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PHARMACOGNOSTICAL STUDY OF TECOMA ARGENTEA BER. & SCHUM CULTIVATED IN EGYPT

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ABSTRACT

The macro and micromorphology of the stem and leaf of Tecoma argentea (Ber. & Schum) are presented to show the diagnostic characters of these organs by which they could be identified and differentiated in the entire and powdered forms. The lipid content is also studied using TLC techniques revealing the presence of B-sitosterol and --amyrin.

From the methanolic extract of the leaves seven flavonoids were isolated. Their structures were established by physical, chemical and spectral methods, proved to be: luteolin, scutellarein, auercetin, 6-hydroxyluteolin-7-0-glucoside, chrysin-7-0-glucoside, scutellarein-7-0-rhamnoside and rutin.

INTRODUCTION

Tecoma argentea (Ber. & Schum.) Family Bignoniaceae was introduced from Argentina and South America and cultivated as an ornamental plant in Aswan Botanic Island.

Tecoma argentea as well as other Tecoma species contain several monoterpene-derived alkaloids of the pyrindone skeleton 1-4. Some of these compounds were reported to have antidiabetic effect 5, 6. It was also used to relieve pain, pneumonia, high fever and to produce sleep. For bleeding of the gums the powder is rubbed round the teeth.

Reviewing the current literature, no further informations could be traced regarding the macro and micromorphology of the different organs of the plant as well as phytochemical screening.

In the present work; the macro-as well as micromorphological features of the stems and leaves, are illustrated, also the lipid and flavonoid content are studied.

EXPERIMENTAL

Material:

Collection was made from plants cultivated in Aswan Botanic Island in May 1981, authentication of the plant was kindly confirmed by Director of the Botanic Island.

1- Study of the lipid content of the leaves:

l kg. of the dried leaves were extracted with pet. ether. The pet.ether extract was evaporated, the residue was saponified with N/2 alco. KOH⁷. The unsaponifiable fraction was extracted with other and the ether was evaporated. The residue was investigated using TLC and benzene-ethylacetate (86:14 V/V) alongside with authentic samples. The plates were sprayed with antimony trichloride in chloroform. The unsaponifiable fraction reveals the presence of B-sitosterol and α -amyrin.

2- Isolation of flavonoids:

The defatted powdered leaves were extracted with methanol and concentrated under reduced pressure. The residue '300g.) which remained after alcohol removal was extracted with ether and ethyl acetate in a separatory funnel successively.

The ethyl acetate (200 g.) was chromatographed over silica gel column (800 g.E"Merck") repared by slurry method. Elution was started with chloroform and chloroform methanol gradient (500 ml.) each fraction.

The flavonoid compounds were eluted in fraction N. 10. The subsequent preparative paper chromatography of the eluted fractions using Whatman N.3 MM and 15% HOAc system 1. led to isolation of the compounds T1-T4.

Acid hydrolysis:

10 mg. of the glycoside in question were subjected to acid hydrolysis by refluxing with 10 ml. of 2N H₂SO₄. The hydrolysate was extracted with ether, the ether extract was tested for the presence of aglycones. The liberated aglycones were chromatographed on Whatman I.1 using n-butanol-acetic acidwater (4:1:5 V/V) system 2 ... developing system. The aqueous phase remaining after that was neutralized with barium carbonate and examined by PC for the liberated sugars using n-butanol-pyridine-water (5:3:3 V/V) as solvent system and appropriate authentic sugars.

RESULTS AND DISCUSSION

From the ether soluble fraction three aglycones(luteolin, quercetin and scutellarein) were isolated using preparative paper chromatography(Whatman N.3 MM) in system 2. Identification of these aglycones was confirmed by colour reactions in UV after and before ammonia solution, m.p., UV-analysis and co-chromatography with authentic samples.

Pharmacognostical stuyd of Tecoma argentea (Eer. & Schum) cultivated in Egypt.

Compound T1:

Pale yellow crystals m.p. 190 c^o,hR_f18 and 15 in systems 1 and 2 respectively, purple colour in UV,purple-brown after exposing to ammonia, exhibited UV-absorption at λ_{max},288,350 nm in MeOH; + NaOMe, 280,400 nm; + AlCl₃,280,440 nm; +AlCl₃/HCl₃,282,378 nm; + NaOAc, 285,354 nm; +NaOAc/H₃BO₃,276,365 nm. On acid hydrolysis as mentioned above, it revealed 6-hydroxy-luteolin m.p. 325 C^oas an aglycone and glucose as a sugar.

The assignment of an oxygen substituent to C_6 and not C_8 was confirmed by the UV spectral data for both the aglycone and the glycoside based on the fact that when flavones and flavonols contain a C_6 oxygen substituent and a free C_5 hydroxy group, Band 1 shows, in presence of AlCl $_3$ /HCl, only about a 25 nm bathochromic shift (relative to MeOH spectral) instead of the usual 40-60 nm shift 8,9 .

By comparing these data with that reported for 6-hydroxy_luteolin-7-0-glucoside 10,11,12 were superimposable.

The compound Tl is 6-hydroxyluteolin-7-0-glucoside.

Compound T2:

It was isolated as pale yellow amorphous substance m.p. 192° c, hR_f20 and 56 in systems 1 and 2 respectively. UV-analysis λ_{max} ,270, 310 nm, MeOH; + AlCl₃,275,380 nm; no shift with NaOAc of either band. It is sparingly soluble in usual organic solvents, soluble in pyridine.

Acid hydrolysis afforded chrysin 13 and glucose.

From the above data it was concluded that the compound T2 is chrysin-7-0-glucoside(4-hydroxyluteolin-7-0-glucoside).

Compound T3:

Yellowish-green crystals, m.p. 233°C, from methanol, upon acid hydrolysis scutellarein was obtained as an aglycone and rhamnose as a sugar, identified by colour reactions, UV-analysis, mixed m.p. and co-chromatography with authentic samples.

From these data it is confirmed that compound T3 is scutellarein-7-0-rhamnoside.

Compound T4:

Yellowish-green needle shaped crystals, by physical, chemical, spectral, mixed m.p. and co-chromatography with authentic samples of rutin was identified.

So that the compound T4 is quercetin-3-0-rutinoside (rutin). The preliminary phytochemical study of the leaves of Tecoma argentea revealed the presence of free and combined flavonoids. Paper chromatographic screening for the ethereal extract proved the presence of at least three flavonoidal aglycones, and four flavonoidal glycosides in the ethylacetate extract.

The 6-hydroxyluteolin was first suspected from the purple-brown colour of the spot on a paper chromatogram under UV light which was unchanged when exposed to ammonia vapour. It was discovered in 1967, isolated from Catalpa and Tecoma species (Bignoniaceae).

However chrysin-7-0-glucoside is very common in several plants belongs to Family Bignoniaceae, although it is very rare to occur in Tecoma species 14.

Habitat:

The plant (Fig. 1) is a tall erect, deciduous tree, attaining 7-8 m. in height and carrying numerous branches.

Pharmacognostical study of Tecoma argentea (Ber. & Schum) cultivated in Egypt.

It bears compound, imparipinnate exstipulate, alternate leaves. The flowering period extends from March to early May.

1- THE STEM

Macromorphology: (Fig. 1).

The main stem is erect; cylindrical to subcylindrical in outline and solid, with monopodial branching; young branches having green colour with smooth surface. The old branches are woody, dark green to brownish green in colour The stem has faint odour and slightly bitter taste.

Micromorphology: (Fig. 2).

A transverse section in the stem (Fig.2A & C) is circular to irregular in outline, showing an epidermis covered with thick smooth cuticle followed by a cortex and a pericycle surrounding a continous ring of vascular tissu; with a wide parenchymatous pith in the centre.

The epidermal cells (Fig. 2B) are square, axially elongated, thin walled with straight anticlinal walls. They measure $80\text{-}90\text{-}160~\mu$ in length and $20\text{-}30\text{-}40~\mu$ in width. Nonglandular trichomes are present being unicellular, bicellular and rarely multicellular uniseriate formed of 2-4 cells covered with warty cuticle. Stomata of anomocytic type are present.

The cortex (Fig. 2C) is comparatively narrow formed of an outer layers of rounded collenchyma cells, followed by parenchymatous cells with intercellular spaces. They contain prisms of calcium oxalate and few small rounded starch grains.

The pericycle (Fig. 2C) is formed of 2-4 rows of parenchymatous cells interrupted by tangentially elongated batches of fibres. The fibres are lignified with wide lumena and accuminate or rounded apices and measuring $15-20-25~\mu$ in

diameter.

The phloem consists of compressed elements with shining thin cellulosic walls. The cambium consists of 3-4 layers of thin-walled tangentially elengated cells. The xylem (Fig.2C) consists of lignified thick walled, radially arranged elements, traversed by narrow medullary rays with slightly lignified walls.

The vessels are arranged in radial rows. They have spiral, pitted and scalar form thickening. They measure 35-40-50 μ in diameter. They are accompanied by tracheids, wood fibres and wood parenchyma. The pith is comparatively wide consisting of large polyhedral to rounded parenchymatous cells with thick, pitted and slightly lignified walls. The powder: (Fig. 2D)

Powdered young stem is green in colour with slight odour and bitter taste. The important diagnostic microscopical features of the powder are:

- 1- Fragments of polygonal axially elongated epidermal cells with nearly straight anticlinal walls and covered with smooth cuticle and showing anomocytic stomata.
- 2- Nonglandular trichomes, unicellular or bicellular, uniseriate covered with warty cuticle.
- 3- Fragments of lignified fibres with wide lumena and accuminate or rounded apices.
- 4- Fragments of vessels of pitted, spiral and scalariform types.
- 5- Fragments of lignified, pitted wood parenchyma, medullary ray cells and pith.
- 6- Fragments of tracheids, pitted and lignified.
- 7- Fragments of parenchymatous cells containing few small rounded simple starch grains and prisms of calcium oxalate, sometimes with pitted lignified walls.

Pharmacognostical study of Tecoma argentea (Ber. & Schum) cultivated in Egypt.

II- THE LEAVES

Macromorphology:

The leaves (Fig. 3) are compound imparipinnate, alternate and exstipulate, consisting of usually 7 leaflets. The leaflets measure 7-12 cm. in length and 5-7 cm. in width, they are oppositly arranged on a nearly cylindrical axis.

The lawina of the leaflets is ovate to nearly cordate in shape with entire margin, assymetric base and acute to blunt apex. The leaflets are shortly petiolate with reticulate venation. Leaflets are whitish green in colour, papery intexture. It has faint odour and slight bitter taste. The leaf rachis is nearly cylindrical with a longitudinal groove on the upper side.

Micromorphology:

A transverse section in the lamina (Fig. 4A) reveals the upper and lower epidermises enclosing in between dorsiventral mesophyll which is replaced in the midrib by the vascular strands and the cortical tissue. The bundle of the vein is cricular, consisting of several groups of fibres surrounding a phloem ring to the outside and xylem ring inwards. It surrounds a central pith.

The epidermis (Fig. 4 B & C) consists of tangentially elongated cells covered with thick smooth cuticle. in surface view the cells appear polygonal, isodiametric with wavy anticlinal walls and measuring 30-35-50 μ in length, and 20-35-40 μ in width.

The lower epidermis is formed of one layer of cells differing from the upper epidermis in being some what square. Ranunculaceous (anomocytic) stomata are present only on

the lower surface, being surrounded by 4-6 cells.

Trichomes of the glandular and non-glandular types are observed on the lower surface only. The glandular hairs consist of unicellular stalk and a multicellular head of 7-15 radiating cells measuring $23-42-50~\mu$ in diameter and $20-35-40~\mu$ in length.

Nonglandular trichomes are observed on the lower surface only. They re usually unicellular, occasionally bicellular and rarely tricellular uniserriate covered with warty cuticle and have blunt apices. They measure $110-\underline{140}-190~\mu$ in length. The upper epidermis is followed by one layer of hypodermal cells. The mesophyll (Fig. 4D) is heterogenous dorsiventral, with one layer of palisade cells interrupted by a mass of collenchyma in the midrib region. The palisade cells have nearly straight walls, measuring $20-\underline{60}-70~\mu$ in length and $15-\underline{20}-25~\mu$ in diameter. The spongy tissue is formed of more or less rounded to irregular parenchymatous cells with wide intercellular spaces. It contains few starch grains and prismatic crystals of calcium oxalate which are scattered in the mesophyll.

The cortical tissue of the midrib (Fig.5) shows an upper and lower subepidermal collarchymatous masses. The rest of cortical tissue is formed anded or polyhedral parenchymatous cells measuring $20-\underline{40}-120~\mu$ in diameter, with wide intercellular spaces and contain few starch grains and prismatic crystals of calcium oxalate, measuring 3-5 μ in length.

The pericycle is formed of lignified fibres, possessing wide lumena and acuminate or rounded apices. They measure $15-25~\mu$ in diameter.

The vascular tissue (Fig. 5) consists of an arch of xylem and arch of phloem. The phloem is formed of relatively wide zone of soft elements. The xylem is formed of lignified,

Pharmacognostical study of Tecoma argentea (Ber. & schum) cultivated in Egypt.

spiral, pitted and scalariform vessels, measuring $20-30-35\mu$ in diameter.

A transverse section in the rachis (Fig. 4F) is more or less rounded in outline, with two rounded ridges on its upper side and shallow groove inbetween. It consists of an epidermis surrounding the cortex which is formed of collenchyma from the outside and parenchyma to the inside, containing few prisms of calcium oxalate.

The vascular system (Fig. 4F) consists of centric contionous ring of vascular tissues with wide central pith and smaller two lateral vascular bundle present twards the upper side of the rachis. The vascular tissue consisting of xylem and phloem surrounded by complete ring of fibres.

Two lateral bundles correspond to the two ridges. The pericycle of the main central ring consists of patches of fibres alternating with small groups of parenchymatous cells.

The Powder: (Fig. 4 F)

Powdered leaf is pale green in colour with characteristic odour and slightly bitter taste. It is characterised by:

- 1- Fragments of the upper epidermis from lamina showing polygonal cells sometimes elongated with wavy anti-clinal walls, covered with smooth cuticle.
- 2- Fragments of the lower epidermis with numerous anomocytic stomata and glandular hairs with multicellullar head of 7-15 radiating cells and a unicellular stalk.
- 3- Fragments of nonglandular trichomes which are unicellular, or bicellular rarely multicellular uniseriate covered with warty cuticle.
- 4- Fragments of heterognous mesophyll showing palisade cells and spongy parenchyma.
- 5- Fragments of lignified pericyclic fibres with wide lumena and blunt apices.
- 6- Fragments of spiral, pitted and scalariform vessels.



Fig. 1: Phatograph of the plant X 1/100

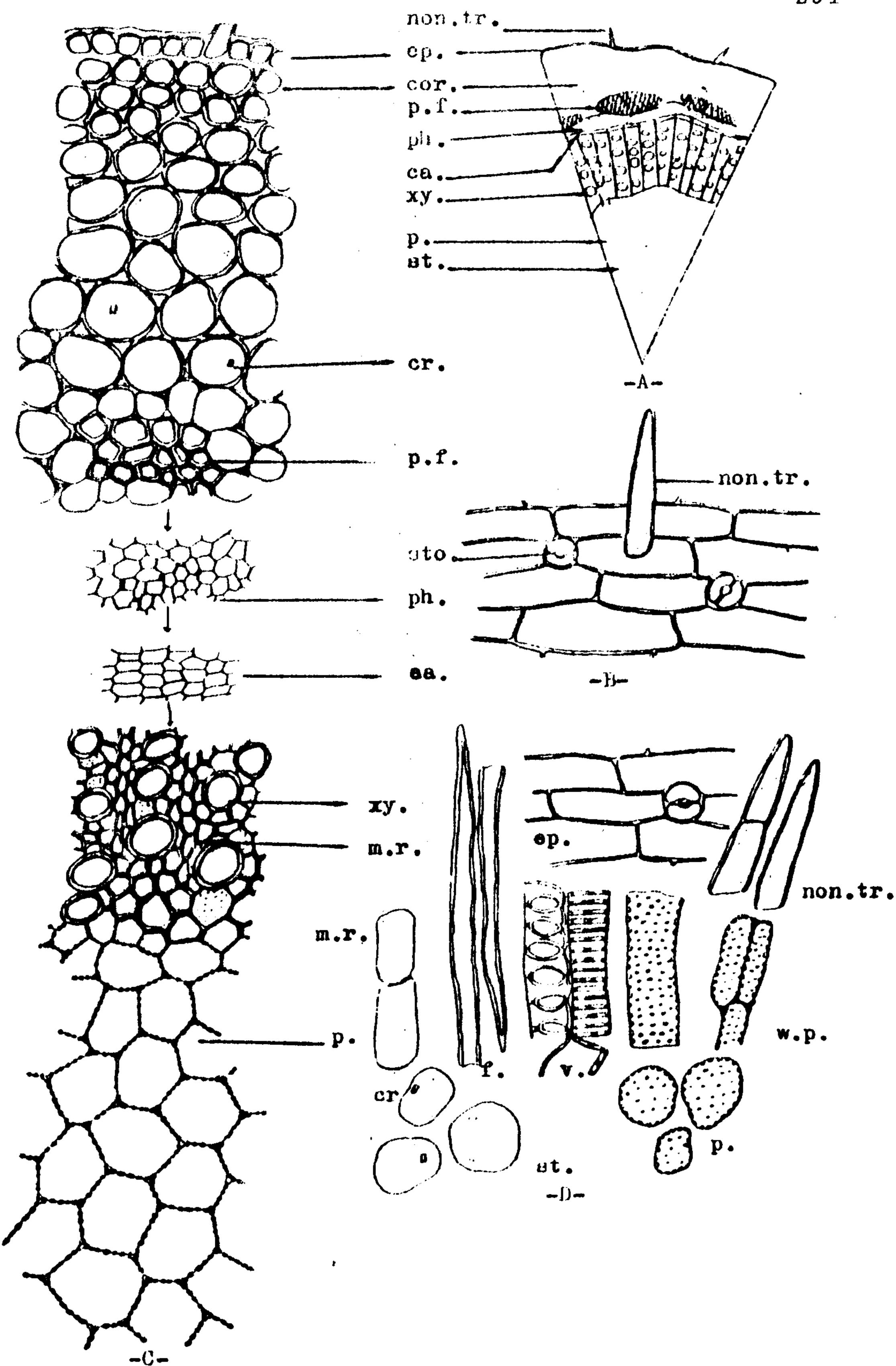


Fig.	2:	A-	Diagrammatic T.S. of the stem	X	10	
		B-	Surface preparation of the stem	X	80	
		C-	Detailed T.S. of the stem	X	80	
		D-	Isolated elements of the stem	X	80	
	ca.	, ca	mbium; cor., cortex; cr., calcium oxalate; ep., epi	d e :	rmis;f.,	
	fibres; m.r., medullary ray; non.tr., nonglandular trichomes p., pith; p.f., pericyclic fib. s; ph., phloem; st., starch grains sto., stomata; v., vessel; w.p., wood parenchyma; xy., xylem.					

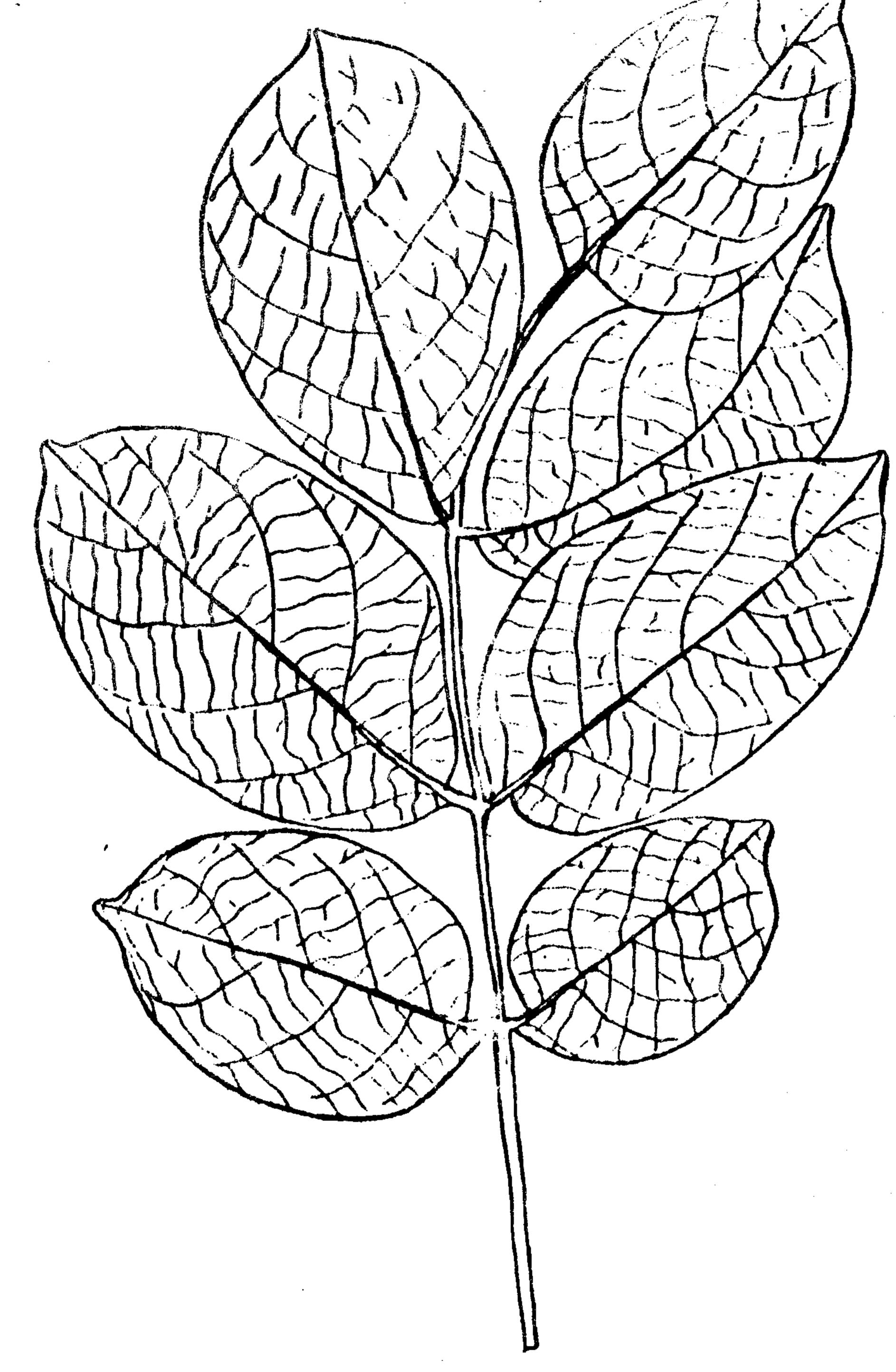


Fig. 3: Sketch of the branch

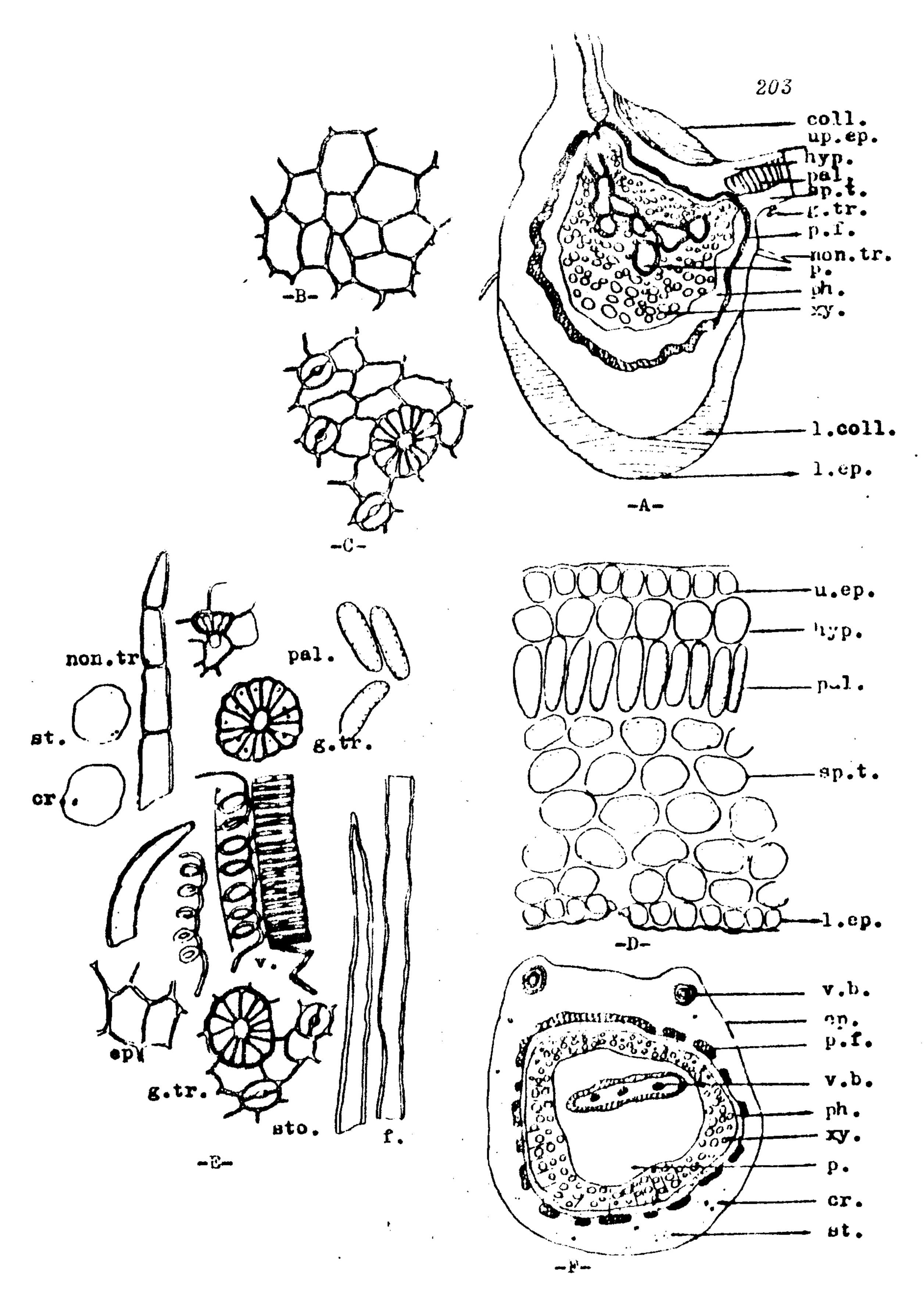


Fig. 4:	A- Diagrammatic T.S. of the leaflet	X 10
	B- Surface preparation of the upper epidermis	x 80
	C- Surface preparation of the lower epidermis	x 80
	D- Detailed T.S. of the lamena	x 80
	E- Isolated element of the leaf	x 80
	F- Diagrammatic T.S. of the rachis	X 10

coll., collenchyma; cr., calcium oxalate; ep., epidermis; f., fibres; g.tr., glandular trichomes; hyp., nypodermis; l.coll., lower collenchyma; l.ep., lower epidermis; non.tr., nonglandular trichomes; p., pith; pal., palisade; p.f., pericyclic fibres; ph., phloem; sp.t., spongy tissue; st., starch grains; sto., stomata; up.ep., upper epidermis; v., vascular bundles; xy., xylem.

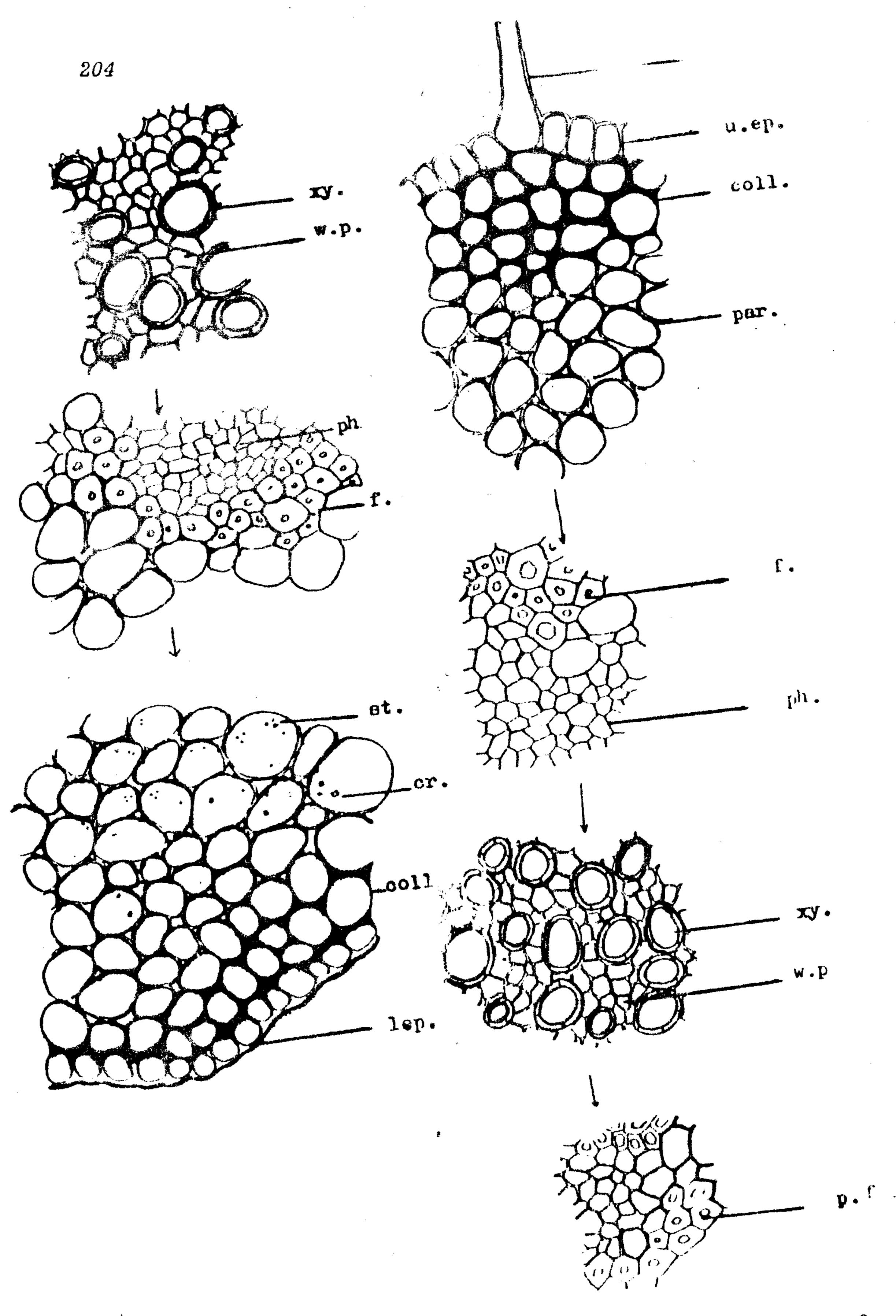


Fig. 5: Detailed T.S. in the midrib region of the leaf X 80 coll., collenchyma; cr., calcium oxalate; f., fibres; l.ep., lower epidermis; non.tr., nonglandular trichomes; par., parenchyma; p.f., pericyclic fibres; ph., phloem; st., starch grains; u.ep., upper epidermis; w.p., wood parenchyma; xy.xylem.

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دراسة عقاقيرية لنبات التيكوما ارجنت بيرواسكم المنزرع في مصر مقبول احمد مقبول - عفاف محمد عبد الباقي - داود ونيس بشاي قسم العقاقير - كليسة الصيدلسة - جامعسسة اسميوطسس

تمت الدراسة العيانية والمجهرية لسيقان واوراق نبات التيكسومسسا ارجنتا وذلك بفرض التعرف عليها كاملة كانت او على هيئة مسحوق •

كماتمت دراسة الجزء الغير متصبن وامكن التعرف على وجسسود بيتساسيتوستيرول والفا اميرين وبدراسة خلاصة الكعول المثيلى تم فمسلال الفلافسونيدات الحسرة وذلك باستخلاصها باثير وامكن التعرف عليها وهسسلالين د اسكيوتلارين وكسوارستين ٠

من خلاصة خلات الایشیل تم فصل ٦- هیدروکسی لیّتیولین -٧- جلوکوزیده، کریزین - ۷ - جلوکوزید ،اسکیوتیلارین - ۷ رامنوزید وروشین ۰

وامكن التعرف عليها بدراسة صفاتها الفيزيائية والكيمائية وطلبرق تحليلها تحت الاشعة الفوق بنفسجية والحلملة الحمضيسة ،