ANCIENT SCIENCE OF LIFE
Vol. No. XXI (1) July 2001 Pages 38 - 50

PHARMACOGNOSTICAL STUDIES ON ORILAITAMARAI -HYBANTHUS ENNEASPERMUS (L) f. MUELL-(VIOLACEAE)

(MRS) T.R. SHANTHA, (MRS) SARASWATHI PASHUPATHY, J.K.P. SHETTY, (MRS) B. VIJAYALAKSHMI, P, KANDAVEL and T. BIKSHAPATHY

Regional; Research Centre, Ashoka pillar, Jayanagar, Bangalore.

Received: 12/6/2000 Accepted: 10/12/2000

ABSTRACT: The paper deals with the detailed pharmacognosy of the whole plant of Orilaitamarai (Hybanthus enneasperms) sold in the local market under the name Purusharatna, Ratnapursuha. The studies include macro, microscopical, histochemical and diagnostic characters of the root, stem and leaf. The physical constants, fluorescence characteristics and chromatographic studies of the whole plant are also presented.

INTRODUCTION:

Orilaitamarai is a Siddha drug used as a karpamoolikai (general tonic), pustikarakka, Balavardini (nutritive), Aanmaiperukki (aphrodisiac) and Tapanashini (antipyretic) [Mudaliar 1988, Venmathiyan 1993]. It is also known as Orithaltamarai and Avviyadam in the Siddha system of medicine (Venmathiyan 1993). Plants possess properties like Suvai-Madhura, Gunam-Varnya, Thanmai-suhna and Perivu-Madhura.

In the Ayurvedic classics this plant is known as Sthalakamalm, Padma (Chunekar 1969) and it is said to be bitter, acrid, used in urinary-calculi, strangury, vomiting, wandering of the mind, urethral discharges, blood disorders, asthma, epilepsy, cough and it also gives tone to the breasts. The leaves and the tender stalks are demulcent and are used in decoction form as electuary (Kirtikar and Basu 1988). The fruit in combination with other drugs has been recommended as an antidote to snake venom and scorpion sting (Chopra 1994). Root is diuretic, used in bowel complaints of children and administered as an infusion in gonorrhoea and urinary tract infection (Asima Chatterjee 1992). Plants possess Katu thikta-rasa, Laghu-Guna, Sheeta-veerya, KatuVipaka properties. Plants have therapeutic indications like Mutra-Krichara, asmari, sula swasa, kasa, vishahara, madhumeha, udara vikara and shirashoola, Kalka of sthalakamalam with mile is used in Pleeha roga (Spleenic disease) and in all sotha rogas (Chunekar 1969).

Literature review revealed that no pharmacognostical studies has been carried out on this plant. Hence an attempt is made to investigate its pharmacognostical features (Iyengar 1975, Roma Mitra 1986). Some work on its phyto chemistry has revealed that the plant contains leucine, isoleucine, tryptohan and phenylalanine (Asima Chatterjee 1992).

MATERIALS AND METHODS:

The drug sold under the name purusharatna, was procured from the local market, and was
identified as H. enneaespermus following gamble (1967), the drug was soaked in 70% alcohol for 24 hours, free hand selections were taken (1967). Preliminary phytochemical tests for the active constituents were carried out by following pharmacopoeia of India (1966). Chromatographic and fluorescence studies were carried out by following block et al (1963) and Chase and Pratt (1949). Organic analysis was carried out by following Dey B.B (1957).

OBSERVATIONS OF THE DRUG:

The drug sold under the name purusuharatna consisted of whole plant with dried, wiry, woody roots, stem with small braches, leaves flowers and fruits.

IDENTIFICATION

The taxonomic characters found in the drug grouped it under the family violacceae. Further analysis of the charcters at generic and species level indicated that the drug may belong to the genus Hybanthus and species H. enneaespermus. It was further confirmed with the authentic herbarium specimen deposited at RRCBI (0398). The taxonomic characters found in the drug were analysed with those found in the classification of families following Gamble’s (1967) FPM.

TAXONOMY:

Hybanthus enneaespermus (L) f. Muell., Fragm, phyt Aust. 10;81. 1876; Tennant in Kew bull 36:103. 1981; Banerjee and Pramanik, Fasc. F1 India 12:2 1983 V. enneaespermma L., sp. P1 937 1753; Ionidium suffreutcosum (L.) R.&S., Syst.Veg 5:394.1819: f1 Brit. India 1:185: FPM 35 V. Suffroticos L., Sp.Pl.937,1753.

FLOWERS NEARLY ALL THE YEAR.

MORPHOLOGY

Branching herbs, leaves 1-3x0.2-0.3 cms, Sub-sessile, linear –laneolate, acute at apex, attenuate at base, glabrescent. Followers pink, solitary; pedicels 0.7-1cm, long. Calyx membranous: lobes 5, 2-3 ,mm. long sub canal, laneolate ciliate. Corolla lobes 5, unequal; upper 2-3 mm. long oblong; laterals 3-4 mm.long falcate; lower 0.8-1x0.6-0.7 cm., orbicular. Stamens 6, connate; filaments short; anterior 2 filaments with filiform appendages, Ovary unilocular, multi-ovuled; style 2-3 mm. long clavate capsule 3-4 mm across, subglobose (Keshav& Yogan 1990).

DISTRIBUTION:

Abundant from Bundelkund and Agra to Bengal and Sri Lanka, Tropical Asia Africa and Australia (Hooker 1875).

Synonyms: (Kirtikar & Basu 1988).

Tamil : Orilaitamarai, Oridaltamarai
Sanskrit : Amburuha, Athichara, Avyatha, Charati, Chariti, Padma, Padmacharini, Sthala padmini, Sthala kamala.

Telugu : Nilakobari, Purusharatham, Suriyakanti, Ratnapurusha
Hindi : Ratapurus

Malayalam : Orelatamara
Bengali : Nunbora

ROOT

MACROSCOPICAL CHARACTERS (PLATE 1):
Roots 10-12 cms. Long wiry slightly woody but easily breaks up, pale creamy yellow in colour with small lateral roots, pleasantly aromatic with slight bitter taste.

MICROSCOPICAL CHARACTERS (FIGS. 1, 2 & 3):

T.s of the root is circular in outline, outer cork layer is composed of twenty to twenty five layers of laterally compressed cells. This is followed by a single layer of broad cork cambium. Secondary cortex is composed of 10 to 12 layers of compactly arranged rounded, thin walled parenchymatous cells, some of the parenchymatous cells contain rosette shaped crystals of calcium –oxalate, square crystals and simple starch grains, phloem cells are well developed with phloem fibres. In between the xylem and phloem a single layer of cambium is present. Xylem vessels are intersected by uniseriate medullary rays. In the centre of the root, thick walled sclerenchymatous cells are present.

MACERATION:

Maceration of the root shows helical to spiral thickened vessels, simple elongated xylem fibres, Tracheids are narrow with simple pits. Xylem parenchyma cells are rectangular with highly thickened walls.

STEM

Macroscopical Characters (Plate 1): Stems are short, slightly woody when dry, pale brown is colour, measures 6 to 10 cms. X0.5 to 0.8 mm., taste sweet, with pleasant aroma.

Microscopical Characters (Figs. 4, 5 & 6): T.S. of the stem is circular in outline and shows a single layer of epidermis with thin wavy cuticle, some of the epidermal cells show simple uniseriate trichomes. Epidermis is followed by 10 to 15 layers of 2 to 3 layers of cells are chloren-chymatous and some of the parenchymatous cells contain oil globules. Phloem is well developed with phloem cells and phloem fibres polygonal stones cells are present with narrow lumen. Walls of the stone cells are highly thickened. Phloem cells are followed by a single layer of cambium. Xylem is well developed and is intersected with uniseriate medullary ray cells, filled with simple starch grains and oil globules in case of young stem. Whereas in case of mature stem, pith is composed of thick walled parenchymatous cells.

MACERATION:

Maceration of the stem shows helical to spiral vessels, thin walled elongated fibres, tracheids with simple pits. Xylem parenchyma cells are rectangular with simple pits (Figs II A-F).

LEAF

Macroscopical Characters (Plate 1): Leaves are small, dorsiventral linear 1-3 x 2-0.3 cm., subsessile, entire, rough to touch, pleasantly aromatic, taste slightly sweet.

MICROSCOPICAL CHARACTERS (Fig. 7 & 8):

T.S. of the leaf passing through midrib region shows plano-convex in our line with upper and lower epidermis comprising of single layered parenchymatous cells covered by striated thin cuticle. Both upper and lower epidermal cells are tangentially elongated. Some of the lower and upper epidermal cells show unicellular trichomes. Cortex consists of 2 to 3 layers of collenchyma, below it palisade tissue usually 2 to 4 layers thick extending up to the laminar region. In laminar region it is
replaced by 2 layers of thickness. Followed by palisade tissue, 2 to 4 layers of collenchyma tissue is present. The centre of the mid rib is occupied by a well developed collateral vascular bundle with protoxylem facing towards the upper epidermis and metaxylem towards the lower epidermis. Phloem is 5 to 6 layered, thin walled and polygonal. Towards the lower epidermis collenchyma is 2 to 5 layered, rounded, thin walled and compactly arranged. Some of the cells of parenchyma and collenchyma show starch grains.

T.S. of the laminar region shows typical dorsiventral leaf structure. Cruciferous type of stomata are present on both lower and upper surface of the leaf, but frequency of stomata is more on the lower surface than on the upper surface.

**MACERATE (FIG.IIIA TO E)**

Maceration of the leaf shows (in surface view), epidermal cells re polygonal with straight walls, the cells of the lower epidermal are striated with cruciferous type of stomata, whereas the epidermal cells of upper are straight walled with stomata. Trichomes are unicellular and xylem vessels are of helical type.

Quantitative ratio of the leaf were also carried out and results are tabulated in Table II. Histochemical tests of root, stem and leaf were carried out and results are tabulated in table III.

**DIAGNOSTIC CHARACTERS:**

**ROOT:** 1. Presence of square to rosette shaped crystals in cortex region
   2. Presence of uniseriate medullary rays.

**STEM:** 1. Presence of simple unicellular trichomes
   2. Presence of 2 to 3 layers of chlorenchymatous cells in the upper region of cortex.
   3. Presence of thick walled, rounded parenchymatous pith in mature stem.
   4. Presence of solitary calcium oxalate crystal in pith and cortex region.

**LEAF:** 1. Presence of subsessile, entire small, linear leaves.
   2. Presence of calcium oxalate crystals (solitary) and starch grains in parenchyma cells
   3. Presence of cruciferous type of stomata on both the surfaces of the leaf, but frequency of presence of stomata is more on lower surface than on upper surface.
   4. Presence of simple unicellular trichomes.

The measurements of different cells and tissues in microns (µ) are tabulated in Table I

**Table 1**
**Measurements for different cells & tissues in microns (µ)**
**ROOT:** (T.S)
- Cork: 8-12-20 x 5-10-15
- Cork Cambium: 5-15-25 x 3-10-20
- Secondary cortex: 15-28-40 x 10-15-25
- Xylem: 10-12-18 x 5-10-18
- Phloem: 5-18-25 x 3-10-20
- Starch Grains: 15-25-30 (diameter)

**MACERATE:**
- Vessel: 15-10-28 x 5-8-12
- Fibers: 25-30-45 x 5-12-18
- Tracheids: 20-25-35 x 15-18-20
- Xylem parenchyma: 20-25-32 x 10-12-20

**STEM: (T.S)**
- Epidermis: 8-15-25 x 5-10-25
- Cortex: 10-15-28 x 8-12-25
- Stone cells: 15-18-30 x 10-15-25
- Trichomes: 10-18-20 x 8-10-18
- Xylem: 15-22-30 x 10-15-20
- Phloem: 5-12-20 x 3-8-15
- Starch grains: 10-15-28 (diameter)

**MACERATE:**
- Vessel: 15-18-25 x 10-12-20
- Fibers: 18-20-25 x 10-12-20
- Tracheids: 20-25-30 x 15-20-25
- Xylem parenchyma: 10-15-20 x 8-12-18

**LEAF:**
- Upper epidermis: 20-30-45 x 10-15-20
- Lower epidermis: 25-28-38 x 18-22-30
- Palisade tissue: 30-38-45 x 25-30-40
- Collenchyma: 20-25-30 x 15-18-20
- Stomata: 10-15-25 x 5-8-10

**MACERATE:**
- Stomata: 10-15-20 x 10-15-20
- Trichome: 15-18-20 x 5-10-18
- Vessels: 15-18-25 x 10-15-20

**Table II**
**Quantitative ratio (value) of leaf**

|                           | Value     | Average value |
|---------------------------|-----------|---------------|
| Stomatal Index            | 10-12-15  | 12            |
| Palisade Ratio            | 7.75-8.5  | 8             |
Table III
Histochemical Test Results

| S. No | Material | Reagent                      | Change          | Test for  | Root | Stem | Leaf |
|-------|----------|------------------------------|-----------------|-----------|------|------|------|
| 1.    | Section  | Iodine                       | Blue colour     | Starch    | ++   | ++   | ++   |
| 2.    | Section  | Ferric Chloride              | No change       | Tannin    | --   | --   | --   |
| 3.    | Section  | Conc. HCl + Pinch of Phloroglucinol | Pink colour | Lignin    | ++   | ++   | --   |
| 4.    | Section  | Sndan III                    | No change in root but pink colour in stem & leaf | Oil globule | --   | ++   | ++   |

PHYTOCHEMICAL EVALUATION OF WHOLEPLANT OF H. ENNEASPERMUS

The whole plants of H. enneaspermus was coarsely powdered for chemical analysis and the results are tabulated in Table IV.

TABLE IV
Physico Chemical Constants (Proximate)

1. Loss on drying at 110C W/W 1.92%
2. Ash content          W/W 10.19%
3. Acid insoluble ash   W/W 5.15%
4. Solubility:
   (a) Ethyl alcohol W/W 9.21
   (b) Water         W/W 18.52
5. Foreign organic matter nil
6. Erective Values:
   (a) Petroleum ether 60-80C W/W 1.38%
   (b) Benzene        W/W 0.7194%
   (c) Chloroform     W/W 0.922%
   (d) Ethyl Alcohol  W/W 6.55%
7. Qualitative Analysis of the Ash:
   Presence of Carbonate, Sulphate, Phosphate and Basic Iron, Calcium, Magnesium, and Sodium.

ORGANIC ANALYSIS:

500 gms. Of air dried, powdered whole plant was extracted in Soxhlet apparatus with different solvents starting from petroleum ether (60-80C) followed by benzene, alcohol (70%) and water, successively for 18 to 20 hours for each solvent. The extracts obtained were subjected to
qualitative chemical test for the identification of various plant constituents and the results are tabulated in Table V.

### Table V

| Constituents       | P.Ether Extract | Benzene Extract | Alcohol Extract | Aqueous Extract |
|--------------------|-----------------|-----------------|-----------------|-----------------|
| Phenols & Tannins  | -               | -               | -               | -               |
| Steroids           | +               | +               | -               | -               |
| Flavonoids         | -               | -               | -               | -               |
| Carbohydrates      | -               | -               | -               | -               |
| Alkaloids          | -               | -               | +               | +               |
| Saponins           | -               | -               | +               | -               |
| Fixed oils         | +               | +               | -               | -               |
| Proteins           | -               | -               | -               | -               |
| Resins             | -               | -               | -               | -               |
| Guns & Mucilages   | -               | -               | -               | -               |
| Glycosides         | -               | -               | +               | +               |

**THINLAYER CHROMATOGRAPHY:**
The details of presence of number of number of constituents with Rf vaklues in both petroleum ether and alcoholic edtracts are shown in Table VI.

**Solvent System:** Benzene: Ethanol

(95:5)

**Developer:** Vannilin – Sulphuric Acid

### Table VI

| Extracts     | Spot 1 | Spot 2 | Spot 3 | Spot 4 | Spot 5 | Spot 6 |
|--------------|--------|--------|--------|--------|--------|--------|
| Petroleum Ether | 0.30   | 0.41   | 0.56   | 0.80   | 0.90   | 0.98   |
| Alcohol      | 0.26   | 0.46   | 0.56   | 0.75   | 0.86   | -      |

Thin layer chromatography of the petroleum ether and alcoholic extracts for constituents gave 6 and 5 spots respectively (Figs. 9and 10)

**FLUORESCENCE ANALYSIS OF WHOLE PLANT:**

For florescence studies the powdered drug was studied under the wave length of 254 um and 365 um UV light and the results are provided in Table VII.

**TABLE VII**
| Treatment          | Visible Light | U-V Light Shortwave (254 um) | U-V Light Longwave (365) |
|--------------------|---------------|------------------------------|--------------------------|
| Powder as such     | Light Green   | Light Green                  | Dark Green               |
| In Methanol NaOH   | Dark Green    | Light Green                  | Dark Ash                 |
| In Ethanol         | Pale Green    | Light Ash                    | White Colour             |
| In Ethanolic NaOH  | Light Green   | Dark Mud                     | Dark Mud with Specks     |
| Dilute HCL         | Light Nud     | Light Grey                   | Dark Grey Ash Colour     |

**SUMMARY:**

Pharmocognostical studies on H. enneaspermus were carried out and the studies revealed that the South Indian Crude Drug market sample consists of whole plant with root, stem, leaf, flowers (few) and fruit, which constitutes the drug orilaitamarai, which is sole under the trade name Ratan Purush/Purusha ratna locally. It has been identified as H. enneaspermus. This drug is mentioned in Bhava prakasha Nighantu (Chunekar 1969) as Sthala Kamalam (H. enneaspermus). Chunekar (1969) is also of the opinion that there are four types of sthala kamalam and this is not mentioned in Charaka, sustutha and vagbhata samhita. In this paper macro, microscopical studies on root, stem and leaf and phytochemical evaluation of the whole plant of H. enneaspermus is discussed under the name Orilaitamarai (Tamil).

**LEGEND:**

| REFERENCE | DESCRIPTION |
|-----------|-------------|
| Plate I   | Market Sample of the Drug |
| Plate II  | Herbarium Speciman of H. enneaspermus |
| Fig. 1    | T.S. of the root (diagrammatic) |
| Fig 2     | Portion of the root enlarged showing Cork and Cortex region |
| Fig 3     | Portion of the root enlarged showing vascular region |
| Fig 4     | T.S. of the stem (diagrammatic) |
| Fig 5     | Portion of the stem enlarged showing epidermis, cortex& phloem. |
| Fig 6     | Portion of xylem region with pith region enlarged. |
| Fig 7     | T.S. of the leaf through Mid-rib region (diagrammatic). |
| Fig 8     | Portion of the leaf, mid-rib region enlarged’ |
| Fig 9     | T.L.C of petroleum ether extract (whole plant). |
| Fig 10    | T.L.C of Alcoholic extract (whole Plant). |
| Macerate: | Root |
| Fig I (A to F) | Parenchyma cells with starch grains and crystals |
| A         | Cork cells |
| B         | Xylem parenchyma |
| C         | Helical vessel |
| Stem Fig II (A to F) | Xylem fibers | Tracheids |
|---------------------|--------------|-----------|
| A                   | Helical vessel |           |
| B                   | Xylem parenchyma |     |
| C                   | Parenchyma with starch grains | |
| D                   | Phloem fibres |           |
| E                   | Stone cells |           |
| F                   | Trichomes |           |

| Leaf Fig III (A to E) | Upper Epidermal cells with stomata (Surface views) |           |
|-----------------------|---------------------------------------------------|------------|
| A                     | Lower epidermal cells with stomata (Surface views) |           |
| B                     | Upper epidermal cells |           |
| C                     | Helical vessel |           |
| D                     | Trichomes |           |

**ABBREVIATIONS:**

| Abbreviation | Description |
|--------------|-------------|
| CAM          | Cambium     |
| CHL          | Chlorenchyma|
| COL          | Collenchyma |
| COR          | Cortex      |
| CRY          | Cortex      |
| CU           | Cuticle     |
| LEP          | Lower epidermis |
| OG           | Oil globule |
| PAL          | Palisade tissue |
| PH           | Phloem      |
| PHF          | Phloem Fibre|
| PI           | Pith        |
| SCL          | Sclerenchyma|
| SG           | Starch grain|
| ST           | Stomata     |
| STC          | Stone cell  |
| TRI          | Trichome    |
| UEP          | Upper epidermis |
| XY           | Xylem       |

**ACKNOWLEDGEMENTS:**

The authors are thankful to the Director, CCRAS, New Delhi for evincing interest in this work. They are also thankful to Dr. S.N. Yoganarasimhan, Sri K.G. Vasanth kumar, and to Dr. K.R. keshava Murthy for their valuable suggestions.
| Author(s)                        | Year | Title                                                                 | Publisher/Details                                                                 |
|---------------------------------|------|-----------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Anonymous                       | 1966 | Pharmacopoeia of India (2nd Eden). Delhi manager of publications, Govt.of India PP 930-990 |
| Asima Chatterjee, Satyesh Chandra Prakashi, Block R.J. | 1992 | The Treatise on Indian Medicinal plants Vol.2, PP 150-151.            |                                                                                   |
| Durrum E.L., Zweig G., Chase C.R. | 1968 | A manual of Electrophoresis (6th print) Academic Press, New York, PP 170-189. |                                                                                   |
| Pratt R.                        | 1949 | Fluorescence of powdered vegetable drugs with particular reference to development of system of identification, J. Am. Pharm. Assn. (Sciendi). 38; PP. 324-331 |
| Chopra R.N., Chopra I.C., Handa K.L | 1994 | Indigenous drugs of India (2nd edition), PP. 221                       |                                                                                   |
| Kapur L.D., Chunekar K.C        | 1969 | Bhavaprakasha Nighantu, Gangasaheb pandya commented by chumekar (in Hind)- Chowkhambha Vidya Bhavan, Varanashi, PP 482 |
| Sitarammn. M.V                 | 1957 | Laboratory manual & Organic Chemistry. S. Viswanath Publication. 3rd Edition. PP. 125-136. |                                                                                   |
| Gamble J.S                     | 1967 | Flora of the presidency of Madras, Vol. I, PP.35                        |                                                                                   |
| Iyender M.A                    | 1950 | Bibliography of Investigated Indian Medicinal Plants, Manipal Power Press ,Manipal. To P.P. 129 1975 |
| Johansen D.A                   | 1940 | Plant Microtechnique McGraw Hill, New York, PP. 204                    |                                                                                   |
| Keshava Murthy K.R., Yoganarasimhan S.N. | 1990 | Flora of Coorg (Kedagu) Karnataka, India PP.204                          |                                                                                   |
| Kirtikar and Basu               | 1988 | Indian Medicinal plants Vo. 1. (2nd edin) PP 204                        |                                                                                   |
| Murugesha Mudaliar, K.S        | 1988 | Materia medica (Veg section ) in Tamil, Tamil Nadu Dept of Siddha Medicine, Madras PP. 138 |
| Roma Mitra                     | 1986 | Bibliography on Pharmacognosy of Medicinal Plants, NBRI, Lucknow. PP.553. |                                                                                   |
| Trease G.E. and                | 1983 | Pharmacognosy (12th edition), Baillere Tindal, London PP.715-724.       |                                                                                   |
| Evans W.C., Venmathiyan G.P    | 1993 | Siddha Vaidya (in Kannada) Rani Chennamma Prakashana, Ulsoor, Bangalore. PP. 126 |
| Wallis T.E.                    | 1967 | Text Book of Pharmacognosy (12th edition), Baillere Tindal, London. PP. 571-582 |
