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Research Article
A Novel Histological Approach for Identification of Alkaloid Bearing Plants

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Abstract

Background and Objective: Alkaloids are only secondary metabolites which participate in plant metabolism and also translocated from one part to another. About 153 families have never been explored for alkaloids and no significant transport channel is reported so far for alkaloid movement from one cell to another after their production in the plant species. The major aim of this study is to sharpen the mind for the histological peculiarities of alkaloid bearing plants and to consider them adequately in forthcoming investigations on translocation of alkaloids. **Materials and Methods:** More than 100 plants/parts were studied histologically and histochemically, to differentiate the alkaloid and non-alkaloid bearing plants. The transverse/longitudinal sections were taken for all the samples using standard methods. These sections were screened for the presence/absence of alkaloids. **Results:** Anatomical studies of different plant parts viz., root, rhizome, stem, bark, leaf, petiole, fruit and seed were conducted. A constant histological feature i.e., pits on the cell wall and in the cell lumen of the tissues other than tracheary elements was noticed in all types of alkaloid viz., tropane, pyridine-piperdine, quinoline, isoquinoline, lupine, indole, steroidal alkaloids, alkaloidal amines and purine bases bearing plants/parts. **Conclusion:** The presence of alkaloids as secondary metabolite in any plant species can be detected by the microscopic structure i.e., cell wall pitting and pits in cell lumen due to plasmodesmata, other than tracheary elements, because this structure was observed in all alkaloid bearing plants only. Secondly, it may also help to locate the translocation of this diverse secondary metabolite. This novel histological finding may be applied to identify the new source of alkaloid from the unexplored plant families and may open new vistas for the chemical and biological point of view. The hypothesis of the finding is that the presence of plasmodesmata and pits in the cells even in sclereids/stone cells can provide a channel for the translocation of these secondary metabolites after their synthesis in the particular plant part. Further, physiological research is required to confirm all the activities related to the chemical and physical processes associated with this hypothesis.

Key words: Alkaloid, histology, pits, plasmodesmata, translocation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alkaloid bearing plants have been found in virtually every habitat in which vascular plants grow and there are no taxonomic characteristics by which a plant may arbitrarily be assigned to a group suitable for alkaloid study. Approximately 35 higher plant families have alkaloids and about 14.2% plant genera contain alkaloids[1]. Twenty most important plant families for the production of alkaloids are the Amaryllidaceae, Annonaceae, Apocynaceae, Asteraceae, Berberidaceae, Boraginaceae, Buxaceae, Celastraceae, Campanulaceae, Fabaceae, Lauraceae, Liliaceae, Loganiaceae, Menispermaceae, Papaveraceae, Piperaceae, Poaceae, Ranunculaceae, Rubiaceae, Rutaceae and Solanaceae[1-3]. These are the only secondary metabolites which have a diverse role in different plants, for instance they (i) Act as regulatory growth factors, (ii) Serve as reserve substances capable of supplying nitrogen or other necessary constituents to the plants or (iii) Participate in plant metabolism over the long term[2,3]. However, qualitative and quantitative variation in alkaloid content is very common in some species. This implies that even though alkaloids have not been regarded as vital to the plant constituents, but they do participate in important metabolic processes. This fact indicates that the presence of transport channels is required for the movement of this important secondary metabolite. Several, sometimes indirect, instances are recorded for plant alkaloids in which the site of biosynthesis differs from the site of alkaloid storage[4]. Therefore, it is assumed that alkaloids are synthesized in a specific organ or tissue and then translocated to other plant parts where storage occurs. The long-distance transport may involve the phloem, xylem or apoplastic space, but virtually nothing is known about the transporters and cellular mechanisms that facilitate the mobilization process[5]. Although several transcription factors of different types, such as AP2/ERF, WRKY and bHLH transcription factors or regulatory factors, such as MAP kinases have been identified in the biosynthesis of various alkaloids[6] but the translocation mechanism of alkaloids is still not known.

It is well known fact that the walls of plant cells have multiple layers and the primary cell wall help the plant for transport and diffusion. The secondary cell wall develops inside the primary cell wall, leaving certain portion, which consist only of primary wall materials of different shapes and are known as pits. The pits of two contiguous cells usually appose one another and are called pit-pair[7]. Physiological significant features of pit-pair are the plasmodesmata traversing the pit membrane and they are thought to provide cytoplasmic continuity between the adjacent cells and appear as small pits/holes in the cell lumen usually in the transverse section. It is reported that plasmodesmata are common in active parenchyma[7].

During anatomical studies I observed pits/holes in transverse and longitudinal sections of different plant parts on cell wall as well as in the cell lumen of alkaloid bearing plants only. Keeping this in mind, detailed histological studies of different plant parts viz., root, rhizome, stem, petiole, leaves, fruit and seed of alkaloid and non-alkaloid bearing plants have been conducted in order to find out histological marker for the identification of alkaloid bearing plants and to establish a co-relation between pits and translocation of alkaloids.

MATERIALS AND METHODS

Plant materials: Authenticated materials of more than 100 plants/parts collected from field or procured from the markets of India during 2000-2015 were studied. All the plant/parts studied were deposited in the 'Drug Museum' of the Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow, India. These plants were grouped as alkaloid and non-alkaloid bearing plants as per the extensive literature survey.

Alkaloid bearing plants: *Abrus precatarius* L., *Aconitum balfourii* Stapf, *A. chasmanthum* Stapf ex Holmes, *A. heterophyllum* Wall., *Acorus calamus* L., *Adina cordifolia* (Willd. ex Roxb.) Hook f. ex Brandis, *Albizia lebbeck* Benth., *A. odoratissima* Benth., *A. procera* Benth., *Allium cepa* L., *Alstonia scholaris* (L.) R. Br., *Andrographis paniculata* (Burm.) Wall. ex Nees, *Aphanamixis polystachya* (Wall.) Parker, *Areca catechu* L., *Artemisia annua* L., *A. dracunculus* L., *A. maritima* L., *A. vulgeris* L., *Asparagus adscendens* Roxb., *A. racemosus* Willd., *A. sprengeri* Regel, *Berberis aristata* Roxb. ex DC., *B. asiatica* Roxb. ex DC., *B. chitria* Lindl., *B. lycium* Royle, *Boerhavia diffusa* L., *B. repens* L., *B. erecta* L., *Butea monosperma* (Lam.) Taub., *Cannabis sativa* L., *Camellia sinensis* (L.) Kuntze, *Capsicum annuum* L., *Cassia angustifolia* Vahl, *Catharanthus roseus* (L.) G. Don, *Chlorophytum arundinaceum* Baker, *C. borivalianum* Sant. And Fern., *Chlorophytum tuberosum* (Roxb.) Baker, *Cinchona officinalis* L., *Cinnamomum camphora* T. Nees, *C. cassia* Blume, *C. tamala* Fr. Nees, *Coffea arabica* L., *Coelogyne cristata* Lindley, *Coscinium fenestratum* (Gaertn.) Colebr., *Crataeva nurvula* Buch-Ham., *Croton tiglium* L., *Curcuma amada* Roxb., *C. longa* L., *C. zedoaria* (Christm.) Rosc., *C. angustifolia* Roxb., *Cyperus rotundus* L., *C. scariosus* R. Br., *Datura metal* L., *Desmodium gangeticum* (L.) DC., *D. triflorum* (L.) DC., *Enicostemma hyssopifolium* (Willd.) Verdoon, *Fumaria*

parviflora Lam., *Gloriosa superba* L., *Glycyrrhiza glabra* L., *Gymnema sylvestre* (Retz.) Schult., *Hemidesmus indicus* R. Br., *Holarrhena antidysenterica* (Roth) DC., *Humulus lupulus* L., *Hyoscyamus niger* L., *Inula recemosa* Hook. f., *Ipomoea batata* (L.) Lam., *I. digitata* L., *Leucas aspera* (Willd.) Link, *L. biflora* (Vahl) R. Br., *L. cephalotes* (Koen. ex Roth) Spreng., *L. clarkei* Hook.f., *Mucuna nivea* (Roxb.) DC, *M. pruriens* (L.) DC., *M. utilis* Wall. ex Wight, *Nelumbo nucifera* Gaertn., *Nicotiana tabacum* L., *Ocimum gratissimum* L., *Ocimum sanctum* L., *Oldenlandia corymbosa* L., *Papaver somniferum* L., *Peristrophe bicalyculata* (Retz.) Nees, *Phyla nudiflora* (L.) Greene, *Phyllanthus amarus* Schum and Thonn., *P. fraternus* Webst., *P. maderaspatensis* L., *Piper betle* L., *P. chaba* Hunter, *P. cubeba* L.F., *P. longum* L., *P. nigrum* L., *P. retrofractum* Vahl, *Punica granatum* L., *Rauvolfia serpentina* (L.) Benth. ex Kurz., *R. tetraphylla* L., *Ricinus communis* L., *Rubia cordifolia* L., *Saussurea lappa* (Decne.) Sch.-Bip., *Sida acuta* Burm., *S. cordata* Burm., *S. cordifolia* L., *S. rhombifolia* L., *Solanum indicum* L., *S. lycopersicum* L., *S. nigrum* L., *S. surattense* Burm. f., *S. tuberosum* L., *S. xanthocarpum* Schrad and Wendl, *Sterculia urens* Roxb., *Streblus asper* Lour., *Strychnos nux-vomica* L., *S. potatorum* L., *Tecomella undulata* (Sm.) Seem, *Theobroma cacao* L., *Tinospora cordifolia* (Willd.) Hook. f. R. Thomas, *Tribulus terrestris* L., *Vitex agnus-castus* L., *V. negundo* L., *V. paniculata* Lam., *Withania somnifera* (L.) Dumal, *Zingiber officinale* Rosc., *Zizyphus jujuba* Mill.

Non-alkaloid bearing plants: *Adiantum capillus-veneris* L., *A. lunulatum* Burm., *A. peruvianum* Klotzsch, *A. venustum* D. Don., *Alcea rosea, Ailanthus excelsa* Roxb., *Althaea officinalis* L., *Anogeissus latifolia* (Roxb. ex DC.) Wall. ex Bedd., *Bacopa monieri* (L.) Pennell, *Boswellia serrata* Roxb. ex Coleb, *Carthamus tinctorius* L., *Centella asiatica* (L.) Urban, *Dillenia indica* L., *Gmelina arboria* Roxb., *Myrica esculenta* Buch.-Ham., *Nardostachys grandiflora* DC., *Oroxylum indicum* (L.) Vent., *Premna corymbosa* Rottle., *Stereospermum chelonoides* (L. f.) DC., *Terminalia alata* Heyne ex Roth., *T. arjuna* (roxb.) Wight and Arn., *T. bellerica* Roxb., *T. catappa* L., *T. chebula* Retz and Willd., *T. tomentosa* (Roxb.) Wight and Arn., *Trewia nudiflora* L.

Sample preparation for microscopy: Hand/cryostat sections of 20-60 mm thickness in transverse (TS) and longitudinal (LS) view were taken for all the plant materials. These sections were histochemically screened for the presence/absence of alkaloids by treating them with different reagents viz., Mayer's (potassium mercuric iodide solution), Wagner's (solution of iodine in potassium iodide), Hager's (saturated solution of picric acid) and Dragendroff's (solution of potassium bismuth iodide)[7]. The purine derivatives did not precipitate with aforesaid alkaloidal reagents; therefore, for its detection, the sections were also screened for Murexide test[7]. The TS and LS were also dehydrated with a successive series of ethanol (i.e., 30, 50, 70 and 80% v/v) before staining with safranin solution[8]. The sections were mounted on glass slides in 50% (v/v) glycerine. All samples were examined under Nikon trinocular microscope and photographs were taken using a Nikon UF-II auto exposure camera, 12 ASA black and white photofilm. The coloured photographs were taken through Olympus trinocular microscope with Magnus digital camera attachment.

RESULTS

Histological studies of different plant parts viz., root, stem, bark, leaf, fruit and seed of more than 100 plants showed the presence of cell wall pitting and pits in lumen due to the plasmodesmata in alkaloid bearing plants only. In the present study attempts have also been made to establish a co-relation between different alkaloid bearing plants and pitting on the cell wall and in the cell lumen due to plasmodesmata. The study was well supported by micro photographs. The presence of pits in different tissue regions of various plant parts, like, root of *Aconitum balfourii* showing pitted parenchyma of cortical and ground tissue (Fig. 1a), rhizome of *Cyperus rotundus* showing pitted parenchyma of ground tissue (Fig. 1b), bark of *Cinchona officinalis* showing pitted parenchyma and stone cells of secondary phloem (Fig. 1c), seed of *Caesalpinia bonduc* showing pitted thick walled cells of testa (Fig. 1d), stem of *Ocimum gratissimum* showing pitted parenchyma of pith region (Fig. 1e), petiole of *Vitex agnus-castus* showing pitted parenchyma of cortical and pith region (Fig. 1f), leaf of *Coelogyne cristata* (an orchid) showing pitted epidermal cells and spongy mesophyll cells (Fig. 1g) were observed. However, significant variation in pit size in different plant parts were noticed for example, very large pits in perisperm cells of *Areca* nuts (Fig. 2a) to minute ones in perisperm cells of *Piper longum* (Fig. 2b). It is interesting to find out that in all alkaloid bearing plants, pits occur in parenchymatous cells of various regions i.e., cortex, endosperm, ground tissue, medullary rays, mesophylls, perisperm, phelloderm, pith and vascular region etc. Sometimes, pitted lumen was also observed in stone cells embedded in cortical, phelloderm and phloem regions of some alkaloid bearing plants. Patches of pitted cells can also be seen in powdered materials of alkaloid rich plants. All the aforesaid-pitted cells gave precipitate or colour when treated

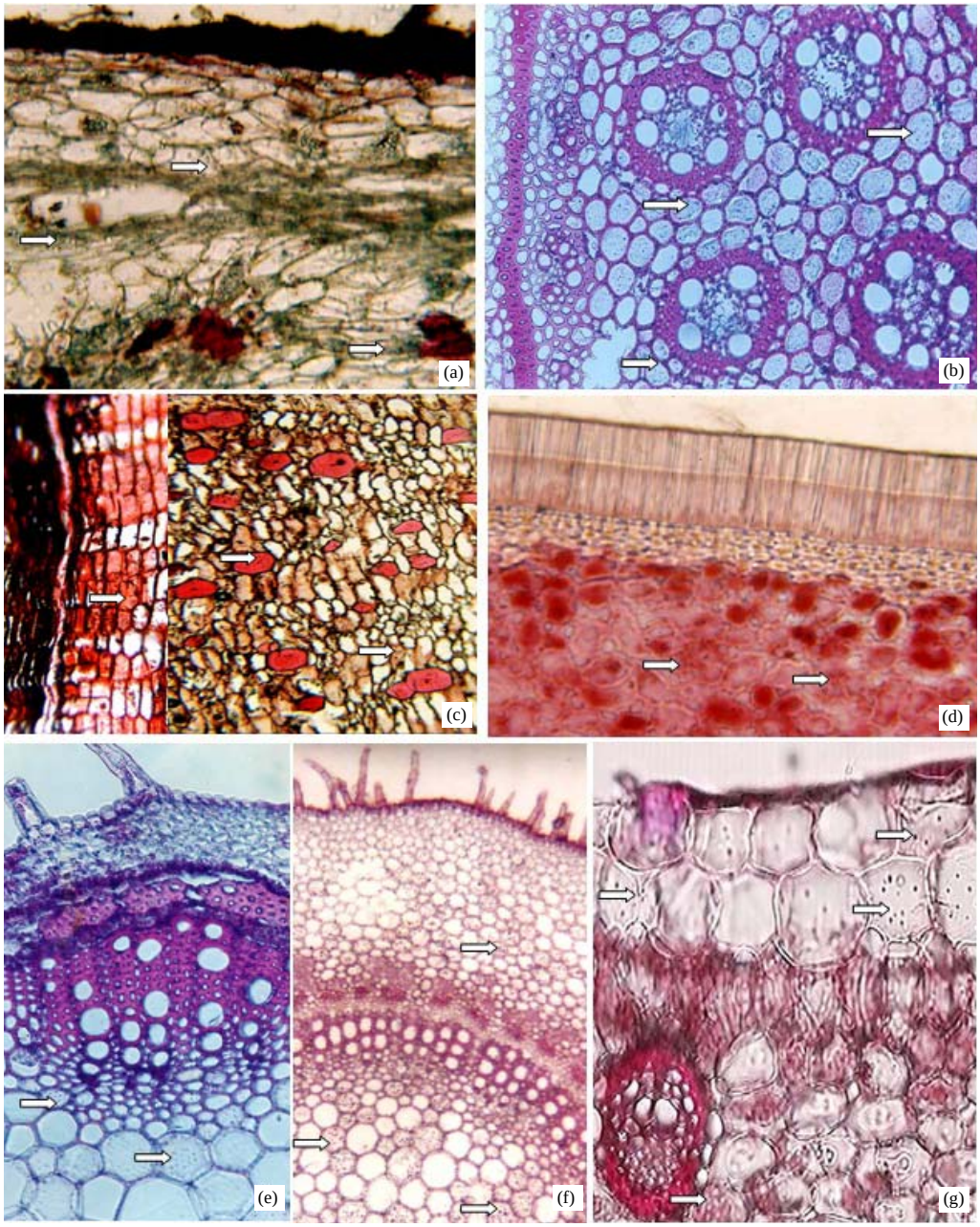

Fig. 1(a-g): TS of various plant parts showing pits on the cell wall and in cell lumen due to plsmodesmata in various tissue region, (a) TS root of *Aconitum balfourii*, (b) TS rhizome of *Cyperus rotundus*, (c) TS bark of *Cinchona officinalis*, (d) TS seed of *Caesalpinia bonduc*, (e) TS stem of *Ocimum gratissimum*, (f) TS petiole of *Vitex agnus-castus* and (g) TS leaf of *Coelogyne cristata*

with different alkaloidal reagents i.e., cream (with Mayer's), yellow (with Hager's) and reddish-brown (with Wagner's and Dragendroff's). However, the pitted cells of tea leaves and coffee beans did not show positive test with usual alkaloid reagents but they gave purple colour with a mixture of potassium chlorate and a drop of hydrochloric acid and exposing the sections to ammonia vapours (Murexide test). This indicates that these plants contain purine bases. In addition, presence of pits in xylem parenchyma of *Capsicum annuum* seedling (Fig. 3a), in epicarp and mesocarp region of poppy fruit (Fig. 3b) and adjacent to laticifers and sieve plate of poppy stem (Fig. 3c) showing a co-relation with alkaloids. It

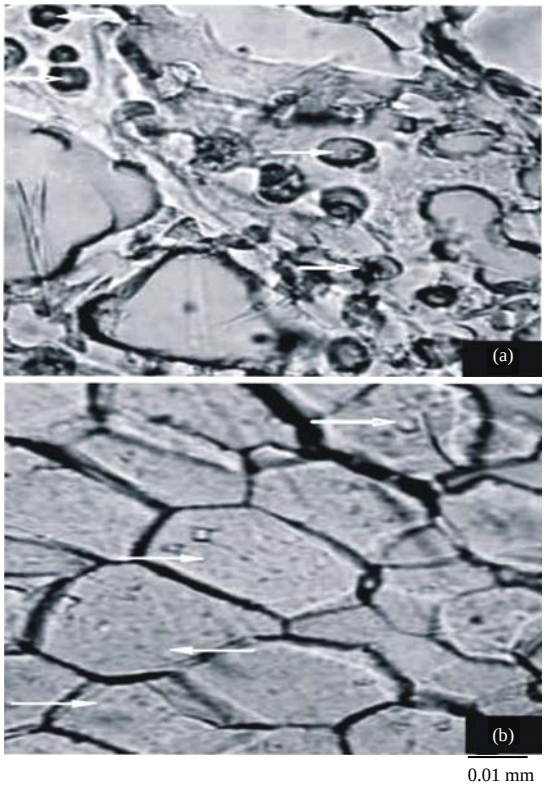

Fig. 2(a-b): TS of plant parts showing various size of pits due to plasmodesmata in lumen of perisperm cells, (a) TS *Areca* nut showing very large pits and (b) TS *Piper longum* fruit showing very small pits

is also notable that this microscopical feature was absent in those plants/parts which are devoid of alkaloids (Fig. 4a-f).

DISCUSSION

The study revealed that the presence of pits on the cell walls and in the cell lumen of different tissue of various alkaloid bearing plants seems to be universal in all types of alkaloid bearing plants for example, plants containing pyrrole and pyrrolidine alkaloids in *Theobroma cacao* seeds; pyridine-piperidine alkaloids in *Areca* nut, *Nicotiana tabacum*, *Ricinus communis*; tropane alkaloids in *Datura* spp., *Solanum* spp., *Capsicum* spp (stem, leaf, petiole and root), quinoline alkaloids in *Cinchona* (bark), isoquinoline alkaloids in *Berberis* spp. (root and stem), *Papaver somniferum* (stem, leaf and fruit), lupine alkaloids in *Phyla nudiflora*, indole or benzopyrrole alkaloids in *Strychnos nux-vomica* (seeds), *Rauwolfia* spp., *Catharanthus roseus* (root, stem and leaf) and *Alstonia scholaris* (stem bark), steroidal alkaloids in *Holarrhena antidysenterica*, terpenoid alkaloids in *Aconitum* spp. (root), alkaloidal amines in *Ephedra* spp. (stem) and *Sida* spp. (Stem and roots) and purine bases in *Camellia sinensis* (leaf and stem) and *Coffea arabica* (seeds). The variation of pit size and density may be due to the quality and quantity of alkaloids in that particular plant/part.

Presence of pits was also reported by other workers in different tissues of various alkaloid rich plant species viz., in the sclerenchymatous cells in *Alstonia scholaris*[9] and in large elongated open cells of wood of *Clematis virginiana*[10], in rectangular pericarp cells of *Capsicum* fruit and beaded seed coat parenchyma of *Paullinia cupana*[11], thick walled cells of xylem parenchyma of *Rauwolfia serpentina*[12], in parenchymatous cells of medullary rays of *Berberis aristata*[13], in stone cells of *Ailanthus excelsa*, *Gmelina arborea*, *Ficus religiosa*, *Saraca asoca* powdered bark[14,15], in cortical parenchyma of *Merremia turpethum*[16], xylem parenchyma of *Convolvulus microphyllus*[17] and even in polyherbal formulations[18,19] but a correlation between pitted cells and presence of alkaloid has not yet been mentioned.

Further, the translocation mechanism of alkaloids is still not fully understood. It has been found to vary from part to part and from plant to plant as reported by earlier researchers. James[20] suggested that translocation of some alkaloids might be through the cell sap by transpiration stream. In tissues such as *Cinchona* bark the alkaloid content was maintained as

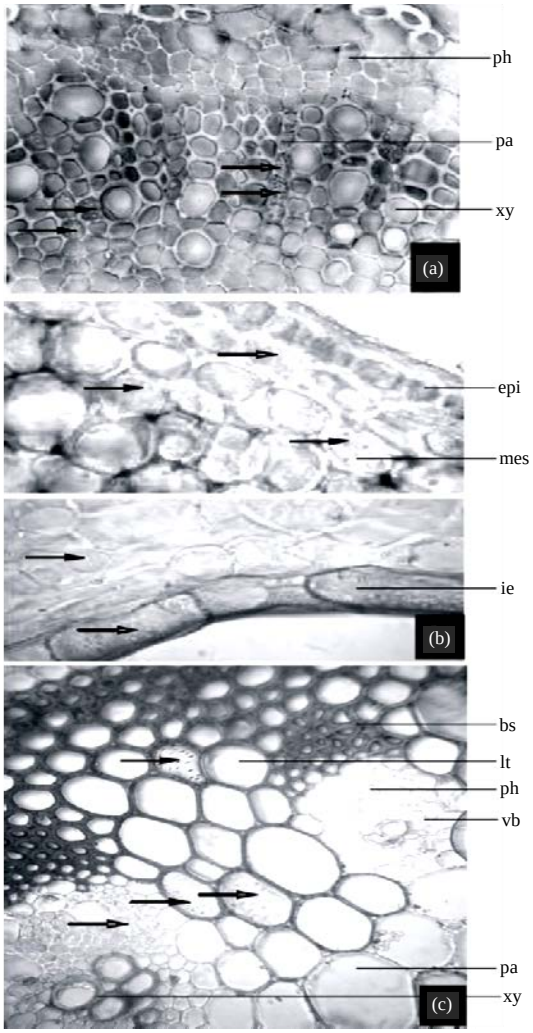

Fig. 3(a-c): TS plant parts showing pits due to plasmodesmata to indicate co-relation with alkaloids, (a) TS *Capsicum annuum* seedling showing pits in xylem parenchyma, (b) TS poppy fruit showing pits in epicarp and mesocarp region and (c) TS poppy stem showing pits adjacent to laticifers and sieve plate, bs: Bundle sheath, epi: Epicarp, ie: Inner epidermis, lt: Laticifer, mes: Mesocarp, pa: Parenchyma, ph: Phloem, vb: Vascular bundle, xy: Xylem

long as the parenchyma remained alive[20]. Similarly, in the germinated *Ricinus* seeds, alkaloids disappear from the endosperm and appear in the cotyledons and the hypocotyls, which show the translocation of alkaloid from endosperm to cotyledons[20].

The presence of plasmodesmata and pits in those cells, which are reported to be rich in alkaloid content, can provide a channel for the translocation of alkaloids from one part to the other of the plant. This is a subject matter of physiological studies. However, in the present study some examples have been undertaken to establish a co-relation between the pits and alkaloids.

In solanaceous plants, tropane esters formed in the roots are translocated to the aerial parts and appear in the earliest stage of seedlings[3]. It was also reported that the hyoscyamine 6-beta-hydroxylase (H6H), an enzyme involved in tropane alkaloid biosynthesis, is localized at the pericycle of the root[21]. In the present study the TS of young root and stem of seedling of *Capsicum annuum* (a solanacious plant) were taken and it was observed that the pitted cells are present at root tip region especially in pericyclic region and parenchymatous cells adjacent to xylem and phloem (Fig. 3a). Thus, the above finding indicates a definite co-relation between cell pitting and alkaloids.

Similarly, Bird *et al.*[22] reported that benzylisoquinoline alkaloids of *Papaver somniferum* accumulate in the cytoplasm or latex of specialized laticifers that accompany vascular tissue throughout the plant and perforation develop

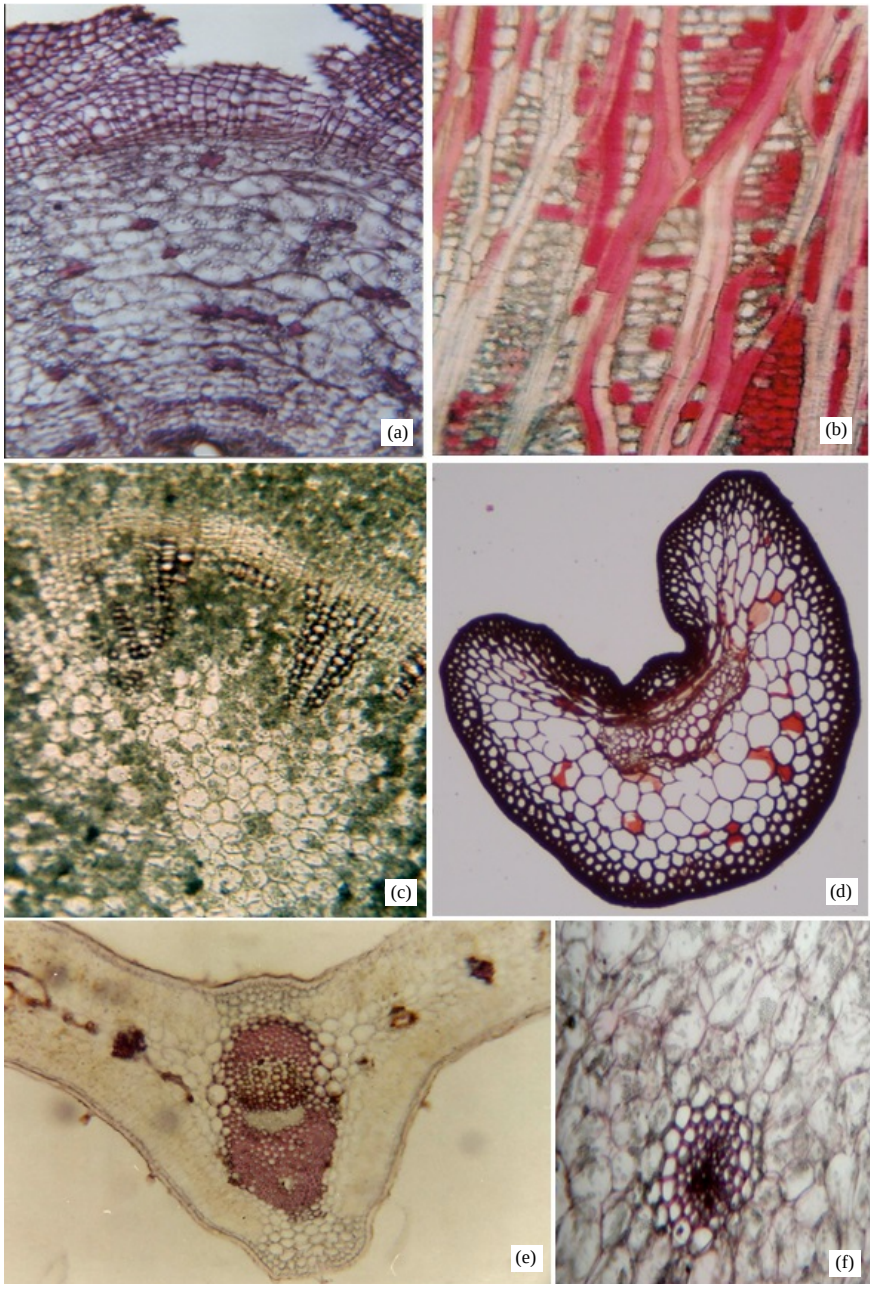

Fig. 4(a-f): TS of non-alkaloid bearing plant parts, (a) TS root of *Stereospermum chelonoides*, (b) LS bark of *Myrica esculenta*, (c) TS stem of *Centella asiatica*, (d) TS rachis of *Adiantum lunulatum*, (e) TS leaf of *Carthamus tinctorius* and (f) TS fruit of *Dillenia indica*

between the lateral walls of adjacent cells ensuring a contiguous network of latex vessels. They also reported that key alkaloid biosynthetic enzymes are found in sieve elements and a field of plasmodesmata frequently found between adjacent sieve elements. Recent findings also showed that those alkaloids which are taken up from the soil are translocated through the xylem, whereas, when genuinely present in plants, they are allocated as N-oxides via phloem[23-25]. I have also observed plasmodesmata and pits in parenchyma adjacent to sieve elements and laticifers in TS of fruit and stem (Fig. 3b, c) of opium poppy. This finding also indicates a positive co-relation between pits and alkaloids.

Alkaloids have complex interactions between plants and their environment, e.g., to protect plants against pathogens

and herbivores and also have ability to diffuse through membranes. Several hypotheses have been proposed regarding the translocation of this secondary metabolite. Previous study showed that the movement of many compounds within the plant is often facilitated through the action of transporters located in the membranes[25]. Yazaki led a hypothesis that ATP-binding cassette (ABC) transporters largely contribute to membrane transport of endogenous secondary metabolites[26]. It was also reported that the movement of many alkaloids within the plant is often facilitated through the action of ABC transporters located in the membranes. The presence of these carriers is often restricted to certain tissues, such as the phloem or particular organelles, e.g., vacuoles[26,27]. Similarly, Nowak and Selmar[25] also reported that the existence of the membrane-impermeable serpentine is required to enable long-distance transport of pyrrolizidine alkaloids within the phloem. Therefore, I proposed a hypothesis that the present histological finding of pits on the cell walls and cell lumen due to plasmodesmata in all type of cells (even the stone cells) and in all regions i.e., epidermal, cortical, perisperm, endosperm, vascular (phloem and xylem) regions of plant parts may provide a continuous channel for the translocation of endogenous alkaloid from one part to another. Further, physiological research is required to confirm all the activities related to the biochemical and physical processes associated with the cell wall pitting and movement of alkaloids through them.

Alkaloids, first discovered in the beginning of 19th century and evolved as an important class of natural product and act on a diversity of metabolic systems in humans and other animals[1]. Many alkaloids are valuable medicinal agents that can be utilized to treat various diseases like, asthma, arrhythmic, bacterial, cancer, cardiac dysfunction, diabetes, fungal, inflammation, malaria, thrombosis etc[28-32]. It is reported that 153 plant families have never been examined for alkaloids[1]. Hence, this histological finding may be utilized for the identification of new sources of alkaloids not only for chemical and biological point of view but also as therapeutic agents.

CONCLUSION

From the ongoing studies it may be concluded that the presence of pits on the cell walls and in the cell lumens due to the plasmodesmata in TS, LS and powdered material of a plant/part is a positive indication of the presence of alkaloids as secondary metabolite in such plants. This histological finding can be applied to identify the new source of alkaloid from the unexplored plant kingdom and also to establish a co-relation between pits and translocation of alkaloids.

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