Bull. Fac. Agric., Cairo Univ., 51 (2000): 175- 190.

PHYTOCHEMICAL STUDIES ON *Noaea mucronata* FORSSK.

(Received: 24.6.1999)

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ABSTRACT

The preliminary phytochemical screening on *Noaea mucronata* showed that it contains: sterols, flavonoids, tannins, chlorides, sulphates, carbohydrates and/or glycosides, terpens, saponins, resin, traces of volatile oil and alkaloids. *N. mucronata* contained glucose and galactose as free sugars, while it contained rhamnose, arabinose, galactose, glucose and xylose as combined sugar in the two localities.

Investigation of free amino acids of *N. mucronata* revealed that proline was absent in El-Arish, while it was present at a relative concentration of 0.2% in Matrouh. The highest concentration of free amino acids was aspartic acid 0.52 and 0.42% in El-Arish and Matrouh samples, respectively, while histidine was the lowest one, 0.11 and 0.07% in El-Arish and Matrouh samples, respectively. Concerning the protein-amino acids, *N. mucronata* contained sixteen amino acids with different concentrations. Lysine was the highest one of the bound amino acids 36.36 and 28.5% in El-Arish and Matrouh samples, respectively. Meanwhile, proline was completely absent at El-Arish sample and present with relative concentration (0.36%) in Matrouh samples.

The fundamental chemical properties of the extracted lipid of *N. mucronata* collected from the two habitats were investigated, where it was observed that the acid values in El-Arish and Matrouh, have no any significant differences, while there were decreases in iodine and saponification values in Matrouh than that found in El-

Arish, resulting from the increasing value of the percentages of the long-chain saturated arachidic acid in Matrouh than that of El-Arish.

The unsaponifiables content of *N. mucronata* was determined and the relative percentages of each component were calculated. There was no significant differences between the two habitats except the presence of decan at El-Arish only. The Gas-Liquid chromatographical analysis of *N. mucronata* lipids revealed the presence of long chain fatty acids, C16, C18 and C20 especially in Matrouh, where arachidic acid was the major fatty acid.

***Key words**: amino acids, fatty acid, Noaea mucronata.*

1. INTRODUCTION

Family *Chenopodiaceae* contains a wide variety of chemical constituents such as volatile oils, organic acids, saponins, pigments, coumarins, sterols, alkaloids, flavonoids, carbohydrates and amino acids (Rizk, 1986 and Ghazanfar, 1994). The pharmaceutical importance of *Chenopodiaceae* plants lies in their production of active organic compounds that possess pharmacological properties. The study of lipids of the four plants *Salsola tetrandra, S. kali, S. longifolia* and *S. rigida* showed that they contain oleic, linoleic, arachidic, palmitic and stearic acids besides eicosanol and β-sitosterol (Karawya *et al*., 1972).

Noaea mucronata (Forssk.), family *Chenopodiaceae* has a wide distribution in Egyptian deserts (Täckholm, 1974). There was little previous chemical investigation on *Noaea mucronata*, which encouraged to investigate its main biochemical constituents, especially carbohydrates, lipid, proteins.

2. MATERIALS AND METHODS

The fresh plant materials of *Noaea mucronata* were collected seasonally from two habitats: El-Arish (North Sinai), at 7PM, far from El-Arish, around the road of El-Arish – Lehfen and Matrouh (North Western Coast), at 4PM, far from Matrouh, around the road of Matrouh-Sidi Barrani. The plants were cleaned, dried in oven at 50°C and ground to fine powder then reserved for plant analysis.

2.1. Preliminary Phytochemical Screening

2.1.1. Water distillation for volatile oils

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

2.1.2. Preparation of the extract for further screening

About 50 g of air-dried plant powder were refluxed with 250 ml of 80% ethyl alcohol for 6 hours, then filtered. The residual powder was then washed several times with hot alcohol. The combined filtrates were concentrated under reduced pressure at 50 °C, then used in the following tests:

Test for tannins: using ferric chloride solution as described by Balbaa (1986). Test for sterols and terpens: using Libermann-Burchard's test according to Fieser and Fieser (1959) and Salkowski reaction's according to Brieskorn and Klinger-hand, (1961).

Test for flavonoids: as described by Shinoda (1928) and Wall *et al.*, (1954). Test for alkaloids: according to Woo *et al.*, (1977) using Mayer's and Dragendorrf's reagent as described by Balbaa *et al.*, (1981).

Test for carbohydrates and/or glycosides: using Molish test as described by Balbaa *et al.*, (1981).

Test for saponins: as described by Wall *et al.*, (1954) and Balbaa (1986).

Chlorides and sulphates: using silver nitrate test for chlorides and barium chlorides test for sulphates according to A.O.A.C. (1970).

Resins and anthraquinones: according to Balbaa (1986).

2.2. Investigation of carbohydrates

2.2.1.Determination of total carbohydrates and soluble carbohydrates

They were determined according to Chaplin and Kennedy (1994).

Insoluble carbohydrates = Total carbohydrates - Soluble carbohydrates.

2.2.2. Extraction of free sugars

Twenty g of the defatted plant powder were extracted with 80% ethyl alcohol, and filtered. The filtrate was clarified by Carrez reagent, filtered and the filtrate was evaporated. The residue was dissolved in 3ml of 10% aqueous isopropanol for chromatographic investigation (Chaplin and Kennedy, 1994).

2.2.3. Paper chromatography of the free sugars

The isopropanol solution of the free sugars was examined chromatographically on Whatmann No.1 paper chromatography by the descending technique, using the solvent systems (1) and (2) in the presence of authentic sugars (Abou-Zeid *et al.*,, 1995).
System(1) n-butanol-pyridine-water-benzene (5:3:3:1).
System(2) n-butanol-acetic acid-water (4:1:5) upper layer.
Spraying reagent: Aniline hydrogen phthalate as described by Partridge (1949).

2.2.4. Extraction of combined sugar

The combined sugars were extracted from the defatted powder of 5 g plant after removing the free sugars. The extraction was carried out by cold method followed by hot method.

2.2.4.1. Cold extraction method: (Laidlow and Percival, 1949)

The plant powder was mixed with acidified distilled water (pH = 4) then left for 12 hour at room temperature, then filtered and the process was repeated till complete extraction. The total filtrate was concentrated under reduced pressure at 40°C and the extracted combined sugars were precipitated by slowly adding 95% ethanol.

2.2.4.2. Hot extraction method: (Laidlow and Percival, 1950)

The previously extracted plant powder by cold method was repeatedly extracted with hot boiling distilled water for 12hr. until complete extraction and the combined sugars were precipitated by 95% ethanol.

2.2.5. Preparation of the combined sugars for hydrolysis: (Hirst and Jones, 1955).

The precipitates of cold and hot extraction were obtained, washed several times with ethanol to remove chloride ions, then the combined sugars were stirred in acetone, filtered and dried in a vacuum desiccator. The total precipitate of combined sugars was heated on a boiling water bath with 0.5 M H_2SO_4 in a sealed tube for 20hr., then the filtrate was freed from sulphates ions by precipitation using barium carbonate, filtered and the volume was completed to 100 ml with H_2O.

2.2.6. Paper chromatography of the hydrolyzed - combined sugars

Few mg of the dried sugar hydrolyzate were dissolved in 10% aqueous isopropanol solution and applied on paper chromatography using the two solvent systems (1) and (2) alongside with authentic sugars (Abou-Zeid *et al*., 1995).

Spraying reagent: Aniline hydrogen phthalate (Partridge, 1949).

2.3. Investigation of amino acids

2. 3.1. Determination of total nitrogen

The total nitrogen content [*i.e.* true protein N, amino N and amide N] was determined using Kjeldahl method (James, 1995).

2.3.2. Identification of free amino acids using amino acid analyzer

According to Anderson *et al.*, (1977) and Pellet & Young (1980).

System condition

Beckman System 7300 High Performance Analyzer.

Column: Na High Performance Column 25 cm.

Injected Volume: 50 µL.

Detector: Visible Light Detector.

Retention time and separated area were plotted using Hewlett Packard 3390 recording integrator.

2.3.3. Identification of protein-amino acids using amino acid analyzer:

Defatted plant powder (1 g) was added to 10 ml of 6 N HCl in a sealed tube, hydrolyzed at 110°C for 24 hours, filtered and the hydrolyzed protein-amino acid were obtained by evaporation of the hydrolyzate till dryness. The residue was dissolved in distilled water, filtered and the volume of the filtrate was adjusted to 100 ml using distilled water. The protein-amino acid was identified according to Anderson *et al.*, (1977) and Pellet & Young (1980).

2.4. Investigation of lipid

2.4.1. Total lipid content

One hundred gm of the powdered plant were extracted with petroleum ether (40-60°C): ether (1:1) for 24 hours using soxhlet apparatus. The lipids were obtained by distilling off the solvent. The last traces of the solvent were removed by heating the liquid sample in a vacuum oven at 50°C to constant weight.

2.4.2. Physical and chemical properties of lipids

The lipid fraction was studied physically with regard to its odour, color and physical nature. It was soluble in petroleum ether, diethyl ether, benzene, chloroform, acetone, carbon tetrachloride and warm alcohol.

2.4.3. Fundamental chemical properties

Acid value (A.V.), saponification value (S.V.) and ester value (E.V.) were estimated according to British Pharmacopaea (B. P.) (1980), while iodine value (I.V.) was determined according to James (1995).

2.4.4. Chromatographic investigation of lipids content

2.4.4.1. Extraction of the fat sample: (Christie, 1982)

The lipids of the plant were extracted according to Christie (1982) method using chloroform: methanol (1:2), saponified for 1 hour, cooled and then extracted by diethyl ether. The ether extract was washed several times with water, dried over anhydrous Na_2SO_4. The non-saponifiable matters (hydrocarbon and sterols) were obtained by removal of ether solvent in a rotary evaporator. The washing water was added to the aqueous layer, which were acidified with 6N HCl and extracted with diethyl ether. The saponifiable matter containing the free fatty acids was obtained after washing the extract with water, drying it over anhydrous Na_2SO_4 and removing the solvent using the rotary evaporator.

2.4.4.2. Identification of fatty acids

Methylation of fatty acid was carried out by Trimethyl silylation reagent. The fatty acid methyl ester was then subjected to Gas-Liquid chromatographic analysis.

A Hewlett Packard gas liquid chromatograph (model 5890) equipped with a flame ionization detector and coiled glass column (1.8m ×0.2m I.D.), packed with 10% DEGS (Diethylene glycol succinate). The gas chromatographic conditions used for isothermal analysis were:

Temperature of column = 170°C, detector = 300°C and injector = 250°C, flow rates: H_2 = 33ml/min., N_2 = 30ml/min. and air = 330ml/min.

The peak areas were measured using a Hewlett Packard integrator model 3392A.

Preparation of Trimethyl silylation reagent

Nine ml of anhydrous pyridine, 3ml of hexamethyl disilazone and 1ml of trimethyl-chlorosilone were mixed together and used for the preparation of TMS derivatives, (Sigma - Sil - A).

2.4.4.3. Identification of unsaponifiable matter

The hydrocarbon and sterol compounds were identified by using a Hewlett Packard gas chromatography, model 5890, equipped with flame ionization detector. The used column size was 25 m x 0.2m I.D., composed from fused silica capillary column coated with dimethyl silicon fluid.

The conditions used for the analysis were

Carrier gas N_2 with flow rate 1ml/min., injection port temperature250°C, oven programmed from:100 to 280°C at 5°C/min. followed by 20min. at 280°C, auxiliary gas nitrogen flow rate: 20ml/min., hydrogen flow rate: 30ml/min. and air flow rate: 400ml/min.

The relative percentage of each compound was determined using triangulation method according to Nelson *et al.*, (1969).

3.RESULTS AND DISCUSSIONS

3.1. Preliminary phytochemical screening of *N. mucronata*

The preliminary phytochemical screening on *N. mucronata*, collected from the two studied habitats, showed that it contains: sterols, flavonoids, tannins, chlorides, sulphates, carbohydrates and/or glycosides, terpens, saponins, resin, traces of volatile oil and alkaloids (Table 1).

Table (1): Preliminary phytochemical screening of *N. mucronata*.

Test	Results*	Test	Results*
Volatile oil	(±)	Saponins	(+)
Tannins	(+)	Chlorides	(+)
Sterols	(+)	Sulphate	(+)
Flavonoids	(+)	Resins	(+)
Alkaloids	(±)	Terpens	(+)
Carbohydrates and/or glycosides	(+)	Anthraquinones	(–)

(–) = Absent (±) = Traces

* (+) = Present

3.4. Investigation of carbohydrates

3.4. 1. Total and soluble carbohydrates

The total carbohdrates were 2.2g% at El-Arish and 2.0g% at Matrouh in winter, while the soluble carbohydrates were 0.86g% at El-Arish and 0.75g% at Matrouh in winter.

3.4. 2. Free sugars

The obtained results of the free sugar extract of *N. mucronata* using comparative paper chromatography by two different systems (Table 2) showed that *N. mucronata* contained glucose and galactose as free sugars in the two localities.

3.4.3. Combined sugar

The obtained results of the hydrolyzed combined sugar extract of *N. mucronata* using comparative paper chromatography by two different systems (Table 2) showed that *N. mucronata* contained rhamnose, arabinose, galactose, glucose and xylose as combined sugars in the two localities.

Table (2): Free and combined sugars of *N. mucronata* at El-Arish and Matrouh.

Sugars	Free sugar	Combined sugar	$R_f \times 100$		Color Reaction
			System 1	System 2	
Rhamnose	(–)ve	(+)ve	45	31	Yellow-brown
Xylose	(–)ve	(+)ve	32	20	Red
Arabinose	(–)ve	(+)ve	28	19	Red
Glucose	(+)ve	(+)ve	23	15	Brown
Galactose	(+)ve	(+)ve	21	14	Brown

3.5 Investigations of free and protein amino acids

3.5.1. The total nitrogen and protein content

The total nitrogen contents of *N. mucronata* were 0.165g% and 0.162g% in winter at Matrouh and El-Arish habitats, respectively, while the total protein were 1.03 and 1.01g% in winter at Matrouh and El-Arish habitats, respectively.

3.5.2. Free amino acids

The free amino acids of *N. mucronata* at the two habitats were investigated using amino acid analyzer. The data present in (Table 3)

show that *N. mucronata* in El-Arish proline was abscent, while in Matrouh proline was found with a relative percentage of 0.2%. It was obvious from (Table 3) that the concentration of aspartic acid was the highest one of the separated amino acids (0.52 and 0.42%) in El-Arish and Matrouh samples, respectively, while histidine was the lowest one (0.11 and 0.07%) in El-Arish and Matrouh samples, respectively.

3.5.3. Protein amino acid

The protein-amino acids of *N. mucronata* were investigated using amino acid analyzer, which revealed that *N. mucronata* contained sixteen amino acids with different concentrations (Table 3).

It is obvious that lysine was the highest one of the separated amino acids (36.36 and 28.5%) in El-Arish and Matrouh samples, respectively, while proline was completely absent in El-Arish sample and present with relative percentage 0.36% in Matrouh samples.

The presence of proline in Matrouh samples may be due to the increase of soil salinity in Matrouh than that in El-Arish, Ali and Sawaf (1992) reported that salinity could inhibit the transmission reactions and hence the glutamic acid is accumulated and transformed to other nitrogenous compounds such as proline.

3.6. Investigation of lipids

3.6.1. Total lipids content

The total lipids content of *N. mucronata* was 1.45g% and 0.9g% in winter at El-Arish and Matouh habitats, respectively.

3.6.2. Physical properties

The obtained lipid was yellowish green in colour, solid, having a faint odour and disagreeable taste. It was soluble in benzene, petroleum ether, diethyl ether, chloroform, acetone, warm methyl and ethyl alcohol.

3.6.3. Fundamental chemical properties

The fundamental chemical properties of the extracted lipid of *N. mucronata* collected from the two habitats are presented in (Table 4), where it is noticed that there were a decrease in acid values in El-Arish and Matrouh (18 and 17.8, respectively) and an increase in ester value in El-Arish and Matrouh (152 and 142.2, respectively).

The saponification values of *N. mucronata* were 170 and 160 in El-Arish and Matrouh samples, respectively, which indicate that the main constituents of lipid were long chain fatty acids having C20, C18 and C16. The result can be confirmed by the saponification value of rape seed oil which its main constituents was C18 and its saponification value ranged between 170 and 180 (Farag, 1995). This was also confirmed by GLC analysis of the fatty acids of *N. mucronata*.

Table (3): Free and protein amino acids of *N. mucronata* (dry matter) at the two studied habitats as mg/gm protein.

Amino acid	Free amino acids		Protein- amino acids	
	El-Arish	Matrouh	El-Arish	Matrouh
Aspartic acid	0.52	0.42	5.2	6.63
Threonine	0.18	0.18	4.2	3.91
Serine	0.16	0.17	4.43	4.8
Glutamic acid	0.5	0.4	4.67	11.4
Proline	–	0.2	–	0.36
Glycine	0.27	0.25	1.47	1.44
Alanine	0.23	0.22	4.15	3.56
Cysteine	–	–	–	–
Valine	0.22	0.27	3.9	1.93
Methionine	–	–	4.9	2.73
Isoleucine	0.17	0.16	5	3.07
Leucine	0.31	0.28	4	2.03
Tyrosine	0.14	0.1	3.47	2.09
Phenylalanine	0.21	0.17	2.08	0.49
Histidine	0.11	0.07	6.2	11.99
Lysin	0.23	0.25	36.36	28.5
Arginine	0.28	0.19	6	8.3

Table (4) showed that the acid values in El-Arish and Matrouh, have no significant difference, while there were decreasing of iodine and saponification values in Matrouh than that in El-Arish, resulting from the increasing value of the percentages of the long-chain saturated arachidic acid in Matrouh than that of El-Arish

Table (4): Acid, iodine, ester and saponification values of lipids of *N. mucronata* at the two studied habitats.

Item	El-Arish	Matrouh	Item	El-Arish	Matrouh
Acid value	18	17.8	Ester value	152	142.2
Iodine value	97.6	80.6	Saponification value	170	160

3.6.4. Investigation of unsaponifiable matter

The unsaponifiables matter content of *N. mucronata* was determined using G.L.C. technique, the relative percentages of each component were calculated and tabulated in (Table 5).

It is obvious from the obtained results that there was no significant difference between the two habitats except the presence of decan at El-Arish only.

Table (5): Hydrocarbons and sterols of *N. mucronata* at the two studied habitats as detected by relative percentage by GLC.

Hydrocarbon and sterol	No. of carbon atom	RRT	Relative %	
			El- Arish	Matrouh
Decane	10	0.064	0.42	–
n-Hexadecane	16	0.31	0.35	0.4
n-Octadecane	18	0.41	2.94	2.6
n-Eicosane	20	0.49	11.89	16.3
n-Docosane	22	0.57	16.2	16.4
n-Tetracosane	24	0.65	17.54	14.1
n-Hexacosane	26	0.71	9.94	10.96
n-Octacosane	28	0.78	3.33	4.76
Stigmasterol	27	0.96	14.28	19.0
β-Sitosterol	27	1.0	21.14	9.0

3.6.5. Investigation of saponifiable fraction of lipid

The Gas-Liquid chromatographical analysis of *N. mucronata* lipids revealed the presence of long chain fatty acids, C16, C18 and C20 especially in Matrouh, where arachidic acid was the major fatty acid (Table 6).

Table (6):GLC of fatty acids of *N. mucronata* collected from the two studied habitats.

Fatty acid	No. of carbon Atom	RRT	Relative %	
			El- Arish	Matrouh
Nonanoic	9:0	0.63	-	0.3
Capric	10:0	0.72	0.434	0.08
Myristic	14:0	0.86	4.12	0.06
Palmitic	16:0	1.0	27.94	26.26
Palmitoleic	16:1	1.46	1.97	1.14
Stearic	18:0	1.57	5.45	7.19
Oleic	18:1	1.7	19.16	9.38
Linoleic	18:2	1.75	19.63	5.63
Linolenic	18:3	2.18	9.86	8.53
Arachidic	20:0	2.24	11.4	41.38

It was observed from the obtained results that the percentages of the unsaturated fatty acids palmitoleic, oleic, linoleic and linolenic were higher at El- Arish than that of Matrouh habitat. Meanwhile, the percentages of capric, myristic and palmitic acids were also higher at El- Arish habitat than that of Matrouh, while only the percentages of stearic and arachidic acids were lower at El- Arish habitat than that of Matrouh habitat.

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دراسات فيتوكيميائيه علي نبات شوك الحنش *Noaea mucronata* (النويا ميكروناتا)

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ملخص

تضم العائلة السرمقيه (الرمرامية) كثيرا من النباتات ذات الأهمية الاقتصادية و الطبية ولذلك فقد تم إختيار نبات شوك الحنش (النويا ميكروناتا) أحد أنواع هذه العائلة لدراسة مكونات النبات الكيميائيه من سكريات وبروتينات ودهنيات وتحليلها لموادها الأوليه وإستخلاصها و التعرف عليها وصفيا وتقديرها

كميا. لنبات شوك الحنش (النويا ميكروناتا) انتشار واسع على امتداد ساحل البحر الأبيض المتوسط في مصر وخاصة في طريق مطروح-براني (16 كيلومتر غرب مطروح) وفى طريق العريش-لحفن (19 كيلومتر جنوب العريش).

وقد إشتمل البحث علي مسح كيميائي أولي للنبات إتضح منه أن النبات يحتوى على استيرولات وفلافونيدات وتانينات وسكريات مختزلة وسكريات و/أو جليكوسيدات وتربينات وصابونينات وراتنجات وكبريتات وكلوريدات بالإضافة إلى زيوت طيارة وقلويدات بنسب ضئيلة جدا.

و قد تم من خلال هذه الدراسة:

1. التعرف على أن نبات شوك الحنش يحتوى على السكريات الحرة جلوكوز وجالاكتوز والسكريات المرتبطة رامنوز وأرابينوز وجلوكوز وجالاكتوز وزيلوز في النبات.
2. أن النبات بمنطقة العريش يخلو من الحمض الأميني الحر برولين بينما يتواجد بنسبة 0.2% بمنطقة مطروح، وأن أعلى نسبة للأحماض الأمينية الحرة كان حمض الأسبارتيك بنسبة 0.52 و 0.42% بمنطقتى العريش ومطروح على التوالى، بينما كان أقلها نسبة هو الهستيدين بنسبة 0.11 و 0.07% بمنطقتى العريش ومطروح على التوالى. وبالنسبة للأحماض الأمينية الداخلة فى تركيب البروتين فقد أوضحت النتائج أن النبات يحتوى 16 حمض أمينى بنسب مختلفة، كان أعلاها الليسين بنسبة 36.36 و 28.5% بمنطقتي العريش ومطروح على التوالي. وقد أختفي البرولين تماما من منطقة العريش بينما تواجد بنسبة 0.36% ببروتين النبات بمنطقة مطروح.
3. تحديد محتوى النبات من الدهون ودراسة خواصها الطبيعية والكيميائية وتقدير الاستيرولات والمركبات الهيدروكربونية والأحماض الدهنية المشبعة وغير المشبعة ونسبها في النبات باستعمال طرق تحليل الكروماتوجرافى الغازي. وقد أوضحت النتائج أنها لا تختلف كثيرا من حيث الصفات في كلا المنطقتين، بينما لوحظ نقص في القيمة اليوديه ورقم التصبن للدهن في منطقة مطروح عن مثيلتها بالعريش كنتيجة لزيادة نسبة حمض الأراشيديك المشبع بهذه المنطقة.
4. تقدير نسب المواد غير المتصبنة وفصل مكوناتها، حيث إتضح عدم وجود فروق معنوية في مكوناتها بمنطقتى الدراسة ماعدا تواجد الديكان بالعريش فقط، كما تم تحديد الأحماض الدهنيه المشبعه وغير المشبعة.