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A Botanical Study and Estimation of Certain Primary Metabolites of *Gymnocarpos decandrus* Forssk

Seham S. El-Hawary[1], Mona M. Okba[1], Rehab A. Lotfy[2] and Mahmoud M. Mubarek[2*]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Cairo University Kasr El-Ainy, 11562 Cairo ;* [2]*Department of Medicinal and Aromatic plants, Desert Research Center, 11753 Matariya, Egypt*

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Abstract

Gymnocarpos decandrus Forssk., of the family Caryophyllaceae, is a well-known grazing wild plant growing in the Middle East region and north of Africa. Recently several medicinal activities such as anti-inflammatory, analgesic, and diuretic were reported for *G. decandrus.* This study presents an investigation of the *G. decandrus*' macro and micro morphological features, to aid in the characterization and differentiation of such a commercially and medicinally important drug either in its entire or its powdered forms. The anatomical characters of the stem revealed the presence of cork cells, cortex, and pericycle traversed by patches of lignified pericyclic fibers. The epidermis of the leaf is characterized by the presence of anisocytic stomata, and absence of hairs. Druse crystals of calcium oxalate were found in the mesophyll. The total protein and lipid content were 10.5 % and 0.8 5% respectively. The analysis of protein revealed that leucine (6.27 %) and aspartic acid (12.87 %) were the major essential and non-essential amino acids respectively. A high level of glutamic acid (11.94 %) was also observed.

Keywords: Amino acids, Caryophyllaceae, Druse crystals, Gymnocarpos decandrus, Macro-Micromorphology.

1. Introduction

Gymnocarpos decandrus Forssk, of the family Caryophyllaceae, is the most extensively distributed species in the Gymnocarpos genus. It is found on the Canary Islands, Morocco, Algeria, Tunisia, Libya, Egypt, Jordan, Syria, Saudi Arabia, Oman, Iran, Afghanistan, and Pakistan (Petrusson and Thulin, 1996).

The original spelling of this species by Forsskal was *Gymnocarpos decandrum*. According to the international code of botanical nomenclature, names with the suffix (carpos) are to be treated as masculine. So the accepted species name was modified to *G. decandrus*. *G. decandrus* is a shrublet with an erect stem, up to 45 cm tall. The bark is greyish or light brown. The leaves are sessile, slightly narrowed to the base, obtuse to subacute at the apex, and the seeds are brown in color (Petrusson and Thulin, 1996).

Traditionally, the aerial parts of the plant are used as food for grazing animals (El-Zanaty *et al.,* 2010). Recently, it has shown potent anti-inflammatory, analgesic, diuretic (Meselhy *et al.*, 1994), and α-amylase inhibitory activities (Sallam and Galala, 2017). The *G. decandrus* aqueous extract exhibited antitumor activity against melanocyte cell lines (Sathiyamoorthy *et al.*, 1999).

The plant has recently showed promising medicinal activities, in addition to being widely used as a grazing plant. The present study aims at the investigation of the *G. decandrus* macro and micro morphological features as well as at the estimation of its primary metabolite content to aid in characterization of such commercially and medicinally important drug either in its entire or powdered forms.

2. Materials and Methods

2.1. Plant Material

Collecting the aerial parts of *Gymnocarpos decandrus*, of the family Caryophyllaceae, was carried out during the flowering stage in April, 2016 from the western Mediterranean coastal region (Alexandria- Mersa Matrooh road 80- 140 km.). The plant was identified by Prof. Dr. Azza El Hadidy, Professor of Taxonomy and Flora-Herbarium, at the Faculty of Science, Cairo University. A voucher specimen of the plant under investigation was deposited with the code no. (7/12/15/1) at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

2.2. Sample Preparation for Botanical Study

Fresh samples of stems and leaves were preserved in 70 % ethanol containing 5 % glycerol. Successive transverse sections (T.S), that are 10-15 μm thick, were performed with a manual microtome and were stained with Safranin

* Corresponding author e-mail: mahmoudmubarek88@gmail.com.

and Fast Green (Ruzin, 1999). The stems' and leaves' powder was stored in dark-colored, tightly closed vessels for the examination of the powdered organs. An optical microscope was used for taking the photographs combined with a digital camera Leica ICC50 HD (Okba *et al.*, 2013).

2.3. Determination of Total Lipid

Twenty grams of the dry plant powder were extracted with petroleum ether (40-60 ºC): ether (1:1 v/v) for twenty-four hours using the Soxhlet apparatus according to (Christie, 1982).

2.4. Determination of Total Nitrogen and Protein

The total nitrogen and total protein content were determined using the Kjeldahl method (James, 1995).

2.5. Methods for Amino-Acid Analysis

The defatted plant powder (0.1 g) was weighed in a tube and 10 mL of 6N hydrochloric acid was added. The tube was sealed and heated in an oven set at 110ºC for twenty-four hours. The solution was centrifuged to precipitate the insoluble components. The hydrolysate was evaporated to dryness on a rotary evaporator at the temperature of 40 ºC. Distilled water (5 mL) was added to the hydrolysate and evaporated to dryness. The residue was dissolved in citrate buffer (pH 2.2) and saved for analysis using an amino-acid analyzer (Arafat, *et al.*, 2009).

2.6. Apparatus and Conditions

Kjeldahl unit and Eppendrof-Germany LC3000 amino acid analyzer. Conditions: the mobile phase is citrate buffer (6.2 M, pH 2.2) with a flow rate at 0.25 mL/min for ninhydrin pump and 0.45 mL/min for eluent pump.

3. Results

Results of the macro and micro-morphological examination of the plant stems and leaves are illustrated in Figures (1-5). Dimensions in microns of the different elements are presented in Table (1)

3.1. Macroscopic Features

The *G. decandrus* Forssk under investigation is a heavily-branched erect shrublet (10-50 cm in height) with greyish or light brown stems (Figure 1A). The stem is erect, up to 45 cm tall and 1-1.5 cm in diameter. The bark is greyish or light brown in color, with glabrous internodes (0.5-3 cm) (Figure 1B). The leaves are fleshy, light green to light brown in color, and are fascicled on younger branches, sessile, linear-terete, slightly narrowed to the base, obtuse to subacute at the apex, oblanceolate. The leaf is 0.4-1 cm in length, and 1-2 mm in width (Figure 1C).

3.2. Microscopic Features

3.2.1. The Stem

The transverse section of stem **(**Figure 2) is almost circular in outline. It demonstrates a cork layer composed of four to five rows of compressed tangentially-elongated cells with suberized walls. The cortex consists of two to three rows of parenchymatous secondary cortex following one to two rows of collapsed parenchyma of the primary cortex. The pericycle is formed of seven to eleven rows of parenchyma traversed by patches of lignified pericyclic fibers.

The phloem is formed of sieve tubes, companion cells, and phloem parenchyma. The phloem parenchyma consists of a soft tissue, formed of ten to twelve rows of thin-walled cellulosic undifferentiated parenchymatous cells.

Xylem is composed of xylem vessels, wood fibers, and lignified wood parenchyma. The medullary rays are formed of lignified axially elongated cells. The pith represents about one third of the diameter of the stem, and is formed of thick lignified parenchymatous cells. The stem powder is brownish yellow in color with a characteristic taste. Microscopically, (Figure 3) it is characterized by the presence of cork fragments which are polygonal isodiametric thick suberized cells. Fragments of polygonal isodiametric epidermal cells with straight anticlinal walls and anisocytic stomata Fragments of lignified pericyclic fibers with wide and narrow lumen, tapering to pointed acute ends were found. Spiral and annular xylem vessels were also detected.

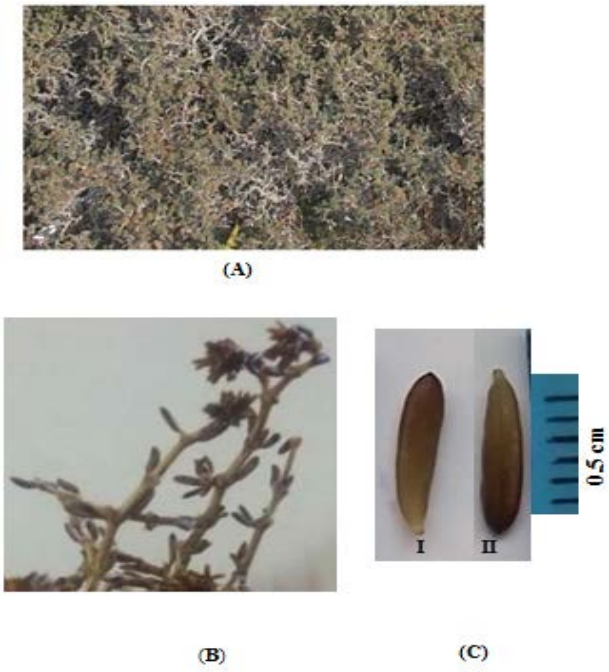

Figure 1. Macromorphology of the *G. decandrus* aerial parts. A: overview of the plant. B: stem carrying leaves. C: leaf (I: upper surface and II: lower surface).

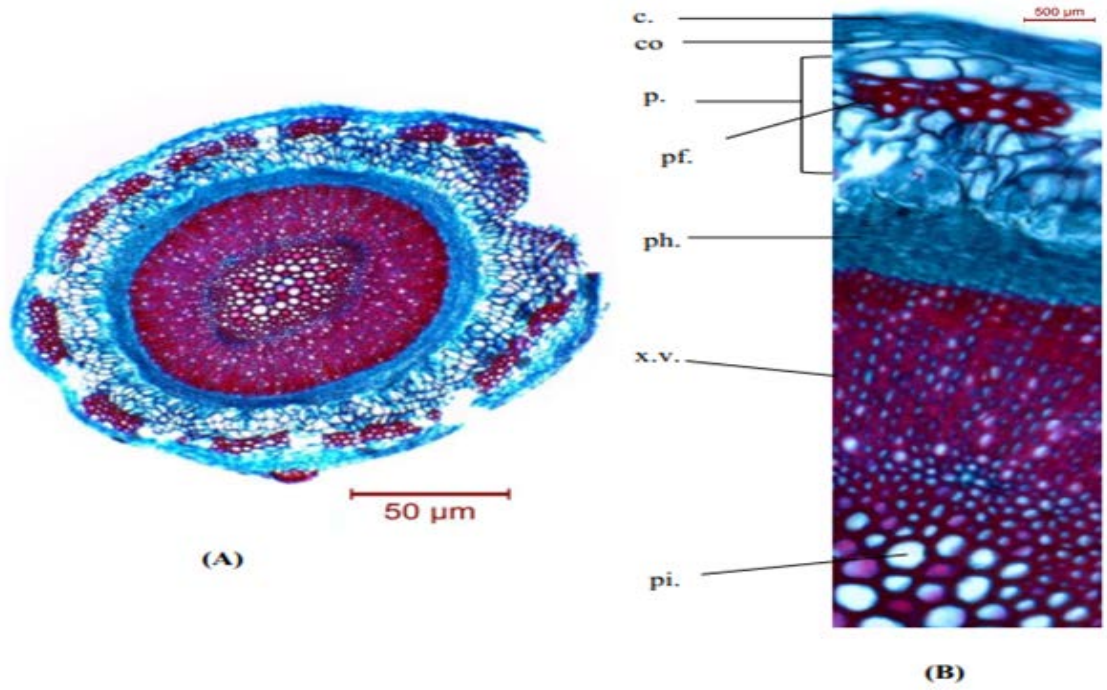

Figure 2. Micromorphology of *G. decandrus* stem. A: T.S. overview. B: T.S. detailed sector. c, cork cells; co, cortex; p, pericycle; pf, pericyclic fibers; ph, phloem; pi, pith; x.v, xylem vessels.

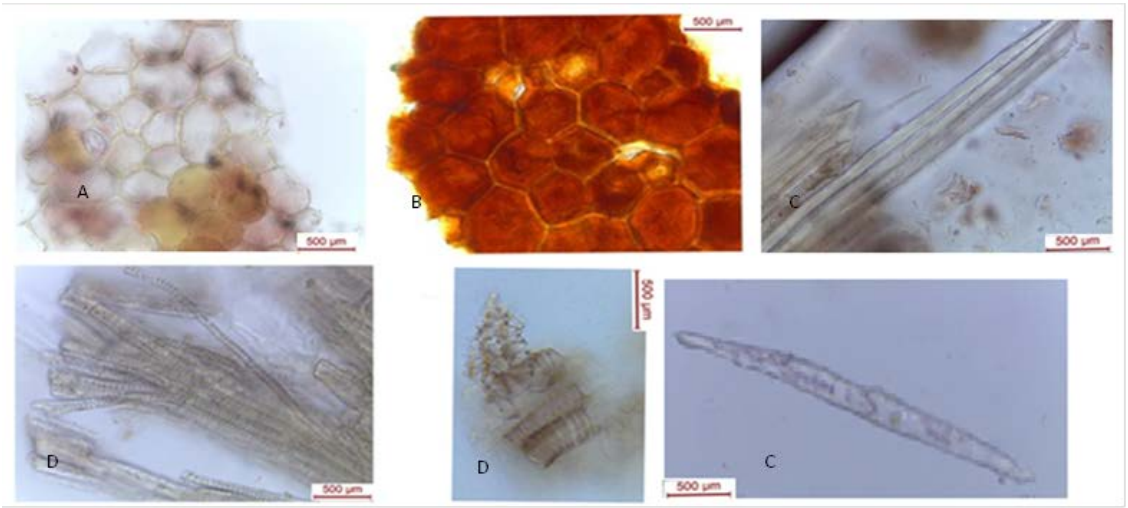

Figure 3. Micromorphology of *G. decandrus* powdered stem. (A) surface; (B) cork cells; (C) pericyclic fiber; (D) xylem vessels.

3.2.2. Leaves

A transverse section of the leaf lamina (Figure 4) shows the epidermis layer. It consists of one row of rectangular epidermal cells without intercellular spaces enclosing the mesophyll. The mesophyll is isobilateral. The palisade layer is formed of three to five rows of elongated columnar closely packed cells, with straight walls having green plastids inside. The palisade layer was extended overall the upper and lower surfaces. The parenchyma cells of the spongy tissue are irregular in shape and formed of five to seven rows of parenchyma cells with wide intercellular spaces containing druse crystals of calcium oxalate and small scattered vascular bundles embedded within. The midrib region consists of four to six rows of slightly thin walled irregular parenchyma cells. The vascular system is composed of a collateral vascular bundle. The phloem is formed of sieve tubes, companion cells and phloem parenchyma. The xylem is composed of spiral, pitted lignified xylem vessels, non-lignified wood parenchyma and wood fibers along with uniseriate medullary rays. The powdered leaf (Figure 5) is green in color, odorless with a characteristic - taste. Microscopically, it is characterized by the presence of fragments of the upper epidermis consisting of polygonal isodiametric straight-walled cells with anisocytic stomata. Fragments of the lower epidermis polygonal isodiametric cells with slightly wavy anticlinal walls and numerous anisocytic stomata were also found. Fragments of neural epidermis along with parenchyma cells containing druse crystals of calcium oxalate and fragments of palisade with columnar, thin-walled cells with green plastids inside were detected. In addition to fragments of wood fiber with wide lumen and blunt ends and tracheid fragments elongated with lignified pitted walls. Fragments of spiral xylem vessels were also observed.

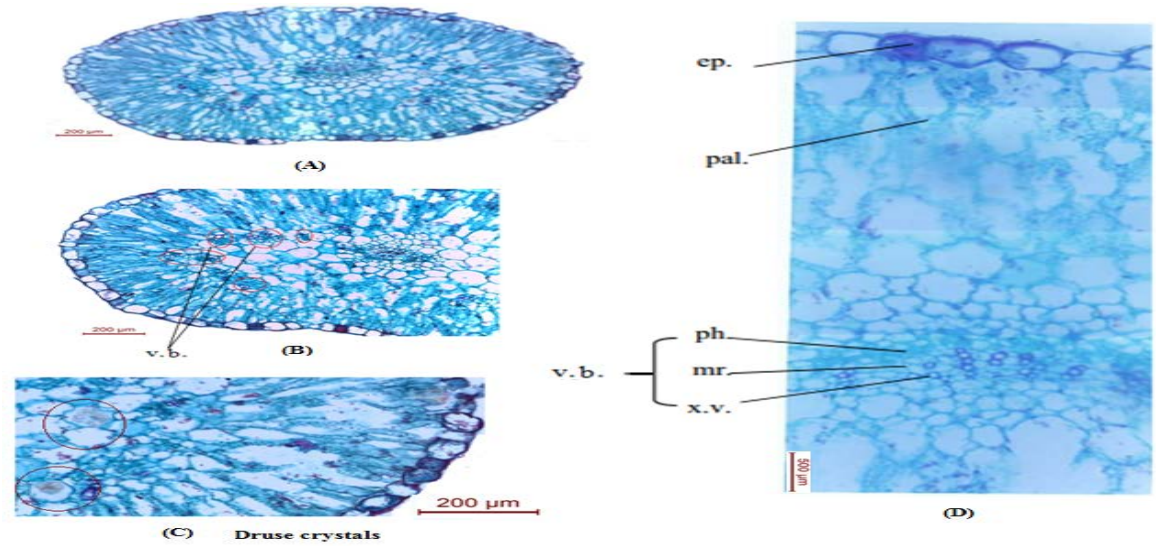

Figure 4. Micromorphology of *G. decandrus* leaf. (A) T.S. overview. (B) T.S. showing vascular bundles. (C) T.S. showing druse crystals. (D) detailed sector. ep, epidermis; mr, medullary rays; pal, palisade; ph, phloem; v.b, vascular bundle x.v, xylem vessels.

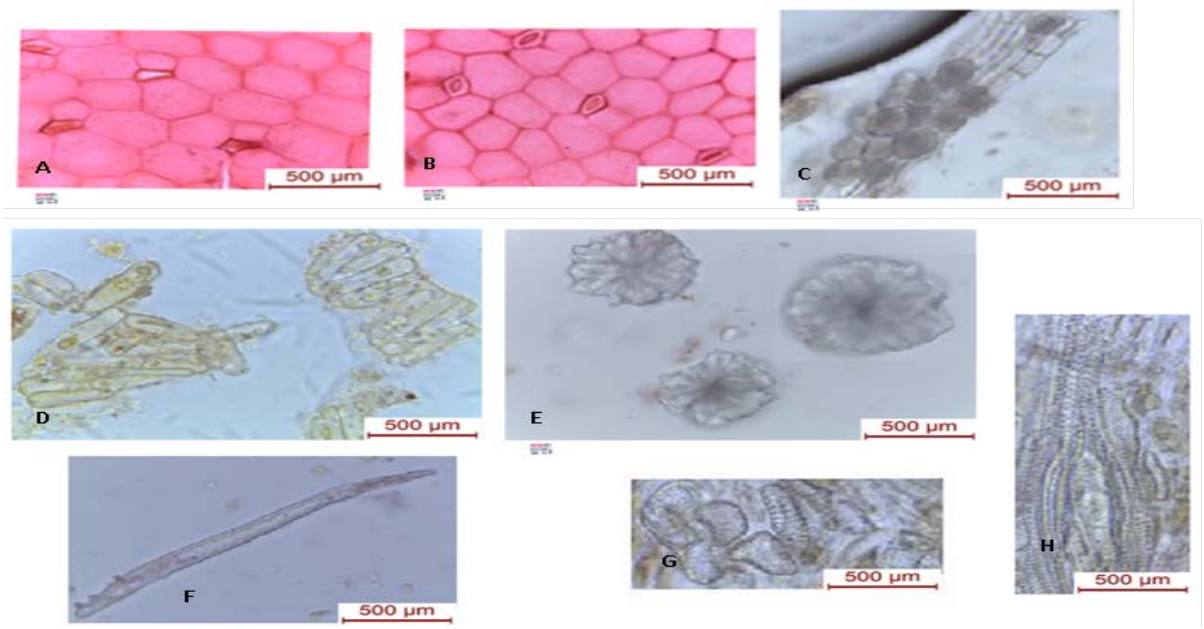

Figure 5. Micromorphology of a *G. decandrus* powdered leaf. (A) upper surface; (B) lower surface; (C) neural epidermis; (D) palisade cells; (E) druse crystals; (F) wood fiber; (G) tracheids; (H) xylem vessels.

Table 1. Dimensions of different elements of *G. decandrus* Forssk stems and leaves in microns.

Item	Length	Width	Diameter
	µm		
Stem			
Cork	20, 38.4, 51.4	22.4, 25.6, 38.4	
Pericyclic fiber	59, 62.5, 68.75	37.5, 50, 56.25	
Xylem vessels			16, 24, 28
Leaf			
Upper epidermis	65, 70, 80	30, 45, 55	
Lower epidermis	55, 90, 95	50, 55, 65	
Palisade	65, 70, 80	11, 15, 35	
Druse crystal			115, 120, 125
Xylem vessels			7.15, 8.19,8.5
Wood fibers	187 ,214, 216	10.5, 13.5,17	

3.3. Estimation of Certain Primary Metabolites

The percentage of total nitrogen, protein, and lipid were 1.68, 10.5 and 0.85 % respectively as tabulated in table (2). The results show that *G. decandrus* contains seventeen protein amino acids (table 3) with leucine (6.27 %) as the major essential amino acid, and aspartic acid (12.87 %) as the major non-essential amino acid. The percentage of the major protein amino acid was that of aspartic acid (12.87 %), followed by glutamic acid (11.94 %) respectively; meanwhile cysteine (0.49 %) was the minor identified protein amino acid.

Table 2. Quantitative estimation of certain primary metabolites in *G. decandrus* Forssk aerial parts.

Item	Percentage
Total nitrogen	1.68
Total protein	10.50
Total lipid	0.85

Table 3. Protein amino acid analysis of the *G. decandrus* Forssk aerial parts.

Essential Amino Acid	%	Non-essential Amino Acid	%
Cysteine	0.49	Arginine	5.07
Isoleucine	3.28	Proline	7.98
Leucine	6.27	Serine	6.41
Lysine	6.00	Glutamic acid	11.94
Methionine	0.94	Glycine	8.98
phenyl alanine	3.96	Alanine	5.61
Threonine	6.07	Aspartic	12.87
Tyrosine	2.49	Histidine	6.62
Valine	5.04		
Total	34.54	Total	65.48

4. Discussion

Different identification methods have been used throughout history including using morphological, anatomical, and chemical profiling. One of the easiest and most economic techniques for plant recognition is the microscopical examination (Singh, *et al.,* 2010). This study presents a complete macro and microscopically identification of the plant.

The current study is in consistence with findings of Metcalfe and Chalk, (1950) who reported the presence of druse crystals of calcium oxalate in this genus. In addition, the presence of anisocytic stomata and the absence of hairs were reported herein for the first time in *G. decandrus* which is not common in Caryophyllaceae.

Amino acids are the building blocks of all vegetable and animal proteins. The current study analyzed and identified the individual amino-acid composition of *G. decandrus*. It is worth noting that the salts of aspartic acid such as magnesium or potassium aspartate have an ergogenic activity either by increasing the metabolism of fatty acids and lowering the utilization of the glycogen in muscles or by reducing the cumulative effect of ammonia in exercise (Williams, 2005). Glutamic acid is necessary for sugar and fat metabolism, and also has a potent antiulcer activity (James and Phyllis, 1997). The high levels of aspartic acid and glutamic acid in *G. decandrus* call for more attention to this important grazing wild plant to explore its new medicinal activities.

References

Arafat MS, Gaafer MA, Amany MB and Shereen LN. 2009. Chufa tubers (*Cyperus esculentus* L.) as new source of food. *World Appl Sci J*. **7(2):** 151-156.

Christie WW. 1982. **Lipid analysis**. Pergamon Press, Oxford.

El-Zanaty RIA, Abdel-Hafez AA, Abdel-Gawad KI, El-Morsy MHM and Abusaief HMA. 2010. Effect of location and growth season on the productivity and quality of some range plants in Wadi Halazien in the North Western Coast in Egypt. *J Nat Sci*., **8(7):** 43-49.

James CS. 1995. **Analytical Chemistry of Foods**. Blackie academic and Professional press. Chemistry. 46: 4358-4362.

James FB and Phyllis AB. 1997. **Prescription for Nutritional Healing**. Avery Publishing Group, 2nd Ed. 38-41.

Meselhy MR, Aboutabl EA and Shoka AAE. 1994. Comparative phytochemical and pharmacological screening of *Polycarpon succulentum*, *Polycarpaea repens* and *Gymnocarpos decandrum* (Caryophyllaceae) growing in Egypt. Bulletin Faculty of Pharmacy (Cairo University), **32:** 399–405.

Metcalfe CR and Chalk L. 1950. **Anatomy of the Dicotyledons**. Clarendon Press, Oxford; London.

Okba MM, Soliman FM and Deeb K. 2013. Botanical study, DNA fingerprinting, nutritional values and certain proximates of *Entada rheedii* Spreng. *Int J Pharm Pharm Sci*, ***5*(3):** 311-329.

Petrusson L and Thulin M. 1996. Taxonomy and biogeography of Gymnocarpos (Caryophyllaceae). *Edinb. J. Bot.*, ***53*(1)**: 1-26.

Ruzin SE. 1999. **Plant Microtechnique and Microscopy**. New York: Oxford University Press.

Sallam A and Galala AA. 2017. Inhibition of alpha-amylase activity by *Gymnocarpos decandrus* Forssk Constituents. *Int J Pharm Phytochem Res*, ***9*:**.873-879.

Sathiyamoorthy P, Lugasi-Evgi H, Schlesinger P, Kedar I, Gopas J, Pollack Y and Golan-Goldhirsh A. 1999. Screening for cytotoxic and antimalarial activities in desert plants of the Negev and Bedouin market plant products. *Pharm Biol*, ***37*(3)**: 188-195.

Singh S, Machawal L and Chauhan MG. 2010. Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on microscopic technique. *Journal of Pharmacognosy and Phytotherapy*, ***2*(5):** 71-75.

Williams M. 2005. Dietary supplements and sports performance: amino acids. *J Int Soc Sports Nutr*. ***2*(2):** 63.