**REVIEW OF LITERATURE**

*Premna* is a genus of the family Verbenaceae consisting of 250 species (Willis and Airy-Shaw, 1973) of which *Premna tomentosa* was much used in the Siddha System of Medicine (one of the Indian Systems of Medicine). Medical literature and research work on this species are scanty. Other species have been investigated by the phytochemists well. The available literature on the species of *Premna* have been reviewed here to have a bird's eye view of the research works carried out so far on the species of this genus.

**ETHNOBOTANY**

Ethnobotanical knowledge is very ancient in India. It can be defined as the total natural and traditional relationship and the interactions between man and his surrounding plant wealth. The term ethnobotany has often been considered synonymous with traditional medicine or with economic botany. It has been the first knowledge which the early man had acquired by sheer necessity, intuition, observation and experimentation. The scope of ethnobotany is expanding at a very fast rate (Jain, 1967, 1986, 1987a, b, 1989).

Sas Biswas and Ahmed (1987) reported 26 medicinal and food plants of Burnihat valley, Assam / Meghalaya. Their uses were collected from the tribals of Mikis, Kacharis, Garos and Khasis. *Premna latifolia* has been reported to be one among the 26 plants. The scraped bark of *Premna tomentosa* is useful in the curdling of milk in Kolli hills, Tamilnadu (Geetha et al., 1996). Young tender leaves of this plant are cooked and eaten as vegetables by the Garos. Leaf extract of *Premna tomentosa* is used as a diuretic by the Chenchus tribe of Mahaboobnagar district, Andhra Pradesh (Dharmachandrakumar and Pullaiah, 1998). Leaf paste of this plant is used to treat scabies, skin rashes and itching in Mahendragiri hills, Orissa (Girah, 2001). Leaf juice of this plant is used to cure abdominal pains by the Chenchus, Yerukalas, Yanadis and Sugalis tribes of the Gundur district, Andhra Pradesh (Muralidhar Rao and Pullaiah, 2001). Except these two species ethnobotanical notes on the rest of the species are not available.
ECOLOGY

Ecological knowledge of a medicinal plant is important for its conservation and development. Ecological notes on the available species of *Premna* are presented here.

Srivastava (1987) revealed that the family Verbenaceae is represented in Madhya Pradesh by 31 species under 17 genera. *Premna tomentosa* is one among them. Rajendran and Daniel (1992, 1995) have reported *Premna wightiana* and *Premna paucinervis* to be endemic to the Southern and Western Ghats of India, respectively. *Premna wightiana* has been found to be an addition to the flora of Nepal. *Premna tomentosa* is reported to be a common plant in the tropical forests of India which is used for medicinal and fuel purposes (Appasamy, 1993). *Premna coriacea* is a medicinal climber, which is found to be reducing in its distribution in the Western Maharashtra (Archana-Godbole, 1995). Siwakoti and Varma (1995) have included *Premna latifolia* var. *viburnoides* as a new addition to the flora of Nepal. Suresh *et al.* (1996) enumerated the tree flora of Mudumalai Sanctuary. The occurrence of *Premna tomentosa* in the drier part of Mudumalai Sanctuary was reported. *Premna tomentosa* and *Premna latifolia* var. *mucronata* are the rare and less known flowering plants of Madhya Pradesh (Anand Kumar *et al.*, 2000). It is evident that the species of *Premna* are components of the tropical forests of India.

PHYTOCHEMISTRY

Phytochemistry is a distinct discipline somewhere in between organic chemistry, plant biochemistry and closely related to natural products. It deals with a variety of organic substances accumulated in plants. The plant may be considered as a biosynthetic laboratory. Not only their chemical compounds such as carbohydrates, proteins and lipids that are used as food by man, but also a multitude of compounds like glycosides, alkaloids, flavonoids, etc., are used as medicines by him in various ways and means. The compounds that are therapeutic are usually their secondary metabolites. The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research (Kokate, 1994).
Premna tomentosa is a less known medicinal plant of high medicinal value. Its medicinal properties are known to be due to the occurrence of an essential oil. It is an aromatic plant characteristic of its family Verbenaceae. The essential oil from this plant obtained in 0.073 per cent yield has been reported to contain limonene (57.8%), β-caryophyllene (17.2%), a new sesquiterpene hydrocarbon (7.8%), an unidentified sesquiterpene tertiary alcohol (5.6%) and an unidentified diterpene hydrocarbon (Lakshmi Narayan and Muthana, 1953). Atal et al. (1978) reported the absence of tannin in Premna tomentosa. Bheemasankara Rao et al. (1979, 1980, 1981, 1982, 1984) isolated from the root bark of Premna latifolia, three new hydroxysandaracopimar-15-enes, a bisnortediterpene (Premnolol) and 3 diterpenes (nelfionol, dehydrorrhleenol and anhyidrorniellol) in addition to the known compounds sandaracopimar-15-en-8 beta-01 and beta-sitosterol. They have also produced additional evidences for the previously proposed structures of the diterpenes prenmosol (I : R = R^1 = H, R^2 = OH, R^3 = CHO) the tetracetate of nelfionol (I : R = R^2 = OAC, R^1 = O, R^3 = CHMeCH_2OAC), the sandaracopimar end derivatives II (R = R^1 = R^3 = H, R^2 = O; R = R^2 = R^3 = H, R^1 = OH; RR^1 = O, R^2 = R^3 = H) (R^4 = CH : CH_2) and sandaracopimarentiol (II : R = R^2 = H, R^1 = R^3 = OH, R^4 = Et) with the help of ^13C NMR spectroscopy. Two more iridoid glucosides, namely, 7-deoxyloganac acid (bisdexoxygennotropein) and geniposidic acid and beta-sitosterol were isolated from the stem bark of this plant. They have also isolated from the root bark of this plant, two new spirosestquerpenes, namely, 2-isopropenyl-6,10-dimethylspiro [4,5] dec-6-ene and 2-isopropenyl-6-formyl-10-methylspiro [4,5] dec-6-ene and were designated premnaspirodiene and premnaspiral respectively. Four pimarenes, 8β-hydroxy, 1β-8β-dihydroxy, 1β,7α,8β-trihydroxy and sandaracopimar-15-enes, besides premnolol and 6-7-dehydrophrenmolol with phytosterols were isolated and reported for the first time from the root bark of this plant. They have also reported three pimaradienols, 14-α-hydroxyisopimmar-7-15-diene, 7α-hydroxysandaracopimar-8(14)-15diene, 7α-hydroxyisocopimar-8-15-diene and ferruginol and taxodione from the root bark of Premna latifolia. Two new flavone glycosides, glycoside-A (apigenin-4'-0-methyl-7-O-arabinopyranosyl rhamnopyranoside) and glycoside-B (5-hydroxy-4'-methoxy-flavone-7-O-trioside) were isolated from the leaves of Premna latifolia (Rao and Raju, 1981). An alkaloid, aphelandrine and the flavonoid, luteolin was isolated from the leaves of Premna integrifolia (Biswanath-Dasgupta et al., 1984). The heartwood of Premna
tomentosa was extracted with methanol, and methanolic extract was triturated with water and extracted with n-butanol. The residue was crystallized from methanol-acetone mixture when Vicenin-3 (6-C-β-D-glucopyranosyl-8-C-β-D-xylopyranosyl apigenin) was obtained (Jyotsna et al., 1984). Methylated iridoid glycosides of the four different plant extract namely, Verbena officinalis, Clerodendrum thomsoniae, Harpogophytum procumbens and Premna lignum-vitae were determined by mass and 1H NMR-spectroscopy (Franke and Rimpler, 1986). Two new phenolic diterpenoids, 11,12,16-trihydroxybieta-5,8,11,13-tetraene-7-one and 11,14-dihydroxy-12,16-epoxybieta-5,8,11,13-tetraene-7-one were isolated and characterized from the root bark of Premna integrifolia (Subba Rao et al., 1987).

Sirutekkone, a chemical component was analysed and established by 2D-NMR from the dried roots of Premna herbacea (Sandhya et al., 1988).

Two new iridoid glycosides, 2" and 3"-caffeoyl-6-alpha-L-rhamnopyranosylcatapol were isolated from Premna odorata (Otsuka et al., 1989a). Two new monoacyl-6-O-alpha-L-rhamnopyranosylcatapols (2"-O-isoferulyol and 3"-O-isoferulyol and four iridoid diglycosides and five monoacyl rhamnopyranoses namely, 2-and-3-O-trans-isoferulol-rhamnopyranoses, 2-and-3-O-trans-P-methoxy-cinnamoylrhamnopyranoses and 2-O-Cis-P-methoxy-cinnamoylrhamnopyranose were isolated from the leaves of Premna japonica (Otsuka et al., 1988b, 1990, 1991). Fridelin, epifridelanol, stearic acid and beta-sitosterol were isolated from the stem of Premna crassa (Wel et al., 1990). A new compound, 3-hydroxy-4-methoxy phenethyl alcohol beta-D (3"-O-alpha-L-rhamnopyranosyl, 4'-O-beta-D-glucopyranosyl-6'-O-ferulyol) glucopyranoside was identified from the 13 compounds isolated from the stem of Premna corymbosa (Yuasa et al., 1993). Two phenylethanoids, namely, premnethanosides A and B and six other compounds were isolated from the leaves of Premna subscandens. From the six compounds, two were identified as megastigmane glycosides. 7-(3,5-dihydroxy-1,5-trimethyl-cyclohexylidene)-9-methylprop\textsuperscript{+}-8enyl-9-O-beta-D-glycopyranoside and 3-hydroxy-5,6-epoxy-beta-ionol-9-O-beta-D-glycopyranoside. The structures of the remaining four new compounds were elucidated to be a 2'-O-beta-D-apiofuranosyl derivative of 3-hydroxy-5, 6-epoxy-beta-ionol-9-O-beta-D-glucopyranoside, benzyl alcohol beta-D (α'-O-beta-D-xylopyranosyl) glucopyranoside, phenethyl alcohol beta-D (2'-O-beta-D-glucopyranosyl) glucopyranoside and 4-4'-dimethoxy-beta-trixinic acid catapol diester by spectroscopic analysis (Sudo et al., 1997, 2000). The volatile
constituents of the flower buds of *Premna serratifolia* were isolated by vacuum distillation from a hexane concrete and fractionated by silica gel chromatography and analysed by GC/MS. 94 compounds were identified. The major components are 1-Octen-3-ol, (Z)-3-hexenol, 2-phenylethyl alcohol (E, Z)-2,4-nonadienal (E, Z)-2-6-nonadienal and linalool (Teai *et al.*, 1998). Two new xanthones named 1-hydroxy-2, 3-methylenedioxy-6-methoxycarbonyl-7-acetyl xanthone and 1,3-dihydroxy-2-methoxy-6-methoxycarbonyl-7-acetyl-xanthone were isolated from the EtOAc extract of the roots of *Premna microphylla* (Wang and Zu, 2003).

**PHARMACOLOGY**

Pharmacology deals with the responses of organisms when subjected to treatment by drugs. It is a prerequisite step before entering into the study of Pharmacy or Pharmaceutics (Wallis, 1985).

Decoction of the leaves of *Premna nauseosa* inhibited the development of liver tumors but not skin tumor (Serrame and Lim-Sylianco, 1995). The dimethylbenzanthracene was used as the initiator and croton oil as the promoter. 67 per cent of the experimental mice developed skin tumors and 50 per cent had liver tumors. The extracts of *Adhatoda vasica, Aegle marmelos, Eclipta prostrata, Melia azadirachta, Nardostachys jatamansi, Oroxylon indicum, Picrorrhiza kurrooa, Premna integrifolia, Pueraria tuberosa, Stereospermum suaveolens, Swertia chirata* and *Valeriana wallichii* were screened for Acute toxicity, behavioural study and effect on the Central Nervous System (CNS) (Debelmas and Hache, 1976). A flavonoid from *Premna integrifolia* showed decrease in prothrombin time during the coagulation of blood (Hamsevani Gopal and Purushothaman, 1984).

The antifeedent and antiovipositional effects of an extract of *Premna integrifolia* (Dixit and Saxena, 1990) showed insecticidal activities against the pest *Callosobruchus chinensis*. Their action persisted for only one week, after which the antifeedent action diminished. Treatment at 50, 30 and 25 mg / 10 g seed had antiovipositional effects. Cytotoxicity of two diterpenes from *Premna schimperi* and *Premna oligotricha* was studied using MTT assay (Habtemariam, 1995). Their cytotoxic activity against three human and two murine (L929 and RAW 2647) carcinoma cell lines varied between 1.5 to 35 µg/ml and was comparable with
azauidine and chlorabucil. The diuretic effect of the alcoholic extract of the root of *Premna latifolia* was reported in rats and dogs (Rema and Vijayamma, 1995). The urine output was more in rats treated with 1 mg/kg and 2 mg/kg body weight, but when compared with a moderately potent diuretic hydrochlorothiazide 2.5 mg/kg, there was only 50 per cent excretion of urine. In dogs, the drug in a dose of 2 mg/kg body weight intravenously produced marked diuretic effect. Twenty five species of Malaysian medicinal plants including *Premna integrifolia* were screened for Platelet – Activating Factor (PAF) and receptor binding (Janton et al., 1996). The results showed significant inhibitory effect. The effect of methanolic extract of the leaves of *Premna tomentosa* was studied against the hepatoprotective activity in acetaminophen induced hepatitis in rats with respect to the activities of some enzymes (Pandimadevi and Devaki, 1998a).

Pre-treatment with the *Premna tomentosa* extract significantly maintained the activities of marked enzymes at near normal. The extract of *Premna tomentosa* was studied for its antioxidant, immunomodulatory, antinociceptive and hypnotic activity by Pandimadevi et al. (1998b, 2003a, b). The activities of various antioxidants decreased in acetaminophen treated rats. Pre-treatment with crude extract of this plant (750 mg/kg body wt. orally) reversed the deleterious effects of acetaminophen. The result indicated that the crude extract of this plant exhibited the hepatoprotective action partly through correction of oxidative stress and validated the traditional use of this plant in hepatic damage. The immunomodulatory activity of this plant was done in chromium induced immunosuppression in splenic lymphocytes. Pre-treatment of the cells with the leaf extract of this plant (at 500 mg con.) resulted in decreased cytotoxicity and ROS levels and restored lymphocyte proliferation similar to that of control cells. The results indicated that the leaf extract of this plant has cryptoprotective and immunomodulatory activities. The leaf extract of this plant showed antinociceptive and hypnotic activity. They were studied in experimental animals by acetic acid induced writhing, tail flicks and tail clip tests. Oral administration of this extract at different doses (100, 200, 400 and 500 mg/kg) led to significant antinociceptive effects. Then treatment with the extract of this plant at different doses (100, 200, 400 and 500 mg/kg) decreased the locomotory activity and potentiality of the pentobarbitone induced sleep time.
The n-BuOH soluble fraction of a methanol extract of leaves of *Premna subscandens* exhibited promotion of collagen network formation by M cells (Sudo *et al.*, 1999). Alcoholic extract of the roots of *Premna herbacea* was investigated for its antipyretic, antinociceptive and antiinflammatory potentials in animal models (Narayanan *et al.*, 2000). The extract, when administered orally to mice has been found to be safe up to a dose of 8 g/kg. A significant antipyretic activity was noted in rabbits while mild antinociceptive effects did not exhibit any antiinflammatory activity in acute but significantly reduced the chronic inflammation.

**ANTIMICROBIAL ACTIVITY**

Medicinal plants possess several curative properties due to the existence of various chemical substances in them as secondary metabolites in one or more parts of these plants (Anil Srivastava *et al.*, 2000). Now-a-days plant medicines are replacing modern medicines due to their safety and lesser side-effects. Determination of their antimicrobial activities against specific pathogens is essential to render proper therapy confidently and also to find out their correct doses. The literature available on the antimicrobial activities of the species of *Premna* are reviewed here.

The diterpene (5R, 8R, 9S, 10R) 12-oxo-ent-3,13(16)-clerodien-15-oic-acid from the leaves of *Premna schimperi* and a novel sesquiterpene 7-alpha-hydroxy-6,11-cyclofanes-3(15)-en-2-one from the aerial parts of *Premna oligotricha* (Habtemariam *et al.*, 1990, 1993) were screened for their antimicrobial activities against some of the gram-positive and gram-negative bacteria, fungi and moulds. The sesquiterpene 7-alpha-hydroxy-6,11-cyclofanes-3(15)-en-2-one was found to be of weak activity against the gram-positive bacteria *Bacillus pumilus, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus faecalis*. The diterpene, 12-oxo-ent-3,13(16)-clerodien-15-oic-acid was active against the gram-positive bacteria. *Staphylococcus aureus* and *Bacillus subtilis* in the concentration range of 20-25 µg/ml. Hexane, ethyl acetate, diethyl ether and methanol extract of the leaves of *Premna tomentosa* (Perumal Samy and Ignacimuthu, 2000) were screened for their antibacterial activities against *Bacillus subtilis, Escherichia coli, Klebsiella aerogens, Proteus vulgaris* and *Staphylococcus aureus*. They were active against *Staphylococcus aureus* and *Escherichia coli*. 
MATERIAL AND METHODS

MATERIAL

Twenty years ago a medicinal plant by the Tamil vernacular name “PUDANGANARI” was said to be sold in the markets of Pattukottai town of Thanjavur district, Tamilnadu by the ‘NARIKURAVA’ community as bundles of twigs and claimed to have therapeutic activity on human paralysis.

“PUDANGANARI” has been selected for the present investigation to find out its medicinal values and to revive its traditional medicinal uses by the ailing people.

It’s botanical identity is Premna tomentosa Willd. (Family : VERBENACEAE).

Premna tomentosa Willd. growing in the wastelands of Pillakurichi village, Alangudi taluk, Pudukkottai district, Tamilnadu. The leaves and stem bark were collected, dried in shade and used for further studies.

METHODS

Field studies

Field trips were undertaken every month to various places in Tamilnadu between January 2002 to January 2003 to locate P. tomentosa, to study its ethnobotany, ethnomedicobotany, economic botany and its life-cycle. The data presented in this work are based on first hand information collected during the period of study. Information was collected from the rural herbal medical practitioners (Nattu Vaidhyas) through interviews, discussions, personal contacts and keen observations.

Localities of P. tomentosa in Tamilnadu are as follows:

- “Pillakurichi” village (plains and wastelands), Alangudi taluk, Pudukkottai district, Tamilnadu.
- “Pacchaimalai” (Hills), Thuraiyur taluk, Tiruchirapalli district, Tamilnadu.

Performance of P. tomentosa in all these localities were observed, studied and reported.
Plant identification

The Tamil vernacular and other Indian vernacular names of this plant were found out and reported (Lushington, 1915).

Botanical identification of the plant was done with the help of floras (Gamble, 1921; Matthew, 1983; Henry et al., 1987).

Identity of the plant was verified with the help of type specimens available in the herbarium of the Botanic Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamilnadu.

Herbarium studies

Herbarium specimens available in the herbarium of the Botanical Survey of India, Southern Circle, Coimbatore and the Rapinat Herbarium, St. Joseph’s College (Autonomous), Tiruchirapalli were observed, studied and the conclusions are reported in order to find out the distribution of the plant and also confirm the identity of the field specimens. Herbarium specimens were prepared following the method of Jeffrey (1984). The specimens were deposited in the herbarium of the Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Thanjavur district, Tamilnadu.

Taxonomy

Taxonomical studies on the plant were carried out and its systematic position has been assigned as per the angiosperm taxonomic classification of Bentham and Hooker (1862-1883).

Ethnomedicobotanical studies

Native medicinemen, herbal medicinemen, Siddha medicinemen and aged people of the villages of the study area where the study plant is available were personally contacted, interviewed and discussed with about the utilization of the plant. The information was gathered during the period from January 2002 to January 2003.
The methods of preparation of drugs from the plant were also noted and reported. The mode of administration of the drug and its dosage are also observed and reported.

Economic utilization of the plant by the local people in various localities of Tamilnadu were also noted and reported.

**Medicinal and economic uses (Literary survey)**

Medicinal and economic uses hitherto published were surveyed and presented (Anonymous, 1969, 1986; Chopra et al., 1986; Kirtikar and Basu, 1984; Murugesa Mudhaliyar, 1988; Yoganarasimhan, 2000).

**Ecological status**

Ecological disturbances to the plant in its habitats were observed, studied and reported. Its present ecological status has been assessed.

**Life cycle**

Performance of the plant in its varied habitats has been observed and reported. Seasons of leaf-fall, new foliage, flowering, fruiting, seed setting and methods of propagation were observed.

**Soil analysis**

Soil-samples were collected from the sites of the plant and analysed for their physicochemical parameters in the Soil Testing Laboratory, Government of Tamilnadu, Tiruchirapalli.

**Meteorological data**

Meteorological data were collected for the varied localities of the plant from the National Pulses Research Centre, Vamban, Pudukkottai district (for the plain-habitat) and Meteorological Research Station, TWAD Board, Perambalur (for the hill-habitat) during the period of observation. The data were studied and correlated with the performance of the plant.
Seed biology

Seed viability test (Hittock and Law, 1968) (Tetrazolium test)

Seeds of *P. tomentosa* were collected from both the plains and hills. Seeds were observed macroscopically and microscopically for their quality. Twenty five seeds collected from each locality for seed viability test.

The seeds were soaked in water in order to initiate the process of germination and make them to get stained easily. Then the seeds were dissected longitudinally through the vertical axis along the narrow width. Seeds were then completely soaked in 1 per cent tetrazolium solution and kept in an incubator at 30° C for 2 h. After staining the seeds were washed and examined and observed under the microscope. Five batches of seeds were considered for seed viability test. Each batch consisted of 25 seeds.

Pharmacognostical studies

Organoleptic characters

Characters such as taste, odour, texture and colour of the leaf and bark were observed for the study plant.

Microscopic studies

Different organs of the plant such as leaf, petiole, stem, stem wood, stem bark, young root, root wood and root bark were fixed in FAA (Formalin 5 ml : Acetic acid 5 ml : 70% Ethyl alcohol – 90 ml).

The materials were left in FAA for a few days and then they were dehydrated employing tertiary butyl alcohol (TBA) series as per the procedure given by Sass (1940). Paraffin infiltration and embedding in wax blocks were done in the usual method (Johansen, 1940). Serial paraffin sections of 10-12μm thickness were prepared with the help of Spencer Rotary Microtome. These sections were stained with Toluidine blue as per the schedule suggested by O’Brien *et al.* (1964). Toluidine blue stain was found to be quite satisfactory for microscopic observation of cells and tissue because of the metachromatic property of the stain. Sections were also stained with Safranin and counter stained with Fast green. For the study of stomata of leaves, small segments of lamina were immersed in Jeffrey’s maceration fluid (10% chromic acid and 10% nitric acid mixed in equal volume) and
kept in Thermostat at 60°C for a few hours. Due to partial maceration, the epidermal peelings get separated from each other. Then the peelings were washed thoroughly, stained with 0.5 per cent aqueous Safranin or 0.25 per cent aqueous Toluidine blue and mounted in glycerine for microscopic examination. For the study of venation pattern of leaf, leaf-bits measuring 1 cm square were boiled in alcohol to remove the chlorophyll contents and then immersed in warm 5-10 per cent sodium hydroxide for several hours. As the leaf bits became transparent they were washed thoroughly, stained with Safranin and mounted in glycerine. Hard stem wood and root wood were boiled in water for a few minutes. Transverse and longitudinal sections of the wood specimens were prepared by sledge microtome.

For studying the wood elements, thin pieces of materials were macerated with Jeffrey's fluid. The separated elements were stained with Safranin and mounted in glycerine. Pollen grains from mature flower were collected and mounted on a drop of glycerine mixed with stain and sealed with cover slip for microscopical observation.

Microscopic observations were made both under normal and polarized lights. Photomicrographs were also taken with NIKON ALPHAPHOTO-2 microscopic unit using normal and polarized lights. The polarised light was very much useful to detect the lignified elements, crystals and starch grains.

Measurement of cells were taken with micrometer. For each element 15-20 measurements were taken. The average values were also calculated.

Quantitative microscopy

Determination of stomatal number and stomatal index (Wallis, 1985)

1. Fragments of the leaves from the middle of the lamina were cleared by boiling with chloral hydrate solution. The upper and lower epidermis were peeled out separately by means of forceps.
2. The mounts of the lower and upper epidermis were separately prepared in glycerin water.
3. A square of known dimensions (0.5 sq. mm) was drawn by means of stage micrometer and camera lucida on a drawing paper.
4. The stage micrometer was replaced by the cleared leaf preparation, focused under the same magnification and the epidermal cells and stomata were traced by looking through microscope. When a superimposed image of the leaf was
seen at the same time. The number of epidermal cells and stomata within the square were counted, a cell being counted if at least half of its area lies within the square, provided two adjacent sides were considered for purpose of calculation.

5. Successive adjacent fields were examined until about 100 cells are counted and the stomatal number was calculated by using the formula

\[ \text{S.I.} = \frac{S}{E+S} \times 100 \]

where,

- S.I. - Stomatal index
- S - Number of stomata per unit
- E - Number of epidermal cells in the same unit area.

**Determination of vein islet and vein termination number (Lala, 1991)**

1. Few leaves were boiled in chloral hydrate solution in a test tube placed in a boiling water bath.
2. The preparation was then mounted in glycerin water.
3. The camera lucida was set up and the black board paper was divided into squares of 0.5 sq. mm by means of the stage micrometer.
4. The stage micrometer was replaced by the cleared leaf preparation and the veins were traced in 4 continuous squares. The vein islets and vein terminations were traced by looking the microscope when a superimposed image of the leaf portion and paper were seen at the same time.
5. The number of vein islets and vein terminations present within the square were counted also by taking into consideration incomplete vein islets on any two adjacent sides of the square.
6. The value for 1 sq. mm was calculated 10 sets of such counts were taken. The observations were recorded in the form of the range.

**Determination of palisade ratio (Wallis, 1985)**

1. Pieces of leaves (about 2 sq. mm) were cleared by boiling with chloralhydrate solution. The sample was mounted in glycerin water and focused under high magnification. A camera lucida was attached and outlines of four continuous epidermal cells were traced.
2. The palisade cells lying immediately beneath the same epidermal cells were focused by slightly lowering the draw tube and traced.

3. The palisade cells in each group were counted, including those cells in the count which are more than half covered by the epidermal cells. The figure obtained was divided by four to obtain the palisade ratio.

4. The palisade ratio was determined for both the surfaces of the leaf. The average of twenty five such determinations were considered for the calculation of range and average.

Preparation of powder (Harborne, 1973)

The shade dried leaf and bark were mechanically powdered after keeping in an electric oven at 35°C for 24 h. These powdered material were used for further microscopical, physicochemical, phytochemical and fluorescence analysis.

Microscopic characteristics of powder (Anonymous, 2003)

Forty g of the powders of leaf and bark of *P. tomentosa* was cleared with chloral hydrate and

(i) stained with iodine for locating starch grains.

(ii) stained with Phloroglucinol and hydrochloric acid for observing lignified tissues.

Fluorescence studies (Chase and Pratt, 1949)

Fluorescence of the leaf and bark powders of the *P. tomentosa* was observed in day and UV light (254 nm) with solvents like sodium hydroxide, hydrochloric acid and 50 per cent conc. sulphuric acid. When the powder and their solvents receive UV light they fluoresce and produce characteristic colour. The day and UV light colours were noted.

Physicochemical studies (Anonymous, 1966)

Determination of moisture content (Loss on drying)

Two g of the powdered drug was taken in a tared weighing bottle and weighed accurately. Dried at 105°C for 5 h allowed to cool in a desiccator and weighed accurately. The drying was continued at 105°C and weighed at 1-hour intervals. When the weight of the sample became constant, the loss in weight and the percentage of loss on drying were calculated.
Determination of total ash value

Two g of accurately weighed and ground samples of leaf and bark were taken in a tared silica dishes previously ignited and weighed; the ground drugs were scattered in a fine even layer at the bottom of the dish and incinerated gradually by increasing heating, not exceeding dull red heat until free from carbon, cooled and weighed. As a carbon free form was not obtained, the charred mass was digested with hot water and the residue was collected on an ashless filter paper. The residue with the filter paper was incinerated and the filtrate was added, evaporated to dryness and ignited at a low temperature. The percentage of ash with reference to the air dried powder was calculated.

Determination of acid-insoluble ash value

The ash was boiled for five minutes with 25 ml of 2N hydrochloric acid (10% w/w) and filtered through an ashless paper (Whatmann No.41). The filter paper was ignited in a silica crucible, cooled, weighed and the percentage of acid-insoluble ash was calculated with reference to the air dried powder.

Determination of water-soluble ash value

The ash was boiled for five minutes with 25 ml of water; the insoluble matter was collected in an ashless filter paper, washed with hot water, dried and ignited at 1000°C temperature. The difference in weight of the ash and the insoluble matter represents the water-soluble ash with reference to the air-dried powder.

Extractive value (Anonymous, 1985)

Alcohol-soluble extractive

A 5.0 g of the air-dried drug was macerated with 100 ml of alcohol of the specified strength and kept in a closed flask for twenty four hours shaking frequently during six hours and allowed to stand for eighteen hours. Filtered rapidly taking precautions against loss of alcohol 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105°C, and weighed. The percentage of alcohol-soluble extractive with reference to the air dried drug was calculated.

Water-soluble extractive

The above said procedure was followed but water was used for extraction.
Chloroform-soluble extractive
The above said procedure was followed but chloroform was used for extraction.

Hexane-soluble extractive
The above said procedure was followed but hexane was used for extraction.

Phytochemical studies
Preparation of extracts
Successive solvent extracts
A 100 g of shade dried powdered plant materials were extracted successively using the following solvents in a Soxhlet extractor.
(a) Hexane (68°C)
(b) Chloroform (60°C)
(c) Alcohol (78°C)
(d) Water (Aqueous) (100°C)
Each time before extracting with the next solvent, powdered material was dried in a hot-air oven below 50°C. Finally, marc was macerated with chloroform water for 24 h to obtain the aqueous extract. The extract was concentrated by distilling of the solvent and then evaporated to dryness on a water bath. The extracts were used for qualitative phytochemicals, HPTLC analysis and pharmacological studies.

Qualitative chemical studies (Kokate, 1994)
Detection of carbohydrates and glycosides
Small quantity of the alcoholic extracts were dissolved in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's test.
Another small portion was hydrolysed with dilute hydrochloric acid for a few hours in water bath and the hydrolysate was subjected to Legal's test.

Detection of proteins
Small quantities of alcoholic extracts were dissolved in a few ml of distilled water and the solution was subjected to Millon's, Biuret and Ninhydrin tests.
Detection of alkaloids

Small portion of the solvent free alcoholic extracts were stirred with a few drops of hydrochloric acid and filtered. The filtrate was treated with various alkaloidal reagents such as Mayer’s, Dragendorff’s, Hager’s and Wagner’s.

Detection of phenolic compounds

Small quantities of alcoholic extracts were dissolved in water and treated with dilute ferric chloride solution (5%), 1 per cent solution of gelatin containing 10 per cent sodium chloride, 10 per cent lead acetate and aqueous bromine solutions.

Detection of flavonoids

Small quantities of the alcoholic extracts were treated with sodium hydroxide and conc. sulphuric acid.

Detection of saponins

One ml each of the alcoholic extracts were diluted with distilled water and made-up to 20 ml and shaken in a graduated cylinder for 15 min. One centimeter height of foam was formed.

Estimation of chlorophyll a, b and total chlorophyll (Arnon, 1949)

A 0.1 g of leaf material was homogenized with 80 per cent acetone using mortar and pestle. The macerated green extract was centrifuged at 2000 rpm for 15 min, supernatant was saved. The residue was extracted with 80 per cent acetone each time until it became colourless. All the supernatant were collected and utilized for chlorophyll estimation. The chlorophyll content was read at Spectronic-20 colorimeter at 645 m and 663 m.

\[
(12.7 \times D663) - (2.69 \times D645) (V/1000 \times 1/w) = \text{Chl. a (mg g}^{-1}\text{ DW)}
\]

\[
(22.9 \times D645) - (4.68 \times D663) (V/1000 \times 1/w) = \text{Chl. b (mg g}^{-1}\text{ DW)}
\]

\[
(20.2 \times D645) - (8.02 \times D663) (V/1000 \times 1/w) = \text{Tot. Chl. (mg g}^{-1}\text{ DW)}
\]

where,

- D - Length of light path in the cell (usually 1 cm)
- V - Volume of the extract in ml
- W - Dry weight of the sample
Estimation of total protein (Lowry et al., 1951)

Extraction

A 0.1 g of the sample was homogenized with 20 per cent trichloroacetic acid (TCA). Then the homogenate was centrifuged at 2000 rpm for 15 min. The supernatant was discarded. The pellet was re-extracted with 5 ml of 0.1 N sodium hydroxide solution. Again it was centrifuged at 2000 rpm for 15 min. The supernatant was saved and made-up to 5 ml with 0.1 N sodium hydroxide.

Estimation of protein using Folin-Ciocaltaeu's reagent

To 0.5 ml of protein extract, 5 ml of alkaline copper solution and 2 ml of distilled water were added and the solution was allowed to stand for 10 min at room temperature and then 0.5 ml of Folin-Ciocaltaeu's reagent was added and mixed vigorously. The mixture was again allowed to stand for 30 min. The blue colour was read with Spectronic-20 colorimeter at 600 nm. The protein content was calculated from the standard graph.

Reagents

1. Alkaline sodium carbonate solution

Two g of sodium carbonate was dissolved in 100 ml of 0.1 N sodium hydroxide.

2. Copper sulphate and sodium potassium tartarate solution

Five per cent copper sulphate in 100 ml of 1 per cent sodium potassium tartarate solution.

3. Alkaline copper sulphate solution

Fifty ml of alkaline sodium carbonate solution was mixed with 1 ml of copper sulphate and sodium potassium tartarate solution prior to use.

4. Folin-Ciocaltaeu's reagent

One part of commercial Folin-Ciocaltaeu's stock solution was mixed with one part of distilled water.
Estimation of total lipid (Sato and Murata, 1988)

A 500 mg of samples were taken and ground separately with chloroform and methanol (2:1 v/v) in a pestle and mortar. The samples were centrifuged at 5000 rpm for 10 min. The supernatant was taken and a pinch of sodium sulphate was added and vortexed. They were centrifuged at 5000 rpm for 10 min. The supernatant was saved in a preweighed bottle. The content was dried and then weighed. The total lipid content was determined by subtracting the final weight from the original weight.

HPTLC analysis (Sethi, 1996)

High Performance Thin Layer Chromatography

HPTLC was performed on aluminium backed silica gel 60F_{254} HPTLC plates (Merck). The mobile phase was benzene: methyl alcohol: acetic acid (7:2:1). Samples were applied to the plates as sharp bands by means of a Camag Linomat IV sample applicator. After drying the spots in a current of air the plates were placed in one trough of a Camag twin trough glass chamber. The mobile phase was poured into the chamber left to equilibrate for 10 min. The plate was then developed until the solvent front had travelled a distance of 7 cm above the position of sample application. The plate was removed from the chamber and dried in a current of air. Detection was performed with a Camag TLC scanner.

Chromatographic conditions

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>HPTLC aluminium plate precoated with silica gel 60F_{254}.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent system</td>
<td>benzene: methyl alcohol: acetic acid (7:2:1)</td>
</tr>
<tr>
<td>Separation technique</td>
<td>ascending</td>
</tr>
<tr>
<td>Migration distance</td>
<td>70 mm</td>
</tr>
<tr>
<td>Detection</td>
<td>UV</td>
</tr>
<tr>
<td>Wave length</td>
<td>270 nm</td>
</tr>
</tbody>
</table>

Mineral studies (Harborne, 1973, 1998)

Two g of dried powders of leaf and bark were taken in 250 ml conical flasks and 12 ml of triple acid mixture (nitric, sulphuric, perchloric acids in the ratio of 2:1) was added and the mouth of the flask was covered with a funnel. The contents were digested in the flask over a sand bath till a clear solution was obtained. The
solution was filtered through Whatmann No.40 filter paper and the filtrates were collected in 250 ml volumetric flasks. The conical flask was washed with small increments of hot water and the washing was added to the filter paper. Residue on the filter paper was also washed with hot water till the filtrate runs free of chloride. The volumetric flasks were cooled under tap water and made-up to 250 ml with cold distilled water. This triple acid extract was used for the analysis of mineral constituents. The minerals were estimated using flamephotometer.

‘Kuzhithailam’ preparation (Kuppusamy Mudhaliyar, 1987)

Principle

Heat was generated by slowly burning cow-dung cakes and applied to the leaves kept in a closed earthen pot. This heat expelled the oil from leaves along with water. The oil along with water was collected in the bottom vessel. Water portion of the collection was evaporated in a solar-oven and the oil alone was collected. This oil is called 'Kuzhithailam'.

Materials required

A new earthen pot of 10 litres capacity with holes at the bottom and an earthen lid, a new earthen vessel of 2 litres capacity, cow-dung cakes, paddy straw, cloth, cotton, rope and fresh leaves of P. tomentosa.

Method

A pit of 3 feet depth and 2 feet diameter was dug in the soil. The 2 litres capacity earthenware was kept at the bottom of the pit. Over it the 10 litres capacity earthenware was placed. Into this pot 4 kg of the fresh leaves of the plant was put and covered with the lid. The mouth of the pot was sealed with a cloth soaked in mud. The lower pot was covered with soil. The lid and the sides of the upper pot was covered with cow-dung cakes and paddy straw. The straw was lit with fire. The cow-dung cakes were allowed to burn slowly for 7 h. The cakes were allowed to extinguish. The ashes were removed and the oil collected in the bottom vessel was collected in an amber coloured glass bottle.

Pharmacological studies

Determination of LD$_{50}$

LD$_{50}$ was determined as per the OECD guidelines.
Antidiarrhoeal activity

Castor oil induced diarrhoea method (Awouters et al., 1978)

Extracts of doses of female Swiss albino mice, 20-25 g, were used after 24 h food deprivation. Extracts (100 mg/kg and 200 mg/kg) were administered orally to groups of ten mice 60 min before the administration of the cathartic agent (castor oil : 0.5 ml, p.o.). Following administration the animals were placed separately in polyethylene cages with filter paper previously weighed, at the bottom, which was changed every 30 min. The following parameters were determined; the time elapsed between the administration of the cathartic agents and the excretion of the first diarrhoeic faeces (wet faeces that leave a halo on the filter paper); the total number of diarrhoeic faeces excreted by the animals in 4 h; and the total weight of diarrhoeal stools in that period of time.

Small intestine transit method (Leng-Peschlow, 1986)

Groups of eight female Wistar rats, weighing approximately 200 g each were fasted for 24 h. A suspension containing 10 per cent active charcoal in 1.5 per cent arabic gum was used as a marker. Test solution (1 ml) was administered orally 60 min after the oral administration of extracts at 100 mg / kg and 200 mg / kg doses. The rats were killed after 30 min the small intestine rapidly and carefully removed, and the length traversed by the charcoal marker was calculated as a percentage of the total intestine length. The inhibitory action of extracts on stimulated small intestine transit were also studied by giving castor oil, 1.0 ml per rat, along with the active charcoal-arabic gum suspension.

Diuretic activity (Lipschitz et al., 1943)

Groups of 6 male albino rats, each weighing 150-170 g were fasted and deprived of water for 18 h prior to the experiments. On the day of the experiment, all the animals were given normal saline orally (25 ml / kg body weight. Group I served as the negative control which received only normal saline 25 ml/kg. Group II received Furosemide 100 mg/kg as reference diuretic. Group III, IV, V and VI received the test drugs.

Immediately after dosing, the animals were placed in metabolic cages specially designed to separate urine and faeces and kept at room temperature of 25 ± 0.5°C. The urine was collected in measuring cylinder upto 5 h after dosing.
During this period no water or food was made available to the animals. The total volume of urine collected was measured for the control and drug treated groups. The parameters taken for each individual rat were body weight, total urine volume, urine concentration of Na⁺, K⁺ and Cl⁻. Na⁺ and K⁺ concentrations were measured by flamephotometry and Cl⁻ concentration was estimated titrimetrically.

All the values are expressed as mean ± standard error of the mean. Test of significance was analysed by student's t-test.

Antioxidant activities

Antioxidant activity was carried out by two methods:

1. Ferric thiocyanide method (FTC method)
2. Thiobarbituric acid method (TBA method)

1. Ferric thiocyanide method (FTC method) (Kizukai and Nakatani, 1993)

Principle

FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases.

Procedure

Mixture of 4 mg of the sample in 4 ml absolute ethanol, 4.1 ml of 2.52 per cent linoleic acid in absolute ethanol, 8 ml of 0.05 M phosphate buffer (pH : 7.0) and 3.9 ml of water were placed in a vial (dia : 38 mm, h=75 mm) with screw cap and then placed in an oven at 40°C in the dark. To 0.1 ml of this solution, 9.7 ml of 75 per cent ethanol and 0.1 ml 30 per cent ammonium thiocynate were added. Precisely, 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5 per cent hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm for every 24 h until the absorbance of the control reached the maximum. The control and standard were subjected to the same procedure as the sample except that of the control, only the solvent was added and for the standard, 4 mg of the sample was replaced with 4 mg of vitamin E.
2. Thiobarbituric acid method (TBA method) (Ottolenghi, 1959)

Principle

The formation of Malonaldehyde is the basis for the well known TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100° C) Malonaldehyde binds TBA to form a red complex that can be measured at 532 nm. The increase of the amount of the red pigment formed correlates with the oxidative rancidity of the lipid.

Procedure

Two ml of 20 per cent Trichloroacetic acid and 2 ml TBA aqueous solution were added to 1 ml of the sample solution prepared as in the FTC procedure, and incubated in a similar manner. The mixture was placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was measured at 532 nm. Antioxidant activity was based on the absorbance on the final day.

Antibacterial activity

Well-diffusion assay method (Bauer et al., 1996)

The various solvent extracts of the dried leaf and bark (100 μg/ml) were tested for their antibacterial studies.

The bacterial organisms tested against various solvent extracts were *Alkaligenes faecalis, Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Pseudomonas fluorescens, Streptococcus aureus, Streptococcus lactis* and *Salmonella typhi*.

Test against standard controls

This test was carried out by using commercially available antibiotic discs viz., Amikacin (10 μg), Amoxycillin (10 μg), Ofloxacain (2 μg), Sparfloxacin (5 μg), Vancomycin (30 μg) and as standard controls for all the test microorganisms. The sensitivity patterns were recorded and the readings were interpreted according to the critical diameter given by National Committee for Clinical Standards (NCCLS, 1997).
The bacterial pathogens were obtained from the Microbiology Laboratory, Sea Horse Hospital Pvt. Ltd., Tiruchirapalli. The 24 h old bacterial culture in Muller Hinton Broth (Hi media, Mumbai) was used as the inoculum. The test organisms were seeded over the Muller Hinton agar plates aseptically. Wells were made on the agar surface with 5 mm corkborer. The test drugs (1 ml) were injected into the well using a micropipette. The plates were incubated at 37 ± 2°C for 48 h. The plates were observed for the clearing zone around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. Readings were taken in three different fixed directions in all three replicates and the average values were calculated.

Clinical studies

Clinical trial was conducted in the District Rehabilitation Centre, Thanjavur. Patients of both the sexes belonging to different age groups were treated to find out the therapeutic efficacy of the drug. The ‘Kuzhithailam’ was applied externally once a day in the morning massaged and allowed to remain for 4 h. It was washed with soap-nut powder after treatment.

Mass-multiplication studies (Hartmann and Kester, 1976)

Mass-multiplication of the plant was carried out between August and December 2002. Stem-cuttings of 15-20 cm length were collected from a healthy tree growing of Pillakurichi village, Pudukkottai district. The cuttings were grouped into three batches of 25 each, based on their thickness (diameter). The three groups of stem-cuttings were named Group-A (1-2 cm), Group-B (2-3 cm) and Group C (1-2 and 2-3 cm) with 3-4 nodes. Slant cuts were made below the nodes of cuttings. The cuttings were planted in polybags filled with red soil, goat's manure and sand in a ratio of 1:2:1. Before planting, the polybags were arranged in two groups (A and B) and they were kept in a spacing of 23.5 x 12.5 under the intermittent mist conditions of a low-cost mist-chamber. The third group (C) was kept outside the mist-chamber as control. The cuttings were removed from the chamber after 150 days and observed for the number of rooted cuttings, number of leaves per cutting and number of established cuttings. After 180 days, the plants were removed from the polythene bags and transferred to earthen pots and then planted in the field after 210 days.
Statistical analysis

The experimental data were statistically analysed and the significance of various treatments were calculated using the student’s t-test. All the results were expressed as means ± S.E.M. P-values of < 0.001 were considered as significant.