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HISTOCHEMICAL LOCALIZATION OF PLANT: A BASIC TOOL FOR DETECTION AND CHARACTERIZATION OF PLANT METABOLITE

Laxmishree S.Chengala[1]* & Mandakini Ingle[2]

[1]Department of Botany, G.M. Momin Women's College, Bhiwandi, Dist. Thane, Maharashtra*

[2]Department of Botany, Satish Pradhan dnyanasadhana College, Thane, (MS)

ABSTRACT

Histochemical strategies play a considerable role in the identity and classification of plants. By using diverse staining techniques and observing tissue-particular chemical compounds, these histochemical studies enable a deeper knowledge of the plant's shape and biochemical composition. Additionally, advancements in histochemical staining, virtual imaging, and molecular integration have improved plant identification processes. A low-cost and incredibly informative analytical method, histolocalization makes it simple to map the distribution of metabolites in newly discovered medicinal plants, the drug portion that contains the metabolites that need to be investigated will then require any further effort. This review discusses the standards, methodologies, and applications of histochemical strategies in plant identification, highlighting their importance in taxonomy, ecology, and pharmacognosy. This less invasive method eliminates the need for additional preparation beyond what is needed for previous methods and enables direct work on fresh raw materials and herbal medications.

***KEYWORDS**: Pharmacognosy, Taxonomy, Histochemical, Ecology, Identification Method.*

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INTRODUCTION

The area of histology that deals with identifying the chemical components of cells and tissues is called histochemistry. Although starch deposition happens all over the plant body, it is most frequently found in seeds, tubers, rhizomes, maize, and the parenchyma of the secondary vascular tissues in the stem and root (Kadam, 2021). The protoplast's main ergastic materials are proteins and starch (Kuster, 1956).The diverse class of phenol derivatives known as tannin is typically associated with glucosides. Many plants' leaves (xylem) are especially rich in tannins (Kadam *et al*., 2021). Fats are a common reserve resource found in seeds, spores, and embryos. The breakdown result of the carbohydratess is glucose. Protein breakdown results in amino acids. Numerous woody plants provide secondary products that are useful in medicine (Dhar *et al*., 1968).This review deals with application of histochemical methods.

In comparison to normal technique, histolocalization, which necessitates strong and persistent knowledge, is currently undervalued. It is odd, however, that a phytochemical investigation now begins with intricate chromatographic testing given the escalating ecological concerns of our day. Plant observationusing morphological, anatomical, and organoleptic characteristics may be a more prudent place to start. In addition to this, the analysis of its histolocalization

characteristics helps to provide the most accurate chemical map of the plant's original state. Further chromatographic investigations can only be made possible by this data, which will enable the economical separation of natural products.

The medicinal value of plant source is mainly based on the presence of numerous classes of primary and secondary metabolites which includes carbohydrates, proteins, alkaloids, flavonoids, sterols and phenols, saponins, triterpenes, etc. that induce some specific physiological pathways (Nurit-Silva *et al.,* 2011; Adams *et al.,* 2013). The chemical characterization of the compounds in medicinal plant species is in particular based totally upon ethnobotanical importanceand the fundamental strategies for the validation of its conventional use to obtain new products. (Castro *et al.* 2011).

Various studies have reported that the location of secondary metabolite accumulation varies among plant species and is associated with their chemoecological function (El Babili*et al*., 2021; Wink, 1986). Nicotidine alkaloids are found in the epidermal cells of leaves, petioles, and stems (Wink, 1986).*Nicotiana*species store nicotinein leavesand *Coffea* spp. seeds store caffeine .

The Food and Agriculture Organisation estimated that in 2002 tover 50,000 medicinal plants are being used worldwide to maintain health and/or treat specific illnesses. In 2016, the Royal Botanic Gardens, Kew, investigated that approximately 17,810 plant species out of 30,000 plants have therapeutic use. Traditional medicine uses plants that produce hundreds of chemical compounds to protect against insects, fungi, illnesses, and herbivorous mammals. To date, many phytochemicals have been found to have proven biological functions.

Three species—*Adhatodazeylanica, Ruta graveolens*, and *Vitex negundo* have been used in traditional medicine by folk to treat rheumatism, bronchitis, scrotal swelling, antiperiodic, gonorrhoea, synovitis, and infected wounds. Histochemical analysis of these plants using appropriate reagents has been carried out by various researchers and metabolites such as starch, protein, tannin, saponin, lipids, glucosides, and alkaloids in the tissues, free-hand slices of leaves and stems were obtained (Rishi *et al.* 2014).In another study, alkaloids, saponins, tannins, oils, starch grains, and other phytochemicals were identified and located in different parts of the leaves and rhizome of C*urcuma neilgherrensis's* leaves and rhizome was conducted. The findings of histochemical investigations may be very helpful for chemotaxonomy and drug adulteration detection

Methodology for Detection of Metabolites through Histoanalysis of Plant Tissues

Table: 1 The following tables depicts the test important for histoanalysis of plant tissues

Methods to Detection of Hydrophilic substances	
Tests	**Inference**
I. Carbohydrates: **PAS Reaction (Periodic Acid:Schiff's reagent)** [McManus J F A 1948]. This method is based on the reaction of periodic acid with carbohydrates, forming carbonyl groups revealed by Schiff's reagent 1. Apply 1% sodium tetraborate (freshly pre-pared) for 30 min 2. Transfersectionsto1%periodicacidfor10min. 3. Rinse briefly indistilled water. 4. ApplySchiff'sreagentfor15minin dark. 5. Wash the sections with sodium metabisulfite for10min. 6. Rinseintap water for 10min. 7.Mountttheslidesusingglyceringelatin. 8. Control: Repeat the test excluding step 2 (periodic acid).	Carbohydrates stain magenta .

Aniline Blue Staining (SmithMMandMcCullyME,1978). 1.Apply0.05%anilinebluefor10min. 2.Rinsebrieflyindistilled water. 3.Mountheslideinthesamebufferusedforstaining.	Thisstainingmarkscallose,whichmaybedetect-edbyagreenfluorescenceunderUVlight
II. Proteins Coomassie Blue Staining (FisherDB,1968) 1. Stainin0.25%Coomassiebluefor15min. 2. Differentiatein7%aceticacid. 3. Rinsebrieflyindistilled water. 4. Mountinglycerin gelatin. Control:Putsectionsinasolutionofacetican-hydride and pyridine (4:6, v/v) for 6 h prior tostaining.	Thismethodstainsproteinsblue)andproducesasimilarresulttoanilineblueblack.
AnilineBlueBlackStaining [FisherDB,1968] 1. Dip sections into 1% aniline blue black for 1min. 2. Wash twice in 0.5% acetic acid to remove ex-cessstain. 3. Rinsebrieflyindistilled water. 4. Dehydrate sections passing quickly through90%, 100% ethanol, then a mixture of 100%ethanol and xylene (1:1, v/v), and finally purexylene. 5. Mountslidesusingsyntheticresin. 6.Control:Putsectionsinasolutionofacetican-hydride and pyridine (4:6, v/v) for 6 h prior tostaining.	This stain reveals proteins in blue, whether structural or acting in the primaryor secondary metabolism
III.Mucilage	
Lugol'sReagent [JohansenDA,1940]. 1. Submerge the sections in the Lugol'sreagentfor 10 min. 2. Rinsebrieflywithdistilled water. 3.MountheslidesusingdistilledwaterorLu-gol's reagent itself	Thisreactionhighlightsthestarchgrainsindarkblue to black]. Almost all otherstructuresstainyellow,butthiscolorhasnospecificsignificance.
IV. Lipids **SudanBlackStaining** [Pearse A G E,1985] 1. StainwithSudanblackBfor20min. 2. Rinsebrieflyin70% ethanol. 3. Washindistilledwater. 4. Mountinglyceringelatin. Control: Sections should be kept in the extrac-tion solution [21] for 6 h or more, depending onthe composition of the secretion (determinedempirically). After this time, the sections shouldbe transferred to distilled water and washed ina period of 4 h (4 × 1 h). Then, the staining pro-ceeds as described.	Thisisageneralmethodwhichstainslipidsdarkblue to black.
SudanIVStaining [Pearse A G E,1985] **1.** ApplySudanIVfor30min. 1. Rinsebrieflyin80% ethanol. 2. Washindistilledwater. 3. Mountinglyceringelatin. Control: As with Sudan black B, the sectionsshould be kept in the extraction solution for atleast 6 h.	SudanIValsostainslipids,ingeneral,which become red or red-orange .
NeutralRedStaining (KirkPW,1970). 1. Stainwith0.1%neutralredfor20min. 2. Rinsebrieflyindistilled water. 3. Mountindistilledwater. Control: As with Sudan black B, the sectionsshould be kept in the extraction solution for atleast 6 h.	Thisfluorochromeemitsdifferentcolorsdepending on the lipid composition Under bluelight, the lipids of secretion fluoresce yellow orgreen , cuticle fluoresces yellow andlignified cell walls fluoresce red.
AcidicandNeutralLipids	

Nile BlueStaining[CainAJ,1947]. 1. StainwithNilebluesolutionfor5minat 60°C. 2. Washtwicewith1%aceticacidat60°C. 3. Rinseindistilled water. 4. Mountinglyceringelatin. 5. Control: As with Sudan black B, the sectionsshould be kept in the extraction solution for atleast 6 h.	Since lipids were detected in the material, Nileblue distinguishes acidic lipids, which stain blue,from neutral lipids, which stain pink
V. FattyAcids	
CopperAcetateandRubeanicAcidStaining [CainAJ,1947 and GanterPandJollésG.1969] 1. Treat sections with 0.05% copper acetate for3h. 2. Apply 0.1 M Na2 EDTA (EDTA acid disodiumsalt solution) for 5 min. 3. Washindistilledwaterfor5min. 4. Transfer sections into 0.1% rubeanic acid(freshly prepared) for 20 min. 5. Washin70%ethanolfor5min. 6. Rinseindistilled water. 7. Mountinglyceringelatin. Control: As with Sudan black B, the sectionsshould be kept in the extraction solution for atleast 6 h.	This method for lipids is slightly more specificthantheSudantestsandidentifiesfattyac-ids through the reaction of copper acetate withtheseacidiclipids,whichsubsequently turndarkgreenwhenexposedtorubeanicacid
VI. DetectionofPhenolicCompounds **FerricChlorideStaining** (JohansenDA,1940) 1. Apply10%ferricchloridefor30min. 2. Wash twice in distilled water to remove sur-plus ferric chloride. Mountinglycerin gelatin.	This method highlights phenolic compoundsthrough iron precipitation, producing a darkcolor, usually black , sometimesbrown.
Potassium DichromateStaining[Gabe M.,1968] 1. Apply10%potassiumdichromatefor30min. 2. Wash twice in distilled water to remove surplusreagent. 3. Mountinglyceringelatin.	This method also highlights phenolic compounds;ingeneralproducingabrownor red-browncolor
FerrousSulfate–FormalinFixation [JohansenDA,1940] 1. Thesamplesshouldbefixedintheferroussul-fate–formalin solution under vacuum for 48 h. 2. Wash4×2h(totaling8h)indistilledwater. 3. Dehydratethematerialin30%,50%,70%ethanol for 12 h each. Embed the material according to the chosentechnique (Paraplast, Historesin, or PEG) andthen section in a microtome	Thebestmethodtodetectphenoliccompoundsisto introduce iron salts into the fixative since theironcompoundfixesandstainsthephenoliccom-pounds
TannicAcidandFerricChloride [Pizzolato T D.,1977] 1. Apply5%tannicacidfor20min. 2. Rinsebrieflywithdistilled water. 3. Submergesectionsin3%ferricchloridefor5min. 4. Washtwiceindistilledwatertoremovesur-plus ferric chloride. 5. Mountthesectionsusingglyceringelatin. Control: Compare the staining obtained in thetestwiththatofsectionstreatedonlywithtannic acid or with ferric chloride.	This method is based on the reaction of tannicacidwithmucilagesandpectins,substanceswhicharefurtherrevealedbytheadditionoffer-ricchloride,producingagreytoblackcolor.
Vanillin–HydrochloricAcidStainingforTannins[Mace M E and Howell C R,1947] 1. Treat with 0.5% vanillin for 20 min. 2.Mount the slide using 9% hydrochloric acid.	Thistestismorespecificforsomephenolic compounds, staining tannins red . Use only sections of fresh material.
VII Alkaloids **Dragendorff'sReagent** [SvendsenABandVerpoorteR.1983]. 1. TreatwithDragendorff'sreagentfor20min. 2. Rinsebrieflyin5%sodium nitrite. 3. Mountindistilledwater. Control: Treat sections with 5% tartaric acidin 95% ethanol for 72 h and repeat the stainingprocedure.	This reagent marks alkaloids in red-brown Fresh and fixed material may beused in this method, but fixed material shows aconsiderablylossofthealkaloidsandthestainingcolor when compared to fresh material.

Wagner'sReagent [FurrMandMahlbergPG,1983]. 1. ApplyWagner'sreagentfor20min. 2. Rinsebrieflyindistilled water. 3. Mountindistilledwater. Control: Treat sections with 5% tartaric acidin 95% ethanol for 72 h and repeat the stainingprocedure.	This method also stains alkaloids red or red-brown It is recommended thatfresh material be used for this test.
VIII. Terpenes **NADIreagent** [DavidRandCardeJP,1961] 1. ApplyNADIreagentfor1hinthedark. 2. Wash in sodium phosphate buffer (0.1 M, pH7.2) for 2 min. 3. Mountinthesamebuffer. Control: As with Sudan black B, the sectionsshould be kept in the extraction solution for atleast 6 h.	This reagent produces differential staining withessentialoils(monoterpenesandsesquiterpenes) staining blue and resins (diterpenes, triterpenes, tetraterpenes and deriva-tives) staining red. Mixtures of essential oils andresins produce varied shades of violet to purple,depending on the prevalence of each compound.
IX. Lignin [JohansenDA,1940]. 1. Apply 10% phloroglucinol for 15 min. 2. Mount the slides carefully with 25% hydrochloric acid.	Phloroglucinol in an acidic medium stains lignin in cell walls pink to red It is possible to use either fresh or embedded material

Significance of Histochemical Localization

Histolocalization, which necessitates strong and reliable competence, is currently undervalued in comparison to normal procedure. Nevertheless, it is unusual to see a phytochemical study begin with intricate chromatographic testing in light of the escalating ecological concerns of our day. A more prudent place to start may be by observing the plant as a whole. In fact, its histolocalization research and botanical (morphological, anatomical, and organoleptic) characteristics enable the initial findings to provide the most accurate chemical map of the plant's natural condition. The ability to evaluate and understand observations is thus sufficient. Only with this information will additional chromatographic investigations enable the economical separation of natural products.

Histochemical techniques are used for the localisation of nucleic acids, promoter activity, minerals, metals, proteins, carbohydrates, and lipids within cells and tissues, as well as for the detection of subcellular components, secondary metabolites, and cell wall components such as lignin and cell wall polysaccharides. Using a microscope and photographic recording, their detection can be based on the color-stain reaction.

Many laboratories utilise histochemistry because it's a rather simple and inexpensive approach. When it comes to plant mutants, screening experimental circumstances that affect plant tissues, and selecting particular time points and kinetics for in-depth analyses later on, it provides supplementary information to high-throughput analytical approaches. Furthermore, the sciences of plant taxonomy and medical botany both make use of histochemistry. For instance, an early evaluation of the kinds of secondary metabolites generated in wild and ancient plant species can benefit from the use of histochemistry (Berni *et al.* 2019). The location of particular secondary metabolites in various organs and among several kinds within the same species can be examined by a histochemical analysis.

In the study of plant stress physiology, histochemical approaches are particularly useful because they allow for the evaluation of the effects on organelles as well as the monitoring of the subcellular sites of secondary metabolite, enzyme, and ROS generation in response to an exogenous stimulus. Information about intricate processes in vivo can be obtained

by synthesising tagged building blocks that are used to create biological macromolecules like lignin (see above). Similarly, click chemistry provides crucial information on, for instance, the dynamics of plant membranes in vivo by labelling specific cell components (Voiniciuc*et al.* 2018).

In conclusion we can say that many plants have secondary compounds that have therapeutic use. Consequently, histochemical analyses of various plant sections focus on identifying the chemical constituents of cells and tissues. Additionally, histochemical localisation has a wide range of distinct pharmacological and economic ramifications. This review comes to the conclusion that histochemical localisation may be utilised in a quick field survey to determine whether certain plants contain bioactive chemicals.

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