

**PHYTOCHEMICAL STUDIES ON *Pancratium arabicum*,
(SICKENB)**

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ABSTRACT

The preliminary phytochemical screening of leaves and bulbs of *Pancratium arabicum* showed that the plants contained sterols, tannins, chlorides, sulphates, reducing sugars, flavonoids, alkaloids, resins, saponins, carbohydrates and/or glycosides. Traces of volatile oils were detected in bulbs only.

HPLC investigation of free sugars of *Pancratium arabicum* revealed the presence of glucose, galactose and fructose in both leaves and bulbs. Fructose had the maximum values in leaves and bulbs (2.66 and 1.623 mg/g, respectively). While galactose had the minimum values in leaves and bulbs (0.086 and 0.030 mg/g, respectively). Concerning the combined sugars glucose and galactose were detected in both leaves and bulbs. Glucose had the maximum values 9.283 mg/g for leaves and 88.521 mg/g for bulbs. While galactose values were 5.737 mg/g for leaves and 5.926 for bulbs.

The free amino acids were investigated using amino acid analyzer, where fifteen amino acids were detected in leaves and sixteen amino acids were detected in bulbs of *Pancratium arabicum*. The same free amino acids were detected in bulbs and leaves except serine, which was absent in leaves. It was observed that threonine percentage was the highest in leaves with proportion 22.97% followed by glycine (15.29%) and valine (12.34%), while the lowest

detected amino acid was cysteine (0.03%). On the other hand, glycine was the highest amino acid in bulbs 18.83% followed by aspartic acid (17.32%) and serine (15.92%). The amino acid analysis of protein revealed the presence of 16 amino acids in both leaves or bulbs of *P. arabicum*. Proline was the highest concentrated amino acid in leaves with proportion 16.21% followed by threonine (15.81%) and glycine (8.19%), while the lowest detected amino acid was leucine 0.10%. On the other hand, the highest concentration of amino acid in bulbs was threonine (20.88%) followed by proline (13.54%) and glycine (8.21%), while the lowest detected amino acid was leucine (0.15%).

The chemical analysis of lipids of *P. arabicum* was proceeded. It is obvious from the obtained GLC results that the unsaponified matter contained 5 sterols in each of leaves or bulbs of *P. arabicum*. The leaves were rich in sterols when compared with bulbs except cholesterol, which were 4.655% and 8.303% in leaves and bulbs, respectively. GLC chromatograms of the fatty acids revealed the presence of myristic and linoleic acids in traces in both leaves and bulbs. Meanwhile behenic acid and linolenic acid were present in traces in either bulbs or leaves, respectively. The higher concentrations of fatty acids were palmitic acid (40 mg/g), followed by stearic acid (20 mg/g), linolenic (20 mg/g), eicosanoic acid (19 mg/g) and oleic acid (15 mg/g) in bulbs. Meanwhile stearic acid represented, the higher concentrations of fatty acids in leaves (44 mg/g) followed by palmitic acid (20 mg/g), oleic acid (19 mg/g), behenic acid (16 mg/g) and eicosanoic acid (5 mg/g).

Key words: amino acids, carbohydrates, fatty acids, *pancratium*.
Phytochemical screening,

1. INTRODUCTION

Family *Amaryllidaceae* plants are usually perennial herbs, with a rootstock, either rhizomes, e.g., *Agave* and *Curculigo* or bulbs e.g., *Narcissus*, *Panocratium*, and *Crinum* species. Plants usually reproduce by means of bulbs (Benson, 1967).

Economically the *Amaryllidaceae* contributes a large number

of plants that are important to many activities. The agaves are primary sources of fiber used in cordage, particularly of sisal and henequen (Lawrence, 1969). Karawya *et al.*, (1982) reported that a hydrolysate of bulbs of *P. maritimum* mucilage contained a large amount of glucose, along with arabinose and galacturonic acid.

Sandberg and Agurell (1959) reported that *Pancratium maritimum* and *Pancratium sickenbergeri* contained alkaloids.

Mortenko (1974) isolated three alkaloids base from *Pancratium speciosum* two of them were identified as lycroine and tazattine. The bulbs contained 0.7% and the leaves 0.34% total alkaloids (dry weight). The isolated 9 alkaloids from the underground parts of *Pancratium trianthum* were named trisferidine, tazettine, hippeastrine, pancratine, galanthamine, lycorine, gordenine, zephyranthine and trianthine (new one).

The structure of pancratistatin, which was isolated from the root of *P. litorale* was determined. Ghosal *et al.*, (1984) isolated three new glucosyloxy alkaloids (hordenine-4-0-beta-D-glucoside, lycorine 1-0-beta-D-glucoside and pseudolycorine-1-0-beta-D-glucoside from fluids of flower, stems and bulbs of *Pancratium bilforum*.

Ghosal *et al.*, (1989) isolated two new alkaloids from *Pancratium bilforum* and their structures were established as 4,6-dimethoxyacetophenone-2-0-beta-D-glucoside and 2,6-dimethoxyacetophenone-4-0-beta-0 glucoside (on the basis of chemical transformation, comprehensive spectroscopic analysis and synthesis of the aglycons). Abou-Donia *et al.*, (1992) isolated two 2-oxyphenanthri-dinium alkaloids from bulbs of *P. maritimum* and were identified as ungeremine and Zefbetaine.

Sener *et al.*, (1994) isolated seven *Amaryllidaceae* alkaloids of the Crinine class from the bulbs of *P. maritimum* of Turkish origin. Five of these, namely (+)-haemanthamine (+)- buphanisine (-)-Crinine, (-)-3B-methoxy 6 a, B dihydroxy-1, 2-dehydrocriname, (-) 6, II α , B dihydroxy-3-methoxy-1, 2- dehydrocrinane were described previously, while the other 2(-)-3B-II α dihydroxy 1,2 dehydrocrinane and (-) 8- hydroxy-9-methoxycrinine represent new compounds.

Ali *et al.*, (1981) investigated the distribution of flavonoids in leaves of cultivated and wild *Pancratium maritimum*, *Crinum bulbispermum*, *Hippeastrum vittatum* and *Polianthes tuberosa*. They

reported that *P. tuberosa* contained kaempferol but all the other species were free aglycones. All these plants contained kaempferol-3-O-oxyloside; while wild *P. maritimum* contained also kaempferol-3, 4'-di-O-xyloside. The percentages of total flavonoids in the air-dried leaves of those plant were, respectively, 0.48, 0.44, 0.13, 0.20 and 0.28%.

Ali *et al.*, (1990) isolated a new chromon (5,7 dihydroxy-6 methoxy-2, 8 dimethyl) and a new flavon (4-hydroxy-5, 7-dimethoxy-8-methyl), together with two known chromones and a known flavon were isolated from the bulbs of *Pancreatum maritimum*. The compounds were characterized by spectral analysis. Abou-Donia *et al.*, (1991) isolated a new glucosyloxy phenolic metabolite from *P. maritimum* bulbs.

Meanwhile there were no known phytochemical studies in the literature on *P. arabicum*; hence we aimed to investigate the main chemical constituents, *i.e.*, carbohydrates, proteins and lipids of *Pancreatum arabicum* to clarify the effect of environment on its biochemical constituents.

2. MATERIALS AND METHODS

The leaves and bulbs of *Pancreatum arabicum* used in the present investigation were collected during the growth season 1999 from Abu Lahw El-Bahary (El-Kasr area), Marsa Matruh. Samples were cleaned, dried in an oven at 60°C for 48 hours and ground to fine powder and then used in the following investigation:

2.1. Preliminary phytochemical screening

2.1.1. Steam distillation

About 5 g of fresh plant materials were subjected to steam distillation according to British Pharmacopoeia (1980) method for volatile oil content.

2.1.2. Method of preparing the extract for further screening

About 50 g of air dried powdered plant material were refluxed with about 50 ml of 80% ethyl alcohol for about 6 hours, then filtered.

The residue powder 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 terpenes (Liebermann- 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) and Shinoda (1928), test for alkaloids according to Jenkins *et al.*, (1957), 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), test for resins according to Fahmy (1923).

2.2. Investigation of carbohydrates

Monosaccharides and polysaccharides were analysed according to Chaplin and Kennedy (1994) using HPLC technique.

2.3. Investigation of free and protein-amino acids

The free amino acids and the hydrolyzed protein-amino acids were determined according to the method described by Pellet and Young (1980). Twenty micro liters of the amino acids were loaded in the instrument capsule for investigation. 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 (g/16g nitrogen) was calculated by a special designed program.

2.4. Investigation of lipids

The lipids were extracted and estimated according to the A.O.A.C. (1970).

Acid value (A.V.), ester value (E.V.) and saponification value (S.V.) were determined according to Güenther (1972). Iodine value (I.V.) was estimated according to Mohamed and Amer (1965).

Fractionation of the unsaponifiable matter and fatty acids, as well as identification of their constituents were carried out using GLC technique as followed by Eaton (1989). The extracted fatty acids and

the standard ones were converted to the corresponding methyl esters using ethereal solution of diazomethane (Farag *et al.*, 1986).

The results of Itoh *et al.*, (1973) and Farag *et al.*, (1986) were used as a guide to characterize some of the unknown compounds. The relative proportion of each individual compound was estimated as the ratio of the partial areas to the total area as mentioned by Fryer *et al.*, (1960) and Nelson *et al.*, (1969).

3. RESULTS AND DISCUSSION

3. 1. Preliminary photochemical screening

The preliminary photochemical screening of leaves and bulbs indicated the presence of sterols, tannins, chlorides, sulphates, reducing sugars, flavonoids, alkaloids, resins, saponins, carbohydrates and/or glycosides. No volatile oil was found in leaves but present as traces in bulbs.

3. 2. Investigation of carbohydrates

3. 2.1. Monosaccharides

Table (1) reveals the presence of glucose, galactose and fructose as monosaccharides in the extract of *P. arabicum* leaves and bulbs. Fructose had the maximum values in leaves and bulbs (2.66 mg/g and 1.623 mg/g, respectively). While galactose had the minimum values in leaves and bulbs. Its values were 0.086 mg/g for leaves and 0.030 mg/g for bulbs.

Table (1) HPLC of free and combined sugars of *Pancratium arabicum* leaves and bulbs (mg/g).

Sugars	Monosaccharides conc.		Polysaccharides conc.	
	Leaves	Bulbs	Leaves	Bulbs
Glucose	1.291	0.456	9.283	8.521
Galactose	0.086	0.030	5.737	5.926
Fructose	2.660	1.623	-	-

3. 2.2. Polysaccharides

Data in Table (1) show also that *P. arabicum* leaves and bulbs contained glucose and galactose as polysaccharides, where glucose had the maximum values 9.283 mg/g in leaves and 88.521 mg/g for bulbs. On the other hand, galactose concentrations were 5.737 mg/g in leaves and 5.926 mg/g in bulbs. It is clear from Table (1) that glucose values of monosaccharides and polysaccharides for bulbs were lower than that of leaves. Meanwhile, galactose and fructose values (of monosaccharides) for bulbs were lower than that of leaves, while galactose values (of polysaccharides) for bulbs were higher than that of leaves.

3. 3. Investigation of free and protein-amino acids

3. 3. 1. Free amino acids (using Amino Acid Analyzer)

On investigation of free amino acid using amino acid analyzer fifteen amino acids were detected in leaves and sixteen amino acids were detected in bulbs of *Pancreaticum arabicum* (Table 2). The free amino acids, detected in bulbs were also detected in leaves except serine, which was absent in leaves of *P. arabicum*.

Table (2): Free amino acids in leaves and bulbs of *P. arabicum* using Amino Acid Analyzer.

Free amino acid	Conc. g/100g dry wt.	
	Leaves	Bulbs
Aspartic acid	0.93	17.32
Threonine	22.97	2.98
Glutamic acid	1.35	2.51
Serine	-	15.92
Proline	0.35	1.67
Cysteine	15.29	18.83
Cystine	0.03	0.01
Valine	12.34	7.22
Methionine	0.62	0.12
Isoleucine	4.79	1.79
Leucine	5.20	1.07
Tyrosine	1.11	0.10
Phenylalanine	3.57	0.88
Histidine	7.69	3.88
Lysine	0.49	0.02
Arginine	1.14	0.33

It is clear from Table (2) that threonine was the highest free amino acid in leaves with proportion of 22.97% followed by cysteine (15.29%) and valine (12.34%) while the lowest detected amino acid was cystine (0.03%). On the other hand, the highest amino acid concentration in bulbs was cysteine (18.83%) followed by aspartic acid (17.32%) and serine (15.92%).

3.3.2. Protein amino acids (using Amino Acid Analyzer)

It is clear from Table (3) that both leaves and bulbs of *P. arabicum* contained 16 amino acids. Proline was the highest detected amino acid in the leaves with proportion 16.21% followed by threonine (15.81%) and glycine (8.19%) while the lowest detected amino acid was leucine (0.10%).

On the other hand, the highest concentration of protein amino acid in bulbs was threonine (20.88%) followed by proline (13.54%) and glycine (8.21%). The lowest detected amino acid was leucine (0.15%).

Table (3) Protein amino acid in leaves and bulbs of *P. arabicum* using Amino Acid Analyzer.

Protein-amino acids	Conc. g/100g dry wt.	
	Leaves	Bulbs
Aspartic acid	0.16	0.36
Threonine	15.81	20.88
Serine	4.06	4.07
Glutamic acid	3.92	4.51
Proline	16.21	13.54
Glycine	8.19	8.21
Alanine	6.51	7.43
Cystine	2.57	2.39
Valine	4.38	3.14
Methionine	1.93	1.49
Isoleucine	4.50	5.90
Leucine	0.10	0.15
Phenylalanine	1.83	0.43
Histidine	3.02	4.21
Lysine	5.10	3.17
Arginine	2.23	4.16

3.4. Investigation of the lipids

3.4.1. Total lipid content

Data presented in Table (4) indicated that leaf lipid content reached the maximum value of 5.39% in the autumn and minimum value of 2.99% in the spring. Also, in bulbs lipid content reached its maximum value of 4.46% in autumn, while the minimum value of 3.23 was found in spring.

3.4.2. Physical and chemical properties and chromatographic analysis of lipids

3.4.2.1. Physical properties

The lipids obtained were yellowish green in colour for bulbs and dark green for leaves. Lipids were semi-solid, having a faint odor and disagreeable taste. The lipids were soluble in petroleum ether (B.p. 40-60°C), ether, chloroform, acetone, benzene, warm methyl and ethyl alcohol.

3.4.2.2. Fundamental chemical properties

The fundamental chemical properties of the extracted lipids of *Panocratium arabicum* plant from leaves and bulbs are presented in Table (5). The acid value, ester value, saponification value and iodine value were: 19.2, 176.4, 195.6 and 27.00 in leaves, respectively. The corresponding values were 25.12, 175.01, 200.13 and 17.00 in bulbs, respectively. The *P. arabicum* lipid fraction was characterized by a low iodine value in bulbs, which indicates the presence of high saturated fatty acid content.

Table (4) Total lipid contents in dry powder of leaves and bulbs of *P. arabicum*.

Seasons	Lipid content (%)	
	Leaves	Bulbs
Winter	3.00	3.60
Spring	2.99	3.23
Summer	4.60	3.70
Autumn	5.39	4.46

Table (5): Acid, ester saponification, and iodine values of lipids in leaves and bulbs of *Panocratium arabicum*.

Analysis	Leaves	Bulbs
Acid value	19.2	25.12
Ester value	176.4	175.01
Saponification value	195.6	200.13
Iodine value	27.00	17.00

3. 4. 3. Separation and identification of unsaponifiable matter fraction using Gas Liquid Chromatography (GLC)

The chromatograms obtained from Gas Liquid Chromatographic analysis for *P. arabicum* of leaves and bulbs are shown in Table (6).

It was clear from the obtained results that there were 5 sterols in both the leaves and the bulbs of *P. arabicum*. The leaves were richer in sterols than the bulbs except for cholesterol and cyclosterol.

Table (6): The separated sterols from leaves and bulbs of *P. arabicum* using GLC.

Sterol	Leaves	Bulbs
	%	%
Cholesterol	0.57	1.95
Campesterol	2.25	1.56
Stigmasterol	0.72	0.38
Cyclosterol	6.60	7.50
24Methenecycloastanol	1.92	1.75

3. 4. 4. Separation and identification of fatty acids by Gas Liquid Chromatography (GLC)

The fatty acid compositions of leaves and bulbs of *P. arabicum* as analyzed by GLC are shown in Table (7).

Table (7): Fatty acid content using Gas Liquid Chromatography of leaves and bulbs *Pancreatium arabicum*.

Fatty acids	Bulbs	Leaves
	Conc. (mg/g)	Conc. (mg/g)
Myristic acid	Traces	Traces
Palmitic acid	40	20
Stearic acid	20	44
Oleic acid	15	19
Linoleic acid	Traces	Traces
Linolenic acid	20	Traces
Eicosanoic acid	19	5
Behenic acid	Traces	16

Eight fatty acids were detected in the lipid fraction of leaves and bulbs of *P. arabicum*. It had been found that myristic acid and linoleic acid were present in traces in both leaves and bulbs. Meanwhile, behenic and linolenic acids were present in traces in either bulbs or leaves, respectively. Palmitic acid had the higher concentration of fatty acids in bulbs (40 mg/g) and stearic acid in leaves (44 mg/g). On the other hand, the lowest fatty acid was oleic acid in bulbs (15 mg/g) and eicosanoic acid (5 mg/g) in leaves.

CONCLUSION

The preliminary phytochemical screening of leaves and bulbs of *Pancreatium arabicum* showed that the plants contained sterols, tannins, chlorides, sulphates, reducing sugars, flavonoids, alkaloids, resins, saponins, carbohydrates and/or glycosides. Traces of volatile oils were detected in bulbs only.

The free sugars glucose, galactose and fructose were found in both leaves and bulbs with maximum values of fructose and minimum values of galactose in both leaves and bulbs. Concerning the combined sugars glucose and galactose were detected in both leaves and bulbs with maximum values of glucose in leaves and bulbs.

Pancreatium arabicum contains fifteen free amino acids in leaves and sixteen in bulbs. The same free amino acids were detected in bulbs and leaves except serine, which was absent in leaves. It was observed that threonine percentage was the highest in leaves followed by glycine and valine, while the lowest detected amino acid was cysteine. On the other hand, glycine was the highest amino acid in bulbs followed by aspartic acid and serine. The amino acid analysis of protein revealed the presence of 16 amino acids in both leaves or bulbs of *P. arabicum*. Proline was the highest concentrated amino acid in leaves followed by threonine and glycine, while the lowest detected amino acid was leucine.

On other hand the highest concentration of amino acid in bulbs was threonine followed by proline and glycine, while the lowest detected amino acid was leucine.

The unsaponified matter of *P. arabicum* contained 5 sterols in each of leaves or bulbs. The leaves were rich in sterols when compared with bulbs except cholesterol. *P. arabicum* contains myris-

tic and linoleic acids in traces in both leaves and bulbs. Meanwhile beheanic acid and linolenic acid were present in traces in either bulbs or leaves. The higher concentration of fatty acids were palmitic acid, followed by stearic, linolenic, eicosanoic acids and finally oleic acid in bulbs. Meanwhile stearic acid represented the higher concentration of fatty acids in leaves followed by palmitic, oleic, beheanic and eicosanoic acids.

These results showed mainly the primary metabolites which affect the production of plant secondary metabolites the active constituents of the plant of medicinal and economic values).

4. REFERENCES

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دراسات فيتوكيميائية علي نبات السوسن *Pancreatium arabicum*

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مركز بحوث الصحراء - المطرية - القاهرة

ملخص

- يعتبر نبات السوسن بانكراتيام أرابيكم من العائلة النرجسية وقد تم جمع النبات من الكثبان الرملية بمنطقتي أبولهو البحري (طريق القصر) وكليوباترا بمحافظة مرسى مطروح.
- 1- أوضحت النتائج أن النبات يحتوى على تانينات ، ستيرويدات ، فلافونيدات وقلويدات وجليكوسيدات و/أو سكريات وكلوريدات وكبريتات وسكريات مختزلة وقلويدات وصابونينات.
 - 2- تم تحليل السكريات باستخدام HPLC وأمكن التعرف على سكريات حرة (جلوكوز وفركتوز وجالاکتوز)، وكذلك التعرف على سكريات في صورة مرتبطة (جلوكوز وجالاکتوز) في كل من الأوراق والأبصال.
 - 3- تم التعرف على 9 أحماض أمينية في الأوراق و 10 أحماض في الأبصال باستخدام الكروماتوجراف الورقي بينما تم باستخدام جهاز تحليل الأحماض الأمينية التعرف على 15 حمض أميني في كل من الأوراق والأبصال ، كما تم التعرف على الأحماض الأمينية المرتبطة على هيئة بروتين نباتي بعد تحليلها باستخدام جهاز تحليل الأحماض الأمينية على 16 حمض أميني في كل من الأوراق والأبصال.
 - 4- وجد من الدراسة أن الدهون الكلية تكون في أقصى نسبة لها في الخريف وأقلها في الربيع في كل من الأبصال والأوراق.
 - 5- تبين بدراسة الليبيدات التي يحتويها النبات ودراسة خواصها الطبيعية والكيميائية أن كل من الأبصال والأوراق تحتوى على 5 استيرويدات.
 - 6- تم التعرف على 8 أحماض دهنية في كل من الأوراق والأبصال.

