

## Morphological, ethnobotanical, Pharmacognostical and pharmacological studies on the medicinal plant *Plumeria alba* linn. (apocynaceae)

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Plants have varieties of compounds like glycosides, alkaloids, terpenoids, steroids, which possess important diverse pharmacological activities to alleviate various diseases and disorders. Plants are vital source of drugs from the ancient time asset the scenario of the Indian system of medicine. The medicinal value of *Plumeria* species in the treatment of various human ailments is mentioned in Ayurveda, Charaka Samhita, and Sushrita Samhita. This alternative system of medicine is gaining increasing popularity worldwide. *Plumeria alba* Linn. (Apocynaceae) is an important shrub and widely used in perfumery and used in different traditional systems of medicine in the treatment of various diseases. The plant is mainly grown for its ornamental and fragrant flowers. This paper enumerates the pharmacognostical, morphological and ethnobotanical importance of the *P. alba*, which may help the researchers to set their minds for approaching the usefulness, efficacy and potency. The *P. alba* is small laticiferous tree or shrub is a native of tropical America, commonly known as White Champa. Leaves arrangement is lanceolate to oblanceolate with white flowers, fragrant in corymbose fascicles and fruit is edible. Leaves and stem were tested for its phytoconstituents, which are used in several traditional medicines to cure various diseases like purgative, cardiogenic, diuretic, and hypotensive. Their medicinal actions are often due to their latex which is commonly drastic and corrosive. Latex is applied to ulcers, herpes, and scabies. Seeds possess hemostatic actions and bark is bruised and applied as plaster over hard tumors.

**Keywords:** Ethnobotanicals, phytochemistry, pharmacognostical, pharmacological activities, *Plumeria alba*.

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## 1. Introduction

India perhaps the largest producer of medicinal herbs. Medicinal herbs have been in use for thousands of years, under the indigenous systems of medicine like Ayurveda, Sidha and Unani. In earth, about 3.6 lakh species of medicinal plants are present among these about 1.4 lakh species are present in India (Mehrola, 1990) and about 70000 plants are used in traditional systems of medicines. All over the world, plants were used as main source of medicines by ancestors (Mukherjee, 2001; Jadhav, 2006). The workers have made valuable contributions towards the ethnobotanical knowledge of primitive men, tribes and other folk healers of the state. The natural and traditional relationship between human societies and plants has brings to light various (Dwivedi, 2008). Even today the rural and aboriginal folks are very much in harmony with nature and bio resources (Shah and Singh, 1990). An obvious advantage of the present study is to create awareness towards the species and enumerate their traditional uses. Some interesting information on *Plumeria alba*. *Plumeria* or frangipani in a genus of flowering plant, family Apocynaceae. It contains primarily deciduous shrubs and small trees (Goyal et al., 2012). The flowers are native to Central America, Mexico, Caribbean and South America but can be grown in tropical and sub-tropical regions (Henry et al., 1987). *P. alba* is a small laticiferous shrub, 4.5 m high, occasionally grown in the gardens. The plant is mainly grown for its ornamental and fragrant flowers. Leaves are lanceolate to oblanceolate, flowers white, fragrant in corymbose fascicles (Chopra et al., 1956). The fruit is edible; latex is applied to ulcers, herpes and scabies and seeds possess hemostatic properties. Its bark is bruised and applied as plaster over hard tumors (Hartwell. 1982). The latter taxon finds use as purgative, cardi tonic, diuretic, and hypotensive (Kirtikar and Basu. 1935). Methanolic extract showed antimicrobial activity against *Bacillus anthracis*, *Pseudomonas aeruginosa* (Asolkar et al., 1992). The plant contain amyriacetate, mixture of amyris,  $\beta$ -sitosterol, scopotetin, plumieride, plumieride coumerate, the iriddoids isoplumericin, and plumieride coumerate glucoside (Nargis et al., 1993; Rengaswami and Venkatarao. 1960; Choudhary et al., 2014).



**Figure 1.** 1a. Flower of *P. alba*    1b. Leaf of *P. alba*    1c. *P. alba* tree

### 1.1. Plant profile

*Plumeria alba* Linn. (White Frangipani) is a fast-growing, medium size tree, family Apocynaceae. The plant can reach a height up to 5-8 feet with many branches on the upper part. Small trees with obanceolate leaves, alternate, bounded at twig tips, flowers are white, fragrant, in corymbose clusters. The white flowers bearing five petals and have fragrance. The vernacular names of the plant is Chameli (Hindi), Perungalli (Tamil), Khairchampa (Marathi), Frangipani, (English), Veyyivarahal (Telgu), Dalanaphula and (Benghal) (Nandkarni, 1954).



**Figure 2.** Leaves of *Plumeria alba*. 2a. Abaxial view (lower), 2b. Adaxial view (upper)

**Table 1.** Morphological studies of *Plumeria alba*.

S. N	Parameters	Features
1.	Habit	Medium size tree
2.	Cultivar	Not cultivated, occur wildly
3.	Plant height	5-8 ft.
4.	Plant characteristics	Herbaceous, photosynthetic having aroma, basically in flowers

5.	Foliage characteristics	Medium leaves
6.	Foliage color	Light green
7.	Flower color	White
8.	Status	Occur wildly, not under cultivation
9.	Conservation	By ex & in situ conservation
10.	Propagation	By seeds, also soft branches by planting in rainy season.

**Table 2.** Taxonomic Classification of *Plumeria alba*.

S.No.	Kingdom	Plantae
1	Subkingdom	Viridaeplantae
2	Phylum	Magnoliophata
3	Subphylum	Eaphyllophytina
4	Class	Magnoliopsida
5	Subclass	Lamiidae
6	Order	Apocynales
7	Family	Apocynaceae
8	Tribe	Plumerieae
9	Genus	<i>Plumeria</i>
10	Species	<i>Alba</i>

**Table 3.** General information of *P. alba*

<b>Scientific name</b>	<i>P. alba</i>
<b>Common name (s)</b>	White Frangipani/caterpillar tree/pagoda tree/pigeon wood/nosegay tree/white frangipani
<b>Family</b>	Apocynaceae
<b>Availability</b>	Generally, available in many areas within its hardiness range
<b>Native Range</b>	Puerto Rico, Lesser Antilles
<b>Zone</b>	10 to 12
<b>Height</b>	15.00 to 25.00 feet

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<b>Spread</b>	15.00 to 25.00 feet
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### 1.2. Leaves

Simple, alternate, oblong to elliptic, thick and leathery, many to 14 inches long and 1½ inches wide, strongly recurved margins, hairless on upper surface, whitish below, many lateral veins almost at right angle from the midribs.

### 1.3. Flowers

Salverform, five waxy white petals, with yellow centers, arranged on cymes on branch tips.

### 1.4. Fruits

Not shown. Follicles, brown long-pointed, usually borne in pairs.

## 2. Morphological Characteristics

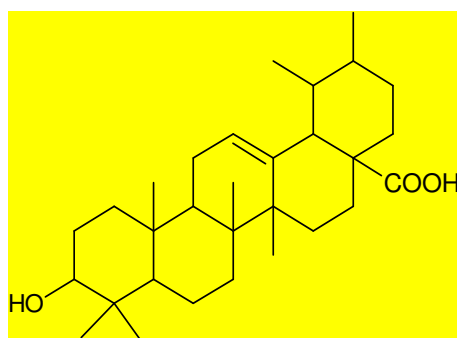
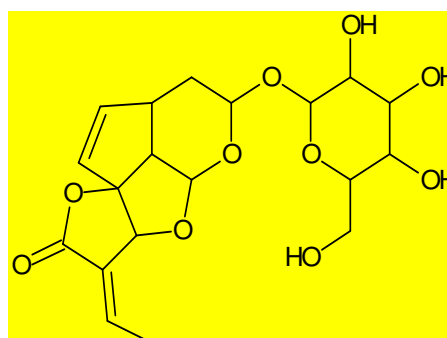
White Frangipani grows as either a small shrub or tree ranging in height from 0.9-6.1 m. The leaves are clustered near the tips of the branches. They are large, 6-22 cm long, 2-7 cm wide, and have an obovate shape and the tip of the leaf is rounded, rather than pointed as it is in other species. The leaves are dark and leathery and tend to be shiny on the upper surface with conspicuous parallel secondary veins that run from the midvein to the margins of the leaves. The flowers of this species are borne in clusters that form at the ends of the branches on a long thick stalk. Each inflorescence contains many white flowers with a small yellow center. Flowers contain five petals that are fused at the base in a short funnel-shaped tube which gradually widens as the lobes of the petals are spread out. The fruit of this species is a dry follicle which splits along one side to release the winged seeds (Mabberley, 2005; Raju, 2000).

## 3. Phytoconstitutes

*P. alba* possesses various bioactive constituents such as sterols, carbohydrates, tannins, triterpenoids, and iridoid glycosides. The aerial part of the plant, i.e., leaves stems, etc., are reported to contain steroids, flavonoids, and alkaloids. The plant is reported to contain mixture of amyris, β-sitosterols copotein, iriddoids isoplumericin, plumeride, plumeride coumerate, and plumeride coumerate glucoside. The fresh leaves and bark contain pluieride, resinic acid, and fulvoplumierin, a mixture of terpenoids, sterols, and plumieride. Bark of the plant contains

cytotoxic iridoids, fulvoplumierin, *Allamcin*, *Allamandin*, 2,5-dimethoxy-p-benzoquinone, plumericin, and lignin liriodinndrin. The root bark of *P. alba* shows the presence of iridoids, tannins, and alkaloids. *P. alba* bark containing alkaloids, carbohydrates, flavonoids, phenolic compounds, and tannins (Siddiqui et al., 1994). The plant is reported as medicinal which contains amyrin acetate, mixture of amyrins,  $\beta$ -sitosterol, scopotetin, the iriddoids isoplumericin, plumieride, plumieride coumerate, and plumieride coumerate glucoside (Gilman and Watson. 1994). The flower oil mainly consists of primary alcohol, viz. Geraniol, citronellol, farnesol and phenyl ethyl alcohol and some linalool. The flowers contain quercetin and kaempferol (Anonymous. 2005).

Rangaswami (1960) reported that the chemical constituent amyrin acetate (210-15°) was derived from powdered bark of *P. alba*. It was extracted with cold pet ether, ether and hot alcoholic residue (58g) from evaporation of pet ether extract. The component was chromatographed on alumina, and eluted with pet ether to give 45.8 g semisolid, from which amyrin acetate 0.3 g was obtained. John (1983) has examined the presence of iridoids such as isoplumericin, plumericin, plumieride, plumieride coumarate and plumieride coumarate glucoside from *Plumeria obtusa*. The study revealed that isoplumericin and plumiericin were rarely found in aerial parts, remaining components highly concentrate in root bark only. Bramadhayalaselvam (1997) isolated ursolic acid (0.06%) and  $\alpha$ -amyrin acetate (0.01%) from bark and plumieride (0.02%) and plumieride-p-coumarate (0.025%) from the leaves of *P. alba*. Kalita (2004) has documented the presence of chemical constituents and energy content in the presence of some latex bearing plants including *P. alba*. The plant parts (leaf, stem, bark) were analysed for elemental composition, oil, polyphenol, hydrocarbons, crude protein, alpha cellulose, lignin and ash. The plant species might be suitable as alternative source of hydrocarbons and other phytochemicals. The bark of *P. alba* containing alkaloids, carbohydrates, flavonoids, phenolic compounds, and tannins (Gilman and Watson. 1994). The plant is a medicinally important being having amyrin acetate, mixture of amyrins,  $\beta$ -sitosterol, scopotetin, plumieride, plumieride, coumerate, the iriddoidsiso plumericin and plumieride coumerate glucoside (Anonymous. 2005). The flower oil mainly consists of primary alcohol, such as geraniol, citronellol, farnesol and phenyl ethyl alcohol, and some linalool. The flowers contain quercetin and kaempferol (Siddiqui et al., 1994).

**Ursolic Acid****Allamacin Glycoside**

### 3.1. Ethnobotanical uses

The various parts of the plant *Plumeria alba* are used in the Indian systems of medicine for various ailments. The latex of the former taxon is applied to ulcers, herpes and scabies (Chopra and Nayar 1956). Its bark is bruised and applied as plaster over hard tumours (Hartwell 1982), whereas the latter taxon finds use as purgative, cardiogenic, diuretic and hypotensive (Kritikar and Basu 1935). Leaves are made into powder and taken twice a day to treat jaundice. Bark powder taken after night meal act as purgative. Latex obtained from the plant is used to treat ulcer. Root bark paste applied in rheumatic pain and root powder taken twice a day used as carminative.

### 3.2. Economic uses

The milky sap of stem and leaf is applied to skin diseases such as herpes, scabies and ulcers. Seeds are haemostatic. Latex is also used as purgative. The tree is grown in temple yards of South India for offering its flowers to temple deities.

### 4. Pharmacology activity

Different part of the *P. alba* was believed, have been useful in a variety of diseases, namely, the diseases of Malaria, Leprosy, Rheumatism, and abdominal tumors. The milky sap of the stem and leaf is applied to skin diseases such as herpes, scabies, and ulcers (Prajapati *et al.*, 2004; Raju. 2000). Its bark is used as plaster over hard tumors, the seeds in hemostasis while the latex is used as purgative, cardiogenic, diuretic, and hypotensive (Rengaswami and Venkatarao. 1960). *P. alba* is also used in the treatment of ulcers, herpes, scabies, and seeds possess hemostatic properties. The bark is bruised as plaster over hard tumors (Raju. 2000). *P. alba* has various type of pharmacological activities. These activities are given below.



#### 4.1. Anticancer activity

The anti-cancer property of the triterpemoids isolated from several plants has been well documented. The *P. alba* has not been investigated for its anti-cancer activities in detail though the plant is reported to contain triterpenoids. Hence the study is directed towards exploring the anti-cancer activity as well as any new molecules that may possess anti-cancer activity in the plant.

#### 4.2. Antimicrobial activity

*P. alba* appears to have significant antimicrobial capacity resembling a broad spectrum antibiotic against the common uro-gastro pathogenic *Escherichia coli*, one of the common bacteria with pathogenic strains and are relatively resistant towards synthetic drugs. This aromatic plant can be a potential source of evolving newer antimicrobial compound and as a non-toxic antibiotic producer agent. The extracts of frangipani have a potential as a natural anti-toxic antibiotic producer, especially against *E. coli* (Zahid *et al.*, 2010). Antibacterial activity of *P. alba* (Frangipani) petals methanolic extracts were evaluated against *E. coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *P. aeruginosa*, *S. saprophyticus*, *Enterococcus faecalis*, and *Serratia marcescens* using disk diffusion method. Frangipani extract also showed high antibacterial activity against *S. saprophyticus*, *P. vulgaris*, and *S. marcescens*, but not more than the zones of the positive control used (Radha *et al.*, 2008; Syakira and Brenda. 2010). The extracts of root, stem and leaves of the Peruvian timber tree *Plumeria alba* yielded the known glucoside, Plumieride. The alcoholic extracts of the leaves also gave rutin. They have antibiotic activity but no cardiotonic activity on isolated guinea pig heart. The extracts are non-toxic in mice (Siddiqi *et al.* 1970).

#### 4.3. Anti-fungal activity

Antifungal activities of methanolic extract and the isolated fraction of the plant *P. alba* was assessed. The zone of inhibition was compared with that of Standard antibiotic ciprofloxacin (5 mg/disc) by disc diffusion method. The anti-fungal activity was assessed by standard dilution technique using Sabouraud dextrose agar medium. The results are compared with standard Clotrimazole (125 µg/ml) (Radha *et al.*, 2008).

#### 4.5. Hepatoprotective Activity



Hepatoprotective activity of *P. alba* extract against paracetamol induced hepatotoxicity in rats. The methanol extract at different doses (100, 200, and 400 mg/kg) of the plant *P. alba* Lam. syn. *Plumeria acutifolia*. Poir were tested for its efficacy against paracetamol-induced acute hepatic damage in Wistar rats. Methanolic extract of *P. alba* did not produce any toxic symptoms or mortality up to the dose level of 2000 mg/kg body weight in mice, and hence the extract was considered to be safe and non-toxic for further pharmacological screening. The normal control group showed a normal liver architecture, hepatocytes very well arranged, central vein without alterations (Chowdhur *et al.*, 2010).

#### 4.6. Antiarthritic activity

The anti-arthritic potential of ethyl acetate and n-butanol fractions (100 and 200 mg/kg, p.o.), respectively of hydroalcoholic extract from leaves of *P. alba* were evaluated *in vivo* models of rodents by using formaldehyde-induced acute non-immunological and Freund's Complete Adjuvant-induced chronic immunological arthritis in Sprague-Dawley rats. Antiarthritic potential of fractions may be due to the protection of synovial membrane, vascular permeability, prevention of cartilage destruction.

#### 4.7. Antioxidant Activity

Free radicals generated either exogenously or endogenously inside the body have been implicated in causation of several diseases such as liver cirrhosis (Slater, 1987), inflammation, atherosclerosis (Halliwell and Gutteridge, 1985), diabetes, cancer (Dreher and Junod, 1996), neurodegenerative disease (Knight, 1997). The link between free radicals and disease processes has led to considerable research into non-toxic drugs that can scavenge the free radicals. Several plant extracts and plant products have been shown to possess significant antioxidant potential (Soudhamini and Kuttan, 1989). The plant *P. alba* was traditionally used for treating ulcers, scabies and hard tumours (Chopra *et al.*, 1956). These biological uses might be related to antioxidant activity. The *in vitro* antioxidant activity of the methanolic extract through the 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and nitric oxide radical scavenging activity (Aquino *et al.*, 2001; Green *et al.*, 1982).

#### 4.8. Larvicidal activity

Leaves extract of *P. alba* found (LC<sub>50</sub> 218.8 ppm) against *Aedes aegypti* mosquitoes (Goyal *et al.*, 2012; Kaushik and Saini. 2009).

## 5. Pharmacognostical studies

The dried and stored powder of leaves of *P. alba* was subjected to standard procedure for the determination of various parameter according to the Ayurvedic Pharmacopoeia, 1999. Omina (1996) had carried out taxonomy, anatomy, cytology and palynology work on Apocynaceae species in east Africa. He had noted that the *Plumeria* species had medicinal properties. Marimuthu (1998) reported that the preliminary phytochemical screening was carried out on 12 laticiferous members belonging to family Apocynaceae, *Plumeria alba* and *Plumeria rubra* (white and pink varieties). He has proved the presence of carbohydrate and amino acids through qualitative analysis. Kennedy *et al.* (1999) have evaluated 24 non-conventional flower species for ascertaining the source of perfume. He had proved that plumeria species contained eugenol (31%) and geraniol (13%) through chromatographic analysis. Kamariah *et al.* (1999) reported that the essential oil of fresh flowers from *Plumeria obtuse*. The study also identified 47 components representing 97 % of the oil. Praveena *et al.* (2001) reported a new host of the rust pathogen uredo *Plumeriae*. It was 1-2 mm in diameter, yellowish orange, powdery mass of spores on the corresponding lower surface. The powder of bark was found to be brown in color with slightly bitter taste and odorless. On microscopically examination, the powder showed sclerenchyma, parenchyma cells, phloem fibers, starch grains, and cork cells. Different parameters, i.e., Total ash, acid insoluble ash, water soluble ash, loss on drying, and swelling index were found to be 6.0, 2.3, 1.8, 1.33, and 20.0, respectively (Goyal *et al.*, 2012). The hot water soluble extractive was high in the bark of *P. alba*. Preliminary phytochemical screening reveals the presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, and tannins in this plant (Rengaswami and Venkatarao. 1960).

### 5.1 Macroscopic Features

The plant is a small tree with dense, spreading branches. The leaves are deciduous leaving only a coloral of leaves at the shoot-tip. The leaves are obovate-elliptic; leaf base is slightly oblique; leaf apex obtuse. Lateral veins are 32-35 pairs; no intra marginal veins are evident (Fig. 1 and 2). The fruits are double follicle, green, woody cylindrical and long. The tree from which leaves

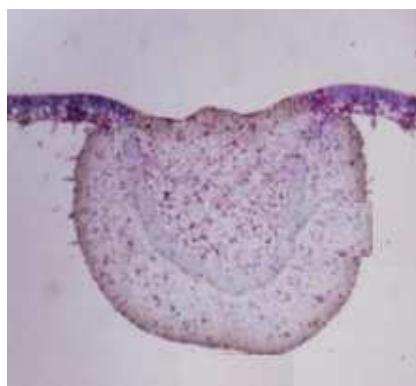
were collected for the present study, exhibits a peculiar periderm like structure on the thick branches. These structures seem to be rhytidome produced in the form of thick, continuous vertical bund, restricted both adaxial sides of the leaning branches (Fig. 3). It appears like sedimentary rock and is firmly attached to the stem (Fig. 2). It seems to be dead tissue and breaks into powder when crushed.



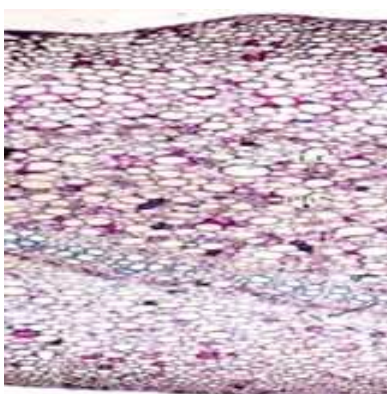
**Fig. 3** Surface feature of the thick lateral branches and Rhytidome of old lateral branch.

### 5.2. Microscopic Features: Leaf

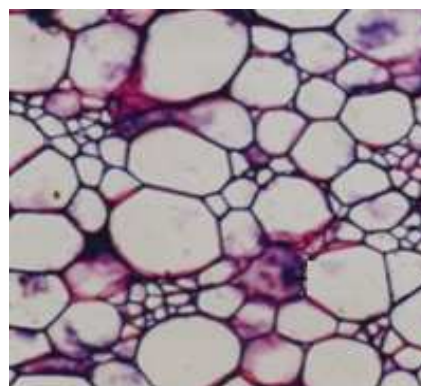
The leaf has very thick midrib and thin lamina arising from the adaxial-lateral portion of the midrib. The midrib is flat on the adaxial side with a short central, less prominent ridge and the abaxial side is wide, prominent, even and semicircular. The midrib is 1.8 mm vertically and 2.5 mm horizontally (Fig. 4). It consists of a thin epidermal layer which is not much distinct from the inner tissues. The ground tissue is differentiated into outer zone of smaller collenchyma cells and remaining portion being parenchymatous. The collenchyma zone is 150  $\mu\text{m}$  wide in the upper part and 50  $\mu\text{m}$  wide in the lower part. Narrow, thick walled, circular or lobed laticifers are seen randomly dispersed in the ground tissue. Some of the laticifers are wide and thin walled and no contents are seen in the laticifers. The vascular system consists of a wide, bowl shaped thin strand and two small, less prominent accessory adaxial lateral strands. The main bowl shaped strand is 1.5 mm wide, 150  $\mu\text{m}$  thick. It consists of short, radial file of 3-5 angular xylem elements and a thin layer of phloem along the outer metaxylem side. Within the concavity of the vascular arcs there are numerous small nests of phloem elements which are known as inner phloem (Fig. 5 and 6). The xylem elements are 30  $\mu\text{m}$  wide. The laticifers are 70  $\mu\text{m}$  wide.



**Fig. 4** Anatomy of leaf (T.S of leaf midrib with lamina)



**Fig. 5** T.S of midrib-a portion enlarged



**Fig. 6** Phloem nest and laticifers in ground tissue of midrib

### 5.3. Lamina

The lamina is trichomatous on the abaxial side and smooth and glabrous on the adaxial side. The major lateral veins project prominently into conical abaxial part. The vein-lets are thick and prominent, but do not project much beyond the surface. The lateral veins and veinlets have a small cluster of xylem elements and a thin arc of phloem. The vascular bundles are subtended by thick mass of parenchyma cells which extend into adaxial column up to the upper epidermis. The adaxial epidermis of the leaf has large cells with thick cuticle. The cells are mostly squarish or rectangular. The abaxial epidermis is thin and the cells are narrow and cylindrical. The mesophyll tissue consists of wide adaxial palisade zone which is single layered, compact and the cells being columnar in shape. They are 150  $\mu\text{m}$  in height. The spongy mesophyll consists of several lobed parenchyma cells which are interlinked with each other around the air chambers (**Fig. 7**).

### 5.4. Epidermal Trichomes

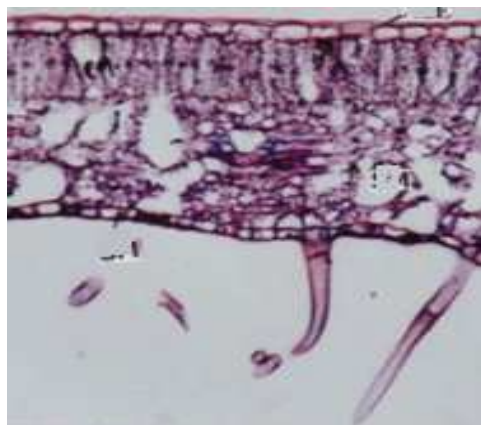
The trichomes are non-glandular or covering type and arise from a group of dilated epidermal cells. The trichomes are multicellular, uniseriate and unbranched. They are narrow and thick walled with smooth surface (**Fig. 8**).

### 5.6. Leaf margin

The marginal part becomes slightly thin with rounded bent down edge. The epidermal layer of the leaf margin is thin, the cuticle is very thick and smooth. The palisade – spongy parenchyma



differentiation is absent in the marginal portion. It consists of 4 or 5 layers of small, thick walled compact parenchyma cells (**Fig. 8**). The marginal part of the lamina is 150  $\mu\text{m}$  thick.



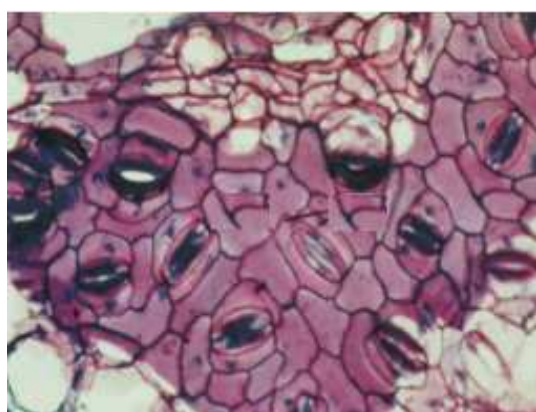
**Fig. 7** Anatomy of the lamina



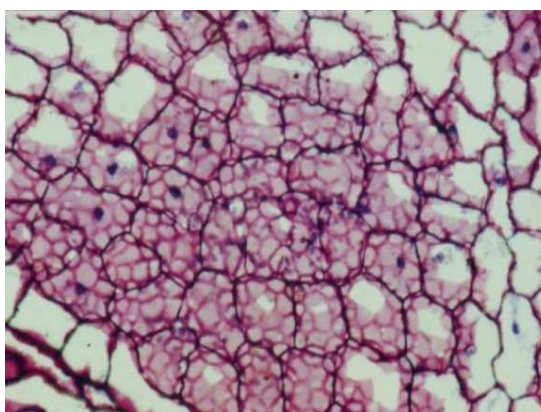
**Fig. 8** Anatomy of the thick lateral vein and leaf margin

### 5.7. Epidermal cells and Stomata

The epidermal cells are rectangular to polyhedral in shape. The cells have straight or slightly wavy anticlinal walls. The walls are moderately thick. The stomata are paracytic type; there are two subsidiary cells, lying on the lateral sides of the guard cells. The subsidiary cells may be equal or slightly unequal in size. The guard cells are elliptical with wide stomatal aperture. The guard cells are 40 x 70  $\mu\text{m}$  in size. Adaxial epidermis is non-stomatiferous (**Fig. 9** and **Fig. 10**). The epidermal cells are uniformly polyhedral and random in distribution. The anticlinal walls are thin, straight or slightly wavy. Some of the cells possess prominent nuclei.



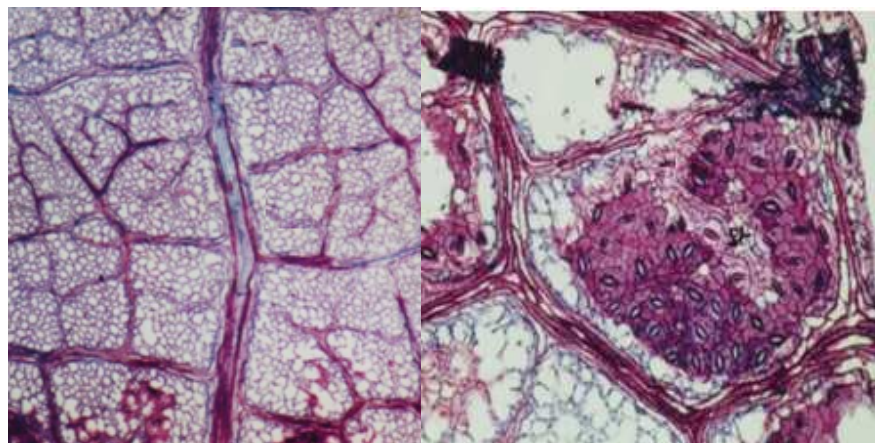
**Fig. 10** Stomatal morphology



**Fig. 11** Epidermal morphology

### 5.8. Venation Pattern

The lateral veins are fairly prominent forming dense reticulations. The vein islets are distinct. They are variable in shape and size. The shape of the islets ranges from square to polyhedral. The islets are random in orientation. The vein terminations are fairly distinct. They are uniformly thin and slender. Most of the terminations are branched into dendroid configuration (Fig. 11).

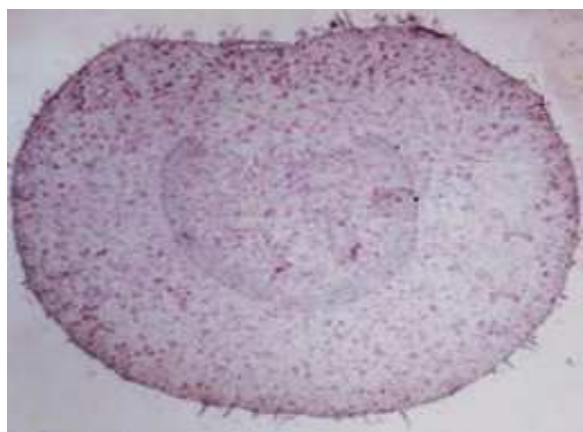


**Fig. 11** Venation pattern

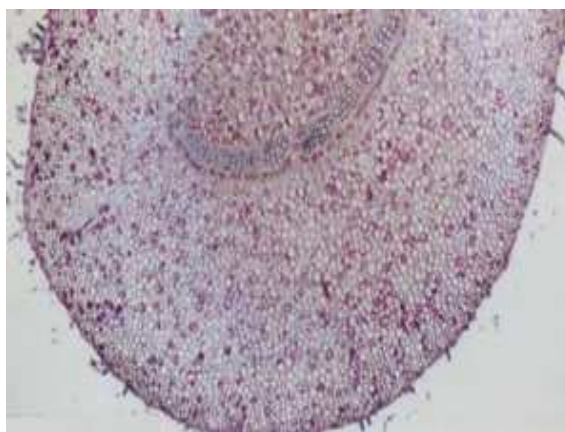
### 5.9. Petiole

The cross sectional outline of the petiole differs from the basal (proximal) part to the terminal (distal) part. The basal part of petiole is circular with a slight concavity on the abaxial side. It measures 3.1 mm horizontally and 2.6 mm vertically. It has dense epidermal trichomes all-round. The epidermal layer is narrow with small squarish cells. The ground tissue occupies the major portion of the petiole. It consists of outer wider zone of small, compact, fairly thick-walled cells and remaining regions have larger, circular, less compact parenchyma cells (Fig.12). The vascular tissues form a wide bowl shaped out-lines which are of 1.5 mm wide and 60  $\mu$ m thick (Fig.13). The xylem elements are in short radial lines with two or three elements in each row. Phloem is in a continuous line. Laticifers are distributed throughout the ground tissue; they are more abundant in central portion, inside the vascular strand. Apart from the outer phloem, there are numerous scattered irregular masses of phloem strands distributed along the inner boundary of the xylem arc (**Fig.12**). Distal part of the petiole has two thick cylindrical adaxial wings and wide, shallow adaxial semicircular round. It is 3.1 mm in horizontal plane and 2.6 mm in vertical plane. The adaxial wings are 500  $\mu$ m in height and 300  $\mu$ m in thickness. The petiole has thin epidermal layer of small squarish cells. The ground tissue has outer zone of smaller, dark,

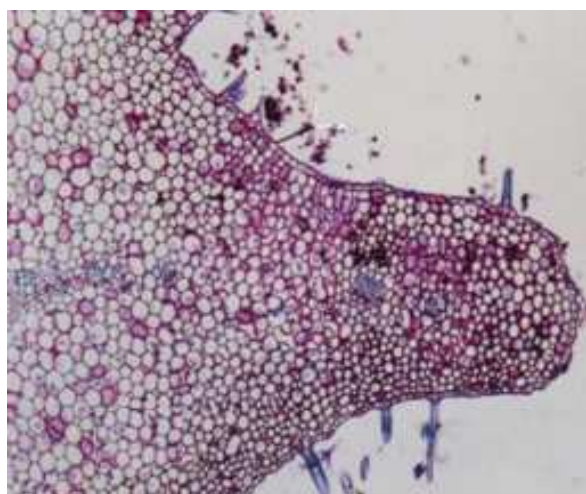
compact cells and inner zone of larger circular thin walled cells. The outer region is gradually transformed in to inner region. The vascular system is similar to that of basal part of the petiole. It is deeply urn- shaped (Fig. 14), comprising of numerous radial parallel short rows of xylem elements and a thin ring of outer phloem elements. Within the concavity of the vascular arc are seen numerous nests of phloem elements mixed with wide, thin walled laticifers. The two margins of the vascular arc give rise to two or three small, circular vascular strands placed along the wings. Wide circular thin walled laticifers are seen widespread in the central ground tissue. In the central part of the ground tissue, these are dark, lobed structures surrounded by rosette of ground parenchyma (Fig. 15).



**Fig. 12** Cross section of proximal part of the petiole



**Fig. 13** T.S of petiole through proximal region (outline and vascular tissues).

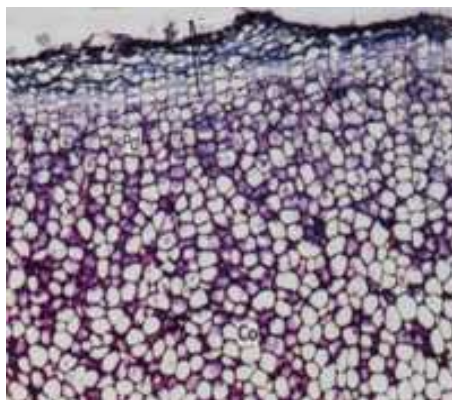
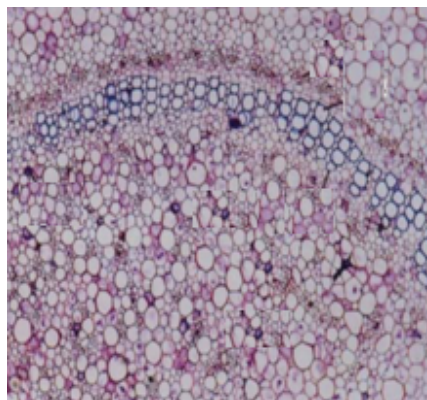




**Fig. 14** T.S of petiole through distal region **Fig. 15** Structure of the petiole through distal region

### 5.10. Stem

Both young and old stems were studied. The young stem has thin periderm, wide cortex and a wide stele enclosing the central pith (**Fig.16**). The periderm is thin and continuous. It is formed by three or four cells to the cortex. The periderm is with initial stage and masses about 150  $\mu\text{m}$  wide. It consists of three or four layers of phellem being situated in between equal number of phelloderm and phellogen. Phelloderm merges gradually within cortex which is quit wide, homogenous and parenchymatous. Densely distributed with cortex are laticifers which are wider than the neighbouring parenchyma cells. Some of the laticifers are seen in horizontal orientation (**Fig.17**).The stele or the primary vascular tissues are seen in the form of thin continuous hollow cylinder encircling the wide pith. Endodermis and pericycle are not evident. The stele consists of radial, parallel rows of two or three xylem elements. On the metaxylem side occurs a ring of small, discrete phloem strands. In the central portion of the pith, there are numerous, small, clusters of phloem scattered all throughout. These phloem strands are the medullary phloem strands. Narrow, thick walled, darkly staining laticifers are seen diffusely distributed in the pith.

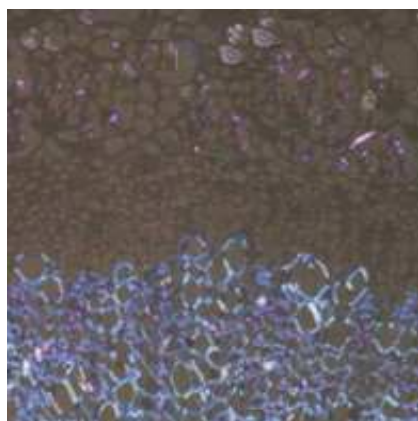


**Fig. 16** Anatomy of the young stem **Fig. 17** Anatomy of the old stem

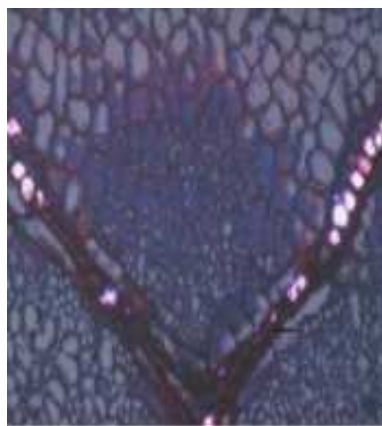
### 5.11. Stem

The stem has well-developed wide periderm and thick cylinder of secondary vascular tissues (Fig. 18). The periderm has replaced the cortex and epidermis forming a thick cylinder on the surface of the stem. It is differentiated into wide phellem and distinct phelloderm zones. The phellem consists of several, compact, tabular, suberised cell layers. The phellem is 200  $\mu\text{m}$  wide.

Phelloderm is 100  $\mu\text{m}$  wide. It consists of thin walled, rectangular, radial files of cells resembling the cortical cells. The cortex is homogeneous and parenchymatous. The secondary vascular cylinder comprises of thick secondary xylem, outer secondary phloem and inner medullary phloem. The secondary xylem cylinder is 450  $\mu\text{m}$  thick. It consists of long radial multiples of vessels and fairly wide radial rays of xylem fibres. The vessels are thick walled, angular and measure 40-50  $\mu\text{m}$  wide. The xylem fibres are thin walled and radially elongated. Some of the xylem fibres especially those that are towards the outer zone of the secondary xylem are gelatinous fibres; they are narrow, solitary or in groups of three or four and have gelatinous inner walls (Fig. 19). Secondary phloem occurs both on the outer and inner portions of the secondary xylem. The outer phloem is in radial files; the inner, medullary phloem is in wide circular masses abutting the xylem cylinder. Laticifers are abundant in the inner cortical zones of the young and old stems. Most of these are circular or slightly angular, wide and thin walled; they are up to 70  $\mu\text{m}$  wide. The laticifers are surrounded by a ring of parenchyma cells which are smaller than neighbouring parenchymatous ground cells (Fig. 19). However, these ensheathing cells do not possess dense cytoplasm or prominent nuclei. The laticifers are random in distribution. In some of the sections, the laticifers appear in horizontal orientation.



**Fig. 18** Anatomy of the stem



**Fig. 19** Distribution of the laticifer

### 5.12. Starch grains and Crystals

Starch grains are fairly common in the cortical cells of the stem. The grains are mostly circular with x or y shaped dark lines. The starch grains are 5-10  $\mu\text{m}$  in diameter. Several starch grains occur within the cell (Fig. 20). Calcium oxalate crystals are abundant in the leaf, midrib, stem

and bark. Prismatic type of crystal is located along the veins of the lamina. Prismatic as well as druse (sphaero crystal) types are seen sparingly in the outer cortical cells of the mid rib (Fig. 21). Prismatic type mostly of rhomboidal shape is located in abundance in the cortical cells of the stem. Prismatic crystals are also seen in xylem parenchyma, especially in the outer zone of the secondary xylem (Fig. 22). The crystals in the leaf are larger than those in the midrib and xylem parenchyma. The crystals are 20.1  $\mu\text{m}$  in size.



**Fig. 20** Starch grains in the stem



**Fig. 21** Crystal distribution in the leaf



**Fig. 22** Crystal distribution in the stem

## 6. Quantitative Microscopy

The observed values for Stomatal number, Stomatal index, Vein islet, Vein termination numbers and linear measurements are given in Tables 4 and 5.

**Table 4.** Leaf constants of *Plumeria alba*.

Parameters	Values
Stomatal number	11.7 per Sq.mm
Stomatal index	21.07 %
Vein islet number	15.4 per Sq.mm
Vein termination number	29.4 per Sq.mm

**Table 5.** Linear measurements of *Plumeria alba*.

Parameters	Average Value ( $\mu\text{m}$ )	
	Length	Width
Trichome (Unicellular covering)	28.5	0.2

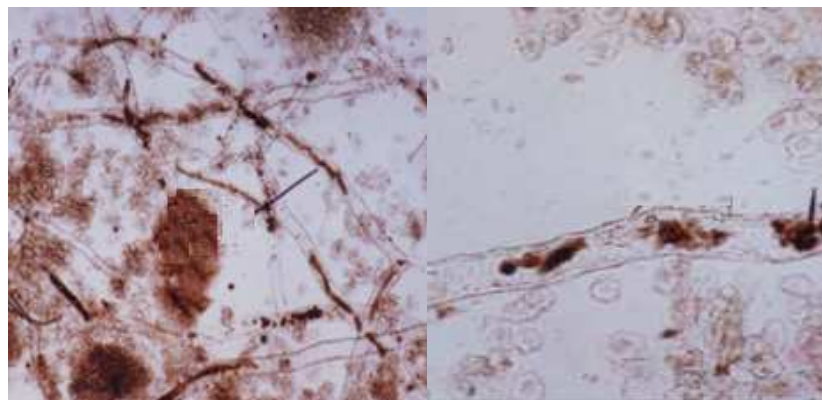


To localise the compounds specific dyes are employed which will selectively stain the compounds depending upon the reaction affinities between the compound and the dye. The relevance of histochemical studies in biological sciences is gaining momentum because of the usefulness of the technique not only in retrieving information on the biochemical pool of the cells but also for diagnostic purpose of the organism studied. In the present study, starch, tannins and lipids were localized in the petiole, lamina, midrib, stem bark and wood. The results are given below in Table 7 and Fig. 22-25.

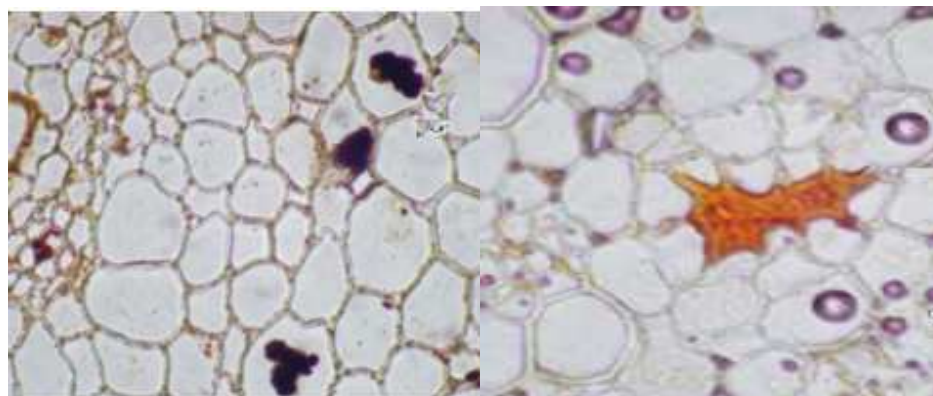
**Table 7.** Histochemical tests on different organs of *P. alba*

S. No.	Tests	Lamina	Midrib	Petiole	Stem	Bark	Wood
1	Starch (IKI)	-	+	+	+	+	+
2	Tannins (FeCl <sub>3</sub> )	-	-	-	-	-	-
3	Lipids (Sudan III)	+	+	+	+	+	+

Note: (+) Present, (-) Absent

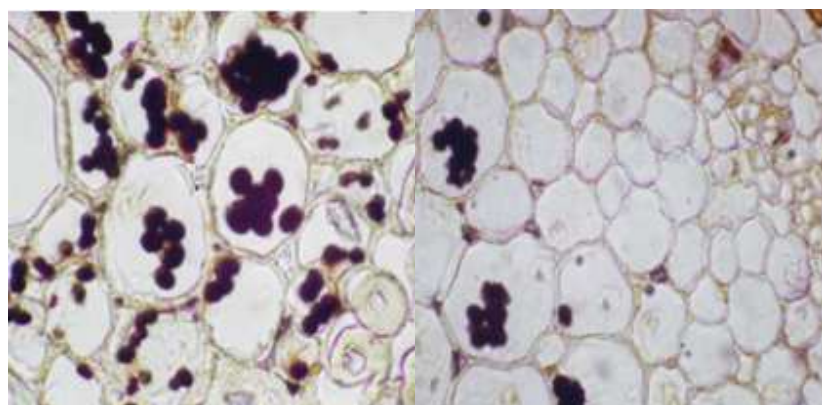


**Fig 22.** Localized in the petiole, lamina, midrib.

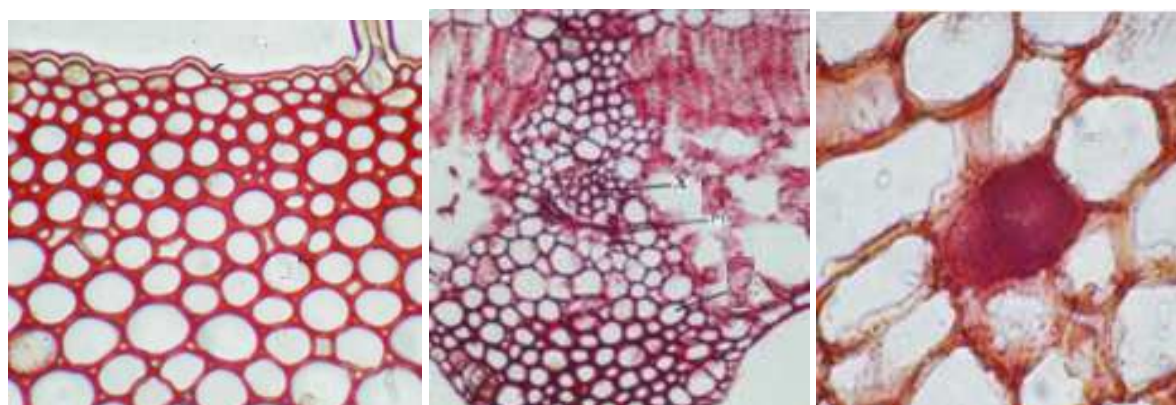




**Fig 23.** Starch grains in the cortical parenchyma nearer to phloem fibres and Starch grains in the cortex of stemwood



**Fig 24.** Midrib stained with ferric chloride showing darkly stained cell walls of the collenchymas, Collenchyma tissue of the petiole stained with ferric chloride shows darkly stained in the cell walls



**Fig 25.** The walls of the laticifer are stained dark showing the localization of lipids

## 7. Qualitative Chemical Analysis

Phytochemical analysis for the presence of phytoconstituents in n-Hexane, Chloroform, Ethyl acetate and Methanol extracts of *P. alba* are given in Table-8.

**Table8.** The preliminary phytochemical test of various extracts of *Plumeria alba*

Test for	n-Hexane			Chloroform			Ethyl acetate			Methanol		
	L	S	W	L	S	W	L	S	W	L	S	W
Triterpenes	+	+	+	+	+	+	+	+	+	+	+	+

Alkaloids	-	-	-	-	-	-	-	-	-	+	+	+
Glycosides	-	-	-	-	-	-	-	-	-	+	+	+
Gums and Mucilage	-	-	-	-	-	-	-	-	-	-	-	-
Fats and Oils	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	+	+	+	+	+	+
Phenols	-	-	-	-	-	-	-	-	-	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	-	-	-	-	+	+	+

L – Leaf, S – Stem bark, W – Wood **Note:** (+) Present; (-) Absent.

### 7.1. Inorganic Mineral Analysis

The amount of Sodium and Potassium estimated by flame photometry and copper, zinc, lead, etc., estimated by atomic absorption spectroscopy present in 100 g of dried plant material are presented in Table 9.

**Table 9.** Amount of minerals present in dried materials of *P. alba*

S.No.	Parameter	Unit Sample <sup>-1</sup>	S.No.	Parameter	Unit Sample <sup>-1</sup>
1	Copper (DB)	mg/kg 118	7	Arsenic (WB)	mg/kg <0.001
2	Zinc (DB)	mg/kg 63	8	Manganese (DB)	mg/kg 72
3	Lead (DB)	mg/kg 11	9	Total chromium (DB)	mg/kg <0.01
4	Nickel (DB)	mg/kg 100	10	Cobalt (DB)	mg/kg 92
5	Cadmium (DB)	mg/kg <0.0008	11	Sodium	mg/kg 0.01
6	Cyanide (DB)	mg/kg <0.005	12	Potassium	mg/kg 0.01
< - Indicates less than minimum detection limit.			DB - Dry basis WB- Wet basis		

### 8. Discussion

To combat the ever increasing new and little known human ailments and to save humanity from the clutches of such diseases, a large number of lifesaving herbals have been introduced in the market. There is no ambiguity in any sense in the efficacy of herbal remedy. However, the herbals are handled mostly by the pharmacognosists or phytochemists who may not possess any botanical knowledge of the drugs they use. Many pharmaceutical laboratories purchase the crude



drugs from the commercial sources and the identity of the drugs is based on the local/ trade names. Regional names or commercial names of the phyto drugs are highly misleading. Added to these problems adulteration or substitution is another menace in the market drugs. Ultimately phyto drugs without botanically confirmed identity may lead to adverse or unwanted results. To establish the botanical identity of a phyto drug, morphological, anatomical and histochemical parameters are being employed. Preliminary phytochemical knowledge of the drug provides both an insight into the chemical background of the plant and chemical standards of the primary constituents. *P. alba* is a complex taxon having a large number of varieties, cultivars and hybrids. In the present study, particular attention is given to provide well defined macroscopic and microscopic standards for the identity of the species used. *Plumeria* is a complex genus with subtle variation among the species. Many cultivars add more difficulty in circumscribing the species. There is a lot of confusion prevailing in flora which provide the taxonomic characters of a few species that occur in India. One species namely, *P. rubra* has dark red or purple flowers while the *P. alba* has white flowers with or without yellow at the throat of the corolla. Yellow spot at the throat of the corolla is invariably seen in most of the plants observed. The variety chosen for the present study has purely white or light shade of yellow in the throat of the corolla. Metcalfe (1979) has established that certain anatomical features of the plant are not affected or modified by the environmental stress. Howard (1979) has shown that microscopic characters of plants combination prove to be of diagnostic values in the study of sterile fragmentary samples. The midrib, its structure as seen in cross sectional view, is specific for each species. In *P. alba* the midrib is quite thick projecting as prominent semicircular structure with wide bowl shaped vascular strand. The adaxial part is flat with a short central conical part. According to Howard (1979) and many others who have done extensive studies on the petiolar anatomy and its bearing on the systematic studies, have shown that the petiole structure is one of the potential sources of diagnostic features. The cross sectional outline and the vascular patterns of the petiole may vary at different levels of the petiole. In *P. alba* the petiole is circular with shallow depression on the adaxial side. It is circular with two thick horns on the adaxial part in the distal part. The vascular strand remains urn-shaped along different levels. Further, numerous phloem strands are scattered throughout the ground tissue, mixed with wide, circular lactiferous canals. Phytochemistry has

developed into a distinct discipline and is closely related to natural product organic chemistry and plant biochemistry. It deals with a variety of secondary metabolites that are produced by plants, their chemical structures, biosynthesis, metabolism, natural distribution and biological functions. For these operations, certain methods are needed for separation, purification and identification of different constituents present in plants. Thus advances in our understanding of phytochemistry are directly related to successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they come. One of the challenges of phytochemistry is to carry out the above operations on small amounts of material (Mukherjee, 2001; Jadhav, 2006).

The qualitative chemical analysis indicated the presence of triterpenes in all successive extracts namely n-hexane, chloroform, ethyl acetate and methanol. Alkaloids, Glycosides and Phenols are present only in methanolic extract. Flavanoids are present in ethyl acetate and methanol extracts. This indicated the presence of a variety of secondary metabolites in the plants, which may be responsible for the wide spectrum of pharmacological activity. In nutrition, minerals are those elements for which the body's requirements are at least 100 mg/day and trace minerals are those elements that are needed in smaller amounts. The minerals include calcium, chloride, magnesium, phosphorus, potassium, sodium and sulphur. There are fourteen essential trace minerals. They are chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, nickel, selenium, silicon, tin, vanadium and zinc. However, evidence for the requirement of nickel, silicon, tin and vanadium are still incomplete. The inorganic mineral analysis by flame photometry and atomic absorption spectrometry indicated that Copper (118 mg), Zinc (63 mg), Lead (11 mg), Nickel (100 mg), Manganese (72 mg) and Cobalt (92 mg) were present in trace quantities in 100g of the plant material (Prajapati *et al.*, 2004; Raju. 2000; Rengaswami and Venkatarao. 1960; Raju. 2000).

Among 17 globally available species of *Plumeria* in addition to several horticultural varieties, *P. alba* may be characterized by the following salient diagnostic features. The plant is a small tree with spreading branches and persistent leaves. The tree from which the leaves were collected for the present study, exhibits an unusual development of longitudinal, vertical thick bund of periderm on the adaxial side of the leaning branches. The leaves are thick, leathery, glabrous and

shining on the adaxial side. They are obovate-lanceolate, obtuse at the apex and oblique at the base. The leaf is distinctly dorsiventral with quite prominent abaxial midrib and lateral veins. The midrib has wide, thick bowl shaped vascular strand and densely crowded large number of phloem nests in the central ground tissue and scattered, wide circular laticifers (Mabberley, 2005; Raju, 2000). The lamina has adaxial epidermal layer of tabular cells and thin abaxial stomatiferous layer of epidermis. Palisade zone is wide, compact with columnar cells. Lateral veins have thick walled, wide bundle sheath with adaxial and abaxial extensions. The stomata are large and broadly elliptical; they are predominantly paracytic type; the epidermal cells have thick, straight or wavy anticlinal walls. Vein islets are distinct and the vein terminations are mostly branched. The petiole is circular with wide, thin deep bowl shaped vascular strand, the ground tissue being occupied by a large number of discrete phloem strands and laticifers. Young stem has thin, wide circular, continuous cylinder of primary vascular tissues with wide pith in which a large number of phloem strands and laticifers are scattered. Fairly old stem has a superficial continuous periderm wide homogeneous parenchymatous cortex, thick cylinder of secondary xylem consisting of radial multiples vessels and files of fibres and phloem on the outer and inner faces of the xylem cylinder. The stem powder exhibits cylindrical narrow, tailed or tailless vessel elements, wide thin walled fibres and anastomosing, non articulate laticifers with dark latex content. Two types of cell inclusions are evident in the leaf and midrib. Prismatic types of crystals occur on the veins of the lamina; druse type of crystals are sparsely seen in the ground parenchyma of the midrib. Prismatic crystals are also abundant in the cortex of the stem and xylem parenchyma. Starch grains are seen in the cortex of the stem. The qualitative chemical analysis, inorganic mineral analysis, showed the presence of sodium, potassium, magnesium etc., and which could be a useful parameter for deciding the nutritive value of the plant. The methanolic extract of *P. alba* give pentacyclic triterpenoid acid, viz. Ursolic acid and Allamacin glycoside (Prajapati *et al.*, 2004; Raju. 2000; Rengaswami and Venkatarao. 1960; Raju. 2000).

## 9. Conclusion

Medicinal plants are rich sources of therapeutic agents without serious side effects, for prevention and cure of various ailments of human being. The immense use of medicinal plants

and their utility in various medicinal systems of the world in general and India in particular, has prompted this research work on known plant source for certain drugs. Herbal medicines are generally perceived as safe products. Hence, Apocynaceae was chosen and a brief review on the existing chemical data of this family was carried out, which is of academic interest. This review brings to light the biological activities of the various compounds isolated from different members of this family. Potential anti-bacterial, anti-fungal, antioxidant and anti-cancerous compounds were found to be present in many members of Apocynaceae. The demonstration of the presence of natural products viz., polyphenols, alkaloids, triterpenoids, flavonoids and other secondary metabolites in medicinal plants will provide a scientific validation for their popular use and serve as guides which may help in the selection of the plants with anticancer activity. So far, the cytotoxic and anti-tumour properties of the medicinal plant namely *P. alba* L. has not been reported. The presence of a variety of compounds in the various species of *Plumeria* but very few of them have been reported in *P. alba*. The plant *P. alba* belonging to the family Apocynaceae was taken up for the study to screen and give a report on the morphological, pharmacognostical and ethnobotanical studies. It has been observed the plant is effective in the treatment of some disorders as presented in ethnobotanical studies. The dried and stored powder of leaves of *P. alba* was subjected to standard procedure for the determination of various parameter. The total ash, acid insoluble ash and water soluble ash was found to be 6.1, 2.0 & 1.4 % w/w respectively. Moreover, a very little attention has been made by the workers towards its phytochemical and biological screening. Therefore, it requires detailed documentation and standardization for the formulation of valuable drugs of therapeutic importance. The all *P. alba* Linn. Scientifically use for the Medicinal activity. The potential anti-cancer property of *P. alba*; and its anti-cancer molecules, identified as Ursolic acid and Allamacin glycoside, may be newer agents with promising scientific scope in the area of cancer therapy and cancer disease management.

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