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SEED HISTOCHEMISTRY OF *MELIA AZEDARACH* L.

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Abstract: Anatomical investigation of cotyledons of *Melia azedarach* L. shows the presence of parenchymatous cells along with large sized secretary cells. Secretary cells could be distinguished by specific histochemical stains like Sudan III and Iodine Potassium Iodide (I_2KI). Cotyledons are also filled with starch and oil bodies. The present study on secretary cells has paved way towards understanding the distribution of various chemicals in particular terpenoids in the cotyledons. These methods, in combination with various microscopic imaging techniques, can be utilized in the study of essential oil secretion in plants.

Key Words: Histochemistry, *Melia azedarach*, Secretary Cell

INTRODUCTION

Medicinal plants are part and parcel of human society to compact diseases, from the dawn of civilization. *Melia azedarach* is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. *Azadiracta indica* A. Juss. and *Melia azedarach* are two closely related species of Meliaceae. The former is popularly known as Indian neem (Margosa tree) or Indian lilac and latter as the Persian lilac. It is an evergreen tree, cultivated in various parts of the Indian sub-continent. Every part of the tree has been used as traditional medicine for household remedy against various human ailments from antiquit. It has been extensively used in Ayurveda, Unani and homeopathic medicine and has become a cynosure of modern medicine. The Sanskrit name of the neem tree is Arishta meaning reliever of sickness and hence is considered "sarbaroganibarani". The tree is still regarded as 'village dispensary' in India. The importance of the neem tree has been recognized by the US national academy of sciences, which published a report in 1992 entitled 'Neem-a tree for solving global problems' (Biswas *et al.*, 2002).

There are about 15 species of shrubs and trees under this genus dispersed in paleo tropical and subtropical region. Two species are native in paleotropical and sub-tropical region. Two species are native to India. Melia is a Greek name for the 'ash tree' and this genetic name is an allusion to the similarity of leaves. These plants are commonly known as Bead tree leaves are pinnate and alternate, Leaflets are entire and toothed flowers are borne in axillary panicles white or purple in colour fruit is a berry (Fig. A-D). Two species occur in India. There are (Battacharjee, 2000)

1. *Melia azedarach* L.
2. *Melia composite* Willd.

A moderate-sized deciduous tree, 9-12m high. Bark dark grey with shallow longitudinal furrows, leaves bi or occasionally tripinnate; leaflets ovate or lanceolate, serrate; flowers lilac, fragrant, in axillary panicles; fruit an ellipsoidal-globose drupe with 4-5 seeds.

The heart wood tough and durable and it is used for making boxes, sports wares, agricultural implements and for the roofing material. Fruits of the plant are used for the production of alcohol because these contain glucose and starch. Goats and birds have been observed by eat the fruit without any ill effect. The stones of the fruits are used in making necklace and rosaries. Its oil is used for preparing the candle wax, for pest control and lice killing.

Chemical constituents of seeds including β-Sitosterol, vanillin, benzoic acid, vanillic acid, daucosterol, β-D- glucopyranose, liminoid glycoside viz 6, 11- diacetoxy- 7-oxo-14 beta-epoxymeliacin (1,5,-diene- 3-o- beta- D- glucopyranoside) and melianol meliacin, meliacarpin, meliartenin vanillin, hydroxyl -3-methoxcinnamaldehyde and (+-) pinoresinol. Stem bark contain limonoids such as 7α-Acetoxy-14 β, 15β-epoxygedunan- 1 ene3-oβ-D- glucopyranoside.

The seed contains up to 40% of a drying oil. It used for lighting, varnishing etc. The must scented seeds are used as beads in rosaries. The fruits are a source of a flea powder and an insecticide. The leaves repel mosquitoes and other insects.

The plant is known to contain a number of organic molecules i.e., terpenoids, flavonoids, steroids, acids and anthraquinones (Kalidhar, 2003).

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MATERIALS AND METHODS

Source of Plant material

Fruits were collected from plants growing in Sri Parasakthi College, Courtallam. Seeds were isolated from the fruits, washed in running tap water, air dried and stored in refrigerators for future use. Fruits were collected during the months of December and January.

Anatomical studies

Thin free hand sections were made with cotyledons. Sections were stained with Saffranin, Sudan III and Iodine solution to localize the storage components in the cotyledons.

Stain Preparation (Krishnamurthy, 1988)

i. Sudan III-0. 4g of Sudan III dye was dissolved in 100 ml of 70% ethanol. The dye solution was filtered after about 12 hours.
ii. I_2KI-4g of Iodine dissolved in 6% potassium Iodide solution.
iii. 0.5% Saffranin-0. 5g Saffranin was dissolved in 2 ml of 95% ethanol and made up to 100 ml with distilled water.

RESULTS AND DISCUSSION

Melia azedarach is a native of west Asia, but naturalized throughout the warm countries. In India it is often cultivated in the plains and lower elevations as an ornamental avenue tree. The tree is valued for its medicinal properties. The present study was under taken to localize the storage components and secretary cells by suitable histochemical stains and reagents using light microscopic techniques.

Histochemical methods have been developed for qualitative and quantitative analysis of virtually all cellular components, including proteins, carbohydrates, lipids, nucleic acids and the range of ionic elements occurring in cell solutions (Gahan, 1984; Kiernan, 1999). These methods, in combination with various microscopic imaging techniques, can be utilized in the study of essential oil secretion in plants.

Sites of terpene synthesis and accumulation, components of essential oils, and structural features of the secretary tissues have been identified. Histochemical investigation of terpene biosynthesis usually involves a test for one of the enzymes specific to the synthetic process for example the localization of (-) trans-carveol dehydrogenase, an enzyme involved in the conversion of (-) trans-carveol to (-) carveon, the principle monoterpene in *Mentha spicata* (Gershenzon *et al.,* 1989). In the present study terpene secreting cells were localized (Fig. E & F). Anatomy of cotyledons shows the presence of parenchymatous cells along with large sized secretary cells (Fig. G & H). Cotyledons store various kinds of reserve food materials, starch being the most abundant one. In *Melia* cotyledons starch grains were localized with I_2KI stain (Fig. E & F). A histochemical method using Sudan III as a reagent was used to detect and localize oil bodies in *M. azedarach* seeds.

The secretary cells in *Melia* occur in groups of 3-5 (Fig. G, H). These cells are known to occur in other members of Meliaceae and all those families which accumulate secondary metabolites (Metcalfe and Chalk, 1950; Esau, 1965). Apart from the starch and the secretary cells oil bodies could also the localized by using lipids specific stain Sudan III (Fig I & J). Secretary cells are reported to occur in a number of dicotyledonous families, including Meliaceae (Metcalfe and Chalk, 1950). According to Dayanandan *et al.* (1993b) Secretary Cells (SC) in neem are the specialized structures, where neem chemicals are synthesized and stored. These structures occur in stem, roots, leaves, cortical and pith region and other ground tissues of stamina tubes, more abundantly in cotyledons. Cotyledons, which are considered to be the store house of triterpenoids, contain a large number of SC. Dayanandan *et al.,* (1993) used terpenoid reagents such as iodine vapour (Harborne, 1973), Carr-Price reagent (Jork *et al.,* 1990) and Libernmmann-Buchard reagent (Oser, 1965) to stain the contents of SC.

Several published reports demonstrate the use of histochemistry to locate essential oil, such as the localization of citral accumulation in *Cymbopogon citratus* (Lewin sohn *et al.,* 1998) Where the aldehyde-specific Schiff's reagent was used to detect the monoterpene aldehydes neral and geranial (Citral) and the lipid stains Sudan Red and Nile blue were used to locate essential oil in leaves of *Salvia aurea* (Serrato - Valenti *et al.,* 1997).

A histochemical method using nitrous acid as a reagent has been used to detect and localize phenols in the secretary structures of plant species from 26 families (Beckman *et al.,* 1972), and in the conifer *Picea abies* (Pinaceae) (Bilkova *et al.,* 1999); however, in both examples, the phenols are referred to collectively and no identification of individual compounds was attempted.

Medicinal plants are used in traditional treatments to cure variety of diseases. In last few decades there has been an exponential growth in the field of herbal medicine. Thus for the above mentioned reason and its medicinal importance, the plant species *M. azedarach* was selected and analysed for secretory cells by histochemical methods.

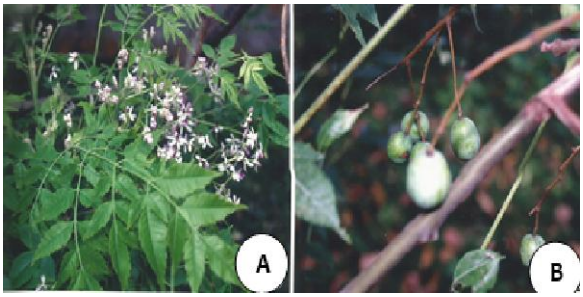

Figure A & B: A flowering and fruiting twig of *Melia* azedarach L.

Figure C & D: Mature dried fruits with the pericarp removed and seed split open

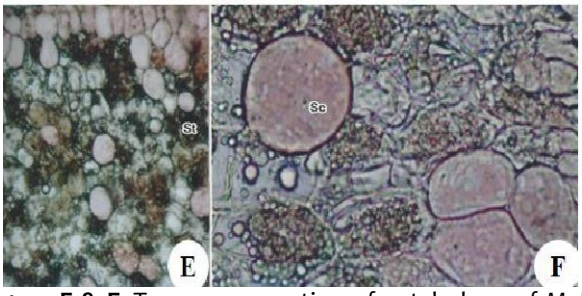

Figure E & F: Transverse section of cotyledons of *Melia azedarach* stained with saffranin and I_2KI reagent show the secretary cells (SC) and presence of starch (St) in the storage respectively. Fig. E X 210; F X 840.

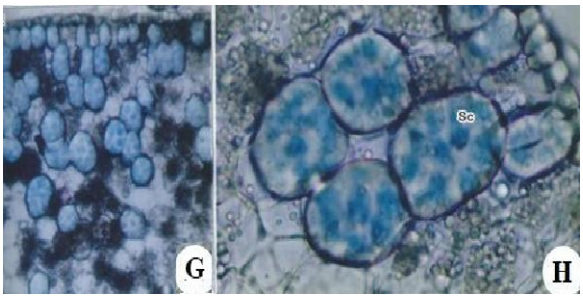

Figure G & H: Cross section of cotyledon stained with TBO to localize the presence of abundant secretary cells filled with meliacine and azadirachtins. Fig. G X 210; H X 840.

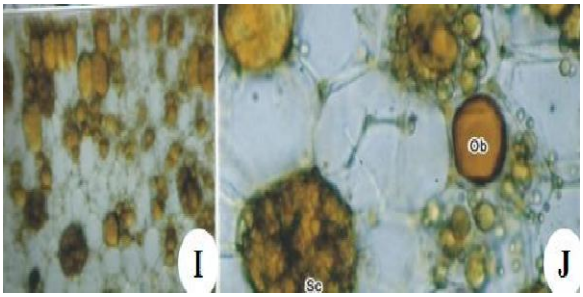

Figure I & J: Cross section of cotyledons stained with Sudan III to exclusively localize the occurrence of oil bodies in the hypodermal and storage regions. Fig. I X 210; J X 840.

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