SRI LANKAN MEDICINAL PLANT MONOGRAPHS AND ANALYSIS

VOL – 12

MUNRONIA PUMILA

Lakshmi Arambewela

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Preface

Studies on medicinal plants of Sri Lanka have been carried out in the Herbal Technology Division of Industrial Technology Institute (former Ceylon Institute of Scientific and Industrial Research) for almost two decades. This monograph which is the twelfth in this series incorporates information collected from literature surveys, researches and also experiences of the Herbal Technology Division staff. This monograph is intended for a varied reading public, herbal drug manufacturers who need to identify their herbal raw materials, Ayurvedic physicians who need some scientific information on medicinal plants, research workers requiring some quick background information on a plant, industrialists or entrepreneurs pondering on commercial ventures and the inquiring lay readers. We hope this monograph fulfils some requirements of each of them.

The authors wish to thank the members of the Herbal Technology Division for their contribution, the Information Service Center for providing information, Department of Plant Sciences and Department of Zoology of the University of Colombo for assisting in anatomical studies, Food Technology Division of the Industrial Technology Institute for helping in the analysis of powdered plant materials and the Microbiology Laboratory for photographing the slides. They also gratefully acknowledge the sponsor National Science Foundation for the research grant (RG / 2004 / TM / 01).

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Colombo 07
Sri Lanka.

Munronia pumila Wight.

Family

Meliaceae

Synonyms

Munronia piñnata (Wall.) Theob.[1]

Munronia hainanensis How. & Chen. [1]

Melia pumila Moon [2, 3, 4]

Selected Vernacular Names

Sinhala - Binkohomba [1,2,3,4,5]

Tamil - Nela vempu [1,5]

Sanskrit - Bhu nimba [1]

Pharmacopoeia

Ayurveda Pharmacopoeia [6]

Distribution

An endemic species, common but not abundant, grows in intermediate and wet zone forests and on rocky places, 0-700 m in the low country in Sri Lanka, in Sigiriya, Ritigala, Balangoda, Lunugala, Wellawaya. Also seen in Southern to North-eastern India, Nepal to Southern China, Vietnam, Thailand, Malaysia, Burma, Sumba, Bali and Timor. It is also cultivated under green house conditions in Europe. [1,2,3,4,5,7]

Morphology[1]

A very small hardy shrub with unbranched stems 5-15 cm long, a whitish bark and long woody roots; leaves rather crowded, pinnate, petioles 1- 4.5 cm long, hairy; leaflets 1-3, pairs and a large terminal one, stalked, oval, acute, entire or coarsely lobed, the terminal leaflet 2-5 cm long, 1.5-3 cm broad, lateral leaflets 0.8-2.7 cm long, 1.5-3 cm broad,

scantily pubescent above, densely hairy below; flowers regular, bisexual, white, scented, 2.5 cm long and as much across when open, bracts linear, 2.5 mm long, hairy, bracteoles smaller, paired and also hairy, pedicels 7 mm long broader at the top, ridged and hairy ; sepals 5, linear or linear- oblong, 4.5-5 mm long, acute or subacute, hairy on both surfaces; corolla 5, basal portion fused to the stamen-tube to a length of about 1 cm, ridged, hairy inside and outside, broadly lanceolate,1.5-1.6 cm long, 0.6-0.75 cm broad subacute, hairy outside especially along the midrib, glabrous inside; stamens 10, filaments connate into a tube which adheres to a corolla tube at the base and terminating in 10 filiform teeth at the mouth, hairy on both surfaces, base recurved into a glabrous sheath round the ovary and part of the style, anthers almost sessile, erect, alternating with the teeth, 2 mm long, narrow, sterile at the top and hairy outside; ovary superior, pyramidal, 1.2 mm long, 5-locular with 2 superposed ovules in each loculus, style 2 cm long, hairy 3/4 way up from base, stigma capitate; fruit a depressed globes 5-lobed capsule, 1.2 cm diameter and hairy, seeds pyriform, narrowly winged, smooth, brown.

Fig-1. *Munronia pumila* plant

1. Leaf 2. Stem

(Source - Compendium of Medicinal plants, A Sri Lankan study. Vol. 2 (2002))

Plant Material of Interest

Whole plant is used but the root is said to be the most powerful part.[1]

Official Drug

Dried whole plant.[4]

Pharmacognostic Features*

Anatomy

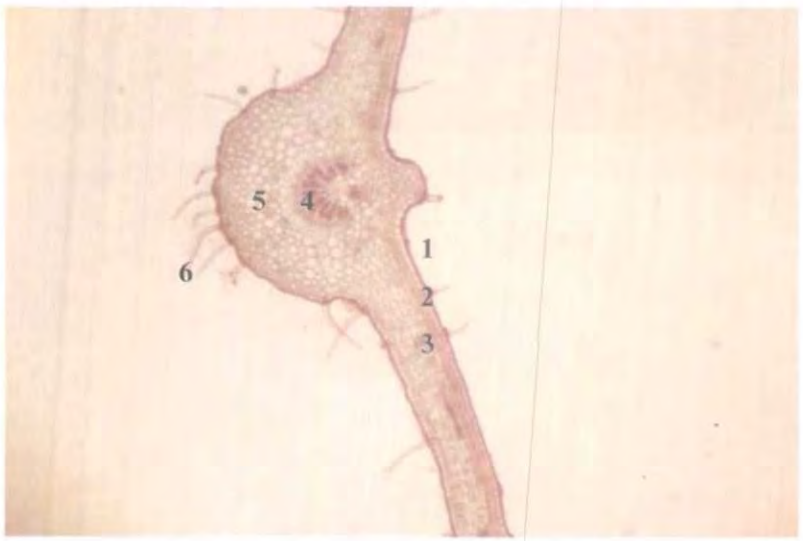

Fig-2. Cross section of *Munronia pumila* leaf (stained with safranine (10 x 10))

1. Epidermis 2. Palisade parenchyma 3. Spongy parenchyma 4.Vascular bundles
5. Parenchyma 6. Trichomes

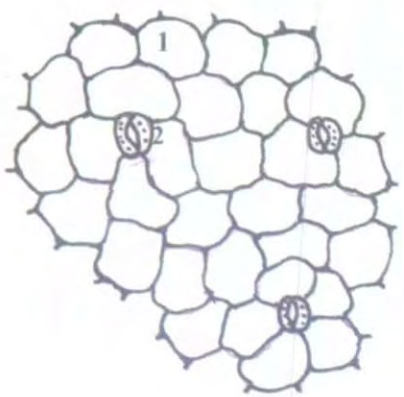

Fig-3. Schematic diagram of epidermis of leaf

1. Epidermis cells 2. Guard cells

Fig-4. Cross section of *Munronia pumila* stem (stained with safranine (10 x 10))

1. Cork 2. Cortex 3.Xylem

Fig-5. Cross section of *Munronia pumila* root (stained with safranine (10 x 10))

1. Cork cells 2. Cortex 3.Xylem 4. Phloem

Powder analysis

Analyzed part – Aerial part of the plant

Organoleptic properties

Colour – Yellowish green

Odour – Odourless

Taste – Bitter

Microscopic characters

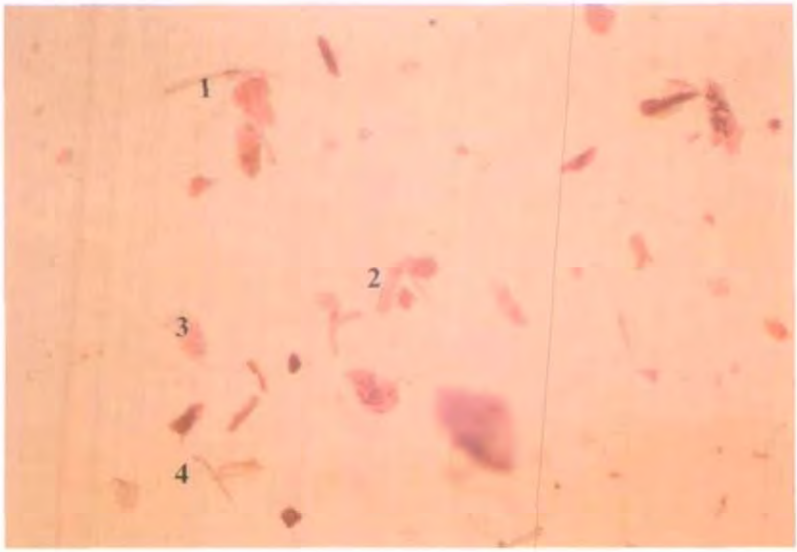

Fig-6. Powder of *Munronia pumila* aerial part under the microscope (10 x 4)

1. Part of a fiber 2. Tracheid 3. Parenchyma cells 4. Trichome

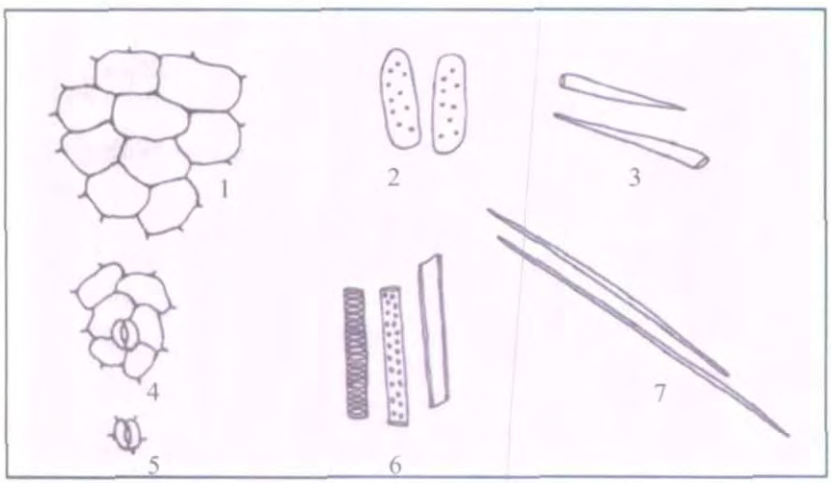

Fig-7. Schematic diagram of powder microscopy of aerial parts

1. Epidermis cells 2. Palisade cells 3. Trichomes 4. Epidermal cells with stomata

5. Stomata with guard cells 6. Vessel segments with different thickenings 7. Fibers

Analyzed part – Roots of the plant

Organoleptic properties

Colour – Light brown

Odour – Odourless

Taste – No special taste

Microscopic characters

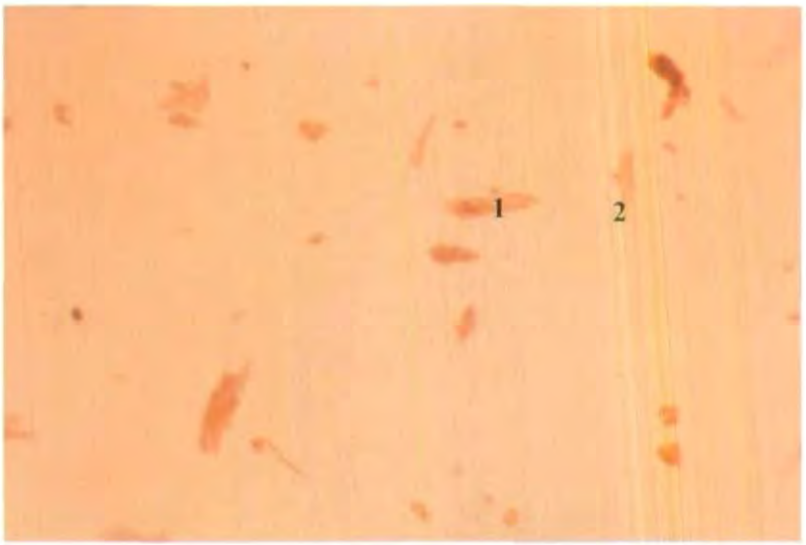

Fig-8. Powder of *Munronia pumila* root under the microscope (10 x 4)

1. Parenchyma cells 2. Xylem vessel segment

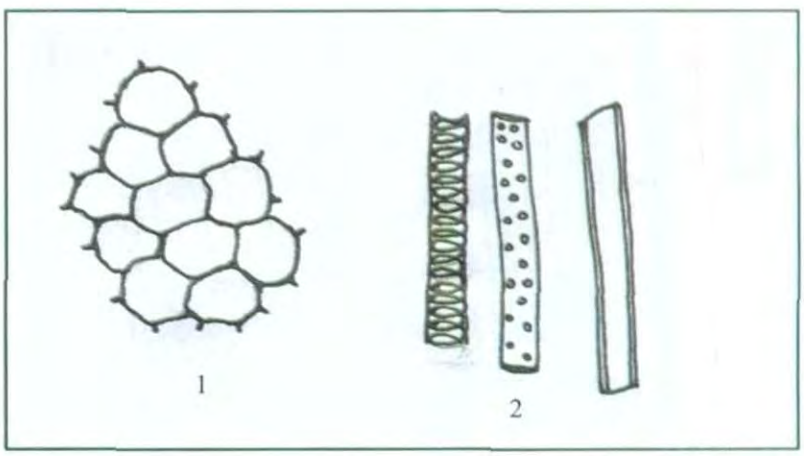

Fig-9. Schematic diagram of powder microscopy of root

1. Pith parenchyma cells 2. Vessel segments with different thickenings

* These analysis were carried out by the authors at Industrial Technology Institute and the Dept. of Plant Sciences and Dept. of Zoology of University of Colombo.

Physico-chemical Analysis[12]

Extractable matter

Crushed, air dried plant material (about 4 g) was weighed to a glass-stoppered conical flask. Solvent (100 mL) was added, weighed, shaken well and allowed to stand for 1h. It was then boiled for 1h and cooled. The weight was readjusted with specified solvent and filtered. Filtrate (25 mL) was taken, solvent was evaporated and oven dried at 105 ^{0}C for 6 h, cooled in a desiccator and weighed.

Total ash

Crushed, air dried plant material (about 4 g) was weighed to a previously ignited crucible. The material was ignited by gradually increasing the temperature to 550 ^{0}C until it was free from carbon. The crucible was cooled and weighed.

Acid insoluble ash

Hydrochloric acid (25 mL, conc. ~70 g/L) was added to the crucible containing total ash, covered with a watch glass and boiled gently for 5 min. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible and ignited to a constant weight.

Water soluble ash

Water (25 mL) was added to the crucible containing total ash, covered with a watch glass and boiled gently for 5 min. The insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the insoluble matter was transferred to the original crucible and ignited for 15 min. at a temperature not exceeding 450 ^{0}C. Water soluble ash is the calculated difference in weight between the total ash and the residue remaining after treatment of the total ash with water.

Moisture content of the samples was estimated and all the calculations were done on dry weight basis.

Table 1. Physico-chemical parameters of *Munronia pumila* plant**

Physico-chemical parameter	Amount%
Ethanol extract of aerial parts	7.7-8.2
Ethanol extract of roots	4.4-5.1
Water extract of aerial parts	16.1-20.5
Water extract of roots	17.1-18.2
Total ash content of aerial parts	7.0-8.2
Total ash content of roots	3.7-3.8
Acid insoluble ash content of aerial parts	6.5-6.8
Acid insoluble ash content of roots	3.6-3.8
Water soluble ash content of aerial parts	2.7-4.3
Water soluble ash content of roots	1.1-1.4

(Results are expressed as percentages on dry weight basis)

Thin Layer Chromatographic Profile**

***Munronia pumila* water extract of aerial parts**

Sample preparation	: *M. pumila* aerial parts (4 g) were boiled for one hour with water (100 mL) and the extract was filtered and evaporated to dryness. Six microliters (6 µL) of the diluted extract (25 mg in 4 mL) was spotted on TLC plate.
Absorbent	: Silica gel-GF_{254}
Solvent system	: Ethyl acetate : Methanol : Chloroform (1.5 : 3.3 : 0.2)
Detection	
Direct evaluation	: R_f values ($UV_{254\ nm}$) - 0.17, 0.46, 0.57, 0.80
Scanning	: Densitometer at 254 nm (before spraying) and 450 nm (after spraying)
Spray reagent	: Anisaldehyde

Fig-10. TLC finger print profile of water extract of *Munronia pumila* aerial parts

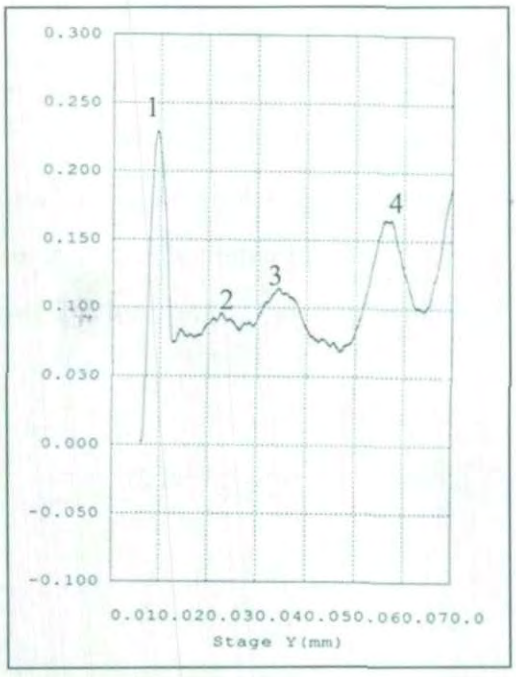

Table 2. Description of densitogram (Fig-11)

Peak no.	Y (mm)	Relative area%
1	9.42	28.47
2	22.78	20.71
3	34.39	19.55
4	56.35	18.69

Fig-11. Densitogram of TLC finger print profile of water extract of *Munronia pumila* aerial parts at 254 nm

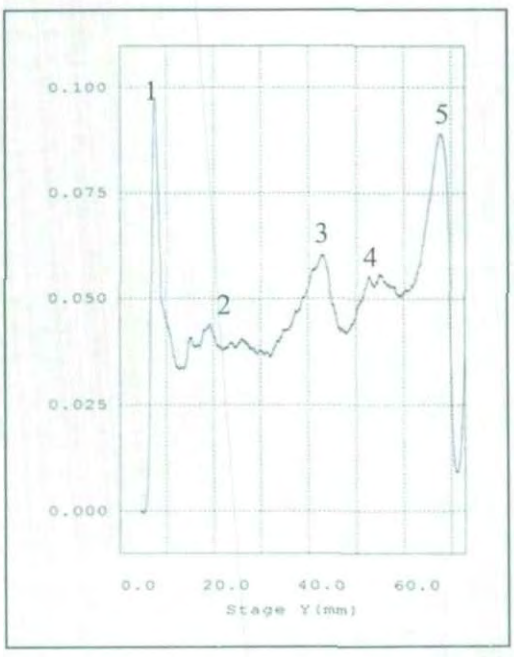

Table 3. Description of densitogram (Fig-12)

Peak no.	Y (mm)	Relative area %
1	7.55	18.45
2	19.28	2.31
3	42.86	17.30
4	55.04	22.04
5	67.85	35.61

Fig-12. Densitogram of TLC finger print profile of water extract of *Munronia pumila* aerial parts at 450 nm

***Munronia pumila* ethanol extract of aerial parts**

Sample preparation : *M. pumila* aerial parts (4 g) were boiled for one hour with 95% ethanol (100 mL) and the extract was filtered and evaporated to dryness. Six microliters (6 µL) of the diluted extract (25 mg in 4 mL) was spotted on TLC plate.

Absorbent : Silica gel-GF_{254}

Solvent system : Ethyl acetate : Methanol : Chloroform (1.6 : 3.2 : 0.2)

Detection

Direct evaluation : R_f values ($UV_{254\ nm}$) - 0.22, 0.65, 0.78, 0.98

Scanning : Densitometer at 254 nm (before spraying) and 450 nm (after spraying)

Spray reagent : Vanillin sulphate

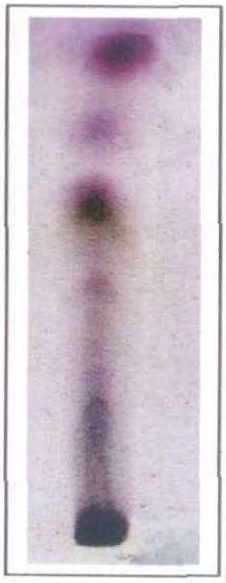

Fig-13. TLC finger print profile of ethanol extract of *Munronia pumila* aerial parts

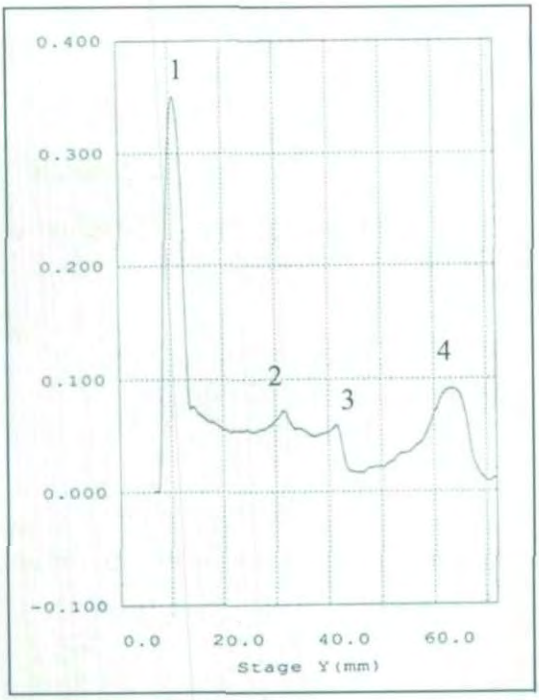

Table 4. Description of densitogram (Fig-14)

Peak no.	Y (mm)	Relative area %
1	10.76	62.03
2	31.59	3.32
3	41.47	3.33
4	63.54	30.56

Fig-14. Densitogram of TLC finger print profile of ethanol extract of *Munronia pumila* aerial parts at 254 nm

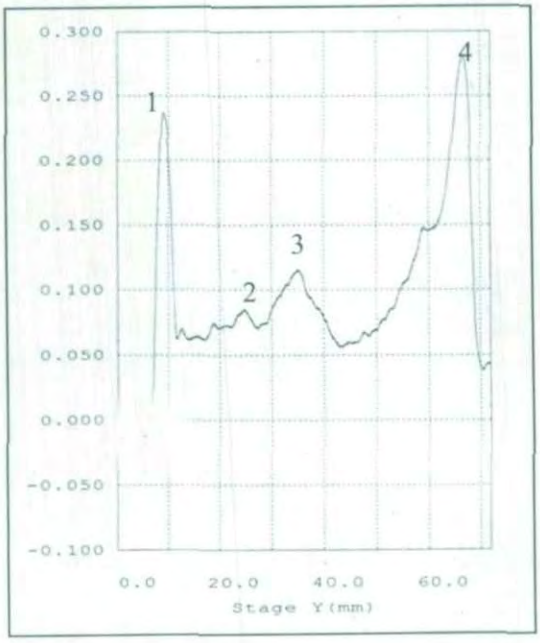

Table 5. Description of densitogram (Fig-15)

Peak no.	Y (mm)	Relative area %
1	9.11	14.38
2	24.84	6.30
3	35.14	20.65
4	66.65	44.98

Fig-15. Densitogram of TLC finger print profile of ethanol extract of *Munronia pumila* aerial parts at 450 nm

High Pressure Liquid Chromatographic Profile**

Munronia pumila water extract of aerial parts

Sample preparation : *M. pumila* aerial parts (4 g) were boiled for one hour with water (100 mL) and the extract was filtered and evaporated to dryness. The diluted extract (5.2 mg in 5 mL) was purified using Sep-pak C18 cartridge.

Injection volume : 20 µL

Apparatus : Shimadzu LC – 10 ADvp pumps and Shimadzu SPD – M 10 Avp uv / vis photodiode array detector.

Column : Inertsil 5U ODS – 2 reverse phase column, (250 mm x 2.6 mm).

Solvent system : Acetonitrile : Water (40 : 60)

Flow rate : 1 mL/min

Detection : 254 nm

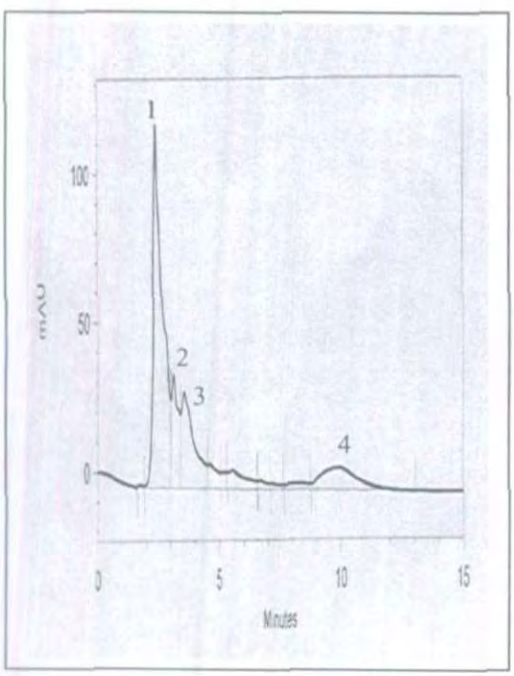

Fig-16. HPLC finger print profile of water extract of *Munronia pumila* aerial parts

Table 6. Retention times of main peaks

Peak no.	Retention Time (min)	Relative area %
1	2.39	47.15
2	3.14	10.79
3	3.57	17.47
4	9.95	11.39

***Munronia pumila* ethanol extract of aerial parts**

Sample preparation	: *M. pumila* aerial parts (4 g) were boiled for one hour with 95% ethanol (100 mL) and the extract was filtered and evaporated to dryness. The diluted extract (6.5 mg in 5 mL) was purified using Sep-pak C18 cartridge.
Injection volume	: 20 µL
Apparatus	: Shimadzu LC – 10 ADvp pumps and Shimadzu SPD – M 10 Avp uv / vis photodiode array detector.
Column	: Inertsil 5U ODS – 2 reverse phase column, (250 mm x 2.6 mm).
Solvent system	: Methanol : Water (70 : 30)
Flow rate	: 0.6 mL/min
Detection	: 254 nm

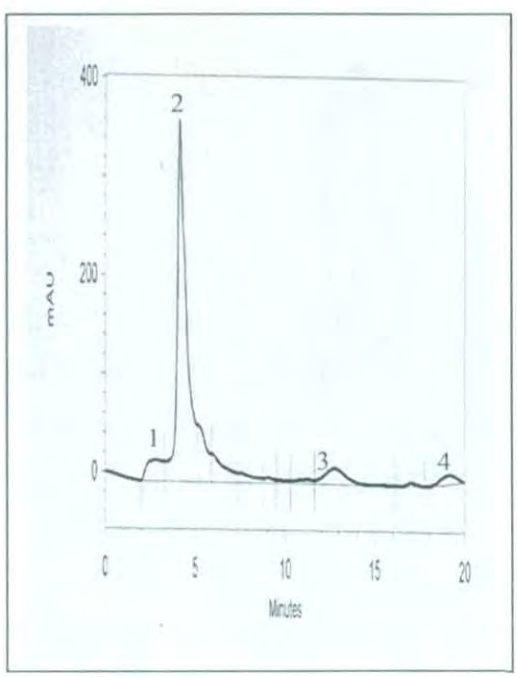

Fig-17. HPLC finger print profile of ethanol extract of *Munronia pumila* aerial parts

Table 7. Retention times of main peaks

Peak no.	Retention Time (min)	Relative area %
1	2.77	6.21
2	4.13	70.40
3	12.75	6.92
4	19.18	2.89

***Munronia pumila* water extract of roots**

Sample preparation : *M. pumila* roots (4 g) were boiled for one hour with water (100 mL) and the extract was filtered and evaporated to dryness. The diluted extract (4.9 mg in 5 mL) was purified using Sep-pak C18 cartridge.

Injection volume : 20 µL

Apparatus : Shimadzu LC – 10 ADvp pumps and Shimadzu SPD – M 10 Avp uv / vis photodiode array detector.

Column : Inertsil 5U ODS – 2 reverse phase column, (250 mm x 2.6 mm).

Solvent system : Acetonitrile : Water (30 : 70)

Flow rate : 1 mL/min

Detection : 254 nm

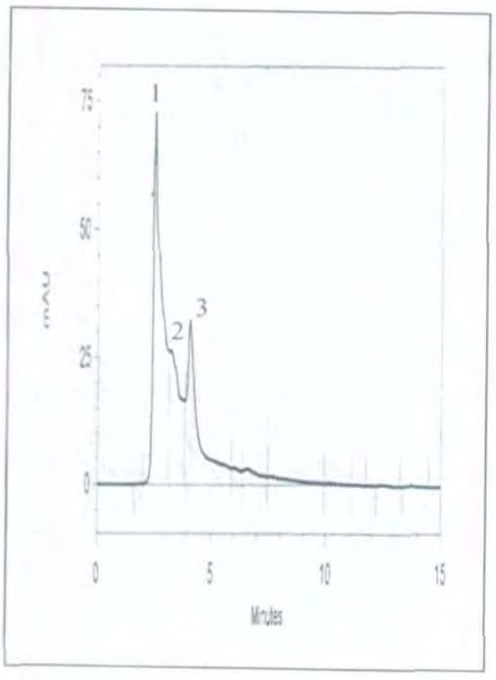

Fig-18. HPLC finger print profile of water extract of *Munronia pumila* roots

Table 8. Retention times of main peaks

Peak no.	Retention Time (min)	Relative area %
1	2.48	43.92
2	3.21	19.47
3	4.03	26.70

***Munronia pumila* ethanol extract of roots**

Sample preparation : *M. pumila* roots (4 g) were boiled for one hour with 95% ethanol (100 mL) and the extract was filtered and evaporated to dryness. The diluted extract (4.8 mg in 5 mL) was purified using Sep-pak C18 cartridge.

Injection volume : 20 µL

Apparatus : Shimadzu LC – 10 ADvp pumps and Shimadzu SPD – M 10 Avp uv / vis photodiode array detector.

Column : Inertsil 5U ODS – 2 reverse phase column, (250 mm x 2.6 mm).

Solvent system : Acetonitrile : Water : Methanol (40 : 40 : 20)

Flow rate : 0.9 mL/min

Detection : 254 nm

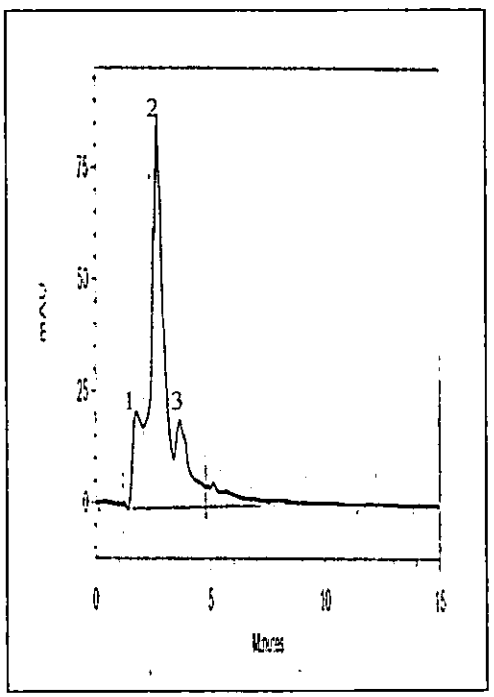

Fig-19. HPLC finger print profile of ethanol extract of *Munronia pumila* roots

Table 9. Retention times of main peaks

Peak no.	Retention Time (min)	Relative area %
1	1.76	11.93
2	2.67	60.32
3	3.64	16.99

** These analysis were carried out by the authors at Industrial Technology Institute.

Phytochemistry

Two compounds have been identified from the methylene chloride fraction of ethanol extract of *M. pinnata* plant. One of them is a fatty acid with 15 carbon atoms and the other compound is a triterpenoid with 34 carbon atoms[13].

Medicinal Uses

Uses described in pharmacopoeia and other traditional systems of medicine

The drug has the following medicinal properties - wound purifying, anthelmintic, carminative, laxative, improving digestive power, reducing dermatitis, promoting lactation, purifying pitta, destroying worms and decreasing thirst.
It is also used for the treatment of following conditions - polyuria, cough, oedema.[1, 6, 8]

Uses in folk medicine

It is given for fever, dysentery and purification of blood.[2]
It has been used in the treatment of leprosy and other skin diseases.[4]
Since it is very effective in bringing down body temperature, it is often used in the treatment of malaria.[1]
Also used to prevent hiccups and vomiting and for sore throats.[1]

Other Uses

A decoction of this plant is an excellent bitter tonic often used as a substitute for Chirata (*Swertia chiretta*).[2, 4]

Ayurvedic/Traditional Medicinal Preparations

Used as a constituent of following Ayurvedic medicines according to Ayurveda pharmacopoeia.[6]

Ahuadi kasaya, Arkananthadi kasaya, Arka-grathiadi kasaya, Kantakaryadi kvathaya, Kirathaadi kasaya, Kiratha-thikthadiya, Kalingadi kasaya, Gudukyadi kasaya, Drakshadi

kvathaya, Duralahadiya, Duruparpatadi kvathaya, Denimbha-debatuadi kasaya, Nagaradi kvathaya, Puncha-mulyadi kvathaya, Punchathitha kvathaya, Patadi kvathaya, Payvanamruthra kvathaya, Pathyadi kvathaya, Phala-thrikadiya, Piththa–shleshmalahara Ashtadashanga kvathaya, Musthadi kvathaya, Mahathithaka ghathaya, Loadhrasamaya/Madhasavaya, Vandana savaya, Ushirasamaya, Chandra-phrabha-vati, Sarasvathi powder, Maha-nimba powder, Bhu-nimba powder.

Propagation[7,9,10,11]

The plant can be grown satisfactorily in dry zones and intermediate zones. Shady areas and humus rich soil with loam are suitable for growth. Vegetative propagation i.e., rooting of stem cuttings is possible in this species but it is not economical as only a single cutting could be obtained from a plant. Nodal cuttings cannot be prepared because this species has very short inter-nodal distances. Traditional propagation is through seeds.

For collection of seeds, a stock of mother plants should be maintained preferably under a greenhouse. Seeds are dispersed after splitting of mature pods of this species during the daytime. The adopted method for collection of seeds in some areas is by covering the mature fruits individually using small polythene bags before splitting. This method is not possible for plants with rosette appearance with leaves and flowers crowded at the terminal. Alternatively, maintenance of plants in larger polythene bags (12" X 10") and collection of dropped seeds under the plants before watering was found to be suitable for this species and more economical. Even seeds collected a few days later will not lose their viability when mother plants with moist soil in the polythene bags are used.

Seeds are some times soaked in water before planting to speed up germination. This procedure facilitates the initiation of seed germination before planting. The advantages of seed soaking treatment are, shortening the time of emergence, improving the infirmity of seedling and circumventing some adverse conditions in the seedbed. Seeds of most herbaceous species could benefit from 8 hour soaking but may be injured by longer soaking periods such as 24 hours or more. Imbibition of water is a primary requirement for activation of seed germination. The dry seeds absorb water and the moisture content

increases rapidly (40-60%) at the early stage. Water softens the seed covering and causes hydration of protoplasm. The seeds swell and the seed coat may break.

Ideal conditions for seed germination are soaking for 12 hours in water and potting in 1:2:2 (surface soil: sand: compost) soil mixture in sterilized media.

Common pests are mites and mealy bugs. Mealy bugs were identified as the most serious pest of *M. pumila.* Mealy bugs crowd in the upper part of the stem with leaves. This pest spreads more rapidly under greenhouse conditions than out side. The insecticide "Dimethoate" is used to control this pest. It is also observed that fresh seeds fallen from mother plants are eaten by "Red Ants". Maintenance of mother plants on a bench and application of grease or burned oil on supporting legs was found to be a suitable remedy.

The plant is ready for harvesting in 9-18 months and the total plant should be harvested. Seed propagation is not sufficient for commercial scale cultivation due to poor production of seeds and their viability. Therefore large scale propagation will be difficult through seeds alone. There is no practice of using stem cuttings. Tissue culture techniques were investigated and successfully used to propagate *M. pumila.* The techniques involved are shoot tip culture, callus cultures, *in vitro* organogenesis and somatic embryogenesis. There is a possibility of propagating *M. pumila* Wight through *in vitro* callus cultures. The studies suggest that the hypocotyls callus of the seed is the best for plant regeneration. Potential also exists for plant regeneration from the leaf callus.

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