. fistulosus* L. using paper chromatography.

Sugars	R <sub>f</sub> × 100	Colour
Raffinose	8	Brown
Maltose	10	Brown
Galactose	14	Brown
Glucose	15	Brown
Sucrose	18	Brown

### 3.2.2. Combined sugars

Investigation of the hydrolyzed combined sugar extract of *A. fistulosus* using Whatmann No.1 comparative paper chromatography, solvent system n-butanol: acetic acid : water(4 : 1 : 5) and aniline hydrogen phthalate as spraying reagent revealed the presence of Rhamnose, raffinose, glucose, galactose, maltose and sucrose as combined sugars are shown in Table (3).

**Table(3):** Combined sugars of *A. fistulosus* L. using paper chromatography.

Sugars	R <sub>f</sub> × 100	Colour
Raffinose	8	Brown
Maltose	10	Brown
Galactose	14	Brown
Glucose	15	Brown
Sucrose	18	Brown
Rhamnose	31	Yellow-brown

### 3.3. Investigation of amino acids

#### 3.3.1. Free amino acids

The free amino acids of *A. fistulosus* were investigated using amino acid analyzer. Data presented in Table (4) showed that *A. fistulosus* contained 15 free amino acids: aspartic acid, threonine, serine, glutamic acid, glycine, alanine, arginine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine and lysine. Glutamic acid was relatively high in its concentration (0.77 %), while histidine was relatively low in its concentration (0.049%) if compared with other free amino acids in the plant.

#### 3.3.2. Protein-amino acids

The investigation of hydrolyzed protein-amino acids of *A. fistulosus* was achieved using amino acid analyzer.

Data presented in Table (5) show that the protein hydrolyzate of *A. fistulosus* contained fifteen amino acids: aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine with different ranges of concentration. Arginine and aspartic acid were



relatively high in their concentrations (2.62 and 2.63 %) if compared with other protein-amino acids in the plant, while methionine was relatively low in concentration (0.12%) if compared with other protein-amino acids in the plant.

On a general view of Tables (4) and (5); *A. fistulosus* enclosed, 15 amino acids which are either found in free state or included in protein structure. These amino acids are classified into 7 essential amino acids, 2 semi-essential amino acids and 6 non-essential amino acids.

**Table(4):** Percentages of free amino acids of *A. fistulosus* using amino acid analyzer.

Amino acids	Conc. (%)
Aspartic acid	0.46
Therionine *	0.07
Serine	0.09
Glutamic acid	0.77
Glycine	0.05
Alanine	0.08
Arginine **	0.21
Valine *	0.10
Methionine	0.07
Isoleucine *	0.18
Leucine *	0.12
Tyrosine	0.08
Phenylalanine *	0.11
Histadine *	0.04
Lysine *	0.07

\* = Essential amino acid

\*\* = Semi-essential amino acid

### 3.4. Investigation of lipids

#### 3.4.1. Physical properties

The obtained lipids were dark green in colour, semi-solid having a faint odour and disagreeable tast. It was soluble in n-hexane, benzene, diethyl ether, chloroform, acetone, warm methyl and ethyl alcohol.

#### 3.4.2. Fundamental chemical properties

The fundamental chemical properties of the extracted lipids of *A. fistulosus* are presented in Table (6).

**Table(5): Percentages of protein amino acids of *A. fistulosus* using amino acid analyzer.**

Amino acids	Conc. (%)
Aspartic acid	2.62
Therionine *	0.54
Serine	1.45
Glutamic acid	2.20
Glycine	0.51
Alanine	0.62
Valine *	1.65
Methionine	0.12
Isoleucine *	0.62
Leucine *	1.20
Tyrosine	0.29
Phenylalanine *	0.89
Histadine *	0.36
Lysine *	0.85
Arginine **	2.63

\* = Essential amino acid

\*\* = Semi-essential amino acid

It is clear from this table that acid and ester values were 18 and 141%, respectively that revealed the presence of fatty acids mainly in ester form. The result of saponification value of *A. fistulosus* was (159%), which indicated that the main constituents of fat were long chain fatty acids.

The results obtained were compared with the saponification value of rapeseed oil as its main constituent was C<sup>18</sup> fatty acid (*i.e.*, Oleic acid, linoleic acid and linolenic acid) and its saponification value ranged between 170 and 180 (Farag, 1995). This was confirmed by GLC analysis of the fatty acid content in *A. fistulosus*. The iodine value of fat content of the plant was 80 which is compatible with the result obtained by GLC analysis of fatty acids.

### 3.4.3. The unsaponifiable fraction (Hydrocarbons and Sterols)

The unsaponifiable matter content of *A. fistulosus* was determined using GLC technique. The relative percentages of each component were calculated and tabulated in Table (7). It was obvious from the obtained results that *A. fistulosus* contained the dodecane, eicosane, heneicosane,



docosane, tricosane, tetracosane, hexacosane, octacosane, squalene, triacontane, dotriacontane, and  $\beta$ -sitosterol.

**Table (6):** Acid, iodine, ester and saponification values of lipids of *A. fistulosus*.

Items	Percentage
Acid value	18
Iodine value	80
Ester value	141
Saponification value	159

### 3.4.4. The unsaponifiable fraction (Hydrocarbons and Sterols)

The unsaponifiable matter content of *A. fistulosus* was determined using GLC technique. The relative percentages of each component were calculated and tabulated in Table (7). It is obvious from the obtained results that *A. fistulosus* contained the dodecane, eicosane, heneicosane, docosane, tricosane, tetracosane, hexacosane, octacosane, squalene, triacontane, dotriacontane, and  $\beta$ -sitosterol.

**Table (7):** Hydrocarbons and sterols of *A. fistulosus* as detected by relative percentage by GLC.

Hydrocarbons and sterols	No. of carbon atoms	Relative percentage
Dodecane	12	1.52
Eicosane	20	1.55
Heneicosane	21	4.00
Docosane	22	3.75
Tricosane	23	27.01
Tetracosane	24	7.85
Hexacosane	26	31.74
Octacosane	28	52.25
Squalene	29	40.71
Triacontane	30	19.38
Dotriacontane	32	121.32
$\beta$ -Sitosterol	27	13.86

### 3.4.5. The saponifiable fraction (free fatty acids)

The saponifiable contents of *A. fistulosus* were determined using GLC technique. The relative percentage of each component was calculated and tabulated in Table (8), which revealed that *A. fistulosus* contained 6 saturated fatty acids: Caproic, caprilic, capric, myristic, palmitic and stearic and 3 unsaturated fatty acids: Oleic, linoleic and linolenic. Linoleic and linolenic, the essential fatty acids are claimed to increase the epithelialisation rate of wounds as reported by Ross and Brain (1977).

Table (8): Fatty acids of *A. fistulosus* as detected by a relative percentage using GLC.

Fatty acids	No. of carbon atom	Relative percentage
Caproic	6.0	0.86
Caprilic	8.0	0.16
Capric	10.0	0.60
Myristic	14.0	0.65
Palmitic	16.0	4.34
Stearic	18.0	4.55
Oleic	18.1	3.26
Linoleic *	18.2	5.90
Linolenic *	18.3	6.94

\* = Essential fatty acids

### 3.5. Investigation of alkaloids

The alkaloid extract was subjected to thin layer chromatography technique (TLC) using chloroform: methanol (8 : 2) as a solvent system. After spraying the dried plates by Dragendorff's reagent - (specific for alkaloids), the plates showed four separated alkaloidal compounds. Three of the compounds (B, C, and D) were minor while compound (A) was present in a considerable amount, which can be separated by preparative thin layer chromatography technique, (Table 9).

Table (9): R<sub>f</sub> values and colour reaction of alkaloidal compounds of *A. fistulosus*.

Compound	R <sub>f</sub> in chloroform: methanol (8 : 2)	Colour reaction with Dragendorff's reagent
A	0.26	orange
B	0.45	orange
C	0.50	orange
D	0.64	orange

### Preparative layer chromatography (PLC) for compound (A)

The total alkaloidal extract was subjected to (PLC) activated plates using silica gel G 245 as a stationary phase and mobile phase chloroform : methanol (8 : 2). After the system was developed, the plates were air dried and the first 1 cm from each plate was sprayed by Dragendorff's reagent to determine the band limits. The major band (scraped silica) was collected and eluted completely by chloroform, and the obtained fraction was then tested by (TLC) precoated plates (Merk). The plate declared that compound (A) was present and accompanied with a small amount from compound (B).

### Purification of compound (A)

The obtained fraction was subjected to further purification using silica gel column chromatography as a stationary phase and mobile phase chloroform : methanol, starting with chloroform and gradually increase the polarity towards methanol.

### R<sub>f</sub> values of compounds

R<sub>f</sub> values of compound (A) were measured in two different systems, it was (0.2) in chloroform : methanol. (95 : 5) and (0.26) in chloroform : methanol (8 : 2).

### Identification of compound (A)

Identification of compound (A) occurred using <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and EI mass spectrum.

#### 1) <sup>1</sup>H-NMR (δ)

When compound (A) was subjected to <sup>1</sup>H-NMR analysis in CdCl<sub>2</sub>, the following signal was obtained, 8.57 (d, J = 2 Hz → H2), 7.74 (6 lines, J = 8 Hz → H4), 7.25 (6 lines, J = 8 Hz → H5), 8.47 (4 lines, J = 5 Hz → H6), 2.9 - 3.1 (broad → NH proton) and 3.1 - 3.7 (multiplet → piperidine ring [9 proton]).

#### 2) <sup>13</sup>C-NMR (δ)

The obtained <sup>13</sup>C-NMR spectral data of compound (A) in CdCl<sub>2</sub> show the following signals: δ 148 (C<sub>2</sub>, C<sub>6</sub>), 140.36 (C<sub>4</sub>), 135.5 (C<sub>5</sub>), 123.5 (C<sub>3</sub>), 47.8 (C<sub>7,11</sub>), 34.5 (C<sub>8,10</sub>), 25.53 (C<sub>9</sub>).



### 3) EI mass

The EI mass spectral data of compound (A) show the molecular ion peak M-I at 161 m/z and M-79 at 84 m/z as a base peak.

The  $R_f$  value of compound (A) was nearly similar to that of anabasine alkaloid.

- The  $^1\text{H-NMR}$  spectral data of compound (A) showed that:
- The presence of two hydrogen's ( $\text{H}_2$  &  $\text{H}_6$ ) at  $\delta$  8.57 and 8.47, respectively are corresponding to the hydrogen in a conjugated system near to nitrogen group.
- The presence of  $\text{H}_5$  at 7.25 and  $\text{H}_4$  at 7.74 were corresponding to the hydrogen at meta and para position in pyridine nucleus.
- The presence of broad band at  $\delta$  2.9 - 3.1 for NH-group and the presence of Multiplet signals at 3.1 - 3.7 were a good indication for piperidine ring.

The  $^{13}\text{C NMR}$  spectral data of compound (A) showed that:

- The presence of 2 carbons at  $\delta$  148 that corresponding to the 2 carbon which adjacent to the nitrogen atom in the unsaturated ring.
- The presence of 3 carbons ( $\text{C}_3$ ,  $\text{C}_4$  and  $\text{C}_5$ ) in the unsaturated ring from  $\delta$  123.5 - 140.36.
- The presence of five carbons from 25.53 - 59.74 in the saturated cyclic ring containing nitrogen.

The previous obtained data of compound (A) show that it could be identified as anabasin, which could be in agreement with (Awaad, 1995 and Dean, 1995).

### 3.6. Investigation of the quaternary bases

The dried quaternary bases as their hydrochloride were dissolved in ethanol 50% then subjected to (TLC) using n-butanol: acetic acid : water (4:1:5) (upper layer) as the solvent system, one major spot (compound E) appeared after the plate was sprayed by Dragendorff's reagent.

### PCL for compound (E)

The total quaternary bases extract was subjected to (PLC) activated plates using silica gel G 254 as a stationary phase and mobile phase n-butanol : acetic acid : water (4:1:5) (upper layer). After the system was developed the plates were air dried and the first 1 cm from each plate was sprayed by Dragendorff's reagent to determine the band limits. The major

band was scraped, the scraped silica was collected and eluted completely by methanol, and the obtained pure compound was spotted on pre-coated silica gel plates along with different authentic samples including choline hydrochloride. The obtained result showed close similarity of (compound E) to choline hydrochloride, Table (10).

**Table (10):**  $R_f$  values of choline hydrochloride (compound E) in different solvent systems.

System used	Proportion	$R_f$	Dragendorrf's test
Methanol	100%	0.02	+ve
Benzene : methanol	30 : 70	0.01	+ve
Methanol : water	60 : 40	0.01	+ve
Butanol : acetic acid : water	4:1:5 (upper layer)	0.12	+ve

Further confirmation was carried out to confirm the identification of compound (E) as choline hydrochloride.

#### Infra red spectral data of compound (E)

IR-spectral data of compound (E) show complete similarity to IR-spectral data of choline hydrochloride, (British Pharmacopoeia, 1980 and Awaad, 1995).

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دراسات فيتوكيميائية على نبات *Asphodelus fistulosus*

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ملخص

يتبع نبات الأسفوديلس فستبولوسيسالعائلة الزنبقية وهو نبات حولي، عشبي، برى يتواجد في صحراء شبه جزيرة سيناء (شمال وجنوب سيناء) ويزهر في الربيع بعد نزول الأمطار العزيرة. لذلك فقد تم دراسة النبات في منطقتين بشمال سيناء، الأولى في وادي أم الصورة (طريق العريش - العوجة، الكيلو ٦٢ جنوب غرب العريش) والثانية في أبو عجيلة (طريق العريش - أبو عجيلة، الكيلو ٤٩ جنوب العريش) لدراسة مكونات النبات الكيميائية. وقد تم من خلال هذه الدراسة عمل مسح كيميائي أولى للنبات اتضح منه أنه يحتوى على مركبات الأنتراكينونات والفلافونيدات والقلويدات والإستيرولات والتربينات. كذلك فقد تم استخلاص وفصل المواد الكربوهيدراتية حيث تم التعرف على ستة أنواع من السكريات وهى رافينوز، مالتوز، جالاكتوز، جلوكوز، سكروز، ورامينوز. وبالإضافة فقد أجرى استخلاص وفصل الأحماض الأمينية حيث تم فصل خمسة عشر حمض أميني إما فى الصورة الحرة أو الصورة المرتبطة ومن بينها سبعة أحماض أساسية واثنان شبه أساسيين. كما تم فصل تسعة أحماض دهنية بينها اثنان أساسيان وهما لينوليك و لينولينيك وتم التعرف على إحدى عشر مركب هيدروكربوني ونوع واحد من الإسترولات هو  $\beta$ -Sitosterol وذلك باستخدام الفصل الكروماتوجرافى الغازى.

تم فصل وتعريف اثنان من القلويدات كولين و أنابسين، وقد تم التعرف عليها من خلال دراسة  $^{13}\text{C-NMR}$  و  $^1\text{H-NMR}$ ، Mass spectroscopy.

المجلة العلمية لكلية الزراعة - جامعة القاهرة المجلد (٥٢) العدد الرابع (أكتوبر ٢٠٠١): ٦٠٧-٦٢٤.



