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PHYTOCHEMICAL STUDIES ON METABOLIC CONTENTS OF *Euphorbia paralias* L.

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ABSTRACT

The preliminary phytochemical screening of *Euphorbia* showed that the plant contained sterols, tannins, resins, flavonoid, sulphates, reducing sugars, saponin, carbohydrate and/or glycosides, chlorides and alkaloid. The paper chromatography investigation of free sugars showed that *E. paralias* contained arabinose, xylose, maltose, glucose and sucrose in the leaves and stems at winter and summer,beside rafinose in the leaves at both seasons, fructose in the leaves and stems at winter and cellobisoe in the stems at both seasons.While it present in the leaves at summer only.

Meanwhile, the chromatographic investigatioin of combined sugars revealed the presence of arabinose, rafinose, xylose, maltose, glucose in the leaves and stems at winter and summer, beside fructose in the leaves and stems at winter and cellobiose in the stems at both seasons, while it present in the leaves at summer only.

The obtained Amino Acid Analyzer showed the presence of free amino acids; aspartic, therionine, serine, glutamic, proline, glycine, alanine, valine, isolucine, lucine, tyrosine, phenylalanine, histidine, ammonia and arginine in the leaves and stems at both seasons , beside lysine in the leaves and stems at summer. The data of protein amino acids hydrolysate revealed the presence of fifteen amino acid with different of concentrations at both winter and summer.

The total lipids and the fundamental chemical properties of lipids of *E. paralias* were determined. It was obvious from the obtained G.L.C. results that the unsaponified matter of leaves and stems at winter and summer contained 10 hydrocarbons, beside cholesterol, β-sitosterol and stigmasterol. G.L.C. chromatograms of the saponified matter revealed the presence of the saturated fatty acids caproic, caprylic, capric, lauric, myristic, palmitic, stearic and arachidic acid beside the unsaturated fatty acids, palmitoleic, oliec, linoleic, linolenic and eicosenoic acid in the leaves and stems at winter and summer, palmitoleic acid in the stems atsummer. While arachidic acid was defected in the leaves at summer and in stems at winter ,beside erucic acid in the leaves at winter stems at summer as saturated. The results revealed marked qualitative and quantitative difference in the chemical constituents of the leaves and stems of *E. paralias* at winter and summer seasons.

Keywords: Euphorbiaceae-Euphorbia- Phytochemical studies

INTRODUCTION

Euphorbiaceae is a large family, which of about 7950 species with 312 genera, cosmopolitan in distribution. Species belonging to this family are trees, shrubs, lianes, herbs and succulents, monoecious or dioecious. Most species contain a milky or coloured latex. (Ghazanfar, 1994).

Watt and Breyer (1962), stated that *Euphorbia* comprises several genera which are reputed by folklore medicinal uses as an astringent for blood vessels, antimicrobial remedies, antimalaria, insecticide, purgative etc.

Täckholm (1974), reported that the family Euphorbiaceae comprises about 7 genera and 52 species. *Euphorbia* is the largest genus of this family,

it includes about 39 species, it is milky poisonous plants of much variable shape.

Mann (1987), showed that biological compounds were identified as product of primary metabolism, i.e. carbohydrates, amino acids and lipids, or as products of secondary metabolism, i.e. phenolics, terpenoides, steroids, and alkaloids. Primary metabolites are essential uniquitous and certainly essential for life, whilst the secondary metabolites are of restricted occurrence and apparent utility. This divisionis useful but somewhat arbitrary. testosterone, for example, is of limited occurrence, but has vital hormonal activity, and nicotine from tobacco plant and few other species has a definite role as insect feeding deterrent.

Lwu (1993), stated that the latex of *Euphorbia hirta* contains 1-inositol, pyrogallic, and catechuic, tannins, and an aikaloid xanthorhamnine. Taxerol, friedelin, β-sitosterol, myricyl alcohol, ellagic acid, and hentriacontane have been isolated from the stem extracts. A number of amino acids and ellagic gallic, chlorogenic and caffeic acids, have been reported as occurring with the flavonoids kaempferol, quercitol and quercitrin in the plant, and they also isolated iso-inositol, glucose, and sucrose.

In spite of the fact that, botanical and phytochemical studies on certain species of *Euphorbia* have been carried out, nothing could be traced concerning primary constituents, i.e. carbohydrates, amino acids and lipids, of *Euphorbia paralias*. So we decided to investigate its main primary compounds, i.e., carbohydrates, proteins and lipids to clarify the effect of area circumastance on the main biochemical constituents.

MATERIALS AND METHODS

Euphorbia paralias was collected during winter and summer(2002) from Abu Lahw El-Bahary at area Matruh .

The collected plants (leaves and stems) were cleaned from impurities, dried in an oven at 60°C for 48 hours and ground to fine powder, then used in the following investigation.

Preliminary phytochemical screening:

1. Steam distillation.

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

2. Preparing the extract for further screening:

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

Tests for tannins according to Balbaa (1986), sterols and terpens according to Balbaa *et al.*, (1981) and Salkowski reaction's according to Brieskorn and Polonius (1961), flavonoids according to Wall *et al.*, (1954), carbohydrates and/or glycosides using Molish's and Fehling reagents according to Harper, (1975), chlorides and sulphates according to (A.O.A.C.

1970), alkaloids according to Woo *et al.*, (1977), saponins according to Wall *et al.*, (1954) and resins according to Balbaa (1986).

Investigation of carbohydrates:

1. The percentage of total, soluble and insoluble carbohydrates were determined according to Chaplin and Kennedy (1994).
2. The chromatographic separation of free and combined sugars were determined according to Chaplin and Kennedy (1994).

Investigation of amino acid:

1. The percentage of total nitrogen content were determined according to James (1995).
2. The separation and identificatiion of free and protein-amino acids were determined using Amino Acid Analyzer according to Pellet and Young (1980).

Investigation of lipids:

1. The percentage of total lipids were determined according to (A.O.A.C., 1970).
2. 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 Mohamed and Amer (1965).
3. Investigation fatty acids and unsaponifiable matter.
   The extracted lipids of *E. paralias* were saponified and purified according to Gûenther (1972) and subjected to G.L.C. investigation.

3.1. G.L.C. of fatty acids :

The fatty acids components were determined according to Nelson *et al.* (1969) and Farag *et al.*, (1986).

3.2. G.L.C. of unsaponifiable matter:

The unsaponifiable matter were determined according to Eaton (1989) and Nelson *et al.* (1969).

RESULTS AND DISCUSSION

Preliminary phytochemical screening:

The preliminary phytochemical screening of *Euphorbia paralias* collected from Abu Lahw El-Bahary Matruh at winter and summer seasons revealed the presence of sterols, tannins, resins, flavonoids, sulphates, reducing sugars, saponin,glycosides and/or carbohydrate, chlorides, and alkaloids (Table 1).

Table (1): Preliminary phytochemical screening of *Euphorbia paralias*

Test	Results	Test	Results
Sterols	+	Saponins	+
Tannins	+	Alkaloids	+
Resins	+	glycosides and/or Carbohydrate	+
Flavonoids	+	Chlorides	+
Sulphates	+		
Reducing sugars	+		

Investigation of carbohydrates:

1. Contents of total, soluble and insoluble carbohydrates

Table (2) indicated that the percentages of total, soluble and insoluble carbohydrates reached their maximum values (3.70& 2.59 %), (1.26 & 0.45 %) and (2.44 & 2.14 %) at winter and its minimum values of (1.86 & 1.57%), (0.35& 0.34 %) and (1.51 &1.23 %) at summer in leaves and stems, respectively, Table (2).

Table (2): Mean values of total, soluble and insoluble carbohydrates of *Euphorbia paralias* at winter and summer.

Items %	Winter		Summer	
	Leaves	Stem	Leaves	Stem
Carbohydrates	3.70	2.59	1.86	1.57
Soluble carbohydrates	1.26	0.45	0.35	0.34
Insoluble carbohydrates	2.44	2.14	1.51	1.23

2. Free sugars :

The paper chromatograms of free sugars of the leaves of *E. paralias* at winter revealed the presence of arabinose, rafinose, xylose, maltose, fructose, glucose and sucrose, while stems contained also cellobiose except the presence of rafinose. On the other hand, paper chromatograms of the leaves of *E. paralias* revealed the presence of arabinose rafinose, xylose, maltose, glucose, sucrose,and cellobiose, while it revealed the abscent of rafinose in its stems in summer (Table 3).

Table (3): The separation of free and combined sugar of *Euphorbia paralais* by using paper chromatographic at Abu Lahw El-Bahary in winter and summer.

Sugars	Free sugars				Combined sugars			
	Winter		Summer		Winter		Summer	
	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
Arabinose	+	+	+	+	+	+	+	+
Rafinose	+	-	+	-	+	+	+	+
xylose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Fructose	+	+	-	-	+	+	-	-
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	-	-	-	-
Cellobiose	-	+	+	+	-	+	+	+

3. Combined sugars:

The paper chromatograms of combined sugars of the leaves of *E. paralias* at winter revealed the presence of arabinose, rafinose, xylose, maltose, fructose and glucose, while ,stems contained also cellobiose. Meanwhile at summer arabinose, rafinose, xylose, maltose, glucose and cellobiose were detected as combined sugars in the stem and leaves of *E. paralias* (Table 3).

The variable changes in the accumulation of carbohydrate fractions in the different organs of the plant may be induced by various levels of drought and salinity as mentioned by Shaddad and Zidan (1989) and Ali (1991). These results are in agreement with the conclusion that the adverse effects of salt stress on plant metabolism include increases in soluble sugars (Downton, 1977 and Hawker, 1980).

Investigation of nitrogenous compounds:

1. Investigation of total nitrogen content:

Data presented in (Table 4) indicated that the percentage of total nitrogen of *E. paralias* reached its maximum values of (4.60& 3.37%) in leaves & stems , respectively at winter and its minimum values of (3.30 & 3.00%) leaves & stems , respectively at summer. Data also indicated that the total nitrogen content was slightly higher at leaves than that of stems. The higher amount of total nitrogen content may due to the higher metabolic rates of *E. paralias* as a result of high water resources of the soil during winter months than that of summer, which accounts to Stocker's assumption (1960).

The increase in the soil moisture stress may be remarkably decreased the assimilatioin and accumulation of nitrogenous compounds in *E. paralias* a behaviour similar to the response of carbohydrates to soil drought condition. These results are in agreement with those obtained by Abd El-Rahman *et al.*, (1971) and El-Monayeri *et al.*, (1981).

Table (4): Mean values of total nitrogen of *Euphorbia paralias* at Abu Lahw El-Bahary in winter and summer.

Seasons	Total nitrogen %	
	Leaves	Stems
Winter	4.60	3.37
Summer	3.30	3.00

2. Free amino acids :

The separated free amino acids of both leaves and stems parts of *E. paralias* at Abu Lahw El-Bahary at winter and summer are given in (Table 5). The obtained results of Amino Acid Analyzer showed the presence of aspartic, therionine, serine, glutamic, proline, glycine, alanine, valine, isolucine, lucine, tyrosine, phenylalanine, histidine, ammonia and arginine as free amino acids ; in the leaves and stems at both seasons with different ranges of concentration beside lysine in the leaves and stems at summer. It was clear that the maximum value of free amino acid of serine (1.05& 0.85& 0.77 mg/100 ml) at winter in leaves and stems and in leaves at summer, while tyrosine (1.47 mg/100 ml) at summer in stems. While lucine 0.04 and glycine 0.08 mg/100 ml at winter, beside lysine (0.08 & 0.11 mg/100ml) at summer in leaves and stems, respectively were the lowest value.

Table (5): Free amino acids of *Euphorbia parallas* at Abu Lahw El-Bahary at winter and summer seasons.

Amina acids %	Winter		Summer	
	Leaves	Stems	Leaves	Stems
Aspartic	0.07	0.30	0.55	1.26
Therionine	0.50	0.22	0.19	0.53
Serine	1.05	0.85	0.77	1.25
Glutamic	0.35	0.37	0.20	0.71
Proline	0.35	0.28	0.49	0.35
Glycine	0.10	0.08	0.09	0.13
Alanine	0.36	0.29	0.33	0.59
Valine	0.55	0.44	0.40	0.78
Isolucine	0.27	0.18	0.18	0.60
Lucine	0.04	0.45	0.12	0.35
Tyrosine	0.48	0.36	0.35	1.47
Phenylalanine	0.49	0.29	0.19	0.75
Histidine	0.36	0.23	0.24	0.61
Lysine	-	-	0.08	0.11
Ammonia	1.21	1.12	1.01	1.08
Arginine	0.25	0.22	0.21	0.62

The physiological significance of proline accumulation may be due to its role in osmoregulation (Barnet and Naylor 1966) provision of both carbon and nitrogen for post stress relief (Thompson *et al.*, 1966), or removal of ammonia (Henkel, 1964 and Abdel Basset, 1992).

Ali and Sawaf (1992) reported that salinity could inhibit the transmination reactions and hence the glutamic acid is accumulated and transformed to other nitrogenous compounds such as proline. They also stated that the content of total free amino acids in the main organs of *Datura innoxia* plant, other than proline increased significantly with the rise of salinization level. In (1991) Ali found that the contents of proline in both shoots and roots of *Trigonella foenum graceum* L. increased progressively with the rise of salinization level.

3. Protein-amino acids:

The investigation of protein-amino acids of *E. paralias* at winter and summer were achieved using Amino Acid Analyzer (after protein hydrolysis) and the obtained results presented in Table (6), showed that *E. paralias* leaves and stems contained fifteen amino acid with different ranges of concentration at both winter and summer. High ration of soluble /insoluble proteins may be due to increased proteolytic activity for osmoregulation. The adverse effects of salt stress on plant metabolism include increased stimulation of reduction in the rates of synthesis of protein (Cusido *et al.*, 1987) and accumulation of amino acid as proline (Ali *et al.*, 1992).

Table (6): Protein-amino acid of *Euphorbia paraliasat* at winter and summer seasons.

Amino acids %	Winter		Summer	
	Leaves	Stems	Leaves	Stems
Aspartic	0.80	0.43	0.99	0.71
Therionine	0.40	0.17	0.38	0.24
Serine	0.42	0.185	0.37	0.26
Glutamic	1.00	0.56	1.00	0.81
Proline	0.83	0.38	0.47	0.42
Glycine	0.43	0.21	0.43	0.29
Alanine	0.34	0.19	0.33	0.24
Valine	0.52	0.24	0.49	0.34
Isolucine	0.45	0.18	0.42	0.27
Lucine	0.62	0.26	0.60	0.35
Tyrosine	0.25	0.84	0.22	0.11
Phenylalanine	0.49	0.18	0.47	0.27
Histidine	0.25	0.14	0.29	0.17
Lysine	0.49	0.30	0.42	0.29
Ammonia	0.94	0.51	0.83	0.73
Arginine	0.39	0.15	0.36	0.20

Investigation of lipids:

1. Total lipid contents:

Total lipid contents of *E. paralias* of reached maximum value of 7.15% and 6.30% in winter on the contrary minimum value of 5.73% and 4.74% wewe obnserved in summer for leaves and stems respectively,(Table 7).

Table (7): Mean values of total lipid content of *Euphorbia paralias* winter and summer seasons.

Seasons	Total lipids %	
	Leaves	Stems
Winter	7.15	6.30
Summer	5.73	4.74

2. The fundamental chemical properties:

The fundamental chemical properties of the extracted lipids of *E. paralias* are presented in (Table 8). It is clear from Table (8) that the percentages of acid value, ester value, iodine value and saponification values were higher in the leaves than those of stems and slightly during winter higher than those obtained during summer.

Table (8): Acid, ester, iodine and saponification values of lipid of *Euphorbia paralias* at winter and summer.

Item	Winter		Summer	
	Leaves	Stems	Leaves	Stems
Acid value	21.35	25.62	20.78	22.97
Ester value	139.21	132.02	103.52	132.86
Iodine value	43.94	45.73	42.04	43.67
saponification value	160.56	157.64	124.30	155.83

1. Saponifiable fraction of lipids:

The fatty acids composition of *E. paralias* were determined using G.L.C. technique. The relative percentage of each component was calculated and Tabulated in (Table 9), which revealed the presence of the saturated fatty acids; caproic, caprylic, capric, lauric, myristic, palmitic, stearic and arachidic acid ,beside the unsaturated fatty acids, palmitoleic, oleic, linoleic, linolenic and eicosenoic acid in the leaves and stems at winter and summer beside, palmitoleic acid in the stem at summer. While arachidic acid was detected in the leaves at summer and stems at winter and erucic acid in the leaves at winter and in stems at summer as saturated.

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

Table (9): G.L.C. of fatty acid of *Euphorbia paralias* at winter and summer seasons.

Fatty acid	Number of carbon atoms	Winter		Summer	
		Leaves	Stems	Leaves	Stems
Caproic acid	C6	0.2	0.1	0.3	0.2
Caprylic acid	C8	1.4	0.2	0.7	0.6
Capric acid	C10	4.1	13.4	4.2	6.8
Lauric acid	C12	2.1	6.2	1.3	3.2
Myristic acid	C14	7.4	11.8	2.8	2.7
Palmitic acid	C16	28.0	22.8	26.8	11.3
Palmitoleic acid	$C16^{/}$	-	-	-	6.8
Stearic acid	C18	0.4	0.1	3.3	0.8
Oleic acid	$C18^{/}$	0.8	0.1	3.7	0.2
Linoleic acid	$C18^{//}$	46.7	0.1	48.4	1.0
Linolenic acid	$C18^{///}$	6.0	40.7	4.5	60.3
Arachidic acid	C20	-	0.1	0.1	-
Eicosenoic acid	$C20^{/}$	2.1	3.6	1.4	5.6
Erucic acid	C22	0.6	-	-	0.2

2. Unsaponifiable matter contents:

The unsaponifiable matter composition of *E. paralias* was determined using G.L.C. technique.

The relative percentages of each component were calculated and recorded in Table (10).

The results at Table (10) syhowed that the leaves and stems of *E. paralias* contained 10 hydrocarbon, cholesterol, β-sitosterol and stigmasterol at winter and summer.

Martin (1985) declared that cholesterol, which occurred widely in animal tissues were found also in some higher plants and algae. Also the wide distribution of cholesterol in plants has recently been shown by (Trease and Evan, 1999).

Table (10): G.L.C. of hydrocarbons and sterols of *Euphorbia paralias* at winter and summer seasons.

Unsapon. fractions	Number of carbon atoms	Relatrive percentages			
		Winter		Summer	
		Leaves	Stems	Leaves	Stems
Hydrocarbons					
Tetradecane	C14	0.2	0.4	0.4	0.2
Hexadecan	C16	0.6	1.7	1.1	0.1
Octadecan	C18	9.6	6.6	2.3	1.4
Eicosane	C20	2.6	9.2	2.0	1.4
Docosane	C22	3.6	2.9	2.0	2.2
Tetracosane	C24	1.7	2.5	1.5	3.0
Hexacosane	C26	11.9	8.0	6.0	6.6
Octacosane	C28	5.4	4.2	5.2	3.1
Triaontane	C30	3.5	6.6	4.5	3.8
Dotriacontane	C32	1.7	7.3	19.3	5.5
Sterols:					
Chlosterol		2.5	4.6	9.3	6.2
β-sitosterol		2.5	7.1	6.2	9.1
Stigmasterol		55.4	37.8	28.7	56.8

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دراسات فيتوكيميائية على المحتوى الأيضى لنبات الايفوربيا براليز"

ايناس عبد المعطى محمد طلبه – حنونة سامى يعقوب

مركز بحوث الصحراء – المطرية – القاهرة

تضم عائلة الايفوربيا كثيراً من النباتات ذات الأهمية الاقتصادية والطبية ولذلك فقد تم اختيار نبات الايفوربيا براليز أحد أنواع فصلية الايفوربيا لدراسة مكونات النبات الكيميائية من سكريات وبروتينات ودهنيات وتحليلها لموادها الأولية واستخلاصها والتعرف عليها وصفياً وتقديرها كمياً.

وقد اشتمل البحث على مسح كيميائى أولى للنبات اتضح منه أن نبات الايفوربيا براليز يحتوى على استيرولات وفلافونيدات وتانينات وصابونينات وسكريات مختزلة وسكريات و/أو جليكوسيدات بالاضافة إلى كبريتات وكلوريدات .

وقد تم من خلال الدراسة التى أجريت على النبات فى بيئة الساحل الشمالى الغربى منطقة أبو لهو البحرى بمرسى مطروح فى فصلى الشتاء والصيف مايلى :

١- التعرف على السكريات الحرة والمرتبطة فى النبات فى كلا من الأجزاء الخضرية والسيقان فى فصلى الشتاء والصيف بواسطة الفصل الكروماتوجرافى الورقى.

٢ - التعرف على الأحماض الأمينية الحرة التى يحتويها النبات والأحماض الأمينية الداخلة فى تركيب البروتين وتقدير النسب المئوية لكلاهما فى فصلى الشتاء والصيف باستخدام جهاز تحليل الأحماض الأمينية.

٣ - دراسة الخواص الطبيعية والكيميائية للدهون الموجودة فى كلا من الأجزاء الخضرية والسيقان للنبات وتقدير الاستيرولات والمركبات الهيدروكربونية والأحماض الدهنية المشبعة وغير المشبعة ونسبها فى فصلى الشتاء والصيف باستعمال طرق تحليل الكروماتوجرافى الغازى وقد أوضحت النتائج أنها تختلف من حيث النوع والنسبة فى فصلى الشتاء والصيف.