Domestication Studies on Jeevakom

(*Malaxis rheedii* Sw.)

(Project No. (T) 005/SRS/2003/CSTE)

PROJECT COMPLETION REPORT

(03.10.2003 to 02.10.2006)

Principal Investigator

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Trivandrum

(English)

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Principal Investigator
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Co-Investigators
Dr. M. Asha Sankar
Dr. E. V. Nybe
Dr. A. Augustin
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CONTENTS

List of Plates / Figures

List of Tables

Preface

'Jeevakom' (*Seidenfia rheedii* Sw. Szlach) is an inevitable constituent drug in many ayurvedic preparations. Known as *rishabhaka* in Sanskrit, it belongs to the 'ashtavarga' group of drugs mentioned in Ayurveda. The drug is costly and most of its requirement is met by the supply from North India. The plant occurs in the forests of Kerala, even though in small quantities. Because of several factors, anthropogenic and otherwise, the natural sources of this drug are getting depleted day by day and at present it is reported to be in the 'rare' category. *In situ* conservation measures are to be adopted urgently. But, in the long run any conservation policy is bound to fail unless we resort to domestication and cultivation efforts also. It is high time the plant is domesticated.

Unlike other crop plants, both quality and quantity are equally important in medicinal plants. Quality is governed by the content of secondary metabolites, which in turn is greatly influenced by the environment in which the plant grows.

Many of the medicinal plants tend to behave differently under domestication or cultivation under artificial conditions. A thorough understanding on the natural habitat and response to domestication is hence essential for any conservation or cultivation programme. Jeevakom has not yet been domesticated. This study is a pioneering attempt on domestication of this valuable drug plant. In a limited three year duration sanctioned, the team collected the traditional knowledge on jeevakom, located its natural habitats in the forests of Kerala and carried out detailed natural habitat analysis, conducted domestication trials and attempted phytochemical and anatomical studies in both wild and domestic plants. Objectives of the study, methodology adopted, experiment wise results, conclusions, summary, contributions made, future line of work, references and appendices are presented chapter wise. During the tenure of study and in the preparation of the report we received generous help and technical support from a large number of institutes and persons. We acknowledge our gratitude to all of them. The sponsor, Kerala State Council for Science Technology and Environment, Tiruvnanthapuram and the host, Kerala Agricultural University always provided the required facilities and services for the successful completion of this project. We hope that the data/information generated in this study may enhance the present day knowledge on the impact of domestication on medicinal plants.

N. Mini Raj
Principal Investigator

Acknowledgement

I would like to express my sincere thanks to

- Kerala State Council for Science Technology and Environment Tiruvananthapuram for financing the project
- The research monitoring committee of Kerala State Council for Science Technology and Environment for their periodic monitoring, critical comments and suggestions during the course of the study
- The Kerala Agricultural University for extending all facilities for undertaking the project
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  rendered during the study
- Dr. E.V. Nybe, Head, Department of Plantation Crops & Spices, College of Horticulture for the help, co-operation and support during the study and for the meticulous editing of the text.
- The Principal Chief Conservator of Forests Tiruvananthapuram for granting permission for forest explorations
- Sri. O.P Kaler, IFS and Sri. P.K. Kesavan, IFS who ensured the co-operation of forest officials
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- Sri. Vimal Kumar, Range Officer Silent Valley; Sri. Sunil, Range Officer Tholpetty; Sri. Vijayan, Range Officer Sendurney ;Sri. Sabi Varghese, Range Officer Karimala., Sri. Thulasidas, Range Officer Periya and Sri. Nelson, Wild life Assistant Parambikulam for making arrangements for the forest explorations
- To Sri. Gopi & Sri. Murali, tribals of Parambikulam, Sri. Maari, tribal guide of Silent Valley, Sri. Chandu, tribal guide of Periya, Sri. Ayyappan, Malayan tribal of Peechi and to Sri. Ajayan, forest watcher, Shendurney whose knowledge of the forest and its flora came in handy for locating the wild habitats of jeevakom.

- To Dr.V. M. Dileep, a freelance nature researcher who accompanied us in our forest trips
- To Mrs. Sincy.A, Senior Research Fellow for full filling her role with sincerity and dedication
- To Dr. Nafeesa, Professor of Botany, Calicut University for the help and services rendered in microtomy work
- To Mrs. Dhanya, A.S, Research Assistant for her expertise in microtomy
- To Dr. N. Sasidharan Scientist, Kerala Forest Research Institute and the Director Botanical Survey of India, Coimbatore for their help rendered in botanical identification
- To Dr.C. Sathish Kumar, scientist, Tropical Botanical Garden & Research Institute, Palode whose expertise on orchids not only enabled us to identify the plant, but opened a whole new world for us as far as orchid study is concerned
- To various Ayurveda physicians who shared their knowledge about jeevakom
- To the co- investigators of the project, Dr. M. Asha Sankar and Dr. Alice Kurian, for their support
- To Dr. A. Augustin , Centre for Plant Biotechnology and Molecular Biology for the help rendered in the phytochemical analysis
- To Prof.. S. Krishnan, Department of Agricultural Statistics for the help rendered in statistical analysis of the data
- To Sri. Kuttikrishnan, driver, Kerala Agricultural University, who worked almost as a member of the team

Last but not least I wish to thank all my innumerable friends, especially in the forest areas I have visited

Date :

N. Mini Raj

EXECUTIVE SUMMARY

For office use only

File No.Documentation No..... /SRS/2006/CSTEName of the scheme: Science Research Scheme (SRS) Year..... to.....

Name of the Programme Officer:

PROJECT COMPLETION REPORT

1.	Title of the project	:	**Domestication studies on Jeevakom (*Malaxis rheedii* Sw.)**
2.	Name of Principal Investigator	:	Dr. N. Mini Raj Assistant Professor (Hort) Dept. of Plantation Crops & Spices College of Horticulture, KAU
	Co-investigators	:	Dr.M. Asha Sankar Assistant Professor (Hort) Dept. of Plantation Crops & Spices College of Horticulture, KAU Dr. E.V. Nybe Professor and Head Dept. of Plantation Crops & Spices College of Horticulture, KAU Dr. A. Augustin Associate Professor (Biochem) Dept. of Biotechnology College of Horticulture, KAU Dr. Alice Kurian Associate Professor (Hort) Dept. of Plantation Crops & Spices College of Horticulture, KAU
3.	Implementing Institution	:	Kerala Agricultural University
4.	Date of commencement	:	03.10.2003
5.	Planned date of completion	:	02.10.2006
6	.Actual date of completion	:	02.10.2006

7. Objectives as stated in the project proposal:

To analyse the response to domestication of the medicinal plant 'Jeevakom' (*Malaxis rheedii* sw.)

8. Deviation made from original objectives if any, while implementing the project and reasons thereof

Nil

9. Project Abstract

The research project entitled " Domestication studies on jeevakom (*Malaxis rheedii* Sw.) was carried out at the Department of Plantation Crops & Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara during 2003-2006. Objective of the study was to analyze the response to domestication of the medicinal plant jeevakom. There were four broad experiments viz plant exploration and natural habitat analysis, domestication trial, phyto-chemical analysis and anatomical studies.

The study indicated that what is sold and used as the two drugs *Jeevakom* and *Edavakom* in Kerala is pseudobulbil of the same plant differing only in size. Larger pseudobulbils are considered as jeevakom and smaller one as edavakom. The plant is botanically *Seidenfia rheedii* (Sw.)(Szlach.) (Basionym: *Malaxis rheedii* Sw.)

Out of the forest areas explored; viz. Wynad, Parambikulam, Peechi, Silent Valley and Shendurney, the species was located at Parambikulam, Peechi and Silent Valley forests. At Parambikulam and Peechi, sufficient population was present whereas at Silent Valley the population was meagre. Contrary to the popular belief of an *abhavadravya* (unobtainable) this study indicated the availability of jeevakom in Kerala forests albeit in small quantities.

Natural habitat analysis revealed that *Seidenfia rheedii* is a lithophytic orchid found on wet rocks amidst moss and grass, in the openings of semi evergreen and evergreen forests at altitudes ranging from 800-1100m above MSL. The natural habitat could be described as **dripping rock ecosystem**. The plant is a short stemmed fibrous rooted herb with a swollen stem forming a conical pseudobubil which is used as drug. The plant regenerates vegetatively through side bubils. After flowering and fruiting which is completed by Dec-Jan, the plant dries up and remains dormant until the next rains.

In the domestication trial the plant responded positively to domestication. Both the growth and yield parameters were high in the domestic crop compared to wild plants. In the first trial where the pseudobulbils were vertically planted, performance was better under 50% shade in terms of both growth and yield parameters compared to fully open conditions. The striking disadvantage in the domesticated crop was very low production of side bulbils, thereby blocking the advancement of generation.

In the second trial where horizontal planting was done, the production of side bulbils was more, thus overcoming the major draw back of vertical placement. Horizontal sowing was also advantageous with respect to other growth and yield parameters.

The pseudobulbils of domestic crop were stored for five months inside earthern pots and sown in the next season to test the viability and evaluate its performance. There was 100 per cent germination, better growth and development. In growth and yield parameters, the 2nd generation crop performed better than 1st generation crop raised simultaneously.

Pseudobulbils from the wild as well as domestic environment were analyzed for the selected phytochemical constituents. Higher values of starch, protein and total free amino acids were recorded in the domestic crop. Presence of high soluble sugars and low amino acids were characteristic of wild samples. High chlorophyll content was recorded in wild samples. Both wild and domestic samples gave negative results for alkaloids and saponins. Overall results indicated the presence of hexosamine/amino sugars or glycosides in the plant. Detailed analysis of these compounds can only elucidate the medicinal property of *Seidenfia rheedii* and the impact of domestication on the ultimate quality of the drug.

In anatomical studies pretreatment of sample for microtomy was standardized. L.S and T.S of root, outer scale, leaf and pseudobulbil were taken. Root cap tissue and root hairs were present which confirmed it as a normal root unlike other orchid roots. In the outer scale, some reticulations were present inside the parenchyma cells which needs further clarification as to whether they are cellular deposits or dead cells themselves. L.S ans T.S of pseudobulbil and leaf were also described.

10. Key words

Seidenfia rheedii Sw. (Szlach), plant domestication, jeevakom, astavarga drugs, medicinal orchids.

11. Achievements:

i. List of Research publications

Sl.No.	Authors	Title of Paper	Name of journal	Volume	Pages	Year
1	Mini Raj, N., Nybe, E.V., Sincy,A.,M,Asha Sankar., Alice Kurien., Augustine,A.	Botanical sources of jeevakom-an ayurvedic drug in Kerala	Proceedings of the 19th Kerala Science Congress	-	206-207	2007

i. **Manpower trained on the project**

a) **Research Scientists or Research Associates**

One Senior Research Fellow

b) **No. of Ph.D produced**

Nil

c) **Other Technical Personnel trained**

One research assistant trained in microtomy

ii. **Innovations/Technology developed**

In the present study the availability of *Seidenfia rheedii* in Kerala forests is confirmed and its status ascertained. The botanical identity of the two drugs, *jeevakom* and *edavakom* which is given differently in various texts is also confirmed in the study. The same plant (larger and smaller bulbils) is used as jeevakom and edavakom in Kerala and the species was identified as *Seidenfia rheedii*. As a long term conservation measure of this rare and valuable drug, domestication trial was attempted and the plant responded positively to domestication. The change in morphological and reproductive behavior of the species upon domestication is quantified. On the quality side, since nothing is known about the phytochemistry of jeevakom, all primary as well as secondary metabolites were analyzed in both wild and domestic plants. Conclusive data are not available on the medicinal quality of jeevakom. But from the present study, amino sugars/glycosides seemed to be the major component imparting quality to the drug. Dynamics of various components upon domestication was also quantified. Techniques of microtomy was standardized for fleshy plant parts.

iv. **Patents taken, if any**

Nil

V **Application potential**

Findings of this study would necessarily lead to conserving the natural population of *Seidenfia rheedii* available in Kerala forests at the same time making the genuine drug available in sufficient quantity for the user industry. Data/information generated from this study would serve as a plat form for the conduct of domestication studies on other medicinal species also, whose supply from the wild is in short of demand.

12. Financial Details

No	Financial position/ budget Head	Funds sanctioned	Expenditure	% of Total cost
I	Man power	1,80,000	1,79,320	41.12
II	Equipment	1,00,000	99,979	22.92
III	Consumables & Contingencies	89,000	88,640	20.32
IV	Travel	30,000	28,539	6.54
V	Overhead charges (10%)	39,930	39,648	9.10
	Total	**4,38,930**	**4,36,126**	**100%**

13. Procurement/ usage of Equipment

a)

Sl. No.	Name of Equipment	Make/ Model	Cost (FE/Rs)	Date of installation	Utilization Rate (%)	Remarks regarding maintenance/break down
1	ACME TLC Equipment	Model No.II	6000.00	04.02.04	100%	In good working condition
2	Digital top pan balance	K. Roy Model No. LCB-E2	10920.00	22.01.04	100%	In good working condition
3	Soxhlet extraction mantle	Rotex Model No. RHM-6	7100.00	29.01.04	100%	In good working condition
4	Water bath	Rotex double walled Cat No. RRW-12	5750.00	29.01.04	100%	In good working condition
5	Applicator, UV lamp 254nm and Sprayer for TLC	Merck	43180.00	13.02.04	100%	In good working condition
6	Soxhlet extraction apparatus-500ml	Vensil	4101.00	30.01.04	100%	In good working condition

7	Soxhlet extraction apparatus-250ml(10 no's)	Borosil	17474.00	30.03.04	100%	In good working condition
8	Micro capillary tube	Merck	4601.00	30.03.04	100%	In good working condition
9	Culture Tube flat bottom with screw	Borosil	853.00	30.03.04	100%	In good working condition

b) Plans for utilizing the equipment facilities in future

The equipments will be utilized for the follow up studies on jeevakom.

Name and signature with date

a. Dr. N. Mini Raj
(Principal Investigator)

1 11 06

b (Co-investigators)
Dr.M. Asha Sankar

Dr. E.V. Nybe

Dr. A. Augustin

Dr. Alice Kurian

Chapter 1
INTRODUCTION

Jeevakom is a medicinal orchid widely used in many Ayurvedic preparations. In spite of wide usage, little is known about it except that it has many rejuvenating properties. It is available in the market in two forms: *Jeevakom* and *Edavakom*. Many Ayurvedic physicians believe that there are two different herbs while many others hold the view that both belong to the same species, the only difference being size. Again many believe that jeevakom does not occur in Kerala and infact, much of our supply comes from other states, especially Punjab.

The aim of the research project is to find out whether jeevakom occurs in Kerala, if so, whether it can be domesticated to ensure adequate supply for Ayurvedic medicines and whether the domesticated plant retains the medicinal properties of the wild plant.

If the project is successful, it will ensure an indigenous supply of Jeevakom and limit our dependence on exotic supply. It will also enhance our understanding of the ecology and pharmacology of this obscure yet widely used orchid. Domestication might also save the species from possible extinction due to over harvesting of the meager population surviving in certain pockets of the Western Ghats

Chapter 2
EXPERIMENTAL SET UP

The present investigation was carried out at the Department of Plantation Crops & Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara. The study period was 2003-2006. There were four experiments in the study namely:

1. Plant exploration and natural habitat analysis
2. Domestication trial
3. Phytochemical analysis
4. Anatomical studies

2.1 Collection of secondary data

Since recorded literature on Jeevakom is scanty, all possible information regarding the drug was gathered from traditional vaidyas, herb gatherers and tribal people. Classic texts of Ayurveda and published flora were also scanned for getting authentic information on the the drug.

2.2 Plant exploration

The Chief Wild Life Warden, Department of Forests and Wild Life, Govt. of Kerala, Forest Head Quarters, Tiruvananthapuram was contacted to get permission for forest explorations. Vide his order dated 6.12.2003 permission was granted for exploring the protected sanctuaries and National Parks for the purpose of the study as per the conditions given in the letter (attached as Annexure).

2.3 Natural habitat analysis

Based on the results of the forest explorations, detailed natural habitat analysis was carried out in the Peechi-Vazhani wild life sanctuary. Details of forest explorations and natural habitat analysis are presented in the next chapter.

2.4 Domestication trial

This experiment was carried out in the experimental fields attached to the Department of Plantation Crops & Spices, College of Horticulture, Vellanikkara. The trial was carried out during 2004-2005 and 2005-2006.Details of the trial are presented in the next chapter.

2.5 Phytochemical analysis

This experiment was carried out in the biochemistry laboratory attached to the Department of Plantation Crops & Spices. Detailed procedures of the analyses are given below:

2.5.1 Estimation of total soluble sugars

Phenol sulphuric acid method

Standard glucose Stock:100mg in 100ml water.Working standard: 10ml stock solution diluted to 100ml with distilled water.

Sample preparation: Homogenized 500mg of the fresh plant sample with hot 80% methanol. Centrifuged the extract. Repeated the extraction and made to 50ml with 80% methanol.

Pipetted out 0.2 to 1.0 ml of working standards and 0.5ml of sample extract into a series of test tubes. Made up the volume in each tube to 1ml with distilled water. Blank was set with 1ml distilled water. To each test tube, 1ml of phenol solution (5%) was added followed by 5ml of 96% sulphuric acid. The tubes were shaken well and kept for 10 minutes. It was then placed in a water bath at 25-30°c for 20 min to develop a light yellowish brown color. Test tubes were cooled and absorbance were read at 490nm.

2.5.2 Estimation of starch

Anthrone method

Standard glucose:Stock-100mg in 100ml water.Working standard-10ml stock solution diluted to 100ml with distilled water.

sample preparation: Homogenized 0.1g of the fresh plant sample with 5ml hot 80% methanol to remove sugars. Centrifuged and the residue was retained. Washed the residue repeatedly with 5ml of hot 80% methanol. Dried the residue well over a water bath. To the residue, 5ml water and 6.5ml 52% perchloric acid were added and centrifuged at 0°c for 20min.Repeated the process. Pooled the supernatants and made up to 100ml in volumetric flask.

Pipetted out 0.2 to 1.0ml of working standards and 0.2ml of extract into a series of test tubes. Made up the volume in each tube to 1ml with distilled water. Blank was set with 1ml of distilled water. To each test tube, added 4ml of anthrone reagent. Heated for eight minutes in a boiling water bath, to develop dark green color. Cooled rapidly and absorbance noted at 630nm.

2.5.3 Estimation of protein

Lowry's method

Standard bovine serum albumin Stock: Weighed accurately 50mg of bovine serum albumin and dissolved in NaOH and made up to 50 ml. Working standard: 20ml of stock solution diluted to 50 ml with NaOH.

Sample preparation: Weighed 500mg of the fresh plant sample and ground well with 5ml of Tris buffer. Centrifuged at 4°c for 10 min at 10,000 rpm. Supernatant was used for estimation.

Pipetted out 0.2 to 1.0 ml of working standards and 0.5ml of sample extract into a series of

test tubes. Made up the volume in each tube to 1ml with distilled water. Blank was set with 1ml of distilled water. Added 5ml of alkaline copper solution. Mixed well and allowed to stand for 10 min. Then added 0.5ml of folin-ciocalteau reagent. Mixed well and incubated at room temp in the dark for 30 min. Blue color was developed. Absorbance noted at 660nm.

2.5.4 Estimation of total free amino acids

Standard L-leucine Stock: Dissolved 50mg leucine in 50 ml of distilled water. Working standard-10ml of the stock diluted to 100ml with distilled water.

Sample preparation: Weighed 500mg of the fresh plant sample and ground well with 5 ml of 10% isopropyl alcohol. Centrifuged and saved the supernatant. Repeated the extraction and pooled the supernatants.

Pipetted out 0.2 to 1.0 ml of working standards and 0.5ml of sample extract into a series of test tubes. Blank was set with 0.1ml of 80% methanol. To the test tubes, added 1ml ninhydrin solution and made to 2ml with distilled water. Heated the tubes in a boiling water bath for 20 min. Cooled and added 5ml diluent solvent and mixed the contents. Blue color was developed. After 15 min, absorbance was noted at 570nm.

2.5.5 Estimation of phenol

Standard catechol: Stock-Dissolved 100mg catechol in 100ml distilled water. Working standard-5ml of stock diluted to 100ml with distilled water.

Sample preparation: Weighed 500mg of the fresh plant sample and ground with 5ml of 80% methanol. Centrifuged at 10,000 rpm for 20 min. Saved the supernatant. Re-extracted the residue with 80% methanol. Pooled the supernatants and evaporated to dryness. Added 5ml of distilled water to this residue.

Pipetted out 0.2 to 1.0 ml of working standards and 0.5ml of sample extract into a series of test tubes. Made up the volume in each tube to 3ml with distilled water. Blank was set with 3ml of distilled water. Added 0.5ml of folin-ciocalteau reagent.

After 3min, added 2ml of 20% Na_2CO_3 solution. Mixed well and heated the test tubes in a water bath for exactly 1 min to develop blue color. Cooled and absorbance measured at 650nm.

2.5.6 Estimation of chlorophyll

Weighed 500mg of fresh plant sample and ground well with 10 ml of 80% acetone. Centrifuged at 5000rpm for 5min and transferred the supernatant to 50ml volumetric flask. Repeated the extraction till the residue was colorless. Volume made up to 50 ml with

80% acetone in amber colored volumetric flask. Absorbance was noted at 645nm, 663nm and 652nm against the solvent (80% acetone) blank.

2.5.7 Estimation of moisture content

Weighed 10g of the fresh plant sample and cut into small pieces. The sample was put in paper bags and kept in oven at medium temperature (55-60°c). Weight of the sample was recorded daily until it attained constant weight. Percentage of moisture in the sample was calculated.

2.5.8 Estimation of total soxhlet extractables

The fresh pseudobulbil was cut into small pieces and dried in the oven at 60°c till it attained constant weight. It was then powdered and used for analysis. Two grams of powdered sample was taken for soxhlet extraction. The solvents used were ethyl acetate, petroleum ether, chloroform, methanol, hexane and acetone. With all the solvents it took four siphonings for the solvent to be colorless. At this point, extraction was stopped and the extract was then transferred to previously weighed beakers and kept aside till the solvent got evaporated completely. Weight of beaker and extract was then taken and crude extractables were calculated.

2.5.9 Thin layer chromatography of phenols

Sample preparation: Weighed 2g of the fresh plant sample and ground well with about 10ml of 80% methanol. Centrifuged. Repeated the extraction , supernatant obtained was pooled and concentrated to 10ml.

The extract obtained was spotted on silica gel coated glass plates using small capillary tubes. The spotted plates were kept in chromatographic chamber containing the solvent system. The solvent was allowed to run ¾ th plates. The plates are taken out of chamber and kept outside. It was then sprayed with spray reagent and kept in oven(110°c)for 30 min. The spots were blue in colour. Rf value was noted.

Solvent system - Chloroform: Acetic acid (10:1)

Spray reagent - Folin's reagent

2.5.10 Thin layer chromatography of free amino acids

Sample preparation: Weighed 1g of the fresh plant sample and ground well with 5ml of 10% isopropyl alcohol. Centrifuged. Repeated the extraction , supernatant obtained was pooled and concentrated to 10ml.

The extract obtained was spotted on silica gel coated glass plates using small capillary tubes. The spotted plates were kept in chromatographic chamber containing the solvent

system. The solvent was allowed to run ¾ th plates. The plates were taken out of chamber and kept outside. It was then sprayed with spray reagent and kept in oven (110°c)for 30 min. The spots were purple in colour. Rf value was noted.

Solvent system used- Butanol: Acetic acid: H_2O (4:1:1)

Spray reagent- 0.1% Ninhydrin

2.5.11 Test for alkaloids

Weighed 5g of the fresh plant sample and ground well with 10ml of 10% acetic acid in ethanol. Centrifuged. Volume made upto 10ml with 10% acetic acid. The supernatant was tested for alkaloids. To 2ml of the sample extract, 1 ml each of the following reagents were added. The reagents used were Dragendorff's, Mayer's and Wagner's. Response to each reagent was recorded.

2.5.12 Test for saponins

Weighed 1g of the plant sample and ground well with about 5ml of ethanol solution. The aqueous alcoholic plant extract obtained was shaken well in a test tube. Noted the formation of persistent foam for the presence of saponins.

2.5.13 Test for unknown sugars

Weighed 5g of the fresh plant sample and ground well with 80% methanol solution. Centrifuged. Repeated the extraction and supernatant made upto 25ml with 80% methanol. This extract was used to carry out Molish's test , Iodine test, Benedicts test, Barford's test, Bial's test and Selvinoff's test and response to each of these tests was recorded.

2.6 Anatomical studies

Fixation

The fresh pseudobulbil was cut into small thin pieces after removing the outer scales.

The material was then treated with FAA (Fomalin(10ml)-Acetic acid(5ml) –Ethyl alcohol 95% (50ml) and water (35ml) solution) for minimum two weeks.

Washing

Dehydrating solution	Absolute ethyl alcohol (ml)	Ethyl alcohol (95%) (ml)	TBA (ml)	Distilled water (ml)
50(%)	0	50	10	40
60(%)	0	50	15	35
70(%)	0	50	20	30

Dehydrating solution	Absolute ethyl alcohol (ml)	Ethyl alcohol (95%) (ml)	TBA (ml)	Distilled water (ml)
80(%)	0	50	30	20
90(%)	0	50	50	0
100(%)	25	0	75	0

Material was washed immediately with water, after decanting FAA solution

Dehydration series

After washing, the material was transferred to the following dehydrating series.

Transferred the plant material from fixative to 50% solution. After 4hrs poured off and replaced with 60% solution again after 4h, it was transferred to 70% solution. It was then kept overnight. In similar ways, next day it was transferred to 80%, 90%, 100%(over night) solutions. On the third day three changes of pure TBA was done and kept overnight.

Infiltration

To the material in pure TBA, equal quantity of paraffin oil was added. After 2 hrs, decanting a little of the TBA-paraffin oil mixture, the material was poured into hot solidified paraffin wax in bottle and kept in oven at the melting temperature of wax(58°c) so that the material is gradually transferred to oven temperature . As the paraffin slowly melted, the material slowly sunk into wax. After 2-3 hrs, complete mixture was decanted and pure paraffin wax (chips or melted) was added and kept overnight. Changes with pure paraffin wax was made for 2-3 days.

Embedding

Paper boats were prepared. Pure melted wax along with the specimen was poured into paper boats. The samples were placed in the boat in correct orientation. Solidified paper boats are floated in a tray containing water. Paper boats are cut into blocks containing the specimen.

Microtomy

Sections of the wax block were taken in a rotary microtome. The sections were taken at 10,12,14 and 20 micron thickness.

Fixing of section

Ribbons obtained from the microtome sections were fixed on cleaned glass slides with Haupt's adhesive. Kept it for 2 days.

Dewaxing

Dewaxed the fixed slides in three changes of pure xylene for 30min each.

Double staining

The fixed slides were then put in the following solutions for fixed time.

Xylene:alcohol(1:1)	: 15-30 min
Alcohol(100%)	: 15-30 min
Water	: 15 min
Safrranin	: 30 min
Water	: 15 min
Alcohol (50%)	: 5-10 min
Alcohol (70%)	: 5-10 min
Alcohol (90%)	: 3-5 min
Alcohol (100%)	: 1-3 min
Fast green (2 drops)	: a few seconds
Alcohol(100%)	: 15 min
Clove oil (2 drops)	: a few seconds
Xylene	: Immersed and took out

Mounting

The slides were mounted with DPX. Kept for a day.

2.7 Statistical analysis

The experimental data were subjected to statistical analysis, wherever there were sufficient values.

Chapter 3
RESULTS

Results of the study "Domestication studies on Jeevakom" (*Malaxis rheedii* Sw) are presented below:

3.1 ABOUT JEEVAKOM

Jeevakom is a herb belonging to the *Ashtavarga* group of drugs mentioned in Ayurveda. According to Susrutha, Jivaka is included in the *Jivanya gana*(vitalizing group of ten drugs) and *Vidaryadi gana*(another group of twenty drugs). Ashtavarga is a group of rare drugs listed by Bhavamisra. Eight tubers or condensed stems obtained from Orchidaceae and Liliaceae families are known as ashtavargas (Dey. 1998). They include *Riddhi, Vriddhi, Kakoli, Kshirakakoli, Meda, Mahameda, Jivaka* and *Rishabaka*. They are well known for their nutritive and tonic properties.

Kerala physicians generally consider astavarga drugs as unobtainable (*abhavadravya*) and they are either deleted or substituted with other permitted drugs. According to Goraksha, if meda, mahameda, jivaka, rishabaka, kakoli and kshirakakoli are not available, then in their places *vasti, vidari, varahikanda* respectively should be used (Mooss,1980). And hence according to old texts *Withania somnifera* and *Sida cordifolia* are the substitutes for Jivaka and rishabaka respectively. Contrary to popular belief, Jeevaka is available in the forests of Kerala, albeit in small quantities.

3.1.1 Uses of jeevakom

Astavarga drugs are well known for their nutritive and tonic properties and are ingredients of *chyavanaprash*, a popular ayurvedic formulation for vitality and strength. According to *Materia Medica* of Ayurveda, these drugs are reported to be cooling, exceedingly spermatopoetic and nourishing. They alleviate aggravated *pitta, daha* (burning syndrome) *asra* (vitiated blood) and *sosa* (consumption). They promote lactation and conception.

Warrier *et al.* (1995) have reported jeevakom to be sweetish, refrigerant, aphrodisiac, febrifuge and also tonic in properties and it is used for the treatment of haematemesis, fever, seminal weakness,

burning sensation, dipsia, emaciation, tuberculosis and general debility. Vaidya and Dhumal (2000) have reported *Malaxis rheedii* for its use for ulcer, healing of wounds, as antiseptic, in amoebic colitis, viper bite and dysentery by the local herb vendors of Mahabaleswar, Maharashtra.

Some of the Ayurvedic formulations containing astavarga/jeevakom are *Dhanwantharam kashayam, Dhanwantharam kuzhambu, Ashtavargam kashayam* etc. Even though generally these are considered unobtainable and deleted, some traditional vaidyas of Kerala still use jeevaka and most of the requirements come from Punjab during the month of December. As the drug is storable, it is kept for the whole year.

3.1.2 Botanical identity of jeevakom

Astavarga drugs listed by Bhavamisra have not yet been satisfactorily identified. In the classic texts of ayurveda, both jivaka and rishabaka are reported to occur in the Himalayan peaks (Dash and Kashyap,1980). Its *kanda* is described as that of garlic and leaves thin and fine. According to another text 'Jivaka has the shape of a *kurcaka*(brush) and rishabaka has the shape of a bulls horn. Botanical description of Jeevaka is given as *Microstylis wallichi* Lindl. and the plant is described as short stemmed, fibrous rooted herb, flowering stem short and swollen at the base, leaves sheathing, flower minute, pale-yellow, green tinged with purple especially near the centre, lip shield like, broadly ovate and tip notched.

As part of the study to ascertain the correct botanical identity of the drug, traditional vaidyas were contacted and it was learnt that the same drug was sold and used as jivaka and rishabaka. Vaidyas consider larger bulbils as jivaka and smaller ones as rishabaka. In the vernacular, jivak is known as *Jeevakom* and rishabaka as *Edavakom*. Vaidyas opined that *edavakom* grows by the side of *jeevakom*. What was sold as jeevakom and edavakom from Punjab was also the same plant differing only in size.

Jeevakom was gathered as a drug in certain pockets of Kerala. The Malayan tribe in central Kerala who gathered it from the forests of Peechi-Vazhani area sold it in the Thrissur market. Tribes of Malabar area collected it from Nilambur forests. In the south, there were reports of collection of jeevakom from the Agasthyamala region. At all these places, mother and daughter bulbils of the same plant were sold as jeevakom and edavakom. In the Kasargode district, the drug was gathered from the sacred groves and was known as *pachilaperumal* on account of the evergreen succulent pseudobulbils. Physicians of North Kerala also certified that there was only one plant for both the drugs.

From all available information it seems likely that what is sold as jeevakom and edavakom in Kerala is the same plant. Botanical identification was done at Kerala Forest Research Institute, Peechi. The latest botanical name of the plant jeevakom is *Seidenfia rheedii* (Sw.) (Szlach.). Synomyms are *Malaxis rheedii* (Sw.), *Microstylis versicolor* Lindl, *Malaxis versicolor* (Lindl), *Microstylis rheedii* sensu auct. non Lindl. (Nair,2000 and Sasidharan, 2004). Plant habit of jeevakom is presented in plate I and fig 1.

3.1.3 Revision of the genus *Seidenfia rheedii* from *Malaxis*

The genus *Seidenfia szlach* was separated from the highly polymorphic genus *Malaxis* Sw. by Szlachetko (1995). Etymology of *Seidenfia* is dedicated to Dr. Gunnar Seidenfaden, an eminent Danish orchidologist. The genus embraces seven species from Srilanka and Deccan and single species from the Seychelles and the species under study is revised as *Seidenfia rheedii* (Sw.)(Szlach) Basionym: *Malaxis rheedii* Sw. Description given to *Seidenfia rheedii* by Margonska and Szlachetko,(2001) is as follows.

Distribution. Srilanka, India(Gujarat, Kerala).Rare. Altitude upto 1860m. *Ecology*. Terrestrial; growing in shady places, on clay, in submontane or midlevel tropical wet evergreen forests, extending to subtropical mountain forests. Flowering July- September, fruiting September-November.

Pseudobulbils fusiform 5.0-256 cm long, 0.9-1.4 cm in diameter. Leaves 4-7, 4.4-18.0 cm long, 2.6-8.4 cm wide. Sessile. The lower one broadly ovate to ovate, broadly round at basal portion; the youngest the narrowest-ovate-lanceolate, plicate, acute to acuminate, margins softly wavy, 5-7 veined, with base continuing into purple-tinged sheaths 2-5 cm long. Inflorescence 60-100 flowered, racemose, peduncle 6.5-10.0 cm long; flower-bearing portion 6-18 cm long, length of rachis between peduncle and lowermost flower increasing with age. Floral bracts 4-8 mm long, 0.9-1.3 mm wide, lanceolate to oblong-lanceolate acuminate, deflexed, 1-veined. Ovary with pedicel about 3mm long. Flowers ca 6.5 mm in diameter, greenish yellow, yellow to orange-yellow or purple to maroon, with sweet pungent smell. Dorsal sepal 3.5-4.0 mm long, 0.8-1.3 mm wide, oblong, linear-lanceolate, obtuse, 3-veined. Petals 3.4-3.7 mm long, 0.6-0.9 mm wide, lanceolate, truncate, sometimes retuse, 1-veined. Lateral sepals 2.4-3.8 mm long, 1.2-1.5 mm wide, obliquely oblong-lanceolate, obtuse, 3-nerved, deflexed. Lip 2.0-

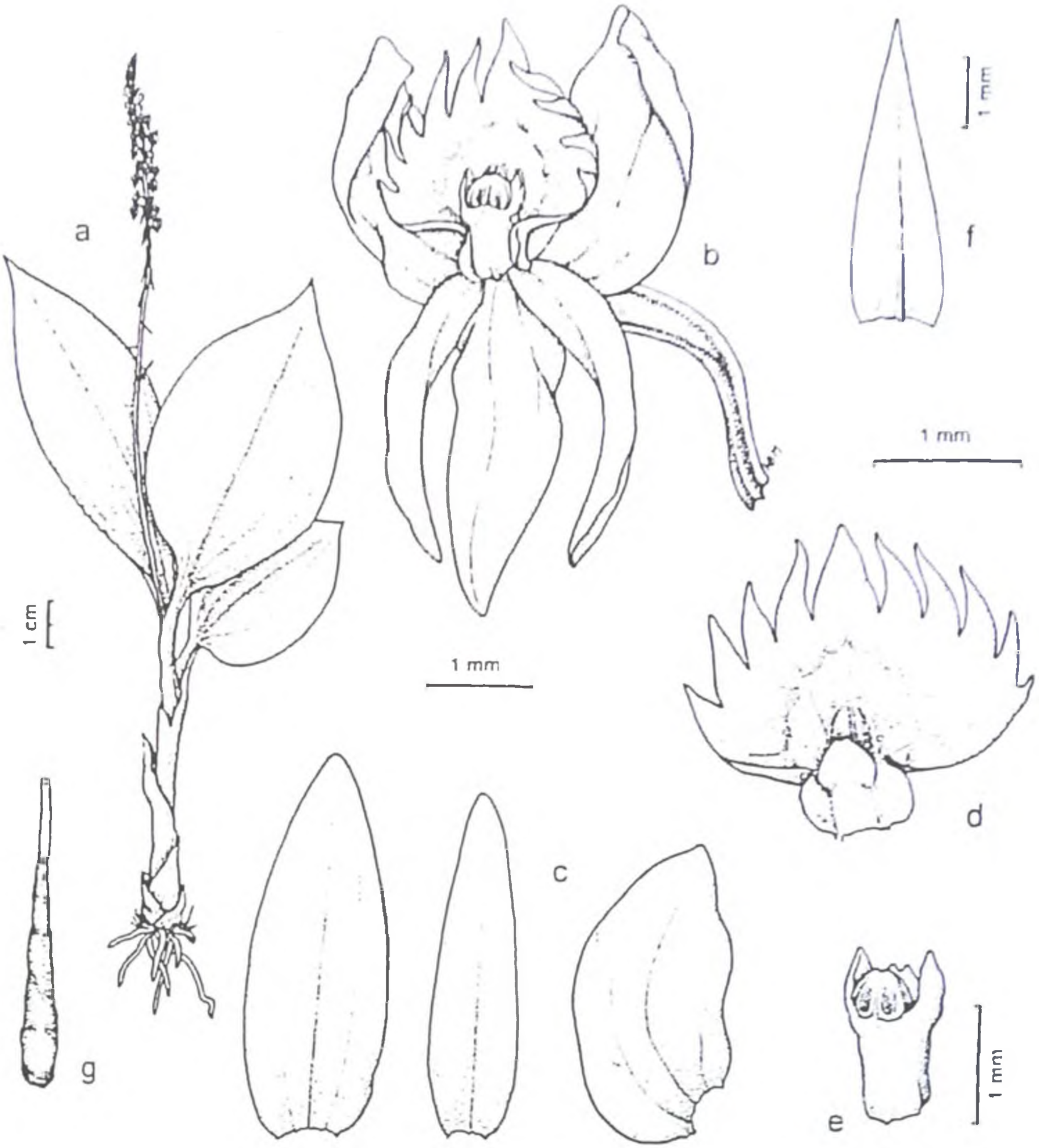


Fig. 1. *Seidenfia rheedii* (Sw.) Szlach. a - plant, b - flower, c- lateral sepal, petal, dorsal sepal, d - lip, e-gynostemium, f-floral bract. g - pseudobulb (drawn from lectotype - Champion s.n. - K-L).

2.3 mm long, 2-2.8 mm wide, broadly cuneate to narrowly flabelliform, pectinate, 9-13 toothed, teeth subequal in length, oblong triangular, commonly acute to obtuse at apex. Gynostemium 1.2-1.8 mm long, 0.8-1 mm wide at tip. Anther 0.34-0.45 mm long, 0.5-0.6 mm wide. Pollinia 0.30-0.35 mm long, 0.1-0.14 mm broad. Capsule 6-10 mm long, 3-5 mm in diameter, pyriform or oblong-obovate.

3.1.4 Jeevakom (*Seidenfia rheedii*) in Kerala forests

Seidenfia rheedii was reported to be present in the forests of Kerala by various workers. Unnikrishnan (1993) recorded its presence in the sacred groves of Malabar and Miniraj and Nybe (1999) recorded it in the Peechi forests. In the Peechi forests, because of several factors-anthropogenic and otherwise, the natural population of this plant was getting depleted day by day and it was reported to be in the 'rare' category (Miniraj and Nybe, 1999). In the manual of non-wood forests produce plants of Kerala, *Seidenfia rheedii* was recorded from the forests of Kannur, Palakkad, Idukky, Kottayam and Thiruvananthapuram districts (Nair,2000). In the biodiversity documentation of Kerala, Sasidharan (2004) reported *Seidenfia rheedii* from Palakkad, Kottayam, Kozhikode, Kollam, Wynad, Kasargode, Kannur, Malapurram, Thiruvananthapuram, Thrissur and Idukky districts.

Published flora of the Wild Life Sanctuaries, National Parks and Periyar Tiger Reserve were scanned to locate the natural habitats of the species and *Seidenfia rheedii* was reported in the flora of Parambikulam, Peechi-Vazhani, Sendurumey and Wynad Wild Life Sanctuaries, Silent Valley National Park and Periyar Tiger Reserve (Manilal, 1988; Mohanan, and Sivadasan, . 2002; Sasidharan 1997; Sasidharan 2002; Subramanian. 1995). In spite of the wide distribution in botanical literature, ayurvedic physicians, ironically still consider it as *abhavadravya* or unobtainable drug.

3.2 FOREST EXPLORATIONS

Based on botanic literature gathered, probable habitats of jeevakom in the Western ghats were explored. Results of the forest explorations are given below:

3.2.1 Parambikulam Wild Life Sanctuary, Palakkad

Parambikulam Wild Life Sanctuary is part of the Anamalai hills. It lies between 76°35' and 76°51'E longitudes and between 10°20' and 10°32'N latitude in the Palakkad revenue district and has an area of 274 km². The altitude ranges from 440m to 1438m. The area

gets both the southwest and northeast monsoons, southwest being the most active. Maximum temperature fluctuates between 24°c and 35°c and minimum temperature between 18°c and 23°c. Natural vegetation of the sanctuary include west coast tropical evergreen forests, west coast semi evergreen forests, southern moist mixed deciduous forests and southern dry mixed deciduous forests.

The sanctuary is rich in wild fauna, abundant being elephants, bison, leopards, bear, boars, sambar, tiger, Nilgiri langur and other animals. Even though part of Kerala (Muthalamada panchayat at Palakkad) the sanctuary is accessible via. Pollachi-Sethumada (inTamil Nadu)-Indira Gandhi Wild Life Sanctuary of Tamil Nadu. The first forest trip was during October 2003. We took permission from the Wild Life Warden's office at Aanappady (entry point to the sanctuary). In spite of the chief wild life warden's letter from Thiruvananthapuram, the wild life warden was sceptic about our research project and his attitude was not at all welcoming. We felt that genuine researcher's deserve a better deal. Finally what convinced him was an official letter from Sri. O.P. Kaler, IFS, Registrar of Kerala Agricultural University introducing the Principal Investigator and vouching her mission.

The exploration team consisted of Ms Sincy.A, SRF in the project, Sri Kuttikrishnan , driver, Kerala Agricultural University, the author and a free lance researcher, Dr. Dileep who was then working at Parambikulam PHC. We were instructed to engage an experienced guide for trekking. Sri. Gopi, an experienced tribal (kadar community) of the Sungam Tribal colony promised to accompany us for the forest trip.

Kadar tribe at Sungam colony and the Eco Development Committee (EDC) guides were not familiar with the plant when a specimen plant of jeevakom and its photographs were shown to them. However, Sri. Gopi was sure of having seen the plant in *Karianchola* Medicinal Plant Conservation Area (MPCA) and the next day we left for Karianchola along with Sri. Murugan (a young inexperienced EDC guide). His ignorance of jungle craft proved costly, for he misled us straight into a herd of wild elephants. We had to run for our lives. After that we decided not to go into the forest till we had the service of an experienced guide.

The very next day we were glad to have the services of Gopi, the most experienced guide in Parambikulam.We trekked along a different route through the ever green/ semiever green patch and reached a thickly vegetated area dominated by huge *cholapoovam*, *churuli*

and *ebony* trees. We were astonished to see Gopi's knowledge of medicinal plants, which he showed us all along the way, describing their uses.The track on both sides were full of medicinal plants, dominant being *Curculigo orchioides, Desmodium gangeticum, Pseudarthria viscida, Clerodendron enermis, Naravelia zeylanica, Adenia hondala, Sida Sp., Chasalia curviflora, Nervilia aragoana* and *Rubia cordifolia.* We were thrilled to hear the noisy and windy wing beats of a large flock of Great Indian horn bills (*Buceros bicornis homrai*) feeding leisurely on fruits of 'cholapoovam'. Gopi, our tracker said it is the most favoured fruit of our state bird.

We continued our search for jeevakom and at last Gopi found it on a '*narivenga*'(the tree on which '*nari*'(tiger) sharpens its claws). A clump of jeevakom-like orchids (150-250) with green hardy pseudobulbils covered with brown scale, with long elongated leaves and with a central long green inflorescence rachis. Flowers were almost shed. Our target plant was a terrestrial orchid with ovate leaves and green fleshy pseudobulbils. But this was an epiphyte. Gopi confidently said this plant could be seen on rocks too. That was a confusing statement. Anyway, photographs and specimens of tree jeevakom were taken for identification.

Later, the botanical features proved it to be another epiphytic orchid. Now it became clear that there are many orchids confusingly similar to jeevakom which could be mistaken for that plant. Back at the field camp site we discussed about the exploration and about the plant with Sri. Nelson, a knowledgeable man and wild life Assistant of Parambikulam Sanctuary and he suggested to explore Karianchola peak, *Pandaravara* and *Karimala* peaks in the *Orukomban* range of the sanctuary.

As the forests were lush green that time with incessant rains and irritating leeches, and as it was totally unsafe and difficult to escape from the wild fauna including elephants, wild boars, bison, bear and leopard we decided to postpone the exploration to December.

3.2.2 Information from tribes of Parambikulam

There are all together 13 tribal settlements in the sanctuary consisting of *kadars, muthuvas, malasars* and *malamalasars.* We contacted the kadar colony at *Kuriarkutty* and *Sungam*, Malasar colony at *Thekkady* and *Kachithode*, malamalasar colony at *Anjamcolony* and *Muppathekkar* and the only Muthuva colcony at *Pooppara*. Next forest trip was in December 2003. Chieftans

and elder people of these tribal groups were contacted to get details about jeevakom and related species.

Traditional knowledge on herbs and their healing properties were more with the malamalasars in the Earth Dam colony. Most of the ethno medicines were for the snake bites, stomach problems and for jaundice like problems. On showing the jeevakom specimen they even pinpointed the areas where it is present. Younger people were so enthusiastic that they gathered several jeevakom like plants next day. But none was true jeevakom.

We gave up all hopes of getting information about the plant from the tribes at Pooppara, Earth Dam, Anjamcolony and left for Kuriarkutty kadar colony on 18.12.2003. It was in the Orukomban range. Mr. Sabi Varghese, Range Officer extended all help to us and arranged Sri. Chinnathambi, a kadar tribal to accompany us to the forest. Our vehicle was parked at the Salim Ali Centre at Kuriarkutty and we began trekking from there.

The Kuriarkutty river was over flowing and we had to take deviations as it was difficult to cross the river. It was a hard trek, but we proceeded with determination. We were frightened to hear bullet shots from near by. Poachers! Country cousins had reached there and they were far more dangerous than wild animals. Since there were no armed forest officials with us, we had to stop midway and return to the camp.

Back at the camp we received another shocking news. Some gossip had gone to forest officials that a KAU team had come to smuggle medicinal plants from the sanctuary. On enquiry it was learnt that it was the aftermath of a news item which came in a TV channel about smuggling valuable flora & fauna from our forests in the name of research. It was a difficult task trying to convince these officials that we were not smugglers. These things are to be expected and we took the incident in our stride.

Next day ie 19.12.2003 Sabi Varghese, the Range Officer along with two armed foresters and Sri. Murali, watchman from kadar colony at Kuriarkutty joined us for exploration. We were told by Sri. Kesavan an elder kadar tribal that this plant is available near small streams along the Kuriarkutty river. We directly went there after trekking four kilometers through the evergreens. All the probable rocky areas around were searched but the species could not be found. Again on a *Narivenga* tree we could locate the jeevakom like epiphyte in clusters of 250-300. The rocks in the Kuriarkutty river was covered with *Kalloorvanchi* (*Rotula aquatica*) a much sought after herb used for urinary stones/kidney stones. There were lot of wild

cinnamon, *Entada scandens*, *Naravelia zylanica* and *Elephantopus scaber* on the way.

The only positive aspect of that days trek was that the forest officials who accompanied us were convinced about the genuineness of our mission and they even had some wild ideas of tracing the plant jeevakom and exploiting it for money, once they came to know that it is a rejuvenating drug.

And that was the final day of the trek. Somehow we had to locate the plant. We chose another track through the evergreens. Destination was *Pezhathode*. The forest was dominated by *Vellapayin* and *Canarium strictum trees* from which the black dammer (*kunthirikkam*) is extracted and *Nilagiranthus ciliatus* (*karinkurunji*).

Six kilometers were over when we reached a rocky patch. There were lot of Nilgiri langurs and gaint squirrels and a lot of birds and butterflies. The forest officials and Murali the watchman were way in front of myself and Sincy, each one competing to locate the jeevakom first. However, much I tried to describe to them about the probable habitats where it could be seen, each of them took their on decisions and directions and were frantically searching for the plant. Only Sincy and Dr. Dileep listened to my words and three of us were looking on wet rocks near the stream.

Suddenly to my delight I located a few tiny bulbils of jeevakom near the stream amidst stones. These are probably washed down from the rocky patch above, I suggested. We decided to search up the rocks. The rocks were almost dry and covered with thick litter. We managed to get some twigs and were removing the litter carefully to see the rocks and there it was!.

On a flat rocky patch up the stream the dried up pseudobulbils of jeevakom were still intact under the litter cover. The plant was in plenty, but all in the dormant stage and covered with scale leaves, but the inner bulbil was still green and succulent. There were thousands of jeevakom plants all over the rock. The forest staff wondered how we could locate these tiny plants.

We were busy preparing our research tools to study about the natural population. Soon, a 1 m^2 wooden frame was ready and in the most ingenious way and we recorded the density of population, counting the individuals per m^2. All other possible morphological observations of jeevakom were taken. The plant community analysis was carried out and the associated plants were identified with the help of tribal and forest staff. Voucher specimens of unknown plants were taken to prepare herbarium for identification. Physiographic features of the

habitat were recorded. Details of natural habitat characteristics would be presented in the next chapter.

3.2.3 Peechi-Vazhani Wild Life Sanctuary

Peechi-Vazhani Wild Life Sanctuary is situated in Thrissur District. The exploration was carried out in Peechi hills which is an extension of the Nelliampathy hills along the Southern tip of Palakkad gap in the Western ghats. It comes under Peechi range. The forest consists of evergreens, semi evergreens and moist deciduous forests. The sanctuary is easily accessible via Pattikkad-Vilangannur-Vellakkarithadam-Thamaravellachal. The wild life warden's office is situated at Vilanganur from where we got permission and instructions to enter the sanctuary.

3.2.4 Tribal knowledge about jeevakom

Malayans are the hill tribes who inhabit the Peechi forests. The *Thamaravellachal* settlement was selected for the study. The tribes earn their livelihood by the collection of Minor Forest Produce (MFP) from the forests. MFP included honey, black dammer and various medicinal plants. Jeevakom gatherers were identified and with their help exploration tracts were finalized. A base camp in the forest was fixed at *Karadippara*, 5 km away from the settlement and forest trips were carried out from there.

About 40 medicinal plants are extracted by the tribes Malayan of Peechi hills on a regular basis. Jeevakom is one among them. Out of twenty families who go for MFP collection, only one family collects jeevakom and that was Sri. Ayyappan and his family who regularly collects jeevakom from the interior and upper reaches of the forest. Neither Sri. Ayyappan nor other tribes were aware of the medicinal properties of the species as it did not come in the limited ethno-medicines practiced by them.

3.2.5 Exploration

As Sri. Ayyappan who regularly collects jeevakom from the forests was thorough about its wild habitats, it was easy to fix the exploration tract. The first forest trip was fixed for January 2004. The vehicle left us at Thamaravellachal and we left for Karadippara, our base camp in the forest with the materials for camp. Four kilometer trek through Moist Decidous Forest (MDF) took us to Karadippara, end of an MDF patch, an extensive rock formation near a stream from where a Semi Evergreen (SEG) patch starts. Tent was ready in the most indigenous ways. It started drizzling soon. *Ayyappettan* managed to get a few logs for the fire and for cooking. At night we crept into our sleeping bags.

Outside silence and occasional call notes of wild fouls and owls and some unknown creatures frightened us a little bit. Around midnight a torch light came towards us. The powerful light was approaching. I looked around. All my team members were sleeping. I tried to wake up Ayyappettan. He went outside, came back after a while with a smile. "No problem". "They are poachers". We spent a sleepless night afterwards.

Next day after a simple breakfast we started trekking by 7.00 am. It was raining heavily which made our trek difficult. Then came a stream which had to be crossed. It was a gushing torrent and risky even for the male members. We were forced to climb a steep mountain since the stream was unaffordable. The climb was arduous, as the path was uncleared and full of leeches. After about 7 km we reached an extensive rock formation. Ayyappettan went up in search of the plant.

The rock was full of moss, grasses and other plants. Soon came the "pooi"-call of Ayyappettan from high up the rocks. Jeevakom was there in plenty.

At last the mission was successful. It was a precarious climb for us as the rock was slimy & slippery. We tried to climb up with both hands & bare foot. The rock was formed into different steps and some how we reached the first step. There was reasonable growth of jeevakom in that step and also an aerial view of the jeevakom population of the entire area. All the larger plants were in bloom and the tiny plants in the vegetative phase. Again the 1 m^2 bamboo frame was ready, we counted the population, collected voucher specimens and recorded other parameters of phyto sociological and community analysis. The plants were abundant over the mossy and moist open rocks and none was present near the streams or in the underground vegetation in the forest. The habitats at Parambikulam and Peechi were almost similar in features. Details of the habitat analysis are presented in the next chapter.

3.2.6 The Silent Valley National Park

The Silent Valley National Park is at the south-western corner of Nilgiris (Lat. 11° 05'N and long 76° 26'E). The plateau slopes towards the south and is practically ringed in by hills. The whole plateau vegetation is shielded from extremes of climate and has its own special microclimate. *Kunthipuzha* originates from the eastern slopes of Silent Valley and takes a relatively gentle course along eight kilometers in the Silent Valley. The forests here show all the known characteristics of tropical rainforests and have a high level of species diversity in flora and fauna and it is one of the

very rich areas for plants of established economic significance, as well as of other special interest. Forests have several unique, endemic, rare and endangered species. On the upper reaches, there are grassland-shola forests and lower reaches evergreen forests. There are no human settlements in Silent Valley, a substantial stretch of forest still remains undisturbed here.

3.2.7 Exploration

The forest trip was planned on May 21-27th 2004. South-west Monsoon had already arrived. The Assisstant Wild Life Warden of the park, Mr. Vimal was an old student of mine and he promised to make all arrangements for our trip. After informing the wild life warden at Mannarkkad we left for Mukkali, the head quarters of Silent Valley National Park. Mr. Vimal was there to extend all possible help and had arranged to accommodate us at *Sairandhri* inside the park, in the Inspection Bunglaw (IB) of the forest department. We were at Sairandhri by around 3 pm.

Appuvettan, the watchman cum cook was there to receive us.*Maari* , a soft spoken young tribal was arranged as the tracker for the next day. We finalized the routes based on the information given in the "Flora of Silent Valley" written by Prof.Manilal. It was rainy, misty and windy.

Next day morning ie. on 22.5.04, we got ready for the trip. As it was raining, a powerful attack of leeches was anticipated and the forest staff spared their anti-leech/ leech-proof socks and trekking shoes and some tobacco-salt mixes to ward off leeches. Maari our tracker friend was so silent, nothing seemed to worry him. Some how I was recollecting the face of an elder tribal (affectionately called *Lachiappan*) who was our regular guide during the nature camps at Silent Valley, which I attended 15 years back. It was a pleasant surprise for me when the forest staff informed me that Maari was the son of *Lachiappan*.

With the paraphernalia for the study, we started trekking towards *Pulippara*, one of the probable natural habitats of jeevakom. Leeches were active from the starting point itself, their members were so large that finally we had to ignore them and decide to donate a few ml of our blood.

The vegetation was so thick and closed and the rains made it a typical rainforest experience and feel. *Kunthippuzha* was so magnificent, as we crossed over it and then we entered another patch of thick evergreen forests. As the canopy was closed there was not much undergrowth. We took the beaten trek path and then entered the grassland zone.

At last some sunlight was there which dried our clothes. The sun became hotter and hotter we felt tired & exhausted. The overgrown grass became thicker and difficult to walk through. Again there were clouds. In a rainforest, the climate changes within seconds. "We have reached Pulippara", Maari told. But where is the 'paara'-? I asked. There were only scattered rocks and that too covered with overgrown grasses. All of us started searching frantically.

'Are there any wet rocks here'? I asked. There was some signs of water dripping in an area, fully covered by grasses.Again I was stressing the point that this is not the typical jeevakom habitat, same time, searching below the grass cover. Soon I noticed a deep maroon inflorescence amidst the dry grass. I went near to have a closer look.

Yes! it is something like jeevakom. With three ovate leaves and an inflorescence. But the bulbil was not clear. We searched for similar plants around and could locate three more but none was in flower. Finally we decided to remove the grass around and the plant was exposed. The pseudobulbil was not prominent and succulent as that of Parambikulam or Peechi jeevakom. The flowers were deep maroon, including the inflorescence rachis. The habitat too was alltogether different. But there was closer resemblance with jeevakom. It could be another species. I took a sketch of the plant. But that was not sufficient for botanical identification.

Finally, we decided to pluck the plant in flower as a voucher specimen. Maari told me that similar plants are there in other areas in the Silent Valley. It was not our intention to locate all the sites. Soon it darkened outside, clouds were running in the stormy winds. The whole forest turned "forbidding". We could not take even a single step against the storming winds. We were on the highest point of Pulippara. Sudden down pour came. Thunderstorm broke out. Raincoats, hats, gherkins could not help. Nothing was visible. I was a little bit scared. Maari was cool as cucumber. That fully wet journey back was not at all easy. All the forest staff heaved a sign of relief when they spotted us. We too were exhausted.

For the identification of the specimen we again scanned the flora of Silent Valley where the description-"basally swollen herbs, leaves 3-5, inflorescence longer than leaves, racemose, flowers maroon colored, lip purple, rounded, reniform, pectinate except for a small portion in the middle"- given by C. Sathish Kumar exactly tallied with our specimen. Even

though there was deviation from the Peechi & Parambikulam specimens with respect to the color of inflorescence, flower and size of the plant, the plant was identified as *Seidenfia rheedii*.

Later, I took the sketch and description to Dr.C. Sathish Kumar, scientist at Tropical Botanic Garden and Research Institute, Palode, Trivandrum and clarified my doubt regarding the botanical identity of the species. He took out his own field notes of Silent Valley flora done way back for his PhD work, which again contained exactly similar description of jeevakom. Most astonishing fact was that he also had described the species from "Pulippara" which ascertained each others sincerity in describing the plant as we saw it and not to go after already reported literature which according to him many researchers practice.

3.2.8 Wynad Wild Life Sanctuary

Situated in the Wynad Plateau, Wynad Sanctuary lies at the junction of the Western ghats, Niligiri hills and the Deccan Plateau, bordering Nagerhola and Bandhipur in Karnataka and and Mudumalai in Tamil Nadu. Total geographical area is 344 km^2. Altitude ranges from 640m to 890m with a mean elevation of 650m. The sanctuary is characterised by gently undulating terrain dotted with 'vayals' or marshes and is drained by large number of perennial streams which flow east into the Kabani river. Vegetation includes mostly the moist deciduous forests except along the western edges and a few other pockets where evergreen forests occur. Much of the sanctuary is highly degraded and/ or heavily disturbed.

3.2.9 Exploration

The forest trip was planned during September 2004, the peak flowering period of jeevakom. Sri. P.K. Kesavan,IFS, Conservator of Kannur was kind enough to do all the arrangements for the trip. Permission was granted by Sri Jaya Prasad, IFS, DFO Manantody and Sri Thulasidas, Range Officer, Periya made the final stage formalities to explore the Medicinal Plant Coservation Area(MPCA) of Periya where the plant was reported to be present. Sri. Joseph and Sri. Gopalakrishnan, forest guards joined our team and Chandu, a Kurichiya tribal served as our guide.

We had a full body coating of neem oil and tobacco powder to ward off leeches, which were abundant on the way. Vehicle was parked inside the MPCA gate. We trekked along the bank of *Chandanathode* and then climbed up. It was an evergreen patch. We searched all rocky patches, the probable

habitats of jeevakom but could not locate the species. The next day we enquired about the plant with the tribes nearby, but could not get any specific information other than that similar looking plants are available in the forests. We proceeded to *Theethunda* a wet rocky area inside the MPCA where jeevakom could be located. The habitat was ideal for jeevakom, we searched all the nook and corners of that extensive rocky area. Lot of grasses, aroids and zingiberaceous plants were present, the common associated plants in other jeevakom habitats. A close relative of jeevakom plant was collected and sent for identification. Later it proved to be another species.

3.2.10 Sendurney Wild Life Sanctuary

This sanctuary is famous for the *Chenkurunji* tree, a tree characterised by its bright colored timber. The Sanctuary established in 1986 is situated in Kollam District and covers an area of 114 km^2. Chenkotta borders this sanctuary on the Tamil Nadu side. From Kollam, we took the meterguage train to Chenkotta and got down at Thenmala and then proceeded to forest IB at Thenmala. The DFO and Range Officer Sri. Vijayan had made all arrangements for our trip. Next day, two forest watchmen and a hired vehicle was arranged for us to proceed to *Rosemala* forests, where we stayed in a dilapidated forest building inside the forest with no water and electricity. Two forest guides from Rosemala colony came as trackers and they made our stay comfortable to the extend possible by bringing essential water from the far away stream and cooking food.

Very next day we started our search in the Rosemala forests where jeevakom was reported to be present. The forest was evergreen with abundant growth of *Piper longum* & *Rauvolfia Serpentina as the* undergrowth of *Chenkurinji* and *Porikambakom* trees. The highly demanded *Aarogyapacha* (*Trychopus zeylanicus* var *travancorensis)* was present in this forest. Exploration began in May 2005 by which time leeches were active, but we had all precautions and none could attack us forcefully. We trekked along the side of a stream, searching all rocks and came back to camp site without any information. Next day we proceeded to *Tharppakulam*, another extensive rocky patch and searched the entire area. Neither jeevakom nor its relatives could be located. As the Rosemala forests is too vast an area and the guides and forest watchmen were totally ignorant about the plant, we wound up the exploration. The specimen plants were given to all the knowledgeable persons there, but no valuable information was received about the plant or its occurrence in the forests.

3.2.11 Summary of forest explorations

The results of forest explorations are summarized below in Table 1.

Table1. Distribution of *Seidenfia rheedii* in the forests of Kerala.

Sl. No.	Name of sanctuary	Altitude	Presence	Habitat characteristics	Plant density/m^2	Status
1	Parambikulam Wild Life Sanctuary, Palakkad	800-1000m above MSL	Present	Extensive wet rocks in the openings of evergreen forests	272	Sufficient population
2	Peechi-Vazhani Wild LifeSanctuary, Thrissur	800m above MSL	Present	Extensive wet rocks in the openings of evergreen forests	200	Sufficient population
3	Silent Valley National Park, Palakkad	1100m above MSL	Present	Scattered wet rocks in grass lands	5	Rare
4	Sendurney Wild Life Sanctuary, Kollam	800m above MSL	Not located	-	-	-
5	Wynad Wild Life Sanctuary,Wynad	600-800m above MSL	Not located	-	-	-

The fact that jeevakom could not be located in Wynad and Sendurney sanctuaries despite positive reports in botanic literatures, leads to point that the population of the plant , if at all available may be scanty.

Contrary to the popular belief of an *abhavadravya* (unobtainable) this study indicated the availability of jeevakom in Kerala forests albeit in small quantities. Jeevakom habitats of the Western Ghats in Kerala are presented in plate 2.

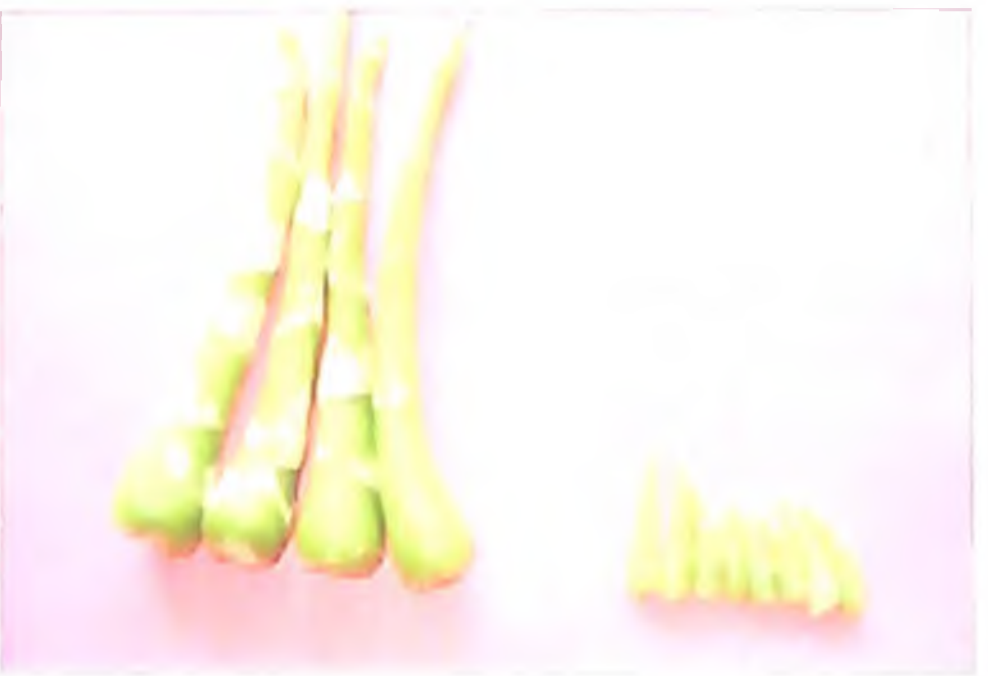
Evergreen forests

Grass lands

Rocky areas with water

Jeevakom Plant

Jeevakom and Udavakom with the outer scale intact



Jeevakom and Udavakom without outer scale

Plate I. Jeevakom- Habit and Habitat

Jeevakom in Parambikulam forests-dormant stage

Jeevakom in Peechi forests-vegetative stage

Kalluvazha - a common association

Dripping rock ecosystem

Jeevakom in Silent Vallley forests-flowering stage

Jeevakom from Silent Valley

Jeevakom from Peechi

Plate 2. Jeevakom in the forests of Kerala

3.3 NATURAL HABITAT ANALYSIS

Based on the results of forest explorations, detailed natural habitat analysis of *Seidenfia rheedii* was carried out in the more accessible Peechi forests, where the specimen population was highest among the areas explored.

3.3.1 Habitat characteristics

Seidenfia rheedii is a terrestrial orchid, which was found to grow on specialized nitches. In all the forests the plant was found to grow on extensive spread out rock formations (*Virieha paara*) in the openings of evergreen or semi evergreen forests. The shade level could be approximately 50 per cent. The only exception was Silent Valley forests where the species was found growing in open grass land over scattered wet rocks which received 100 per cent sunlight. But here, the plants were well protected by the overgrown growth of grasses.

In Peechi forests, at all jeevakom habitats, the species colonized on the decomposed organic matter on wet rocks amidst moss and grass. Though inside evergreen forests, the plant was abundant in the openings which received atleast 50% sunlight. The fibrous roots of the plant cling to the organic matter and moss growth present on the rocks during the active growth phase making the plant grow upwards. The rocks were just wet and not with flowing water. At all locations the species behaved as a typical lithophyte and the natural habitat could be described as **dripping rock ecosystem**. Habitat characteristics of jeevakom are presented in Plate 2.

3.3.2 Plant associations

At all natural habitats studied, the associated plants were bryophytes, grasses, aroids and *Costus* species. In Peechi forests, at all the locations *Ensete superbum* (*Kalluvaazha*) was a dominant species associated with jeevakom.

3.3.3 Plant habitat and phenology

Seidenfia rheedii is a short-stemmed fibrous rooted lithophytic herb. Stem is swollen at the base forming a conical pseudubulbil which is the part used in medicine. Leaves are sheathing, 5-7 in number, covering the entire pseudubulbil. Leaves and the sheath are mostly green, sometimes with a suggestion of brown. Regeneration is through vegetative means, through the side bulbils, which sprout from the mother bulbils. Morphological features of the species in the wild habitat are given in table 2.

The pseudobulbils, which will be still green and succulent covered with the scale

leaves, remained dormant during summer months. With the receipt of summer rains, they bulged, became active and with the onset of south-west monsoon, they began to sprout. Four or five daughter bulbils emerged from a mother plant either from the basal region or from the nodal regions. As soon as the rocks became wet, moss growth began. The emerging roots of sprouts striked the organic matter and each sprout developed into an individual plant. With the decomposition of organic matter and dripping of water, the plant grew fast and the mother bulbil gradually degenerated. Plant attained maximum vegetative growth by July. By August they entered into reproductive phase putting forth inflorescence. It is a long raceme, with light brown rachis bearing numerous tiny flowers. Flower buds are green in color and they are yellow when open. Peak flowering was observed during September. All large plants invariably had flowers while tiny ones remained in the vegetative phase itself. Fruits were also produced but only a few in number. By the month of January-February, as the northeast monsoon receded, the organic matter and the moss growth got dried up and finally the rocks. The plant started senescence with leaves turning yellow and finally drying up. The leaf sheath covering the pseudobulbil also dried up and the bulbil go to dormant phase to tide over the summer season. Under undisturbed conditions, the dried up plants remain on the rock itself. Another way of natural protection to the pseudobulbils is the thick litter cover and at certain points the plants may not be even visible outside. But in the event of an unseasonal torrential rain, the tiny side bulbils may get dispersed, washed off and get scattered. During the next season they regenerate from the new spot. Growth and development of jeevakom is presented in plate 3.

Table 2. Morphological features of *Seidenfia rheedii* in the wild habitat

Sl.No	Character	Mean Value*
1	Plant height (cm)	12.55
2	Number of leaves/plant	5.26
3	Length of leaf (cm)	10.48
4	Leaf breadth (cm)	3.86

table 2 contd.....

Sl.No	Character	Mean Value*
5	Leaf area (cm)	40.45
6	Diameter of pseudobulbil at the base (cm)	1.12
7	Diameter of pseudobulbil at the top (cm)	0.924
8	Number of roots/plant	22.36
9	Number of side bulbils per plant	6.0
10	Dry weight of pseudobulbils (g)	5.10

- The data subjected to statistical analysis are presented in table 5.

The native tribes (Malayans) gathered the bulbils of this drug plant during December-March. They pick only large bulbils and leave the tiny ones for next season's regeneration.

3.4 DOMESTICATION EXPERIMENTS

This experiment was carried out in the experimental fields attached to College of Horticulture, Vellanikkara during 2004-2006.

3.4.1 Domestication trial -1

Propagules (pseudobulbils) of jeevakom were brought from Peechi forests and they were used for the experiment. Seed material was collected in the last week of May, at the dormancy breaking stage. The trial was carried out as a pot culture experiment. There were two treatments.

T1-Fully open condition

T2-50% shade (provided by shade net house)

Potting mixture consisted of sand, soil, well rotten and powdered farmyard manure and vermicompost in equal proportions. Pots were filled with the potting mixture and pseudobulbils weighing approximately five grams were shallowly planted vertically at a depth of one centimetre. Pots were mulched heavily with green leaves and watered daily. There were 50 pots under each treatment. Periodic observations were recorded throughout the growth and development of the plant. Response of the species to domestication as indicated by various parameters is presented in table 3.

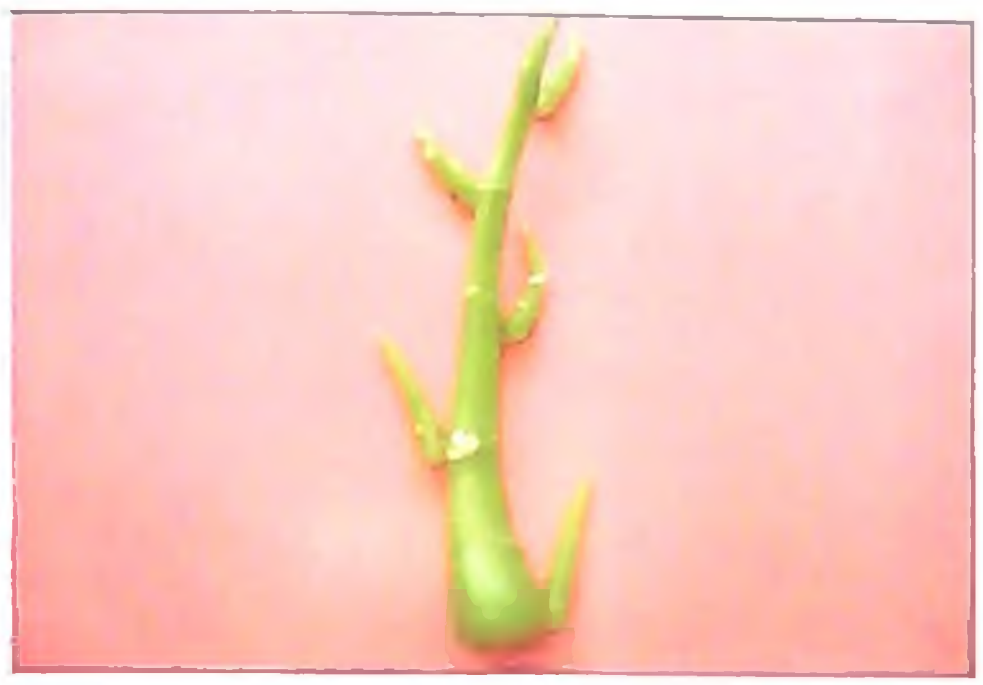
Table 3. Performance of *Seidenfia rheedii* in the domestic environment

Sl No.	Character	Performance (mean value)	
		50% shade	open
1	Days taken for germination	18.37	28.48
2	Germination percentage (%)	86.88	47.05
3	Plant height (cm)	13.26*	8.136*
4	Length of leaf (cm)	8.66*	7.044*
5	Breadth of leaf (cm)	3.96*	2.758*
6	Leaf area (cm)	34.29*	19.36*
7	Percentage of plants which produced side bulbils	49.05	40.62
8	No of side bulbils per plant	1.035*	0.710*
9	Days taken for flowering	50	67
10	Percentage of flowering	5.66	3.1
11	Diameter of pseudobulbil at the base (cm)	4.895*	3.508*
12	Diameter of pseudobulbil at the top (cm)	2.22*	1.744*

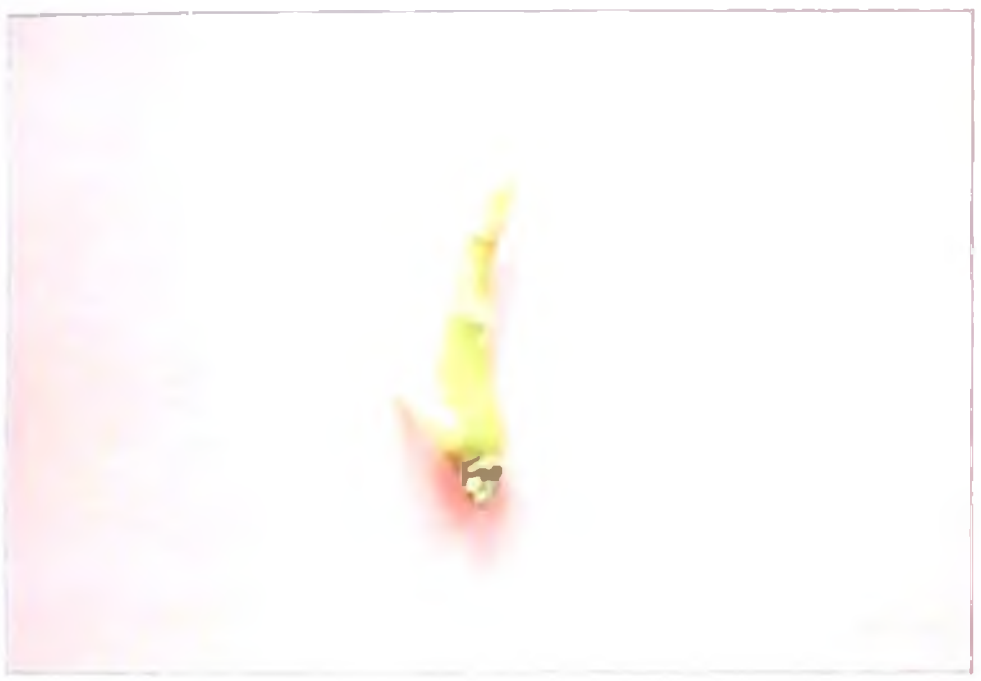
* values subjected to statistical analysis are presented in table 5.

The results indicated that the plant responded positively to domestication. The species performed better under 50% shade as indicated by various morphological characters. About 87 per cent of plants germinated under shaded conditions within 18 days and under open condition within 29 days. There was an increment in plant height by 38.64 %; leaf length by 18.66%; leaf breadth by 30.55% and leaf area by 43.5% under 50% shade compared to open. In the domestic environment only less than six per cent of plants came to flowering. After six months ie., by January the plants started yellowing and finally withered. Fully dried plants were harvested during February after seven months of planting. Harvest observations are presented in table 4.

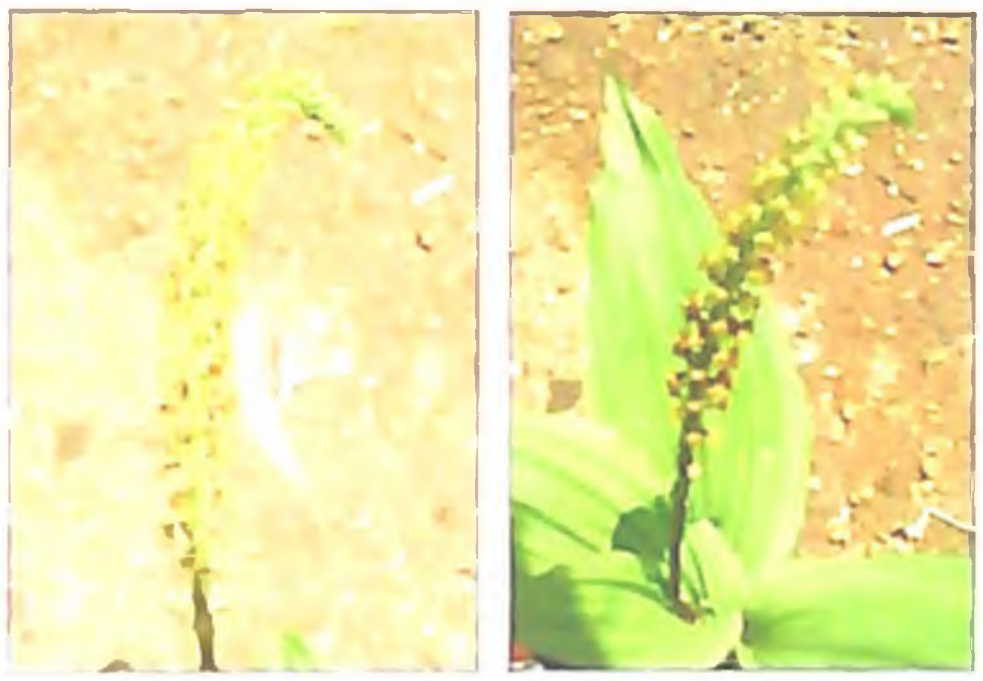
Regenaration from tiny bulbils



Regenaration from nodes

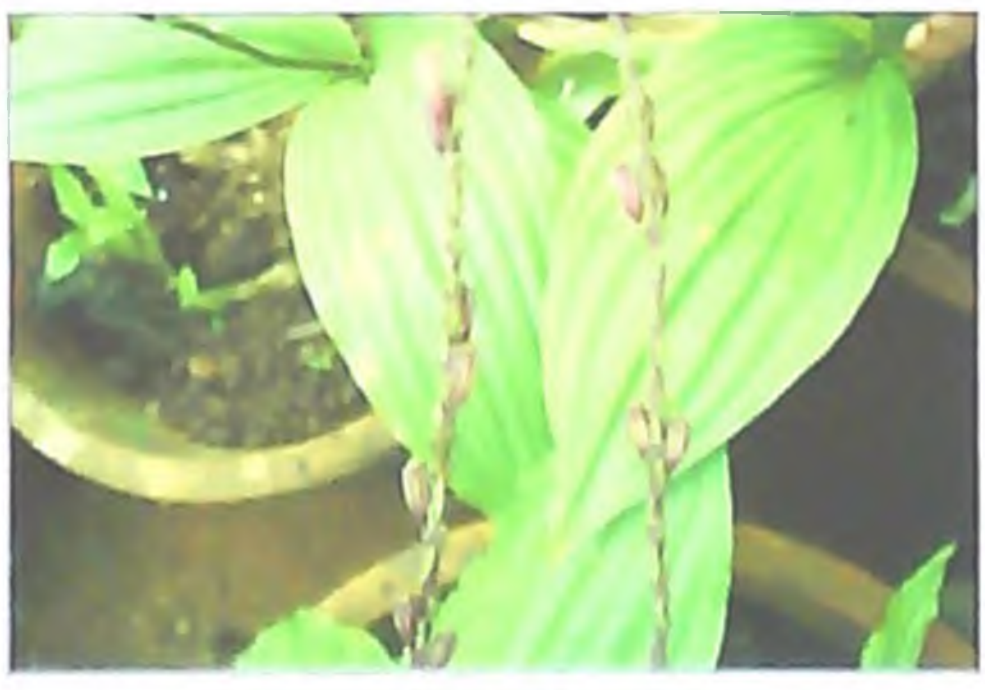


Sprout from tiny bulbil



Inflorecense of Jeevakom

Decay of mother bulbil



Fruitset

Plate 3. Growth and development of Jeevakom

Jeevakom under 50% shade

Jeevakom under open condition

Jeevakom under vertical planting

Harvested pseudobulbils

Crop from open, 50% shade, wild

Plate 4. Domestication Trail - I

Table 4. Yield and yield parameters of *Seidenfia rheedii* in the domestic environment

Sl No.	Character	Performance (mean value)	
		50% shade	open
1	Duration of the crop (days)	205	205
2	Number of roots/plant	11.45*	11.8*
3	Average length of roots (cm)	7.70	4.59
4	Diameter of pseudobulbil at the base (cm)	4.895*	3.508*
5	Diameter of pseudobulbil at the top (cm)	2.22*	1.744*
6	Length of pseudobulbil (cm)	11.3*	8.10*
7	Single plant yield (d/w basis) (g)	7.90*	3.28*
8	Percentage of plants which reached harvest stage (%)	86.89	36.76

* Values subjected to statistical analysis are presented in table 5.

Total duration of the crop was 205 days in the domestic environment. Compared to open condition and 50% shade, crop growth was better under shade. Shade crop had lengthier roots(40.38% increase in root length) and larger pseudobulbils (28.3% increase). Single plant yield as indicated by the dry weight of pseudobulbils was also highest under 50% shade. It was almost double than the open condition. Almost 87 per cent of plants could be harvested from the shade net house whereas in the open field, only 37 per cent reached harvest stage. The striking disadvantage of the domestic crop was low production of side bulbils. It was observed that initially there were sprouts from the upper nodal regions of the pseudobulbil, but these sprouts could not touch the substratum for anchorage and finally they dried up. The sprouts which emerged from the basal region only developed into new plants and the mother bulbil dried up slowly from the tip downwards. Performance of first domestic crop of jeevakom is presented in plate 4.

3.4.2 Comparison of growth of jeevakam under different conditions

The following table depicts the comparative performance of jeevakam in the wild as well as domestic environments

Table 5. Comparision of growth of *Seidenfia rheedii* in the natural, 50% shade and open environment

Sl. No	Environment means	Plant height	Leaf length	Leaf breadth	No of leaves	Leaf area	No of side bulbils	Diameter of bulbil (base)	Diameter of bulbil(top)	No of roots	Wt of mother bulbil
1	Shade	13.3[a]	8.66[a]	3.96[a]	2.68[a]	35.9[a]	1.036[a]	4.695[a]	2.22[a]	3.56[a]	9.56[a]
2	Open	8.14[b]	7.04[b]	2.75[b]	2.52[a]	19.9[b]	0.71[b]	3.51[b]	1.74[b]	3.52[a]	3.28[b]
3	Natural	24.0[a]	10.4[a]	3.77[a]	2.41[a]	39.4[a]	2.54[a]	1.12[b]	0.89[b]	4.52[a]	5.00[a]

* Figures with even number of letters in a column are not statistically different

With respect to plant height, leaf length, leaf area, number of roots and number of sidebulbils wild plants were significantly superior to domestic crop; both under shade and open conditions. Breadth of the leaf was on par under natural and 50% shade. Leaf production and diameter of the bulbil at the base were significantly high under the domestic crop under shade. In almost all characters open condition of the domestic crop either recorded significantly lower values or were on par with the domestic shade crop. With respect to total dry matter production (yield per plant), domestic crop under shade recorded significantly higher values compared to domestic open and wild plants. Considering the critical factors viz. only 37 per cent plants in the domestic open crop reached harvestable stage, significantly lower yields and low side sucker production in domestic open crop, the experiment results were indicative of 50% shade as ideal for the domestication of the crop compared to open. However, the striking disadvantage of the domestic crop (both shade & open) was very low production of side bulbils. In the wild, the plant produced 4-5 side bulbils at from all nodes of the mother bulbil. In the domestic

crop, the lower production of side bulbil was attributed to the vertical planting method adopted in the study, where the sprouts did not get anchorage to develop into separate plants. As the side bulbil production was not sufficient, advancement of the generation was at risk as jeevakom is a vegetatively propagated species. Comparitive performance of jeevakom under different environment is presented in plate 4.

3.4.3 Domestication trial –II

The domestication trial was repeated during 2005-2006. There was a midway modification of the treatment- method of planting in the second year trial to increase the production of side bulbils. Instead of vertical, **horizontal planting** was adopted. Similarly, the open condition was deleted from the treatment, as it did not give desirable results. Since the root system was limited instead of large pots, shallow bonsai pots were used for the trial. The propagules were brought from Peechi forests in the first week of May at the dormancy breaking stage and were planted in shallow bonsai pots filled with potting mixture (sand+ soil+ compost+ FYM in equal proportions). The pseudobulbils were just placed over the potting mixture horizontally and gently pressed. They were then

Table 6. Performance of *Seidenfia rheedii* in the domestic environment (50% shade) under horizontal planting.

Sl. No.	Character	Mean Value
1.	No. of days to germination	10.31 days
2.	No. of days for the opening of first leaf *	13.41 days
3.	No. of days for flowering	19.17 days
4.	No. of side bulbils produced *	4.3
5.	Percentage side bulbil production	98.66 %
6.	Percentage of flowering	34.66 %

table 6 contd.....

Sl. No.	Character	Mean Value
7.	Weight of mother pseudobulbil *	9.38 g plant^{-1}
8.	Weight of daughter pseudobulbils *	5.59 g plant^{-1}
9.	Weight of single daughter pseudobulbil *	1.57g plant^{-1}
10.	Total weight of pseudobulbils*	14.97 g plant^{-1}
11.	Duration of crop	153 days

* Values subjected to statistical analysis are presented in table 8.

immediately covered with leaf mulch and watered daily. There were 50 pots kept under 50% shade provided by the shade net house. Germination, growth, development, flowering and fruit set observations were recorded. Performance of *Seidenfia rheedii* under horizontal sowing in the domestic environment is presented in table 6.

Data given in table 6 clearly indicated that there was more sprouts in each planting unit. Side bulbils sprouted from each node and also from the basal region. Sprouts from the base (1-3 in number) as well as lower nodes were vigorous and fast in growth. Roots of all side bulbils struck soil immediately as the mother bulbil was sown horizontally and each of them developed into separate plants. More vigorous (1-2) sprouts grew into full plants, producing flowers and fruits. Overall germination was faster. On an average each unit produced 4-5 side bulbils. This horizontal sowing proved to be advantageous for production of side bulbils Performance of jeevakom in 2nd domestication trial is presented in plate 5.

3.4.2.1 Comparision of horizontal Vs vertical planting

Performance of *Seidenfia rheedii* in the domestic environment under different methods of sowing is compared in table 7.

Table 7. Performance of *Seidenfia rheedii* under different methods of sowing (50% shade)

Sl. No.	Character	Performance	
		Horizontal planting	Vertical planting
1	Days taken for sprouting	10.31	18.37
2	Sprouting percentage	100%	86.88%
3	No. of days for opening of first leaf *	13.4	21.53
4	Number of leaves per plant	6.76	6.76
5	Days taken for flowering	19.17	50
6	Percentage of flowering	34.66%	5.66%
7	Duration of crop (days)	153	205
8	Total single plant yield (d/w)*	14.97g plant^{-1}	7.9g plant^{-1}
9	Number of side sprouts per plant*	4.3	1
10	Percentage of plants which reached harvest stage	100%	86.89%

* Values subjected to statistical analysis are presented in table 8

Values presented in table clearly indicate the advantages of horizontal sowing against vertical. There was advancement of germination by eight days, unfurling of first leaf by nine days and flowering by 30 days. There was 13.12 per cent increase in germination in horizontal placement. Most significant change was the increase in number of side bulbils per plant. There was 33 per cent increase. Yield advantage was almost double in the horizontal sowing. 100 per cent plants reached harvestable stage, 34.66 per cent flowered (as against 5.66% in vertical) and 11.4 % set seeds. Crop duration was also less in horizontal sowing (42 days earlier).

Comparative performance of jeevakom under different methods of planting is presented in table 8.

Table 8. Yield and yield parameters of *Seidenfia rheedii* under different methods of planting

	Days for first leaf opening	No. of side bulbils	Total yield	Wt. of mother bulbil	Wt. of single side bulbil	Total wt. of side bulbils
Vertical	22.21	1.17	10.57	9.56	1.53	2.39
Horizontal	13.44	3.35	14.97	9.38	1.57	5.59
T-stat	13.49	8.30	2.66	0.113	0.14	3.86
Probability	0.000	0.000	0.013	0.910	0.88	0.000

Except for weight of mother bulbil and single side bulbil, all other parameters recorded significantly higher values in horizontal sowing. Since the total yield and total number of side bulbils were significantly high under horizontal sowing, the earlier mentioned parameters do not matter much

3.4.3 Floral characters

Floral characters of the domesticated crop are presented in table 9.

Table 9. Floral Characters of *Seidenfia rheedii* in the domestic environment (50% shade)

Sl.No.	Charaters	Value
1	Total number of flowers per inflorescence	122.6
2	Length of inflorescence	13-28cm
3	Life of inflorescence	20.3 days
4	Percentage of seed set	11.4
5	No. of pods/inflorescence	6

The stalk of the inflorescence was green or purple in color. The bud was green which changed to yellow on opening. The flowers were variable in size without any particular smell. Sepals linear obtuse and long. Petals one veined and 13-14 teethed. Stamens with anthers two-loculed and sub terminal with four pollinia in pair.

3.4.4 Storage

The fresh pseudobulbils of domestic crop were stored in dry sand inside earthern containers. After a period of five months they were examined. 90% of bulbils turned brown and were shrunk. The bulbils are sown to test their viability. The bulbils were sown horizontally under 50 % shade. There was 100 per cent germination. The growth was vigorous and all the nodes produced side bulbils. There was normal flowering, fruit set and seed set. This clearly indicated that even though there was browning and shrinkage, the bulbils did not loose their viability during storage, up to five months. Growth and development observations were recorded up to October 3, 2006 (till the termination of the project) and are presented in table10. Both the crops ie. the IInd generation crop of wild plants after domestication and storage for five months and the Ist generation crop of the wild plants from Peechi were raised simultaneously with same management conditions and the comparative data are presented below. The crop was maintained in the field even after termination of the project and the harvest details are also presented.

Table 10. Performance of first and second-generation crop of *Seidenfia rheedii* in the domestic environment

Sl.No.	Character	Mean values *	
		Ist generation crop	IInd generation crop
1	Germination (%)	100	100
2	Plant height (cm)	14.9	19.45
3	Length of leaf (cm)	10.47	12.64
4	Breadth of leaf (cm)	4.64	5.75
5	Leaf area (cm^2)	48.58	72.68

table 10 contd.....

		Mean values*	
		I[st] generation crop	II[nd] generation crop
6	Number of leaves	5.3	6.2
7	Inflorescence length(cm)	26.0	20.13
8	Days to flowering	35	52
9	Percentage fruit set	35.6	15.3
10	Average number of fruits/plant	6	1
11	No. of side bulbils/plant	4.6	6.2
12	Weight of mother pseudobulbil(g)	12.59	22.84
13	Weight of single side pseudobulbil(g)	2.08	2.63
14	Total weight of side bulbils (g)	8.87	16.47
15	Total single plant yield(dw)(g)	21.47	39.32

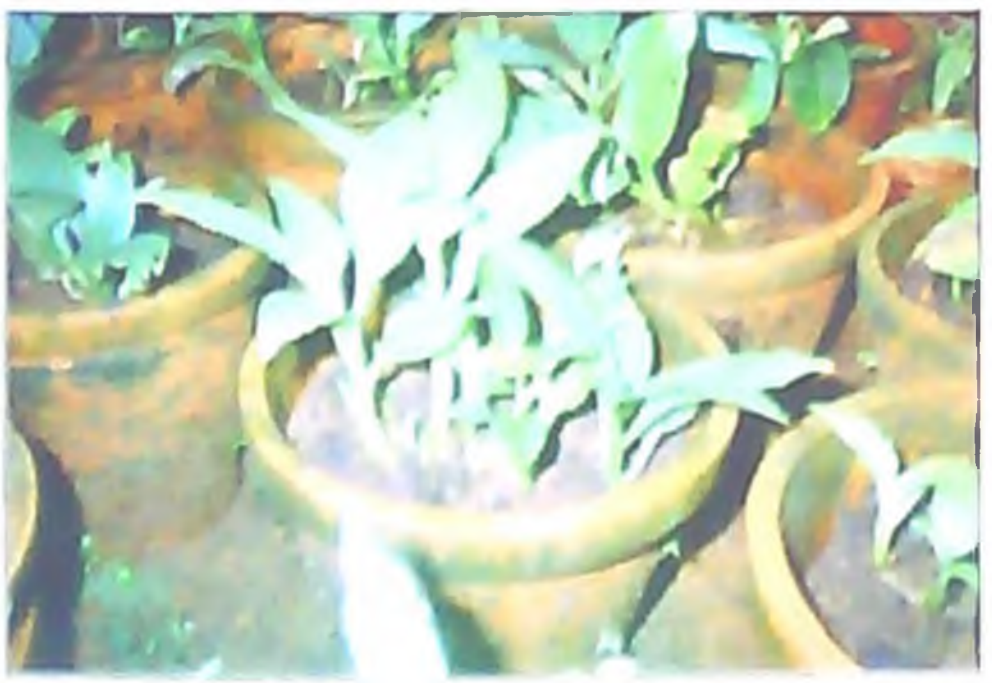
* Values subjected to statistical analysis are presented in table 11

All the vegetative characters recorded higher values in the stored II[nd] generation crop. Flowering and fruit set were better in the I[st] generation crop. With respect to production of side bulbils also, the crop raised from stored pseudobulbils recorded highest values. Yield parameters viz. weight of mother bulbil, total weight of side bulbils and total plant yield recorded higher values in the Ii[nd] generation crop.

Jeevakom under horizontal planting

More sprouts on horizontal planting

Single sprout on vertical planting



Profuse side bulbils

Jeevakom in bonsai pots

Plate 5. Domestication Trail - II

Storage in mud pots

Browning of jeevakom on storage

Sprouts from stored bulbil

Second generation crop

Second and first generation crop-pseudobulbils

Plate6. Storage of Jeevakom

Table 11. Performance of first and second-generation crop of *Seidenfia rheedii* in the domestic environment

Sl. No.	Character	Mean value		probability of t-value
		I st generation crop	II nd generation crop	
1	Length of leaf	10.47 cm	12.64cm	0.000
2	Breadth of leaf	4.64 cm	5.75cm	0.035
3	Leaf area	48.58cm²	72.68cm²	0.001
4	Plant height	14.9cm	19.45cm	0.000
5	Inflorescence length	26.0cm	20.13cm	0.026
6	Number of leaves	5.3	6.2	0.000
7	No. of side bulbils/plant	4.6	6.2	0.024
8	Weight of mother bulbil	12.59g	22.84g	0.000
9	Weight of single side pseudobulbil(g)	2.08g	2.63g	0.248
10	Total weight of side bulbils (g)	8.87g	16.47g	0.017
11	Total single plant yield(dw)(g)	21.47g	39.32g	0.000

All the values except weight of single side bulbil were significant conforming the superiority of the IInd generation crop. Performance of stored bulbils of jeevakom are presented in plate 6.

3.4.5 Summary of domestication trials

Jeevakom (*Seidenfia rheedii*) responded positively to domestication. Performance was better under 50% shade with horizontal placement of bulbils. Harvested bulbils could be stored for five months without loss of viability. Second-generation crop also performed well under 50% shade with horizontal sowing.

3.5 PHYTO-CHEMICAL ANALYSIS

Pseudobulbils from the wild as well as domestic environments were analyzed for

the selected phytochemical constituents and the results are presented in table 12.

3.5.1 Primary metabolites

Content of primary metabolites of *Seidenfia rheedii* from different habitats are presented in table 12.

Table12. Content of primary metabolites of *Seidenfia rheedii* in the wild and domesti environments

Sl. No.	Component	Content		
		Wild	50% shade	Open
1	Total soluble sugar (%)	0.88	0.52	0.72
2	Starch (%)	14.7	16.6	14.20
3	Protein (%)	0.53	0.63	0.85
4	Total free amino acids (%)	0.09	0.33	0.49
5	Phenol (%)	0.030	0.034	0.034
6	Total chlorophyll (mg/g)	0.053	0.022	0.021
7	Chlorophyll a (mg/g	0.025	0.010	0.010
8	Chlorophyll b (mg/g)	0.028	0.011	0.012
9	Moisture (%)	80.7	76.6	NA

Soluble carbohydrates were high in the wild samples compared to domestic, while in the domestic crop high values were recorded under open condition. Starch and protein contents were high in the domestic crop. Free amino acids were high in the domestic crop compared to wild sample. There was not much variation in the phenol content of forest and domestic samples. Chlorophyll a, chlorophyll b and total chlorophyll recorded high values in the wild samples.

Table 13. Contents of phytochemieal constituents of single plant of *Seidenfia rheedii*

Sl.No.	Component	Content		
		Shade	Open	Wild
1	Total soluble sugars(%)	0.81	1.39	1.38
2	Total phenols(%)	0.026	0.051	0.027
3	Total free amino acids(%)	1.45	1.14	0.39
4	Protein(%)	0.85	0.61	0.51
5	Starch(%)	8.95	11.05	14.2

Single plant analysis also gave same pattern of phytochemical constituents except insignificant variation in the starch and phenol contents.

3.5.2 Thin Layer Chromatography of phenolics

Samples were subjected to thin layer chromatography for detecting phenolics. The result obtained are present in the table 14.

Table 14. Thin Layer Chromatography of phenols

Sl. No.	Rf value			Colour of Spot
	Shade	Open	Wild	
1	0.06	0.06	0.05	Blue
2	0.63	0.62	0.63	Blue
3	0.67	0.67	0.67	Blue
4	0.70	0.70	0.70	Blue
5	0.79	0.77	0.79	Blue
6	0.91	0.91	0.91	Blue
7	0.94	0.94	0.94	Blue

* Solvent system - Chloroform : Acetic acid (10:1). Spray Reagent - Folin's reagent

Thin Layer Chromatography of phenolics indicated seven different phenols in all samples. Rf values and color of the spots were same in wild and domestic samples.

3.5.3 Thin Layer Chromatography of free amino acids

Thin layer chromatography of the sample was carried out for amino acids. The results obtained are present in the table 15.

Table 15: Thin Layer Chromatography of Total free amino acids

Sl. No.	Rf value			Colour of Spot
	Shade	Open	Wild	
1	0.20	0.19	0.22	Purple
2	0.34	0.32	0.34	Purple
3	0.38	0.38	0.40	Purple
4	0.52	0.53	0.52	Purple
5	0.64	0.65	0.66	Purple
6	0.92	0.93	0.94	Purple

* solvent system used- Butanol: Acetic acid: H_2O (4:1:1) spray reagent- 0.1% ninhydrin

Six amino acids were identified in both wild & domestic samples. Rf values and color of the spot were same for all the samples.

3.5.4 Test for alkaloids

The qualitative tests for alkaloids were carried out in *Seidenfia rheedii* and results are presented in table 16.

Table 16. Qualitative test for alkaloids

Sl.No.	Reagents used	Results	
		50% shade	wild
1	Dragendorff's Reagent	-ve	-ve
2	Mayer Reagent	+ve	+ve
3	Wagner Reagent	+ve	+ve

Negative results were recorded for alkaloids.

3.5.5 Test for saponins

Saponins were also absent in *Seidenfia rheedii*

3.5.6 Test for unknown sugars

Samples were tested for identifying the sugars present and results are presented in table 17.

Table 17. Detection of unknown sugars present in *Seidenfia rheedii*

Sl.No.	Tests	Results
1	Molish's test	+ve
2	Iodine test	-ve
3	Benedicts test	+ve
4	Barford's test	-ve
5	Bial's test	+ve
6	Selvinoff.s test	+ve

Results indicated the presence of hexoses/glycosides in the plant. Even though there was positive test for fructose, the possibility of ketose sugar was ruled out because of the extremely bitter taste of the cell sap. Presence of hexosamines/aminosugars or glycosides is to be examined.

3.5.7 Total soxhlet extractables

Total crude extractable of *Seidenfia rheedii* was estimated with different solvent systems and results are presented in table 18.

Methanol gave maximum percentage of extraction (3.35%), which indicated the presence of high quantity of soluble carbohydrates and related compounds.

3.5.8 Inference of phytochemical analysis

From the above results of phytochemical analysis following inference could be made. Higher values of starch, protein and total free amino acids recorded in the domestic crop could be the normal response of the plant to added inputs. Presence of high soluble sugar and low amino acids was characteristic of the wild samples. This is an indication of the presence of plant amines and glycosides or similar group of compounds. Absence of alkaloids saponins etc. also supports the same. Detailed analysis of these compounds can only elucidate the medicinal property of *Seidenfia rheedii* and the impact of domestication on the ultimate quality of the drug.

Table 18. Content of total soxhlet extractable of *Seidenfia rheedii* in various solvents

Sl.No.	Solvent used	Total soxhlet extractables (%)
1	Ethyl acetate	1.05
2	Petroleum ether	0.70
3	Chloroform	0.95
4	Methanol	3.35
5	Hexane	0.40
6	Acetone	1.55

3.6 ANATOMICAL STUDIES

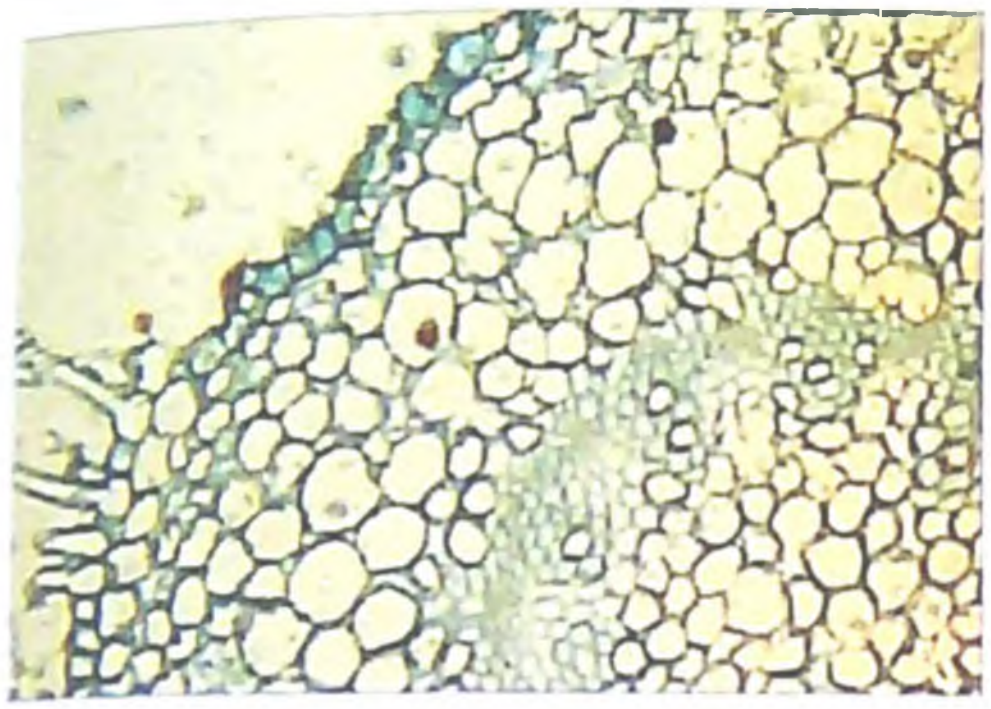
Microtome sections of root, pseudobulbil, outerscale and leaf were taken. Sections are described below and presented in plate 7 and plate 8.

3.6.1 L.S of root

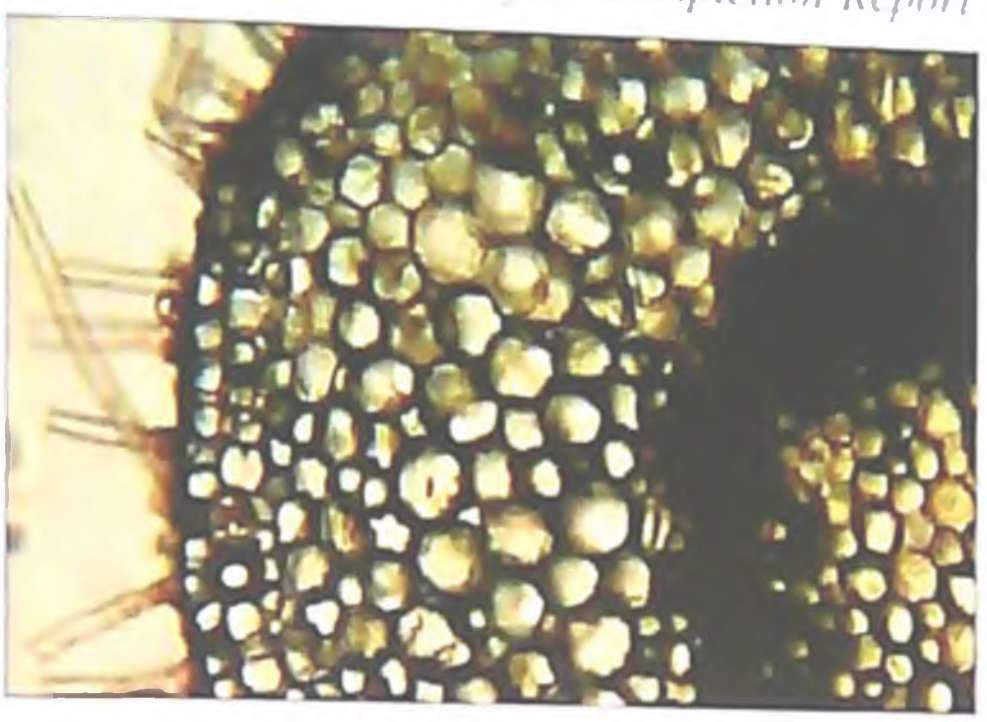
L.S of root is presented in plate 7. Primary aim of taking root section was to ascertain whether the roots are normal or modifications for the lithophytic adaptation. Root tip was sectioned and stained and viewed under microscope. The section clearly depicts four layer of root initials. An outer layer (calyptrogen), followed by dermatogen, periblem and pleurome to the inner side. Calyptrogen gives rise to root cap, dermatogen develop into epidermis, periblem gives rise to cortex and pleurome differentiates into vascular cylinders. Presence of root cap confirmed it as a normal root (unlike most of the orchids) which does functions of absorption of water, nutrients etc from the substratum. This proves beyond doubt the suitability of the species for domestication as any other crop plant without any special management practices usually given to orchids.

3.6.2 T.S of root

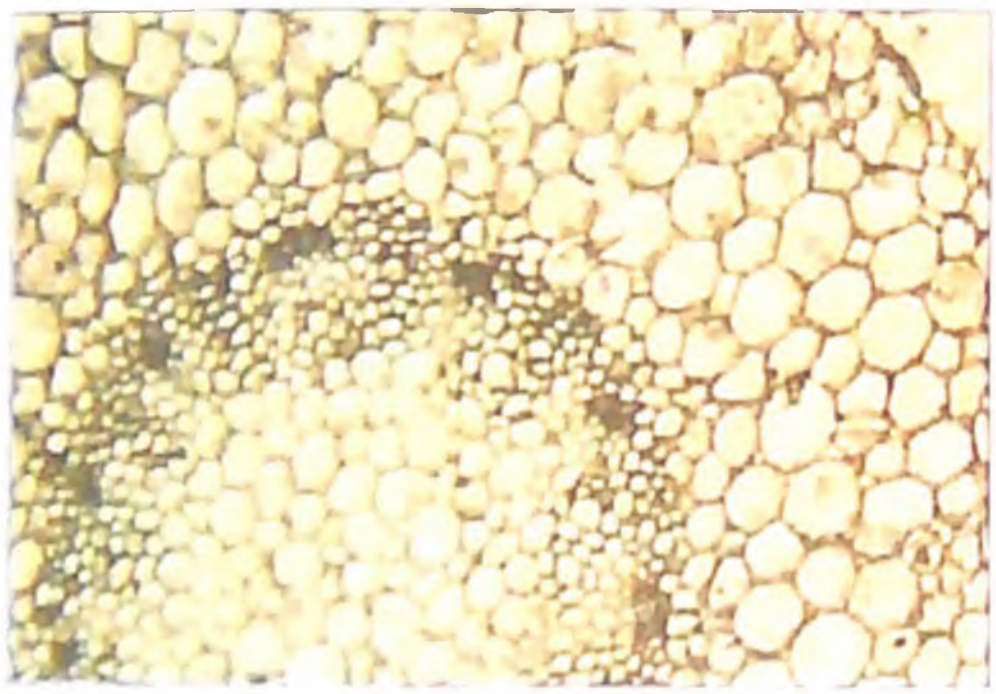
T.S of root is presented in the plate 7. Single layered paranchymatous outer epidermis is visible from which the developing single celled root hairs are clear. Cortical cells are multilayered followed by the single layered endodermis, single layered pericycle and radial vascular tissues. Presence of root hairs confirmed it as a normal root. Presence of more xylem cells was noticed which might be a xerophytic adaptation.



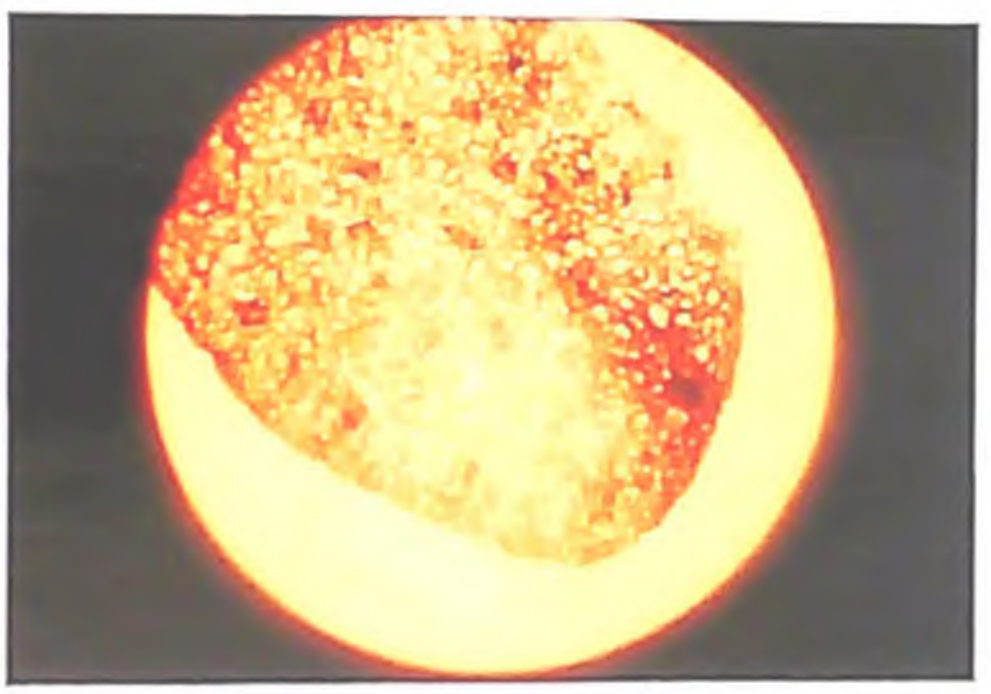
T.S Root



T.S Root

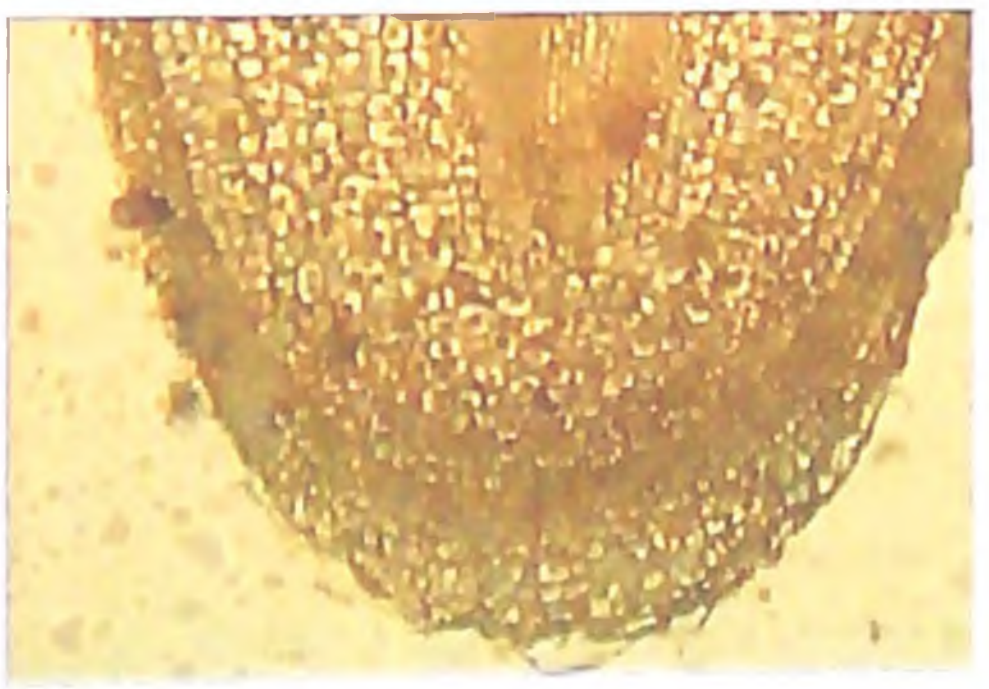


T.S Root



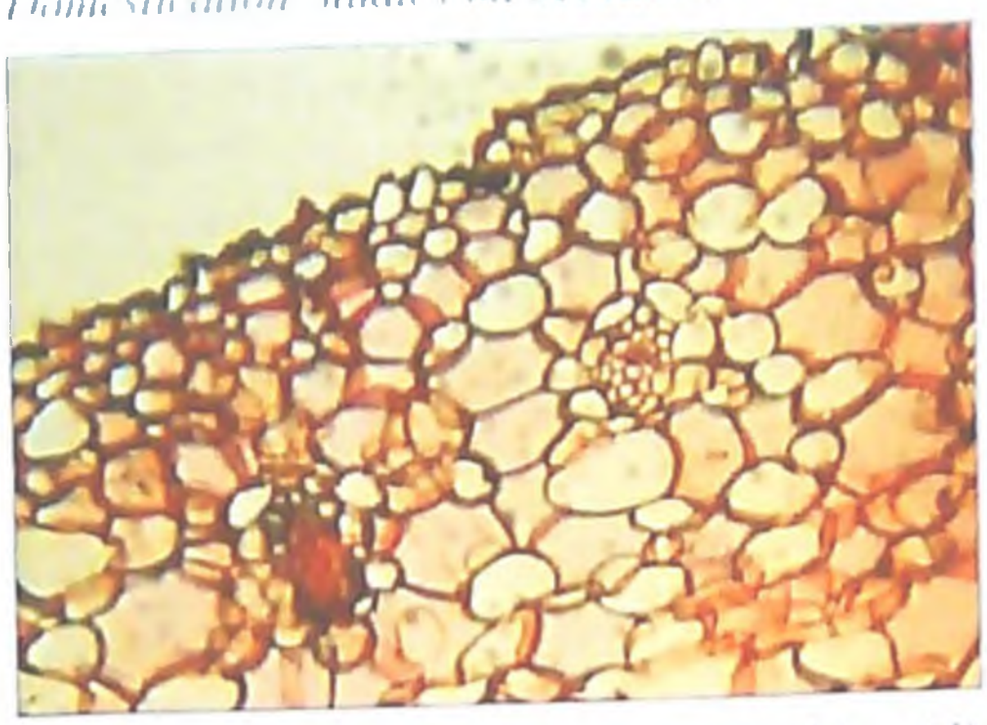
C.S Pseudobulbls

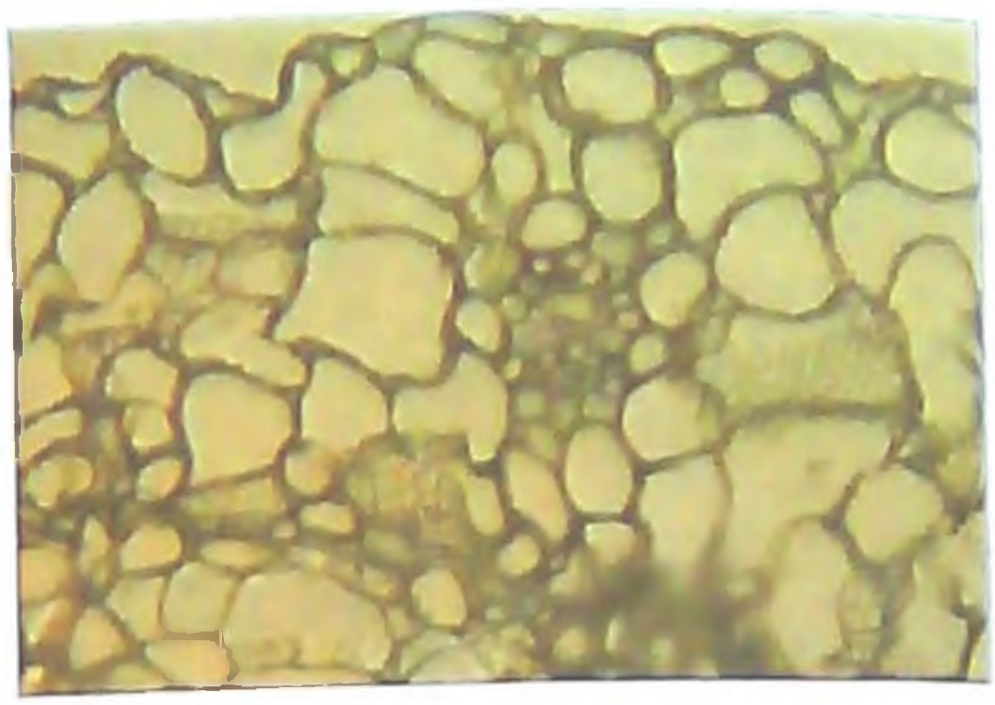
T.S Root



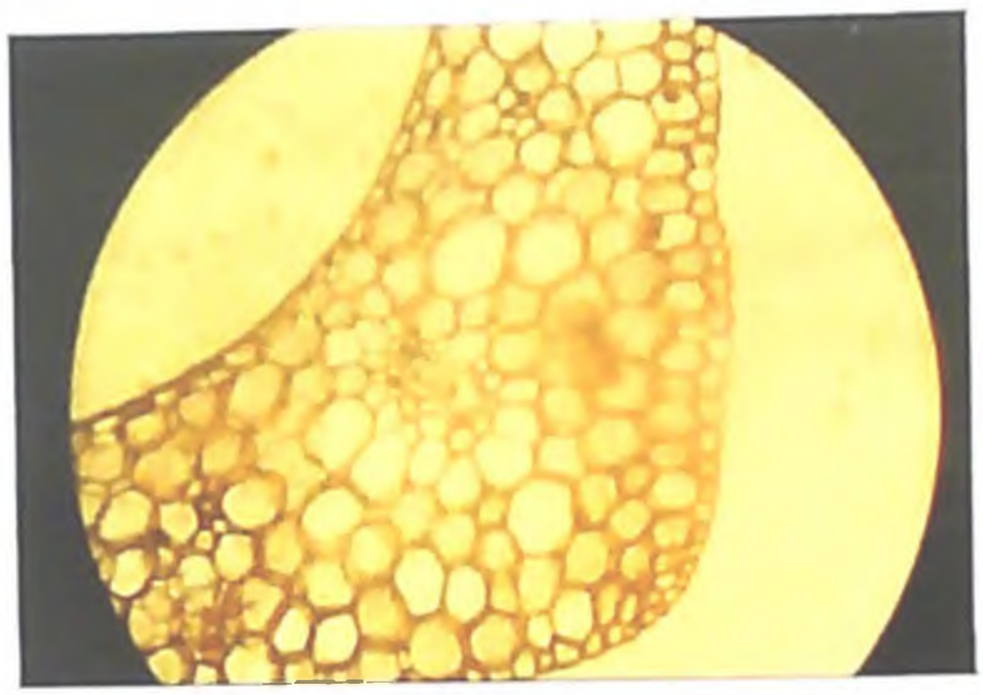
T.S Root

Plate 7. Anatomical features of Jeevakom

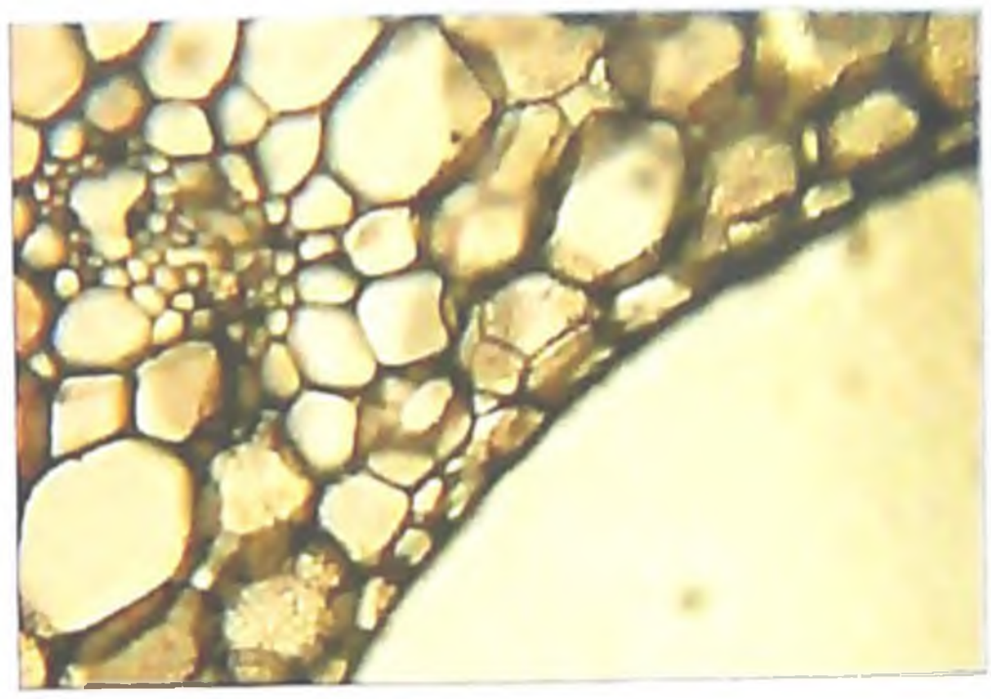




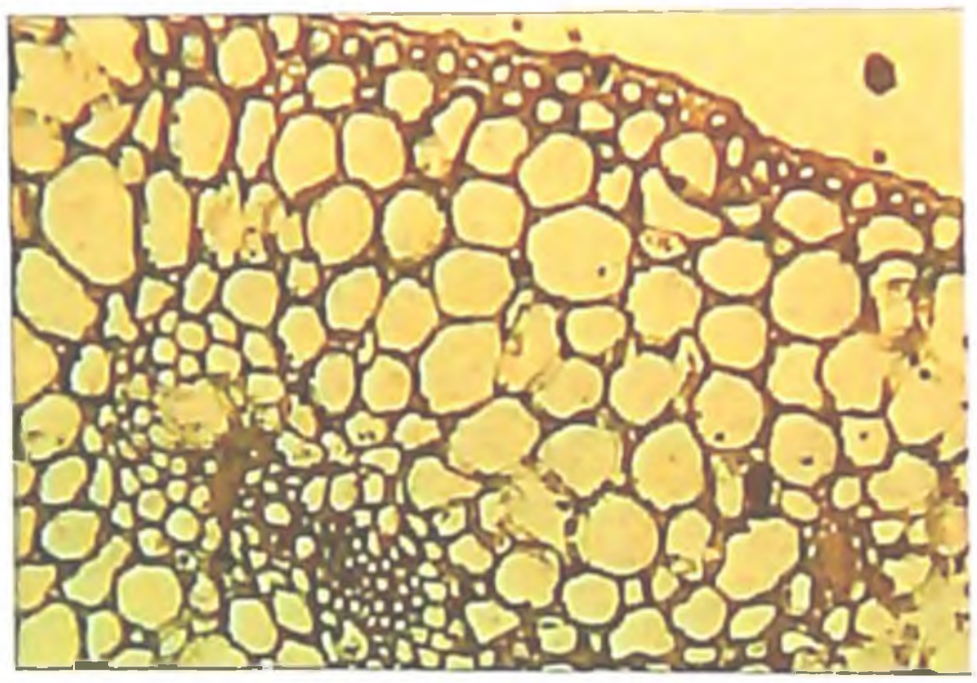
T.S. of Pseudobulbul

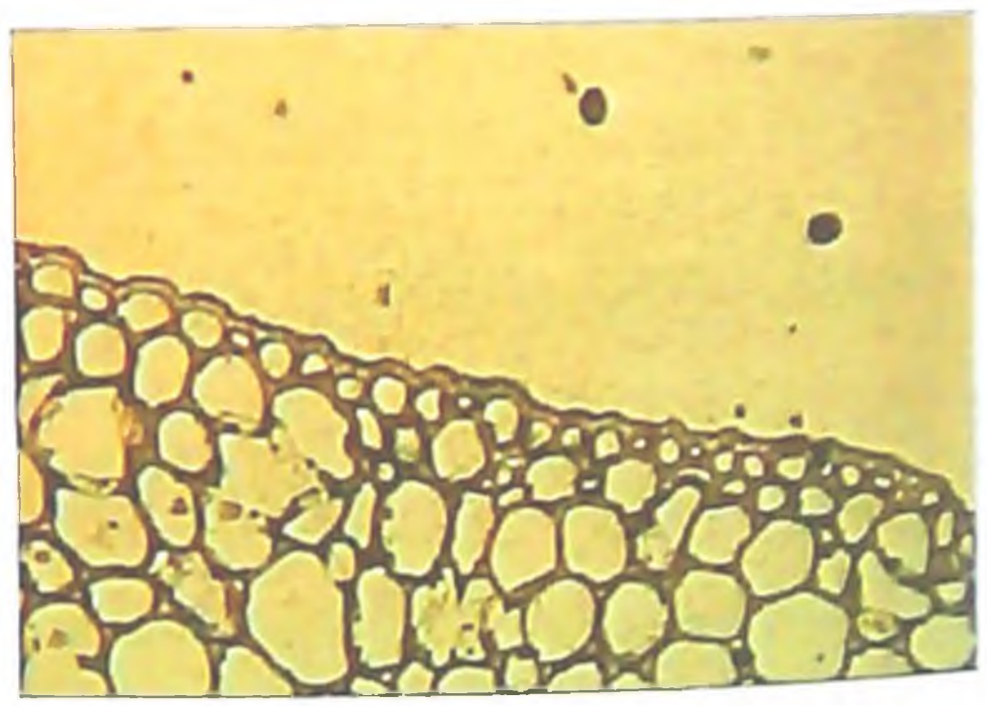


C.S Outer Scale



T.S Outer Scale





T.S of Leaf

Plate 8. Anatomical features of Jeevakom

3.6.3 T.S of leaf

T.S of leaf presented in plate 8 depicts the upper and lower epidermis of single layered isodiametric parenchymatous cells with cuticle. Lower epidermis is interrupted with stomata. Upper and lower epidermis enclosing the mesophyll tissue is not differentiated into spongy tissue and palisade layer. Instead it is full of parenchymatous cells. Vascualr bundles are seen with xylem towards upper epidermis and phloem towards lower epidermis. Surrounding the vascular bundles are the sclerenchyma cells.

3.6.4 T.S of outer scale

T.S of outer scale is presented in plate 8. Upper and lower epidermal layers are made of single layered barrel shaped parenchyma cells. Below the epidermal layer is the multilayered parenchyma cells interrupted with alternating large and small bundles which on either sides have sclerenchyma cells. Phloem is not seen, instead below the upper layer of sclerenchyma cells in the bundle, a cavity is present. Below the cavity xylem is seen in the endarch condition. The parenchyma cells show some reticulations inside which needs further clarification as to whether they are cellular deposits or dead cells. In either case it would be adaptation for a protective covering to the pseudobulbil during the dormant period.

3.6.5 T.S of pseudobulbil

T.S of pseudobulbil presented in the plate 8 shows outer single layered epidermis made of barrel shaped parenchyma cells coated with cuticle followed by multilayered parenchymatic ground tissue embedding many vascular bundles. Each bundle has sclerenchyma cells on both sides, followed by phloem and xylem.

Chapter 4

SUMMARY

Summary of the project "Domestication Studies on Jeevakom" carried out at the Department of Plantation Crops & Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara during 2003-2006 is presented below:

4.1 Botanical identity of jeevakom

From all available information gathered as part of the project, it seems likely that what is sold and used as the two drugs 'Jeevakom' and 'Edavakom' in Kerala is pseudobulbil of the same plant differing only in size. Larger pseudobulbils are considered as jeevakom and smaller one as edavakom. The plant is botanically *Seidenfia rheedii* (Sw.)(Szlach.) (Basionym: *Malaxis rheedii* Sw.)

4.2 Plant explorations

Out of the forest areas explored; viz. Wynad, Parambikulam, Peechi, Silent Valley and Shendurney, the species was located at Parambikulam, Peechi and Silent Valley forests. At Parambikulam and Peechi, sufficient population was present whereas at Silent Valley the population was meagre. Contrary to the popular belief of an *abhavadravya* (unobtainable) this study indicated the availability of jeevakom in Kerala forests albeit in small quantities. In all the forest areas explored the tribal knowhow on the species was limited and only the tribals of Peechi gathered it as a drug plant.

4.3 Natural habitat analysis

Seidenfia rheedii is a lithophytic orchid found on wet rocks amidst moss and grass, in the openings of semi evergreen and evergreen forests at altitudes ranging from 800-1100m above MSL. The natural habitat could be described as **dripping rock ecosystem**. The plant is a short stemmed fibrous rooted herb with a swollen stem forming a conical pseudobubil which is used as drug. The plant regenerates vegetatively through side bubils. After flowering and fruiting which is completed by Dec-Jan, the plant dries up and remains dormant until the next rains.

4.4 Domestication trial

Jeevakom responded positively to domestication. In the first trial where the

pseudobulbils were vertically planted, performance was better under 50% shade in terms of both growth and yield parameters compared to fully open conditions. The striking disadvantage in the domesticated crop was very low production of side bulbils, thereby blocking the advancement of generation.

In the second trial where horizontal planting was done, the production of side bulbils was more, thus overcoming the major draw back of vertical placement. Horizontal sowing was also advantageous with respect to other growth and yield parameters.

4.5 Storage studies

The pseudobulbils of domestic crop were stored for five months inside earthern pots and sown in the next season to test viability and to evaluate the performance. There was 100 per cent germination, better growth and development. In growth and yield parameters, the 2nd generation crop performed better than 1st generation crop raised simultaneously.

4.6 Phytochemical analysis

Pseudobulbils from the wild as well as domestic environment were analyzed for the selected phytochemical constituents. Higher values of starch, protein and total free amino acids were recorded in the domestic crop. Presence of high soluble sugars and low amino acids were characteristic of wild samples. High chlorophyll content was recorded in wild samples. Both wild and domestic samples gave negative results for alkaloids and saponins. Overall results indicated the presence of hexosamine/amino sugars or glycosides in the plant. Detailed analysis of these compounds can only elucidate the medicinal property of *Seidenfia rheedii* and the impact of domestication on the ultimate quality of the drug.

4.7 Anatomical studies

Pretreatment of sample for microtomy was standardized. L.S and T.S of root, outer scale, leaf and pseudobulbil were taken. Root cap tissue and root hairs were present which confirmed it as a normal root unlike other orchid roots. In the outer scale, some reticulations were present inside the parenchyma cells which needs further clarification as to whether they are cellular deposits or dead cells themselves. L.S ans T.S of pseudobulbil and leaf were also described.

4.8 Contributions made towards increasing the state of knowledge in the subject

Jeevakom (*seidenfia rheedii*), is an inevitable constituent drug in many ayurvedic formulations. But Kerala physicians generally consider this as *abhaavadravya* or unobtainable drug. At present, most of the drug

requirement of Jeevakom is met from the annual supply from Punjab. In the present study the availability of *Seidenfia rheedii* in Kerala forests is confirmed and its status ascertained. The study ascertained the availability of the plant for use as drug which at present is either deleted or substituted with other drugs. The botanical identity of the two drugs, jeevakom and edavakom which is given differently in various texts is also confirmed in the study. The same plant (larger and smaller bulbils) is used as jeevakom and edavakom in Kerala and the species was identified as *Seidenfia rheedii*. As a long term conservation measure of this rare and valuable drug, domestication trial was attempted and the plant responded positively to domestication. All these findings would necessarily lead to conserving the natural population in the forests and at the same time making available the genuine drug available in sufficient quantity for the user industry.

Present literature/knowledge on the impact of domestication on medicinal plants is scanty. Unlike other plants both quality and quantity are equally important in medicinal plants. There are reports on influence of environment on the production of secondary metabolites and subsequently on the quality of the drug. In the present study, valuable information on the impact of domestication on jeevakom (*Seidenfia rheedii*) is available. The change in morphological and reproductive behavior of the species upon domestication is quantified. In the preliminary trials, the plant responded positively to domestication with significant improvement in yield. On the quality side, since nothing is known about the phytochemistry of jeevakom, all primary as well as secondary metabolites were analyzed in both wild and domestic plants. From the present study, amino sugars/glycosides seemed to be the major component imparting quality to the drug. Dynamics of various components upon domestication was also quantified.

4.9 Future line of work

Unlike other plants, both quality and quantity are equally important in medicinal plants. Most of the medicinal plants tend to behave differently when they are taken out of their natural habitat. In this study, the plant responded positively to domestication in terms of growth and yield. But the quality aspects of the domesticated crop require further investigation. Biochemical, pharmacological and pharmacognostic studies are required to fully comprehend the impact of domestication on this species. Also, the present study came out with the general packages for domesticating the crop. Refinement of this packages with respect to shade requirement, potting media composition, standardization of

propagation method, storage and seed germination are further needed to develop a complete package of practices for the large scale cultivation of this valuable drug plant.

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Annexure

Permission for Collection of Specimens from the Protected Areas for Scientific Research

No. WL 11-7938/03 *Dated : 06/12/2003*

Title of the Project	Domestication studies on Jeevakom
Principal Investigator	N. Mini Raj
Institution	College of Horticulture, Kerala Agricultural University, Thrissur
Duration of the project	01-12-2003 to 01-07-2006
Funding Agency Environment	Kerala State Council for Science, Technology & Sasthra Bhavan, Pattom, Thiruvananthapuram.

Subject to the provisions of the Wildlife (Protection) Act, 1972, and the Kerala Forest Act, 1961, and the rules made there under Mr. N. Mini Raj, Assistant Professor, Department of Plantation Crops and Species, Kerala Agricultural, Thrissur is granted permission to enter and collect biological materials as specified in the attachment for the purpose of scientific research on the above project on following conditions.

1. The researcher shall collect only specimens of permitted species from such Wildlife Sanctuaries/National Parks / Forest areas and on such dates / time mutually agreed by the researcher and the Divisional Forest Officer / Wildlife Warden having jurisdiction over the area.

2. The Researcher and his team shall be bound to act in accordance with the existing Acts, Rules and directions of the concerned officers.

3. The field programmes and visits of the research team shall be intimated to the concerned Wildlife Warden/Divisional Forest Officer / Asst. Wildlife Warden / Range Officer in advance.

4. The researcher shall collect only minimum number of specimens absolutely required for the research work. The list of specimens collected shall be submitted to the concerned Wildlife Warden / Divisional Forest Officer and Assistant Wildlife Warden / Range Officer immediately after each visit. In the case of inventory of

flora / fauna, since the identity of the specimen collected is not known, a list of number of unidentified specimens collected shall be given.

5. If photography is involved during the course of collection of biological resources, copies of the photographs with identity of plants / animals shall be sent to Divisional Forest Officer or Wildlife Warden as the case may be, who shall maintain a register such cases.

6. A minimum of three copies of the published research reports and one copy of the Final Research Report shall be given free of cost to the Chief Wildlife Warden, on expiry of the period of research.

7. The institution shall be held liable for any damage or loss caused due to the action of negligence or other wise of the members of the research team. The Institution / researcher shall make good to Government in Forest Department for any loss caused and for the destruction or damage to any forest produce, wildlife, forests or the environment. The loss will be assessed by the Chief Wildlife Warden and his decision shall be final.

8. Being an institution under the central / state Government / autonomous body under the central / state Government it is exempted from payment of the Security Deposit.

9. The Chief Wildlife Warden has the full discretionary powers to grant, suspend or reject permission to regulate the field works related to the research project with respect to time and space in view of the protection and management problems in the Protected Areas / Forest areas.

10. The Chief Wildlife Warden or any competent authority shall have the powers to suspend, withdraw or cancel the permission granted, for violation of any of the conditions of permission, or if it is subsequently found that any particulars furnished by the applicant in the application are not true or for any other just and valid reasons to be recorded in writing and, the Government or Department or any Forest Officer shall not be liable to pay any compensation for loss or damages or inconveniences caused due to the suspension, withdrawal or cancellation of the permit.

11. Any vehicle, vessel, weapon, trap or tool that has been used for violation of the conditions specified herein shall be forfeited to the State Government and the

offender will be proceeded against as per provisions of the laws in operation in forest area / wildlife sanctuary / national park / closed area of the State.

12. The study team shall be permitted entry into the forest area only with proper accompaniment of Forest Guides / Staff as the case may be suitable amount as fixed by the Chief Wildlife Warden from time to time will have to be remitted by the study team for such services rendered by the Forest Department.

12/2003

Chief Wildlife Warden,
Kerala

Copy to :
1. Copy to Smt. N Mini Raj, Assistant Professor, Department of Plantation Crops and Spices, College of Horticulture, Kerala Agriculture University, Thrissur - 680 656
2. Copy to Wildlife Warden, Shendurney, Parambikulam, Peechi-Vazhani, Silent Valley, Wayanad.
3. Deputy Director, Periyar Tiger Reserve
4. Copy to Stock File.