VEGETAL STAINS *A GOGÓ*

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INTRODUCTION

Since I practice microscopy as a hobby, one of my favorites activities I like to develop is the confection of tissue preparations from living beings - especially vegetal - using "histological techniques adapted to my kitchen". With modern observation methods (phase contrast, DIC, fluorescence, etc.) and the practice of molecular biology, the techniques used in professional research are no longer as necessary as the beginning of the biology's development on the microscope. However, they have a particular interest in teaching as well as in the field of clinical pathology, where they keep using stains for the diagnosis of many diseases.

It is true that for enthusiasts it is easier to focus their observations on the existing microbiology in stagnant drops of water collected in ponds or infusions made at home. The apparatus and the number of reagents necessary to perform staining are much higher and more expensive, in addition to the difficulty of making the cuts. However, despite the difficulties, it is a field full of challenges from the point of view of laboratory work, which for us give a great deal of satisfaction and hours of pleasant distraction.

In this article I report a small collection of classic stains and some more updated in the field of plant histology, extracted from classic plant micro-technical texts and other recent articles, with the aim of checking the results, already described and expected and eventually, prepare my own collection of preparations.

MATERIALS AND PROCEDURES

For staining, the most immediate thing is to make cross sections of stems between 4 and 6 mm in diameter. Of all species I have practiced with, I have chosen the A*ralia cordata* plant according the good results it offers when it is processed, presenting a large number of tissues and therefore eye-catching colorations. It is also easy to get.

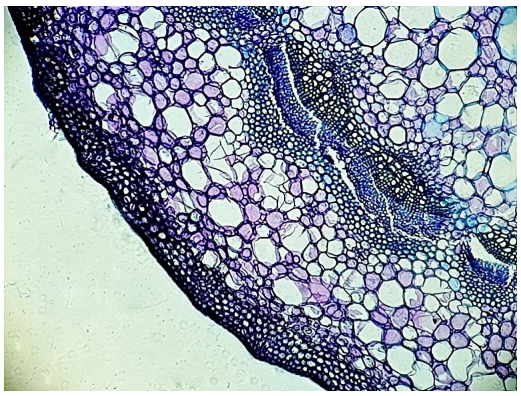
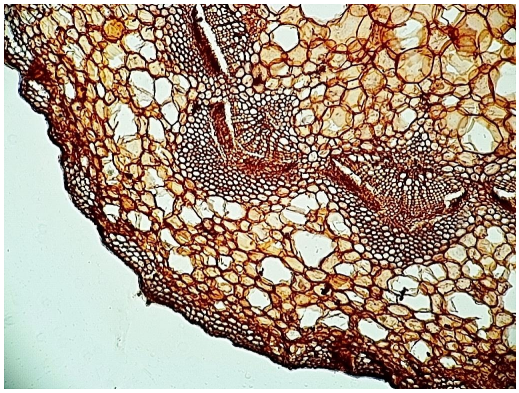
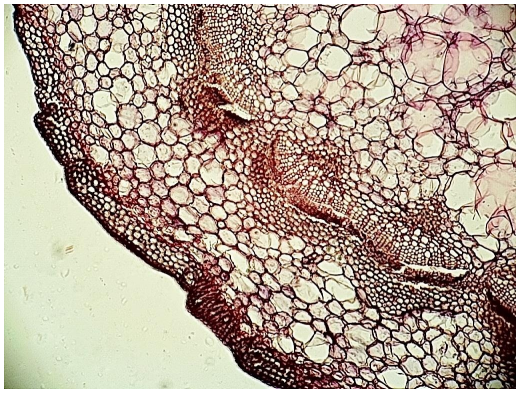
The material, once cut from the plant, was subdivided into pieces of about 2 cm and was fixed in 70º ethanol or FAA between 24 and 48 hours. After washing them in water, they underwent an inclusion process in PEG 1500 to obtain blocks and from them, obtaining cuts with a hand microtome and a histological knife. After that, the sections were kept in water to dissolve the PEG. Finally, each staining described and the most appropriate mount for each staining were performed.

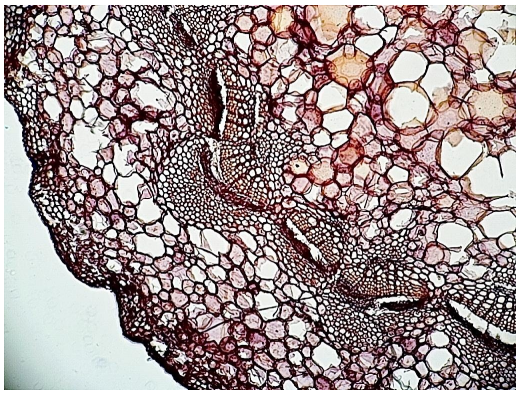
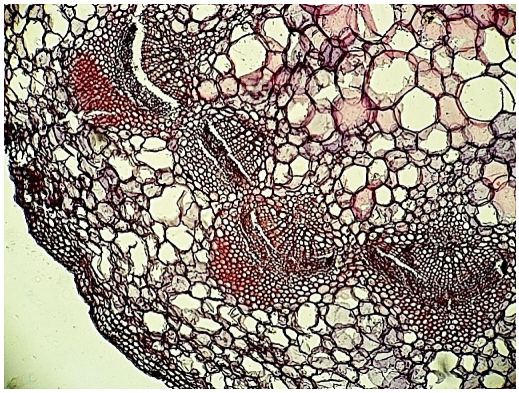
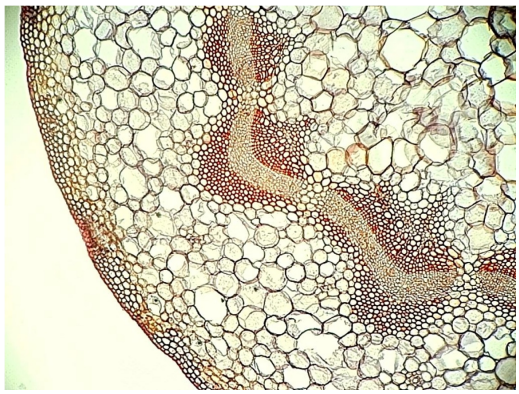
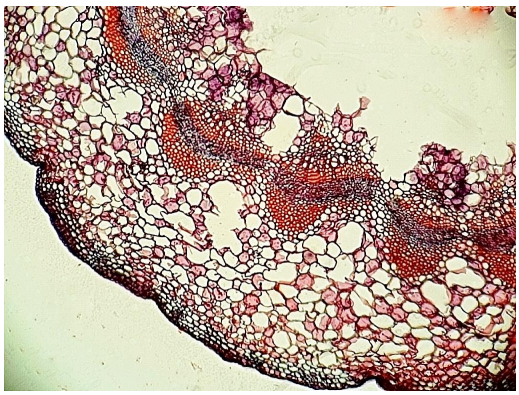
The preparations were left for a few days to stabilize and the mounting medium to solidify at some extent. Subsequently, photomicrographs were obtained. The equipment used was an Olympus CX 31 microscope (fitted with a BX series trinocular head) equipped with a 5 Mp USB camera. For the shots, the MiCam 2.4 and ZP Combine Program was used to stack between 6 and 8 shots.

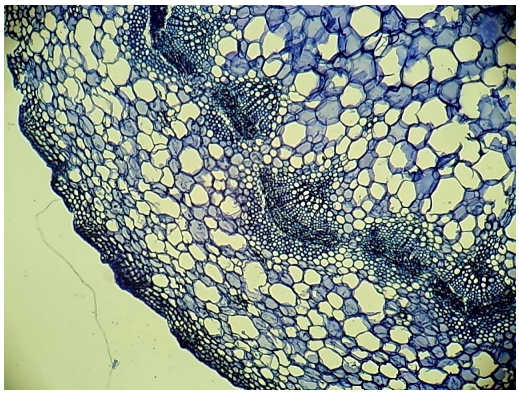
A simplified scheme of each staining is added to each photomicrograph. These have been classified by groups according to the number of dyes and characteristics of the stains. Some of the sections are a little bit damaged, but I preferred to leave them following the objective that all the photos showed the same area and they belonged to the two inclusion blocks used, that is, to the same piece of processed stem.

1. SIMPLE DYES WITH OR WITHOUT COUNTER STAINING

These are stains made with a single dye, generally basic in aqueous solution, which stains walls and cell nuclei. To add optical contrast, a counter stain can be used with another cytoplasmic dye, in aqueous or alcoholic solution with high graduation (70º-96º). According to this second solvent, the mount can be aqueous or resinous if it is alcohol, taking advantage of the fact that the material is practically dehydrated and it only needs to be cleared before assembling.

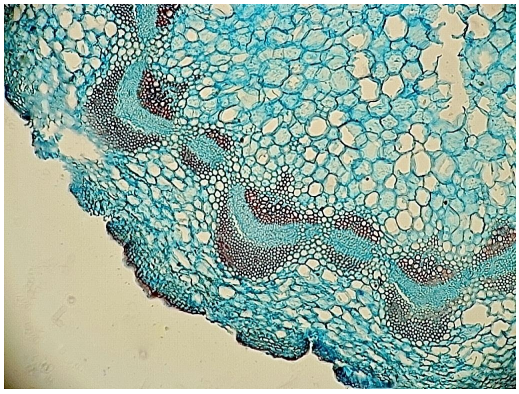
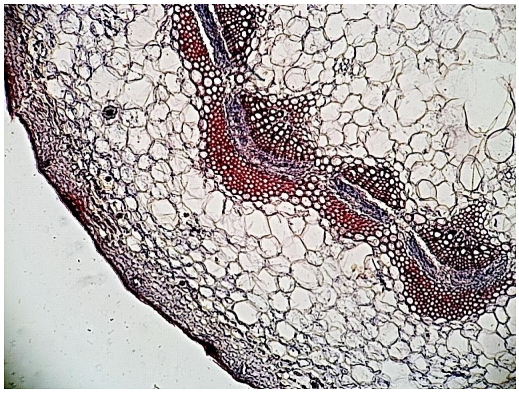
	**Toluidine blue** ≤ Stain in TBO 4 min. ≤ Wash with water. ≤ Mount in aqueous medium.
	**Delafield Hematoxylin (Orange G)** ≤ Stain in DH 10 min. ≤ Wash with water. ≤ Counterstain with OG. ≤ Wash with EtOH 96º. ≤ Clear. ≤ Mount in resinous medium.
	**Delafield Hematoxylin (Eosin)** ≤ Stain in DH 10 min. ≤ Wash with water. ≤ Counterstain with EO. ≤ Wash with water. ≤ Mount in aqueous medium.

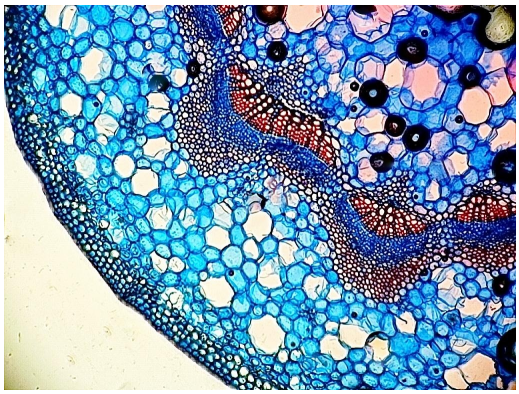
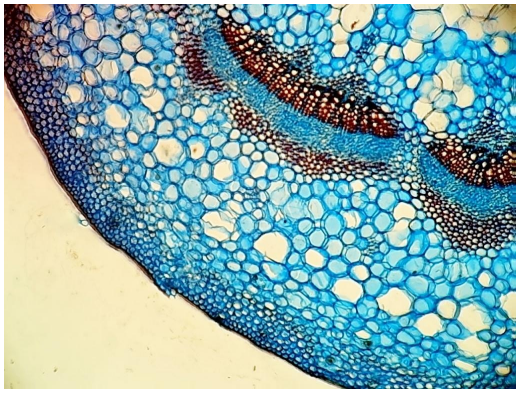
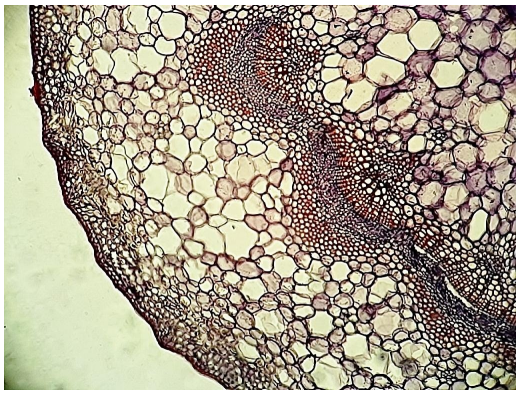
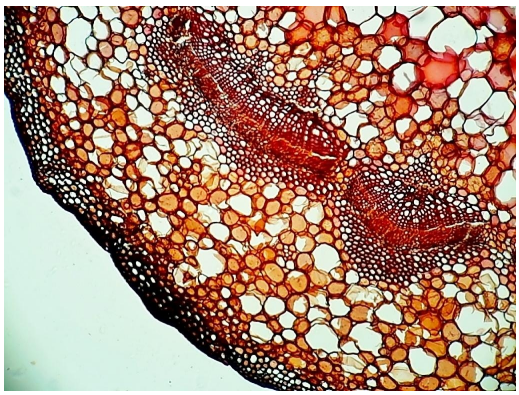
	**Ehrlich Hematoxylin (Orange G)** ≤ Stain in EH 10 min. ≤ Wash with water. ≤ Counterstain with OG. ≤ Wash with EtOH 96º. ≤ Clear. ≤ Mount in resinous medium.
	**Ehrlich Hematoxylin (Eosin)** ≤ Stain in EH 10 min. ≤ Wash with water. ≤ Counterstain with EO. ≤ Wash with water. ≤ Mount in aqueous medium.
	**Gentian violet (Orange G)** ≤ Stain in GV 10 min. ≤ Wash with water. ≤ Counterstain with OG. ≤ Wash with EtOH 96º. ≤ Clear. ≤ Mount in resinous medium.
	**Gentian violet (Eosin)** ≤ Stain in GV 10 min. ≤ Wash with water. ≤ Counterstain with EO. ≤ Wash with water. ≤ Mount in aqueous medium.

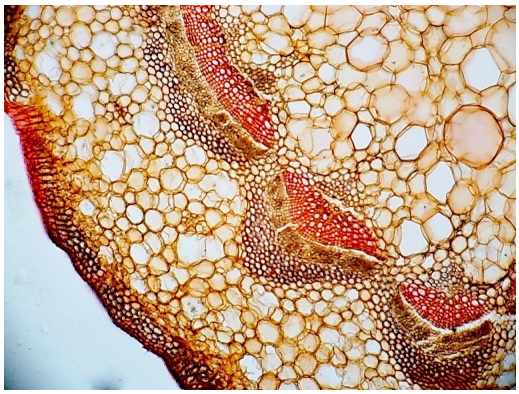
	**Gentian violet (IKI)** ≤ Mordant IKI 15 min, wash with water. ≤ Tinción con GV 15 min. ≤ Wash with water. ≤ Mordant IKI 1-5 min. ≤ Dehydrate and clear. ≤ Mount in resinous medium.

2. DOUBLE STAINING WITH SAFRANIN

Safranin is undoubtedly the most widely used dye in botanical stains. It is normally used in aqueous or alcoholic solutions with low graduation, as the first dye to dye lignified and suberized cellular walls.Subsequently, another dye that has an affinity for cellulose or even lignin is used. It is advisable to differentiate Safranin with acidified alcohol before applying the second dye, so that Safranine stains only what interests us.

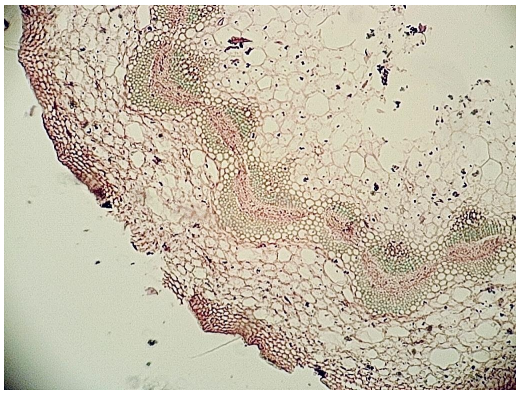
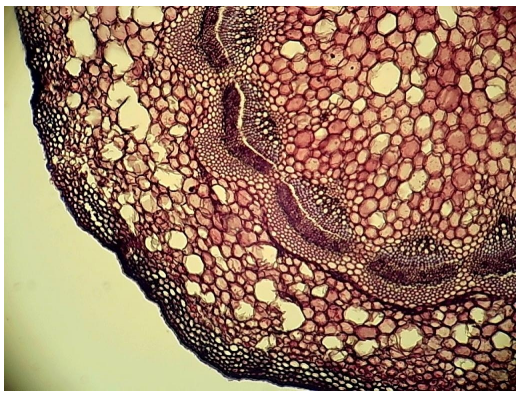
	**Safranin-Fast green FCF** ≤ Stain with SA 30 min. ≤ Wash with water. ≤ Differentiate and dehydrate. ≤ Stain with FG 10 s. ≤ Wash with EtOH 96º and clear. ≤ Mount in resinous medium.
	**Safranin-Anilin blue** ≤ Stain with SA 30 min. ≤ Wash with water. ≤ Differentiate. ≤ Stain with AB 15 min. ≤ Mount in aqueous medium.

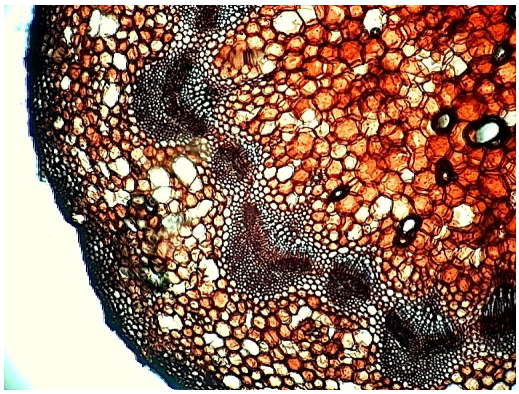
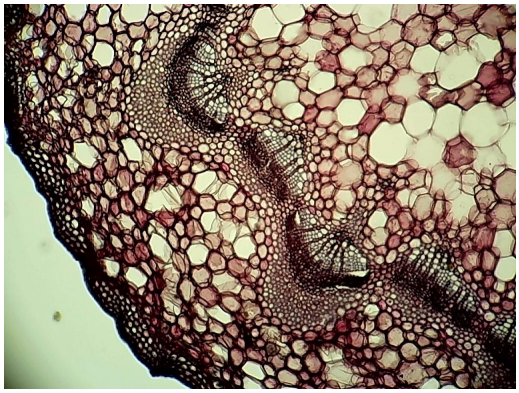
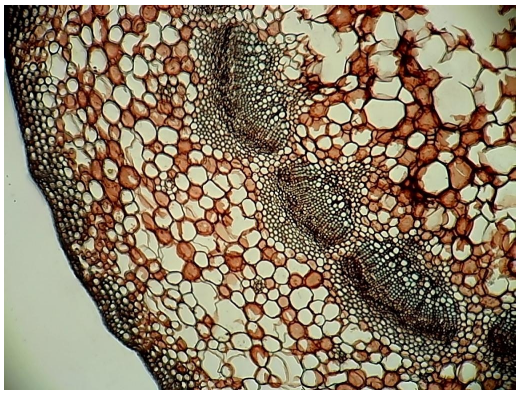
	**Safranin-Alcian blue** ≤ Stain with SA 30 min. ≤ Wash with water. ≤ Differentiate. ≤ Stain with AAl 10 min. ≤ Mount in aqueous medium or... ≤ Dehydrate and clear. ≤ Mount in resinous medium.
	**Safranin-Astra blue** ≤ Stain with SA 30 min. ≤ Wash with water. ≤ Differentiate. ≤ Stain with AA 10 min. ≤ Mount in aqueous medium or... ≤ Dehydrate and clear. ≤ Mount in resinous medium.
	**Safranin-Delafield Hematoxilyn** ≤ Stain with SA 30 min. ≤ Wash with water. ≤ Differentiate with acidulated EtOH. ≤ Stain with DH 15 min. ≤ Wash with acidulated water, tap water and distiled water. ≤ Mount in aqueous medium.
	**Safranin-Gentian violet** ≤ Stain with SA 30 min. ≤ Wash with water. ≤ Stain with GV 15 min. ≤ Differentiate. ≤ Wash with water. ≤ Mount in aqueous medium.

	**Safranin-Orange G** ≤ Stain with SA 30 min. ≤ Wash with water. ≤ Stain with OG. ≤ Wash with EtOH 96º. ≤ Clear. ≤ Mount in resinous medium.

3. DOUBLE STAINS WITH GREEN DYES FOR LIGNIN

Green dyes that have an affinity for lignin are used in these stains (there are also those that have an affinity for cellulose). Specifically, they can be Iodine Green, Malachite Green or Methyl Green. A red dye is usually used for cellulosic walls, giving an inverse coloration to that of SAVR,

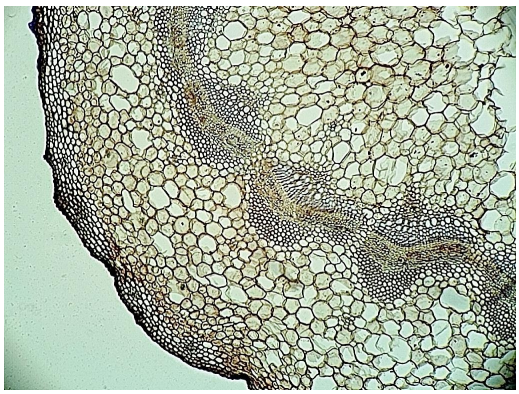
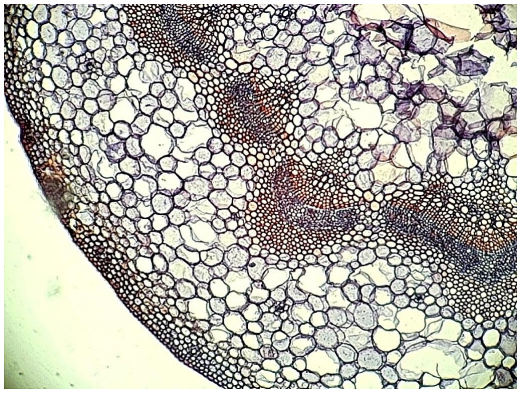
	**Malachite green-Grenacher's Carmin** ≤ Staining with MaG 30 min. ≤ Wash with water. ≤ Differentiation. ≤ Staining with CA1 h or more. Wash. ≤ Aqueous or ... ≤ Dehydration and rinse. ≤ Resin mount.
	**Malachite green-Acid fuchsin** ≤ Staining with MaG 30 min. ≤ Wash with water. ≤ Differentiation. ≤ AF staining 3 min. Wash. ≤ Aqueous or ... ≤ Dehydration and rinse. ≤ Mount in resinous medium.

	**Malachite green-Congo red** ≤ Staining with MaG 30 min. ≤ Wash with water. ≤ Differentiation. ≤ Staining with CR 5 min. ≤ Wash with water. ≤ Aqueous mount.
	**Methyl green-Acid Fuchsin** ≤ MG staining 30 min or more. ≤ Wash with water. ≤ AF staining 3 min. ≤ EtOH wash. ≤ Aqueous or ... ≤ Dehydration and clear. ≤ Resin mount.
	**Methyl green-Congo red** ≤ Staining with MG 30 min. ≤ Wash with water. ≤ Staining with CR 5 min. ≤ Wash with water. ≤ Aqueous mount.

4.TRIPLE STAINING

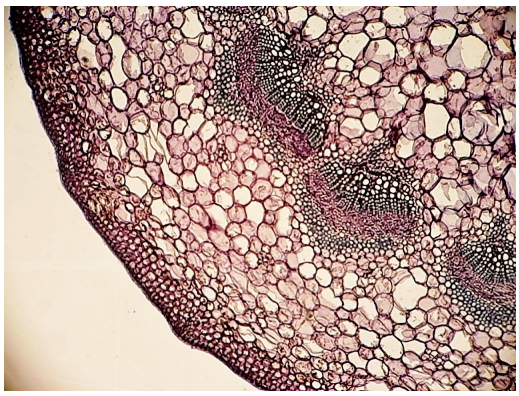
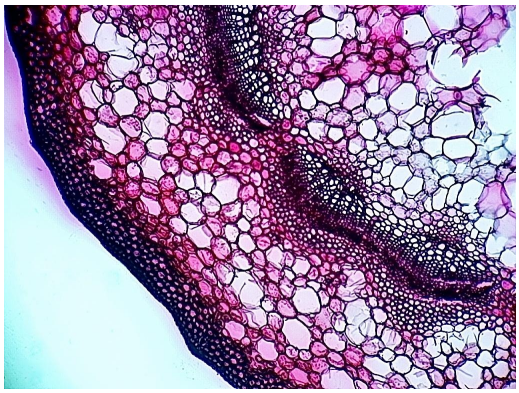
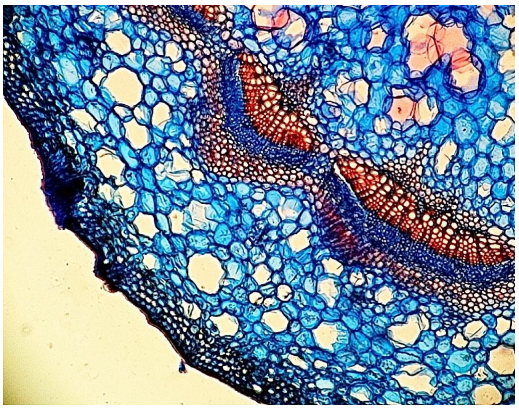
These are stains to color many types of tissues or to reveal different organelles of cells (cytological staining). They are complex to perform but offer very attractive results in thick cuts and with duller shades in very fine cuts.

If they are used as cytological stains, they require a very precise fixation. Some of these stains have multiple variants, such as Flemming's.

	**Methyl green-Acid fuchsin-Eosin (Cooper's triple stain, dyes in aqueous medium)** ≤ Stain with MG 30 min, wash. ≤ Stain with AF 3 min, wash. ≤ Stain with EO 10 s, wash. ≤ Mount in aqueous medium.
	**Safranin-Gentian violet-Orange G (Flemming's triple stain)** ≤ Stain with SA 30 min, wash. ≤ Stain with GV 5 min, wash. ≤ Deshydrate. ≤ Differentiate with OG. ≤ Clear. ≤ Mount in resinous medium.

5. SIMULTANEOUS DOUBLE STAINING

They are double stains that are carried out in a single step, preparing a dye solution mixing the two dyes. These can have different or equal affinity for certain tissues, so in some cases they compete, however, given the application times of the dye solution, very good results are achieved. The advantages of this type of staining are the time in which they are carried out and the elimination of the need for differentiation between the two dyes.

	**Delafield Hematoxilin-Methyl green** ≤ Dye solution: DH and MG aqueous y 1 %, in proportions 5:1 to 10:1. ≤ Leave to act for 10 min. ≤ Wash with slightly acidified water and then distilled water. ≤ Aqueous or ... ≤ Dehydration and rinse. ≤ Resin mount.
	**Basic fuchsin-Methyl green** ≤ Dye solution: BF and MG in aqueous solutions at the same percentage and in a 4: 1 ratio. ≤ Leave to act for 10 min. Wash with water and alcohol alternately. ≤ Aqueous or ... ≤ Dehydration and rinse. ≤ Resin mount.
	**Safranin-Alcian blue** ≤ Coloring solution: SA and AB in aqueous solutions at the same percentage and in proportions between 1: 4 to 1: 7. ≤ Leave to act for 10 min. ≤ Wash with water. ≤ Aqueous or ... ≤ Dehydration and rinse. ≤ Resin mount.

REFERENCES

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3.- Rebecca Luque et col, *Astra Blue and Basic Fuchsin double staining of plant materials*. *Biotechnic & Histochemistry, **73** (1998) 5.*

4.- Tolivia, D. and Tolivia, J., *Fasga: a new polychromatic method for simultaneous and differential staining of plant tissues, Journal of Microscopy, **148** (1987) 1*

5.- Walter Dioni. *Safe microscopic techniques for amateurs. Part two, solidifying media.* MICSCAPE MAGAZINE, January 2003.

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