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PHYTOCHEMICAL STUDIES ON THE HERB *Gomphocarpus sinaicus* BOISS

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ABSTRACT

The preliminary phytochemical screening of *Gomphocarpus sinaicus* Boiss (collected from two localities; El-Qusaima and Saint Catherine)showed that the plants contained sterols, tannins, alkaloids, carbohydrates and/or glycosides, chlorides, sulphates and resins.The chromatographic investigation of the free sugars showed the presence of glucose and maltose in the two studied habitats, raffinose in the plants of El-Qusaima and ribose in Saint Catherine habitat.

The obtained GLC chromatograms of the combined sugars of *Gomphocarpus sinaicus* revealed the presence of fructose, glucose, galactose and maltose in the two studied habitats, rhamnose in the plants of El-Qusaima.

The free amino acids valine, proline and leucine are present in the plants of El-Qusaima habitats, while alanine, phenylalanine and proline are present in the plants of Saint Catherine. The data of protein-amino acids showed that *Gomphocarpus sinaicus* contained sixteen amino acids in El-Qusaima and fifteen amino acids in Saint Catherine habitat with different ranges of concentrations in the two studied habitats.

The fundamental chemical properties of lipids of *Gomphocarpus sinaicus* collected from the two habitats were determined. It was obvious from the obtained GLC results that the unsaponified matter of the lipids contained squalene, stigmasterol and β-sitosterol with different ranges of concentrations in the two studied

areas. GLC chromatograms of the saturated fatty acids revealed the presence of palmitic and arachidic acids beside the unsaturated fatty acids oleic and linolenic in the plants at El-Qusaima habitat. Data also revealed the presence of the saturated fatty acid arachidic beside oleic, linoleic and linolenic as unsaturated fatty acids in the plants of Saint Catherine habitat.

The results revealed marked qualitative and quantitative differences in the chemical constituents of the plants in the two studied habitats.

Key words: *amino acids, asclepiadaceae, carbohydrates, fatty acids, Gomphocarpus sinaicus,.*

1. INTRODUCTION

Gomphocarpus sinaicus Boiss (=*Asclepias sinaica* Muschl.), family *Asclepiadaceae* (milkweed family) is one of the most common shrubs distributed in the warm tropical regions (Ghazanfar, 1994). It grows in the Isthmic desert, Nile region and in Sinai proper. It is known in arabic as Ghalquit el-deeb or Hargel or Gheil (Täckholm, 1974).

Reviewing the current literature, all members of the genus *Gomphocarpus* were chiefly used as arrow poison depending on their digitalis-like action and cumulative effect in the body. All organs of the plant are known to be toxic for man and animals, due to the high content of cardiac glycosides (El-Askary, 1993).

Chopra *et al.*, (1956) mentioned that the root of *Asclepias curassavica* is used as a remedy for piles and gonorrhoea, while the juice of the leaves is used as anthelmintic.

The root of *Asclepias crispas* L. is used as diuretic and its tincture is used for colic. The alcoholic extracts of *Asclepias tuberosa, A. speciosa* and *A. curassavica* are toxic when injected in rabbits and rats. *A. curassavica* and *A. tuberosa* extracts inhibited *Mycobacterium tuberculosis* and the fluid extract of *A. incarnata* seeds raised the blood pressure with no effect on respiration (El-Askary, 1993).

The leaves and the latex of *Calotropis procera* of family

Asclepiadaceae were used for treating wounds, pain, scorpion stings and for strengthening muscles affected by paralysis. In Pakistan, the bark of *C. procera* was used as an expectorants, roots as purgative, dried juice (latex) as an anti-spasmodic and a nerve tonic (Ghazanfar, 1994).

Borovskikh (1966) isolated gomphotin from leaves of *Gomphocarpus fruticosus* and studied the effect of gomphotin on collateral coronary circulation in dogs and respiration rates in rabbits.

Mitsuhashi and Kurumi (1969) isolated cymarose, D-digitoxose, uzarigenin, cynanchogenin and ursolic acid from the whole plants of *G. fruticosus*.

Kretsu (1983) stated that the subterranean organs of the *G. fruticosus* contained quercitin, coumarins, cardiac glycosides, β-sitosterol and rutin, while the roots contained tannins, quercitin and the sugar moiety rhamnose.

El-Askary *et al.*, (1993) isolated five cardenolide glycosides with doubly linked sugars from powdered stems of *Gomphocarpus sinaicus* and in (1994) they isolated 14 β, 17α-epoxy-5, 6-dehydrocalotropin from *G. sinaicus*. In (1995) they stated that 5,6-dehydrocalotropin was found to be the main cardiac glycoside in all organs (except seeds).

Ghazanfar (1994) reported that the leaves and stems of *Calotropis procera* (family *Asclepiadaceae)* contain calotropin and calotropagenin, while the latex contains glycosides, uscharin, calotoxin and calactin.

Our study aimed to investigate the main chemical constituents, *i.e.*, carbohydrates including free and combined sugars, free and protein-amino acids and lipids which include the fundamental chemical properties, fatty acids and unsaponifiable matter of *Gomphocarpus sinaicus* Boiss, to clarify the effect of environmental conditions on its main biochemical constituents, as affected by the severe desert conditions and from which active constituents may be produced.

2. MATERIALS AND METHODS

Gomphocarpus sinaicus collected at 20th February 2000 from the two natural localities; El-Qusaima (North Sinai) and Saint Catherine (Wadi El-Arbiean at km 5- South Sinai).

The collected plants were cleaned from impurities, dried in an oven at 60 °C for 48 hours and ground to fine powder, then used in the following investigations:

2.1. Preliminary phytochemical screening

2.1.1. Steam distillation for volatile oils

About 50 g of fresh plant materials were subjected to steam distillation according to British Pharmacopoeia (1980) method for volatile oil contents.

2.1.2. Preparing the extract for further screening

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

Test for tannins according to Claus (1967), test for sterols and terpens (Libermann-Burchard's test) according to Balbaa *et al.*, (1981) and Salkowski reaction's according to Brieskorn and Klinger-Hand Polonius (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 reagents according to Harper (1975), test for saponins according to Wall *et al.*, (1954), test for chlorides and sulphates according to A.O.A.C.(1970), and test for resins according to Fahmy (1923).

2.2. Investigation of the free and combined sugars by Gas-Liquid Chromatography (GLC)

The free sugars were extracted from the defatted powder of *Gomphocarpus sinaicus* by pyridine (Malpress and Morrison, 1949) at 100 °C for 30 minutes under reflux. The pyridine extract was cooled, filtered and evaporated under vacuum at 45 °C. The residue

was dissolved in 100 ml of 10 % isopropyl alcohol and subjected to GLC technique (Eaton, 1989). The combined sugars were hydrolyzed with 6N HCl after extraction of the defatted air dried plant powder with re-distilled pyridine and subjected to GLC technique as follows:.

1. The sugars were converted to trimethylsilyl derivatives according to Chaplin and Kennedy (1994) by using the silylation method.
2. The silylation reagent used was prepared by mixing pyridine (10 Vol.) with hexamethyldisilazane (2 Vol.) and trimethyl chlorosilane (1 Vol.).
3. The following conditions were used for GLC analysis of the free and combined sugars; Apparatus: GCV Pye-Unicam, Column: GP 3% SP 2330 on 100/120 mesh supelcoport, Column temperature: 100-300 °C, programming rate: 10 °C/min., Initial temperature: 100 °C for 1 min, Final temperature: 300 °C for 7 min, Injector temperature: 280 °C, Detector temperature: 300 °C and Flow rate 40 ml/min.

   Attenuation: 4×10^3, chart speed: 2 cm/min.

2.3. Investigation of free and protein-amino acids

2.3.1. Identification of free amino acids: (Qualitatively)

Ten grams of defatted air-dried plant powder were refluxed with 70% ethyl alcohol. The resulting mixture was filtered and concentrated, then passed through a column of cation exchange resin. Elution was carried out using 70% ethyl alcohol to take all the carbohydrates, then with 2% HCl and 2% ammonia for elution of amino acids. Whatman No. 1 filter paper chromatography was used for the separation and identification of free amino acids of *Gomphocarpus sinaicus*. The solvent used was; n-butanol: acetic acid: water (4:1:5). The colour reagent used was ninhydrin (0.25%), prepared according to Block *et al.*, (1958).

2.3.2. Identification of protein-amino acids (Quantitively)

The hydrolyzed protein-amino acids were determined using amino acid analyzer technique according to the method described by Pellet and Young (1980), summarized as follows:

A known weight of defatted dry sample containing 50 mg protein was hydrolyzed in sealed evacuated pyrex test tube using 5 ml of 5.7 N hydrochloric acid at 110 °C for 24 hours. The hydrolyzate was filtered and the residue was washed with distilled water. The volume of the filtrate was adjusted to 50 ml using twice distilled water. Five ml of the filtrate were evaporated to dryness at room temperature. The obtained residue was dissolved in 5 ml loading buffer (0.2 N sodium citrate buffer pH 2) and the solution was filtered through 0.22 μm membrane. Twenty microliters of the final filtrate were loaded in the instrument capsule to investigate the protein-amino acids. LKB alpha plus high performance Amino Acid Analyzer LKB biochrom. LTD England was used for this purpose. Retention time and area were determined using Hewlett Packard 3390 recording integrator. The concentration of each amino acid GM/16GM, nitrogen was calculated by a special designed programme.

2.4. Investigation of lipids

The lipids were extracted from the plant powder with petroleum ether: ether (1:1 v/v) for 24 hours using Soxhlet apparatus. The lipids were obtained by distilling off the solvent to a constant weight (Güenther, 1972).

2.4.1. The fundamental chemical properties

Acid value (A.V.), ester value (E.V.) and saponification value (S.V.) were determined according to British Pharmacopoeia (1980), Iodine value (I.V.) was estimated according to A.O.A.C.(1970).

2.4.2. Investigation of fatty acids and unsaponifiable matter

The extracted lipids of *Gomphocarpus sinaicus* were saponified and purified according to Christie (1982) and subjected to GLC investigation.

2.4.2.1. GLC of fatty acids

The extracted fatty acids and the standard ones were converted to the corresponding methyl esters using ethereal solution of

diazomethane according to Farag *et al.*, (1986). The methyl esters of the fatty acids were analyzed with a Pye-Unicam gas chromatography apparatus, which was conducted with a coiled glass column (150 mm x 4 mm), packed with diatomite C (100-120 mesh) and coated with 10% poly ethylene glycol adipate (PEGA). The column oven temperature was programmed at 8 °C/min. for 70 °C to 190 °C, then isothermally at 200 °C for 20 minutes with nitrogen at 30 ml/min. Peak identification was performed by comparing the relative retention time of each compound with those of standard materials. The relative proportions of each individual compound were estimated as the ratio of the partial areas to the total area as mentioned by Fryer *et al.*, (1960) and Nelson *et al.*, (1969).

2.4.2.2. GLC of unsaponifiable matter

The combined ethereal extracts and washings were washed with water until free from alkalinity, dried over anhydrous sodium sulphate and filtered. The filtrate was evaporated to dryness under vacuum and the residue was subjected to GLC investigation. The gas-liquid chromatography apparatus, equipped with flame ionization detector, was used in the identification of unsaponifiable matter. The temperatures of injector, column and detector were 250, 300 and 300 °C, respectively. Gases flow rates were 30, 33, 330 ml/min for nitrogen, hydrogen and air, respectively. The chart speed was 2 ml/min and the attenuation was 32 x 10^{-2}. Authentic samples were also injected and the relative retention times (RRT) of unsaponified matter were estimated with the authentic sample. The results of Farag *et al.*, (1986) were used as a guide to characterize some of the unknown compounds. The relative percentage of each unsaponifiable compound was determined using triangulation method according to Nelson *et al.*, (1969).

3.RESULTS AND DISCUSSION

3.1. The preliminary phytochemical screening of *Gomphocarpus sinaicus*

The preliminary phytochemical screening of *G. sinaicus* collected from the two habitats showed that it contained sterols,

tannins, alkaloids, resins, chlorides, sulphates, reducing sugars, carbohydrates and/or glycosides but neither volatile oils, flavonoids nor saponins were present (Table 1).

Table (1): Preliminary phytochemical screening of *Gomphocarpus sinaicus.*

Test	Result	Test	Result
1. Volatile oils	-	7. Saponins	-
2. Tannins	+	8. Chlorides	+
3. Sterols	+	9. Sulphates	+
4. Flavonoids	-	10. Resins	+
5. Alkaloids	+	11. Reducing sugars.	+
6.Carbohydrates and/or glycosides	+		

+= positive - = negative

3.2. Investigation of carbohydrates

The obtained GLC chromatograms of free sugars of *Gomphocarpus sinaicus* at El-Qusaima revealed the presence of glucose, maltose and raffinose (Table 2). On the other hand, GLC chromatograms of *G. sinaicus* at Saint Catherine habitat show that they contained ribose, glucose and maltose, beside some unknown sugars as free sugars (Table 2).

Table (2): Retention times and relative percentages of free sugars of *G. sinaicus* **at El-Qusaima and Saint Catherine using GLC.**

R.T.	Sugar	Relative percentages (%)	
		El-Qusaima	Saint Catherine
2.75	Ribose	-	15.07
3.15	Unknown	8.71	10.98
3.65	Glucose	20.32	35.12
4.70	Unknown	-	13.75
7.15	Maltose	29.50	17.65
10.35	Raffinose	23.79	-
10.40	Unknown	-	7.42
11.15	Unknown	11.37	-
11.30	Unknown	6.30	-

GLC chromatograms of combined sugars of *G. sinaicus* at El-Qusaima habitat revealed the presence of fructose, glucose, galactose and maltose,beside some unknown sugars as combined sugars Meanwhile, at Saint Catherine habitat rhamnose, fructose, glucose, galactose and maltose were detected, beside some unknown sugars as combined sugars in the plant (Table 3).

3.3. Investigation of free and protein-amino acids

3.3.1. Free amino acids

The separated free amino acids of *G. sinaicus* at the two studied areas (as shared amino acids) are given in (Table 4). It was obvious that the plants at El-Qusaima habitat contained valine, proline and leucine. The data also revealed the presence of alanine, proline and phenylalanine as free amino acids at Saint Catherine.

Table (3): Retention times and relative percentages of sugars arising from combined fractions of *G. sinaicus* at El-Qusaima and Saint Catherine, using GLC.

R.T.	Sugar	Relative percentages (%)	
		El-Qusaima	Saint Catherine
2.35	Rhamnose	-	10.75
2.60	Unknown	9.80	-
3.40	Fructose	12.36	18.65
3.50	Unknown	9.52	-
3.65	Glucose	20.95	29.30
3.70	Galactose	30.78	27.15
7.15	Maltose	16.52	10.31
8.30	Unknown	-	3.82

3.3.2. Protein-amino acids

Protein-amino acids of *G. sinaicus in* the two studied areas, were achieved using amino acid analyzer (after protein hydrolysis) and the obtained results are presented in Table (5). Data showed that *G. sinaicus* contained sixteen amino acids with different ranges of concentrations in the two studied habitats. On the other hand, at El-Qusaima habitat, the total determined amino acids of the plant was (3.94 mg/g protein), while it was(3.63mg/g protein) at Saint Catherine

habitat. The clear variation in amino acid contents between habitats may be due to the severe environmental conditions of the desert.

It was obvious from Table (5) that the total amino acids were slightly higher in *G. sinaicus* at El-Qusaima than that of Saint Catherine habitat. High ratios of soluble and insoluble proteins may be due to increased proteolytic activity for osmoregulation (Cusido *et al.*, 1987).

Table (4): The presence of free amino acids in *Gomphocarpus sinaicus* at the two studied areas during winter.

Free amino acid	Locality	
	El- Qusaima	Saint Catherine
Alanine	-	+
Valine	+	-
Phenylalanine	-	+
Proline	+	+
Leucine	+	-

+ = positive - = negative

Table (5): Protein-amino acids of *Gomphocarpus sinaicus* (dry matter) at the two studied areas as (mg/g protein) during winter.

Amino acids	El-Qusaima (mg/g protein)	Saint Catherine (mg/g protein)
Essential amino acids:		
Threonine	0.18	0.17
Valine	0.27	0.24
Methionine	-	-
Isoleucine	0.17	0.18
Leucine	0.31	0.27
Phenylalanine	0.22	0.20
Histidine	0.09	0.10
Lysine	0.20	0.24
Arginine	0.21	0.22
Non-essential amino acids:		
Aspartic	0.33	0.29
Serine	0.20	0.20
Glutamic acid	0.63	0.56
Proline	0.45	0.43
Glysine	0.25	0.23
Alanine	0.26	0.23
Cysteine	0.07	-
Tyrosine	0.10	0.07
Total determined amino acids	3.94	3.63

3.4. Content of the lipid fractions

3.4.1. Total lipid contents

The total lipid contents of *Gomphocarpus sinaicus* reached a maximum value of 6.11% in winter and a minimum value of 3.05% during summer at El-Qusaima (Table 6), while at Saint Catherine habitat the maximum value of the total lipid contents was 5.96% in winter and a minimum value of 2.11% during summer.

3.4.2. Physical properties of lipids:

The lipids obtained were yellowish green in colour at El-Qusaima, while they were dark green in colour at Saint Catherine, semi-solid having a faint odour. They were soluble in ether, petroleum ether, chloroform, acetone, benzene, warm methyl and ethyl alcohol.

Table (6): Mean values of total lipid contents of *Gomphocarpus sinaicus* in the two studied habitats during the growth season 2000.

Seasons	Total lipids (%)	
	El- Qusaima	Saint Catherine
Winter	6.11	5.96
Spring	4.53	3.17
Summer	3.05	2.11
Autumn	3.90	2.53

3.4.3. Fundamental chemical properties:

The fundamental chemical properties of the extracted lipids of *G. sinaicus* collected from the two habitats are presented in Table (7). It is clear from the data obtained that, acid value (A.V.) was higher at El-Qusaima than the other locality. While iodine value (I.V.), ester value (E.V.) and saponification value (S.V.) were higher at Saint Catherine area than the other locality.

Table (7): Acid, iodine, ester and saponification values of lipids of *Gomphocarpus sinaicus* in the two habitats.

Item (mg%)	El-Qusaima	Saint Catherine
Acid value (A.V.)	19.65	8.91
Iodine value (I.V.)	23.11	28.75
Ester value (E.V.)	98.23	161.40
Saponification value (S.V.)	117.88	170.31

3.4.4. Unsaponifiable matter contents

The unsaponifiable matter composition of *G. sinaicus* was determined using GLC technique. The relative percentages of each component were calculated and recorded in Table (8). From the results, the plants of *G. sinaicus* at El-Qusaima and Saint Catherine contained, 12 hydrocarbons, squalene, stigmasterol and β-sitosterol.

3.4.5. Fatty acid contents

The saponifiable contents of *G. sinaicus* were determined using GLC technique. The relative percentage of each component was calculated and presented in Table (9). Data reveal the presence of saturated fatty acids palmitic, arachidic acids beside the unsaturated fatty acids, oleic and linolenic acids in *G. sinaicus* at El-Qusaima habitat. Data presented in Table (9) reveal the presence of arachidic acid as saturated fatty acid beside the unsaturated fatty acids oleic, linoleic and iinolenic acids in plants growing in Saint Catherine habitat.

Table (8): GLC of hydrocarbons and sterols of *Gomphocarpus sinaicus* at the two studied areas during winter.

Number of Carbon atom	Name	Relative percentage %	
		El-Qusaima	Saint Catherine
Hydrocarbons			
19	n-Nonacosane	17.31	11.38
20	n-Eicosane	1.91	6.52
22	n-Docosane	1.78	8.96
23	n-Tricosane	1.98	11.42
24	n-Tetracosane	6.91	11.71
-	Unknown	9.36	5.71
26	n-Hexacosane	1.87	4.41
-	Unknown	1.77	6.19
28	n-Octacosane	4.91	4.56
29	Squalene	9.36	3.42
-	Unknown	6.34	1.97
30	n-Triacontane	1.91	0.88
-	Unknown	1.64	4.33
* Sterols:			
27	Stigmasterol	18.99	12.11
27	β-sitosterol	13.68	6.43

Goss (1973) stated that the most abundant fatty acids of desert plants were palmitic, stearic and linoleic acids.

It is obvious from the present data that, palmitic acid represented the higher percentage of fatty acids (30.56%) at El-Qusaima, followed by oleic acid (22.02%). Meanwhile, at Saint Catherine habitat, the higher percentage of fatty acid was linoleic acid (29.23%) and the lowest percentage of known fatty acids was arichidic acid with a percentage of (14.19%).

El-Askary (1993) mentioned that the sap of *Asclepias (Gomphocarpus) cornuti* contains α-amyrin, β-amyrin, while the oil of the seeds of *A. syriaca* contains mainly linoleic and oleic acids.

Table(9): GLC of fatty acids of *Gomphocarpus sinaicus* at the two studied areas during winter.

Number of Carbon atom	Name	Relative percentage %	
		El-Qusaima	Saint Catherine
Saturated fatty acids			
16	Palmitic acid	30.56	-
20	Arachidic acid	16.42	14.19
-	Unknown	6.44	9.05
-	Unknown	6.17	11.63
Unsaturated fatty acids:			
18:1	Oleic acid	22.02	19.29
18:2	Linoleic acid	-	29.23
18:3	Linolenic acid	18.22	15.73

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دراسات كيمو نباتية على غلقة الديب
Gomphocarpus sinaicus

ايناس عبد المعطى محمد طلبة - فاطمة على أحمد

مركز بحوث الصحراء – المطرية – القاهرة

ملخص

تضم العائلة الصقلابية (الاسكليبادية) كثيراً من النباتات ذات الأهمية الاقتصادية والطبية ولذلك فقد تم إختيار نبات غلقة الديب (جومفوكاربس سينايكس)، أحد أنواع هذه العائلة لدراسة مكوناته الكيميائية من سكريات وبروتينات ودهنيات وتحليلها لموادها الأولية واستخلاصها والتعرف عليها وصفيا وتقديرها كميا.

وقد اشتملت الدراسة على مسح كيميائي أولى للنبات اتضح منه أن نبات غلقة الديب يحتوى على استيرولات وتانينات وقلويدات وسكريات مختزلة وسكريات و / أو جليكوسيدات بالاضافة إلى كبريتات وكلوريدات .

وقد تم من خلال الدراسة التى أجريت على نباتات جمعت من منطقتين مختلفتين (القصيمة وسانت كاترين) مايلى :

١ – التعرف على السكريات الحرة والمرتبطة فى النبات فى المنطقتين وصفياً وكمياً بإستعمال طرق التحليل الكروموتوجرافى الغازى .

٢– التعرف على الأحماض الأمينية الحرة التى يحتويها النبات فى كل من منطقتى الدراسة بواسطة الفصل الكروماتوجرافى الورقى والأحماض الأمينية الداخلة فى تركيب البروتين وتقدير نسبتها المئوية فى المنطقتين باستخدام جهاز تحليل الأحماض الأمينية .

٣ – دراسة الخواص الطبيعية والكيميائية للدهون الموجودة فى النبات فى كل من المنطقتين وتقدير الاستيرولات والمركبات الهيدروكربونية والأحماض الدهنية المشبعة وغير المشبعة ونسبها بإستعمال طرق التحليل الكروماتوجرافى الغازي ، وقد أوضحت النتائج أنها تختلف من حيث النوع والنسبة من منطقة إلى الأخرى.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٥٢) العدد الثالث (يوليو ٢٠٠١): ٤٢٧-٤٤٤.