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PHARMACOGNOSTICAL STUDY OF GLADIOLUS SEGETUM KER-GAWL. CULTIVATED IN EGYPT.

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ABSTRACT

Detailed macro-and micromorphology of the root and corm of G. segetum Ker-Gawl. are given to facilitate their identification in both entire and powdered forms.

The pet.ether extract of the leaves, corms and roots of the plant afforded α-amyrin, B-amyrin and B-sitosterol. The fatty acids and the mucilage of the different organs were analysed by GLC. The relative viscosity of each mucilage was determined.

INTRODUCTION

Gladiolus segetum Ker-Gawl. (Iridaceae) is a herbaceous perennial plant cultivated in Egypt as an ornamental plant[1,2,3].

It has been reported that corms of some Gladiolus species were used in folk medicine as a remedy for dysentry, impotence, for relief of rheumatic pains and the smoke from the burning corms is sometimes inhaled for colds[4].

Reviewing the current literature revealed that Gladiolus atroviolaceus was proved to contain anthocyanidins (petunidin, malvidin, delphinidin, peonidin and pelargonidin) and flavonols (quercetin and kaempferol)[5]. Also it was reported that genus Gladiolus contains ascorbic acid[6] and saponin[7]. Nothing, however, was traced in the current literature dealing with the botanical study or the study of lipid and mucilage contents of G.segetum Ker-Gawl. Therefore, it was thought desirable to undertake this study.

EXPERIMENTAL

Plant Material:

Samples were collected from G.segetum Ker-Gawl. cultivated in public and private gardens in Cairo district The plant was kindly identified by Dr.A. Fayed, Assistant Professor, Faculty of Science, Dept. of Botany , Univ. of Assiut. The samples were separated into their individual organs viz: roots, corms and leaves.

a) For the botanical study, fresh material, as well as, material preserved in alcohol 70% containing 5% glycerin were used. For the study of the powder, the roots and corms were air dried separately and powdered (No. 36).

b) For the chemical study, samples were air-dried in shade and powdered (No. 36).

c) For the study of mucilage, fresh samples were boiled with ethanol to destroy the enzyme activity. The ethanol was decanted and the marc was dried in an oven at 60°C and powdered(No. 10).

A- Botantical Study:

a- Macromorphology:

Gladiolus segetum Ker-Gawl. (Iridaceae) is a herb with erect, simple, leafy stem, attaining height of about 30-55 cm.

The leaves are 3-4 in number, simple, sword-shaped, 25 to 40 cm long, and 1 to 2 cm. broad at the middle and many-nerved.

The inflorescence is a spike with 5 to 8 pink flowers. The plant flowers in Egypt at February and March.

The roots (Fig. 1) are adventitious covering the flattened surface of the corm. The root is cylindrical and fleshy, measuring 3-10 up to 14 cm. long and from 0.2 to 0.7 cm in diameter. The young roots are pale brown in colour while the old ones are dark brown. The dried root breaks with a short fracturs exposing a whitish interior, it has a faint odour and a bitter taste.

The corm: (Fig. 1). The fresh corm is globose, swollen underground stem. It varies in size from 2 to 3 up to 5 cm in diameter. The outer surface is rough and brown in colour, while the interior of the corm is yellowish white. The dried corm is brittle and breaks with a short mealy fracture. It has a sternutatory odour and bitter taste.

MICROMORPHOLOGY

The Root:

A transverse section in the root(Fig. 2A) is nearly circular in outline. It shows an outer irregular, brown protective tissue consisting of ruptured epidermis and exodermis, a wide parenchymatous cortex limited with a distinct endodermis surrounding a complete ring of central stele. The stele is surrounded by a parenchymatous pericycle and encloses from 7-12 groups of alternating arcs of primary xylem and phloem on separated radii.

The epidermis (Fig. 2A) consists of a single layer of mostly ruptured subrectangular cells with thickened lignified walls. Arising from the epidermal cells of the young root few unicellular non-glandular unbranched hairs. The hairs measure 490-510-568 µ long and 24-51-80 µ wide.

The exodermis (Fig. 2,A &B) is formed of 3-5 rows of brownish cells with lignified wavy walls. The cells are polygonal, subrectangular, measuring 16-48-88 µ high , 32-112-136 µ long and 32-56-96 µ wide.

The cortex (Fig. 2,A &B) comprises a wide region of polygonal isodiametric to rounded thin-walled parenchyma, The outer zone of the cortex shows small cells but the inner region shows wide cells with very narrow intercellular spaces. Some cells contain masses of mucilage which swell and partially dissolve in water, but they

are insoluble in alcohol and stain with Ruthenium Red (T.S.). Few small, rounded and single starch granules are scattered in the parenchyma and measure from 18-36-75 μ in diameter, No calcium oxalate could be detected.

The endodermis is distinct single layer of usually square to subrectangular cells(Fig. 2B) with U-shaped tnickened, lignified radial and inner tangential walls. These cells are tangentially elongated with thick walls and narrow lumen (Fig. 2 D) measuring 220-360-480 μ long and 30-40-56 μ wide and 32-41-58 μ high.

The stele (Fig. 2A & B) consists of 7-12 altergating arcs of primary xylem and phloem. The pericycle marks the outer-one to two rows of the stele, it consists of cellulosic,thin-walled parenchyma, but the pericyclic cells adjecent to the primary xylem are formed of fibres with thick lignified walls, narrow lumen and measuring 400-456-495 μ long and 20-24-40 μ wide. The phloem is represe-nted by small thin walled elements.

The xylem is polyarch, composed of groups of lignified vessels with reticulate, spiral and pitted thickening and measuring 75-80-180 μ in diameter. The central pith is formed of polygonal isodiametric lignified cells. In surface view, the cells are tangentially elongated with thick pitted walls measuring 96-360-390 μ long and 40-48-103 μ wide.

The Powder:

Powdered root (Fig. 2D) is brown in colour with sternutatory odour and bitter taste. It is characterised by the following:

1- Fragments of irregular brown lignified cells of the exodermis.
2- Fragments of polygonal, subrectangular, brown epidermal cells.
3- Fragments of the endodermis, tangentially elongated cells having thick stratified walls and narrow lumen.
4- Fragments of lignified pericyclic fibres each with narrow lumen and somewhat pointed ends.
5- Fragments of lignified vessels with reticulate, spiral and pitted thickening.
6- Fragments of subrectangular parenchyma with lignified pitted walls.

The Corm: (Fig.3 A & B) A transverse section in the corm is nearly rounded in outline, somewhat irregular. It shows an outer cork enclosing a broad ground tissue. The bundles are scattered throughout the ground tissue. The bundles are closed collateral.

The Cork consists of 5 to 12 regular layers of brownish lignified tabular cells appearing polygonal with straight anticlinal walls in surface view(Fig. 3C) and measuring 24-32-48 μ high, 128-133--168 μ long and

80-104-120 μ wide. The two or three outermost layers of the ground tissue are formed of rounded to oval collenchyma cells.

The ground tissue: consists of polyhedral or rounded parenchyma cells. The cells have thin walls and showing narrow intercellular spaces. Most of these cells are packed with starch granules and styloids of calcium oxalate. The starch granules are simple or compound of 2 to 3 components. The individual granules are round or oval in shape with hardly distinct hilum and measure about 18-36-90 μ in diameter. Styloids of calcium oxalate measure 100-128-152 μ long and 16-24-40 μ wide. Many cells contain masses of mucilage which stain red with ruthenium red (T.S.) and blue with methylene blue and give no colour with coralin soda.

The vascular system is represented by numerous, closed , collateral vascular bundles widely separated from each other. Each bundle consists of lignified vessels nearly surrounding a batch of phloem. The vessels are lignified and showing spiral, scalariform and pitted thickenings and measuring 50-70-100 μ in diameter. The phloem consists of sieve tubes and companion cells.

The Powder:

The powdered corm(Fig. 3D) is brownish in colour having a sternutatory odour and bitter taste. It is charactrised by:

1- 1- Fragments of brownish, lignified, polygonal cork cells with straight anticlinal walls.
2- Fragments of cortical cells containing starch granules and mucilage masses, as well as styloids of calcium oxalate.
3- Fragments of lignified vessels with spiral, reticulate and pitted thickening.
4- Numerous starch granules, either free or in the parenchyma cells.

B- Chemical Study:

Study of Lipid:

The air-dried powdered leaves, corms and roots(200 g each) were separately extracted with pet.ether(b.r. 60-80°C) and each extract was separately concentrated under reduced pressure. The residue obtained in each case(5 g) was separately subjected to saponification[8]. The unsaponifiable matter in each case was screened on silica gel G (E-Merch) plates using choroform-methanol (99.5 : 0.5) as solvent system. Only 5 spots were located in each case. The unsaponifiable matter(2 g) was fractionated over a column of neutral alumina(Prolabo) using the eluents pet.ether and pet.ether-ethyl acetate gradient. As a results α-amyrin (20 mg, m.p. 183-6°C), B-amyrin (40 mg, m.p. 197-8°C) and B-sitosterol (25 mg, m.p. 135-8°C) were isolated. The IR, m.p.,m.m.p. and cochromatography of the three isolated compounds were found to be identical with those reported for authentic samples

The liberated fatty acids in each case were, separately methylated[9] and the resulting methyl esters were separately analyzed by GLC (Table 1)

Study of the Mucilage:

a) Cold extraction mucilage (CEM)[10]

Powdered sample (10 g) of the leaves, corms and roots were separately mixed with one liter of distilled water, stirred for 12 hours at 28°C, and kept at the same temperature for further 2 hr. The solution, in each case, was passed through a folded muslin. The process was repeated, till no cloudiness was produced on the addition of 4 volumes of alcohol to one volume of the aqueous extract. The mucilage, in each case, was precipitated from the combined aqueous extract by slowly adding 4 volumes of 95% ethanol while stirring. The precipitate obtained by centrifugation, was washed several times with ethanol till free from inorganic ions. The mucilage was then vigorously stirred in absolute acetone, then in dry ether, filtered and dried in vacuum desiccator over anhydrous calcium chloride and weighed.

b) Hot Extraction mucilage (HEM)[11]

The marc left after exhaustive extraction with cold water was repeatedly treated with boiling water until complete extraction of the mucilage was affected(tested as mentioned before). The mucilage was precipitated by 95% ethanol and purified as described above. The

percentage of CEM and HEM of each organ are given in Table 2.

Hydrolysis of Mucilage:

Mucilage of each organ(CEM and HEM)(200 mg), was separately heated[12] with 5 ml. 0.5 M H_2SO_4 in a sealed tube for 20 hr. in a boiling water bath. The hydrolysates of the mucilages under investigation were neutralised with $BaCO_3$, filtered and the filtrates were evaporated under reduced pressure.

GLC of Hydrolysates:

The trimethylsilyl ether drivatives of each hydrolysate were prepared[13] and gas chromatographed.The percentage of the different sugars in each case are given in (Table 3).

Viscosity of mucilages:

The relative viscosity of the mucilages under investigation was determined by means of Ostwald viscometer at 28°C, using 1% aqueous solution of mucilage. Relative viscosity is expressed in centipoise with reference to water as solvent. Results are given in Table 4.

RESULTS AND DISCUSSION

The TLC investigation of the unsap. of the different organs of Gladiolus segetum Ker-Gwal. proved

their identity. Only 5 spots were located in each organ. Three of them were isolated and characterised as ∝-amyrine, B-amyrin and B-sitosterol. The Fatty acids from the soap fractions of the pet. ether extracts. of the different organs were methylated and analyzed by GLC. Results presented in Table 1 show that myristic, myristoleic, palmetic, stearic, oleic and linoleic acids are present in lipid fraction of all the investigated organs, but in different percentages. Palmetic acid was found to be the major acid being 45.4% in the leaves, 56.3% in the corms and 52.4% in the roots.

The different organs of the investigated plant can be arranged according to their mucilage contents in a descending order as follows: corms(26.2%g % w/w), roots (10.5%) and leaves (7.2%). Each mucilage was acid hydrolyzed and the silylated hydrolysates were analyzed by GLC. Results in Table 3 showed that galactose is the major constituent in the mucilage (from 30.9-44•4%) followed by arabinose(from 14.9-26.8%), xylose(13.2-23.4%), galacturonic acid (9.7-14.1%) and glucose (6.1-13.7%).

According to the viscosities(Table 4), the different mucilages under investigation can be arranged in a descending order as follows: The corms (5.1), roots(4.8) and leaves (4.3).

Table 1: GLC* analysis of methylated fatty acids present in the lipid fraction of the different organs of Gladiolus segetum Ker-Gawl.

Peak No.	*Fatty acids*	*r*	*Percentage of fatty acids*		
			Leaves	*Corms*	*Roots*
1	--	0.02	0.8	traces	traces
2	--	0.06	traces	traces	traces
3	myristic	0.25	14.4	11.1	10.2
4	myristoleic	0.33	6.1	5.1	4.3
5	palmitic	0.49	45.4	56.3	52.4
6	stearic	0.92	9.7	4.2	8.1
7	oleic	1.00	12.1	13.2	16.1
8	linoleic	1.25	11.3	9.7	8.7

Legend:r: retention time relative to that of oleic acid.

* GLC operating conditions:
column: coiled glass, 5 feet long, 4 mm i.d., packed with Reoplex 400 adsorbed on celite (100-120 mesh); carrier gas, nitrogen at a flow rate of 60 ml/min. hydrogen flow rate, 400/ml/min.; column temp., 200°C; detector oven temp., 230°C; attenuation 50 x 10^{-1}; Chart speed, 1 cm/min.; sample size, 0.02 ml.

Table 2: Precentage of CEM and HEM of the examined organs.

Plant organs	*Percentage**		
	CEM	*HEM*	*Total*
Leaves	5.1	2.2	7.3
Corms	20.9	5.3	26.2
Roots	6.4	4.1	10.5

* The experiment was done in triplicate.

Tabe 3: Percentage of different suger components of mucilages by GLC*

Sugers	*Leaves*		*Corms*		*Roots*	
	CEM	*HEM*	*CEM*	*HEM*	*CEM*	*HEM*
Arabinose	24.4	19.1	18.5	14.9	26.8	19.2
Xylose	19.1	13.2	21.2	23.4	20.6	16.9
Galactose	38.2	42.3	44.4	36.5	30.9	41.2
Glucose	7.3	11.1	6.1	13.7	11.3	8.9
Galacturonic acid	10.9	14.1	9.7	11.4	10.3	13.8

* GLC operating conditions:
column, 3% SE 30; oven programer, 150:200°C with 6°C/min; [illegible] x 10^{-2}; [illegible] oven temperature 255°C; nitrogen flow rate, 60 ml/min; hydrogen flow rate, 60 ml/min; air flow rate, 400 ml/min; chart-speed 1 cm/min.

Table. 4: Relative viscosity of different mucilages of investigated organs

Plant organ	*Viscosity in centipoise*	
	CEM	*HEM*
Leaves	4.3	3.9
Corms	5.1	4.2
Roots	4.8	4.2

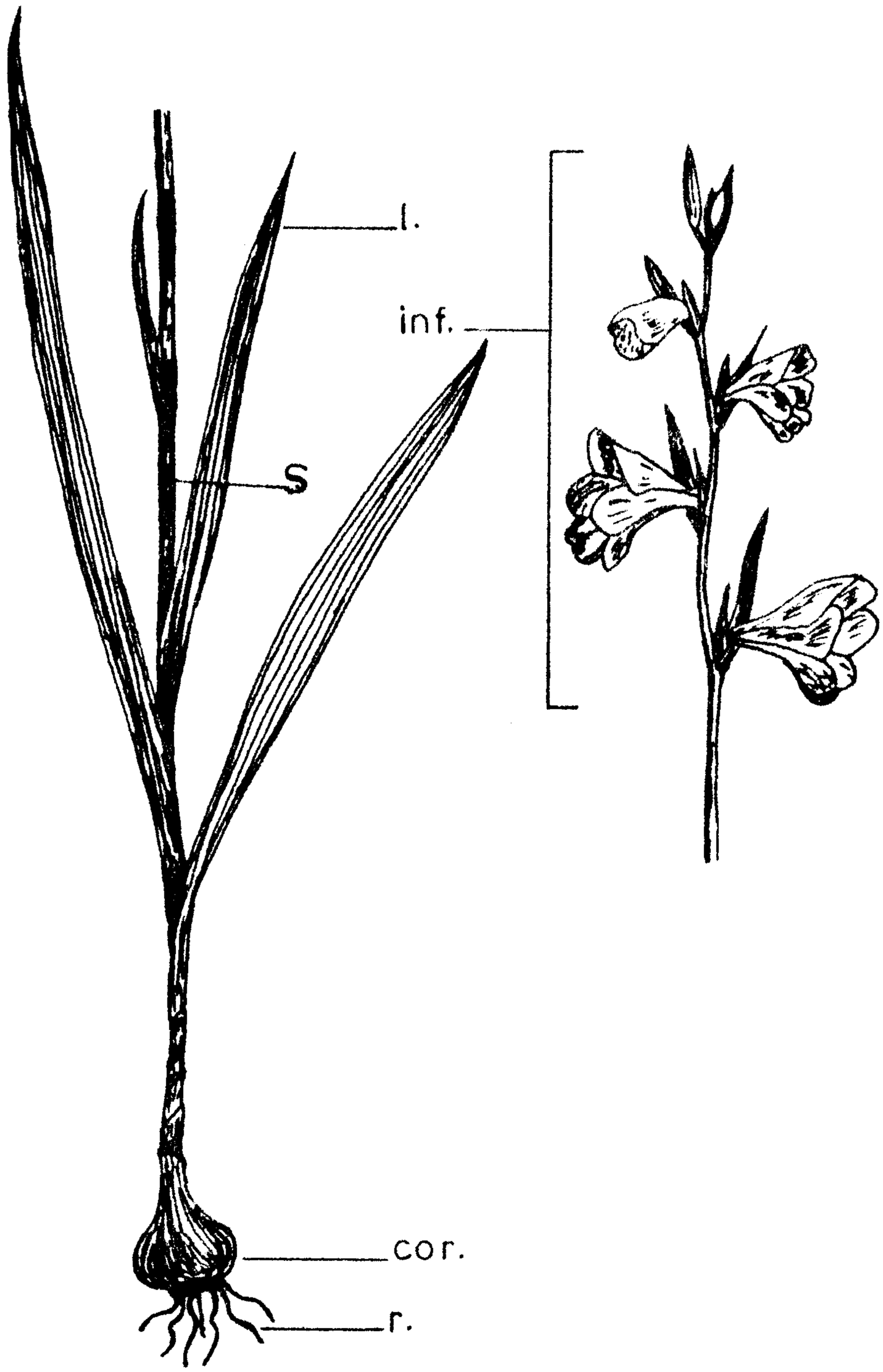

Fig. 1: Sketch of Gladiolus segetum Ker-Gawl X 1/3

cor., corm; inf., inflorescence; l., leaf; r., root; s., stem.

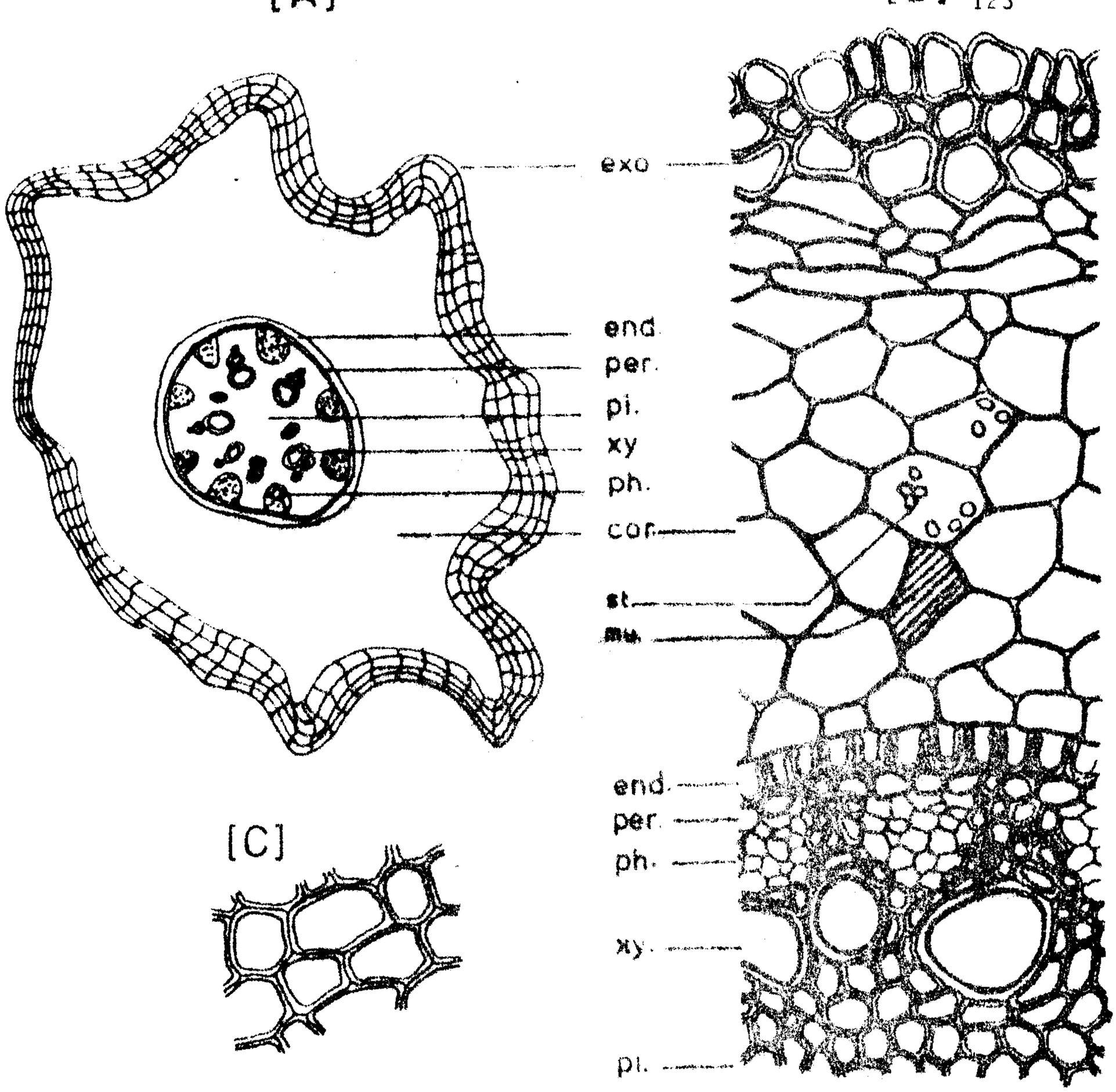

Fig. 2: The Root

A- Diagrammatic T.S. X 70
B- Detailed T.S. X 145
C- Surface preparation X 145
D- Isolated elements X 145

cor., cortex; end., endodermis; exo., exodermis; mu., mucilage; n.g.h. non-glandular hair; per., pericycle; ph., phloem; st., starch granules; pi., pith; xy., xylem.

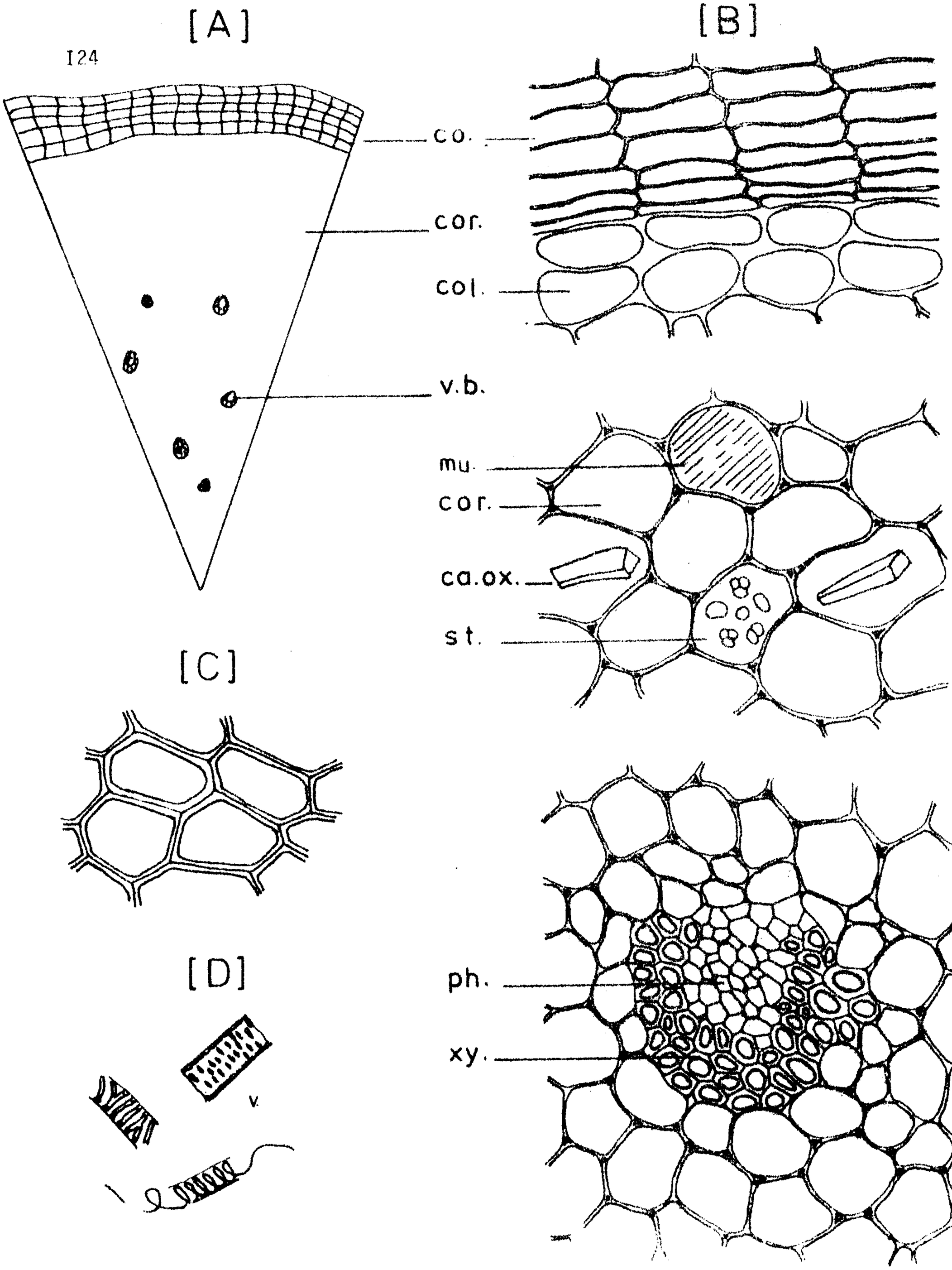

Fig. 3: The Corm

A- Diagrammatic T.S. X 30
B- Detailed T.S. X 125
C- Surface View of the cork X 125
D- Isolated element X 125

caox., calcium oxalate; co., cork; col., collenchyma; cor., cortex; mu., mucilage; ph., phloem; st., starch; v., vessels; v.b., vascular bundle; xy., xylem.

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دراسة عقاقيرية لنبات
جلاديولوس سيجيتم كر ـ جـــــــول.

احمد عبد الرحمن على ـ محمد احمد الشنوانى ـ مصطفى كامل مصباح
وسمير انيس روس
قسم العقاقير ـ كلية الصيدلة ـ جامعة اسيوط

يتعرض البحث الحالى الى:

١ـ دراسة عيانية ومجهرية لكل من جذور وكرومات هذا النبات وذلك حتى يمكن التعرف عليها فى حالتها الصحيحة او على هيئة مسحوق.

٢ـ دراسة خلاصة الاثير البترولى وقد تم فصل والتعرف على مواد الفا أميرين، بيتا اميرين وبيتا سيتوستيرول.

كما تم دراسة الاحماض الدهنية لاوراق وجذور وكرومات هذا النبات وعينت النسبة المئوية للاحماض المختلفة فى اجزاء النبات الثلاثة وذلك بواسطة كروماتوجرافيا الغاز.

٣ـ تم استخلاص ودراسة المواد الهلامية فى اجزاء النبات(الورق ـ الجذور ـ الكرومات) وتحديد النسبة المئوية لموكوناتها باستخدام كروماتوجرافيا الغاز.

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