PHARMACOGNOSTICAL STUDIES ON AEGLE MARMELOS CORR. AND CATHARANTHUS ROSEUS LINN. - (ANTI - DIABETIC PLANTS)

By

MADHUBALA S.
Reg. No. 92PLS05

A THESIS SUBMITTED TO
THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN (DEEMED UNIVERSITY) COIMBATORE-641 043
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN LIFE SCIENCES.

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CERTIFIED AS BONAFIDE RESEARCH WORK.

Signature of the
Head of the Department

Signature of the
Dean of the Faculty

Signature of
the Guide

Acknowledgement

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Contents

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

INTRODUCTION

Since disease and death have always co-existed with life, the study of diseases and their treatment must also have been contemporaneous with the dawn of human intellect. The primitive man is believed to have used the plants as therapeutical agents and remedial measures as they were most easily procured those days. There is no authentic record of medicines used by the primitive man. But the RigVeda supplies curious information on the subject. From it, we learn that the Indo - Aryans used the 'Soma' as a medicinal agent. 'Soma' plant is conjectured by many to be Ephedra pachyclade. The Indo - Aryans used the plant for sacrificial purposes and its juice is described in ancient literature as a stimulating beverage. When the Indo - Aryans came to use the soma plant for therapeutical purposes they came to possess a knowledge of the medicinal properties and uses of herbs and plants.

The knowledge of medicinal plants must have been accumulated in the course of many centuries. In his work on plants and animals under Domestication, Darwin says:-

"From innumerable experiments made through dire necessity by the savages of every land, with the result handed down by tradition, the nutritious, stimulating and medicinal properties of the most unpromising plants were probably first discovered". Thus primitive people of all

ages had some knowledge of medicinal plants acquired largely on a trial and error basis.

Phytotherapy was the ancient system of medicine, practised world wide. In this method of therapy, drugs of plant origin were used in the treatment of various ailments. Phytotherapy proved to be a highly effective and successful mode of therapy, as numerous plant products were found effective against diseases caused by micro organisms.

Historical evidences of phytotherapy are found in the manuscripts of "Eber Papyrus" (16th century B.C.) (Sambamurthy and Subramanyam, 1989). Emperor Shen Nong of China, is popularly known as the "Father of Phytotherapeutic Research". In his work entitled "Shen Nong Ben Cas", the uses of Ephedrine. Chaulmoogra oil, Camphor and Opium are elaborated.

India has a rich tradition of phytotherapy as clearly evident from Vedic literature. The Atharva Veda is one of the oldest written records of the world which describes numerous medicinal plants. Some of these plants find a place in the Ayurvedic, Siddha and Unani systems of herbal medicine.

The Vedic Aryans are acquainted with about a hundred medicinal plants. When a king appoints a purohita, he repeats a prayer in which he entreats that all the herbs of a hundred kinds over which king Soma rules will grant him uninterrupted happiness.

There are great names concerned with the phytotherapic mode of treatment. Hippocrates, the Father of Medicine (500 B.C.) prescribed eating Garlic as treatment for uterine tumors. Hartwell from the National Cancer Institute Central Files, reported that cancer incidence in France is supposed to be lowest where garlic consumption is greatest.

Dioscorides who came with the invading Roman army to Britain left descriptions of how to use such medicinal herbs as staunch-wood. In India, dramatic relief from trigeminal neuralgia is obtained by prescribing an indigenous drug recipe along with isonicotinic acid hydrazide. (Lewis and Elvin - Lewis,1977).

One of the outstanding developments in the field of natural products in recent times has been the discovery of the therapeutic properties of Rauvolfia serpentina for the treatment of hypertension and mental diseases.

Ipecacuanha roots were used in Ancient Brazil and the Far-East Countries for the treatment of diarrhoea and dysentry (Ghosal,1989). Extracts of Pongamia pinnata proved effective in the treatment of leprosy and erysephalis. The extracts of Acalypha indica and Tylophora asthmatica were used in the treatment of asthma. Similarly the leaves of Aegle marmelos Corr. (Ponnachan et. al., 1993) and the whole plant parts of Catharanthus roseus Linn. (Rastogi et al.,1990) have been reported to have anti - diabetic effect.

Diabetes mellitus is a metabolic disorder affecting insulin production and resulting in faulty metabolism giving rise to sugar in the urine and imperfect fat metabolism.

Weakness, weight loss, increased thirst and urination, diabetic neuritis, failure of vision, impotence, itching of the skin and furunculosis are all common manifestations of this disease.

Heredity and obesity are very important predisposing causes of diabetes, although inflammatory and degenerative lesions of the pancreas resulting from pancreatitis, carcinoma, arteriosclerosis and hemochromatosis account for a small number of cases.

At present the total number of patients suffering from diabetes in our country is estimated to be 15.2 million. An epoch making discovery in 1921 that a pancreatic extract (Insulin) would lessen the symptoms of diabetes was a landmark in the area of drugs from natural sources. During world war II when Insulin was not available in many countries, search was made for an insulin substitute from plant sources. 148 plants of 50 families were reported to have hypoglycaemic activity. Two of the most important hypoglcaemic plants mentioned were Aegle marmelos Corr.(Rutaceae) and Catharanthus roseus Linn. (Apocynaceae). The tea from Catharanthus roseus has been

used in S.Africa, Nepal, Australia, S. Vietnam and the Philippines and the proprietary products convinca and vinculin are still marketed as oral insulin substitutes in some of these countries.

Many users of herbal medicines believe that natural remedies are more hormonious with the physiology of living organism than the synthetic chemical drugs. Synthetic chemicals tend to produce severe pathogenic effects within the organism in the course of therapy. Hence, phytotherapy is a simple, harmless and environment friendly mode of treatment. With the increasing advancements in Biochemistry, many research institutions are involved in screening plants for the drugs,chemicals, alkaloids etc., which they contain.

Hence the present investigation was undertaken with the main objective of screening the powder of the above mentioned anti-diabetic plants for their Pharmacognostical characters through the following parameters:-

Taxonomy, Anatomy ; Organoleptic, Microscopic and Fluorescence Evaluation ; Biochemical, Phytochemical, Physical Constant Determination.

Review of Literature

REVIEW OF LITERATURE

The literature pertaining to the work entitled "Pharmacognostical studies on Aegle marmelos Corr. and Catharanthus roseus Linn. (anti-diabetic plants)" is reviewed under the following heads:

I. Pharmacognostic studies couducted on the experimental plants.

II. Pharmacognostic studies conducted on the plants belonging to the family Rutaceae and Apocynaceae.

III. General researches relat ed to the present investigation.

I. Pharmacognostic studies conducted on the experimental plants:

Anatomical and external characters of seed of Aegle marmelos Corr. have been described. Seed kernels contain protein (62%), oil (30.5%), carbohydrate (2.3%), and ash (2.5%) on a dry weight basis. (Banerji et al., 1982).

Pharmacognostical studies were conducted on the roots of Catharanthus roseus Linn. and its adulterants. Macroscopic characters of roots of white and pink flowered forms of Catharanthus roseus Linn. were found to be

different from those of their adulterants in form, colour and odour. Microscopically, both forms were distinguished mainly on the basis of their size of starch grains and vessels. Powdered samples of both authentic drug and the adulterants under UV radiation showed variation in fluorescence properties (Srivastava et al., 1988)

II. Pharmacognostic studies conducted on the plants belonging to the family Rutaceae and Apocynaceae:

Pharmacognostical studies were carried out on Vallaris solanaceae by Mukherjee et al., (1980). Macro and microscopic characters, quantitative values, fluorescence characteristics, chemical tests, ash and extractive values of the root, stem and leaf of V.solanaceae have been reported.

Botanical and chemical characteristics of 4 sps. of Ervatamia viz., E.orientalis, E.daemeliana, E.obtusisuscula and E.lifuana have been investigated. The 4 sps. are distinct botanically as well as chemically. A key for botanical identification of the 4 sps. has been provided (Allorge et al., 1980).

Yuxiu et al., (1981) studied the morphological and histological characters, colour reactions, capillary chromatograms and thin - layer chromatograms of 8 sps. of Rauvolfia, viz., R.yunnanensis, R.latifrons, R.tiaolushanensis, R.vomitoria, R.hainanensis,

R.oblanceolata, R.rubrocarpa, have been compared. Morphology, histology and thin-layer chromatograms of 6 adulterants of Rauvol ia viz., Ervatamia officinalis, E.hainanesis, E.divaricata, Alstonia scholaris, Winchia calophylla and Evodia lepta have also been studied.

Datta and Datta (1982) studied the pharmacognosy of Allamanda bark drugs. Bark samples of A.catharitica have been studied in the chemical, histochemical, anatomical, macromorphological, fluorescence characteristics and other pharmacognostic information have been given.

Pharmacognostic studies were conducted on the leaf of Aganosma dichotoma in 1984 by Wahi et al. The macroscopical and microscopical characters along with the numerical values, fluorescence characteristics and physical constants have been studied with a view to bring out its pharmacognostical characters. Preliminary studies of leaf showed the presence of sterol, glycoside, flavonoids, resins, tannins and reducing sugars.

Pharmacognostic evaluation of the bark drug of Alstonia scholaris has been discussed. Diagnostic features of the apparently similar bark of A.macrophylla have also been reported (Datta et al., 1984)

Hypoglycaemic activity of some medicinal plants such as Aegle marmelos, Salacia reticulata and Momordica charantia have been studied with their aqueous extracts of

root barks and fruit juices taken orally (Karunanayake et al., 1984). Kong et al.,(1986) studied the pharmacognostic differentiation between Murraya paniculata and Murraya koengii..

Pharmacognostic studies were conducted by Ochamendi et al., (1986) on the foreign sps. of Rauvolfia cultured in Cuba. Results of pharmacognostic study of the roots of three foreign species of Rauvolfia: R.caffra: R.serpentina and R.vomitoria are given. Such species were recently introduced in Cuba with the purpose of their possible utilization as a source of reserpine.

Therapeutic importance, macro and micro morphology, chemistry and tissue culture studies on 10 Apocynaceae drugs viz., Aganosma dichotoma, Allamanda catharitica, Carissa caranca, Catharanthus roseus, Ervatamia dichotoma, E.coronaria, Ichnocarpus frutescens, Nerium indicum, Digitalis spp., Rauvolfia serpentina have been reviewed (Sen et al., 1987).

Dey and Das (1988) conducted pharmacognostic studies on the rhizome, root, stem, leaf, flower and fruit of Alstonia scholaris, Musa paradasiaca, Ficus hispida etc. Macroscopic and microscopic observation ware carried out for the authentication of the drug samples. Ash and extractive values were found out. Quantitation of crude alkaloids, total sugar, protein, nitrogen, spectrophotometric analysis of the pigments and fluorescence analysis were also studied.

III.General researches related to the present investigation:

Under ultra violet radiation after treatment with several reagents, numerous powdered vegatable drugs and some powdered animal drugs were examined. The characteristic fluorescence of each drug was observed and data pertaining to colour and intensity were recorded. A similar observation was made by Chase et al., (1949) and a study on "A Nomenclature of colours for Naturalists and Compendium of useful knowledge for Ornithologists" was made by Ridge Way (1986) (Kokoshi et al.,1958).

Atique et al., (1985) found that young leaves of custard apple (A.squmosa) together with black pepper (P.nigrum) are used by tribemen in certain villages of Aligarh district to cure diabetes. Some clinical data and folk claims are also presented here.

A bibliographic survey on the anatomical study of 312 Indian medicinal plants for the period 1970-1984 has been given. For each plant, the botanical names,family, common name, Indian names, plant part investigated and the reference number have been incorporated in a tabular form, Various shortcomings in research papers on anatomical studies of plant parts have also been discussed from the pharmacognostic point of view and suggestions have been made for the proper botanical description, so that the details are helpful in the proper authentication of crude drugs (Bagchi and Puri, 1985)

The article by Lela (1987) reports new ways of looking at Ayurvedic drugs in the treatment of diabetes mellitus, cancer etc. Gymnema sylvestre, Momordica charantia , Tinospora cordifolia, Tribhanga - shila showed significant result in the treatment of diabetes.

Folk-lore uses of 26 anti-diabetic plant sps. occurring in Rayalaseema have been reported. The method of preparation and dose of administration of crude drugs as suggested by tribal and non-tribal herbalists are recorded. Also the known chemical constituents of these plants are reported. (Nagaraju et al., 1989).

The paper presented by Joshi et al., (1989) deals with the phytochemical screening for alkaloids, saponins and tannins of 182 samples consisting of 147 species which are used as medicine either in indigenous system or by aboriginal tribes of Gujarat forests.

The paper presented by Alam, et al., (1990) reports about some medicinal plants used in the treatment of diabetes which includes Aegle marmelos.

Role of crude drugs in supplying the active constituents as well as nutrients like carbohydrates, proteins, fats, vitamins and minerals have been discussed by Siddiqui et al., (1991). These drugs not only cure the body but also nourish it and strengthen the defence system. Active constituents as well as nutrients in Plantago major,

P.ovata, Coriandrum sativum, Aegle marmelos etc., have been tabulated .

Bisht et al., (1992) reported the presence of coumarins, flavonoids, steroids, triterpenoids, and xanthones through phytochemical screening of 27 unexplored plants having medicinal or toxic properties.

Methodology

METHODOLOGY

Different parts of the following species of medicinal plants were used in the "Pharmacognostical studies on Aegle marmelos Corr. and Catharanthus roseus Linn. (anti-diabetic plants)":

Table - 1

S.No.	Plant	Part studied
1.	Aegle marmelos Corr.	Leaf
2.	Catharanthus roseus Linn.	Whole plant

Specimens of the above plants were collected at their flowering stage, from different parts of Coimbatore. The plant specimens were made into herbaria and identified from authentic specimens at the Botanical Survey of India, Southern Circle, Coimbatore. Their voucher specimens were kept at the Herbarium, Botany Department, Sri Avinashilingam Deemed University, Coimbatore.

The fresh specimens - leaf of Aegle marmelos Corr. and the leaf, stem and root of Catharanthus roseus Linn, were fixed in FAA. Free hand and microtomic sections were taken. The sections were stained with safranin (aqueous) amd fast - green by adapting Alcohol - Xylol series and mounted in DPX mountant. (Johanson,1940)

The above mentioned plant parts were shade dried and powdered by using Wiley Mill (0.5 mm) at the Sugarcane Breeding Institute, Coimbatore. These plant powders from the experimental plants were taken for further studies. The powder was sieved through 40 mesh sieve plate and proceeded for pharmacognostical studies.

The various methods used in the study includes:

1. Organoleptic Evaluation
2. Microscopic Evaluation
3. The behaviour of the powder with various chemicals
4. Fluorescene Analysis
5. Biochemical Analysis
6. Physical Constant Determination
7. Preliminary Phytochemical Screening

1. ORGANOLEPTIC EVALUATION

This includes the study of the colour, texture and taste of the powdered samples. (Jackson and Snowdown,1968). The results of the above study are presented in Table 2.

2. MICROSCOPIC EVALUATION

The powdered plant samples were bleached by boiling in 5% Chloral hydrate, and were mounted in glycerine. Later the slides were observed under the microscope to find its constituents. The materials seen were drawn in cameralucida diagrams (Fig.5 and 6).

3. BEHAVIOUR OF THE POWDER WITH DIFFERENT CHEMICALS

The sieved plant powders were treated with a number of chemicals in order to study their behaviour. The different chemicals used include:

1. 1N NaOH in Methanol
2. Picric Acid
3. Acetic Acid
4. Conc. Hcl
5. 5% Iodine solution
6. 5% Ferric Chloride
7. Conc. HNO_3
8. Iodine

The behaviour of the powders with different chemical reagents were studied and the results are presented it Table 3.

4. FLUORESCENCE ANALYSIS

To study the fluorescence properties of the powdered samples, they were exposed to the visible light as well as UV radiation as per Kokoshi et al., (1958) and Chase and Pratt (1949) method and the colour of fluorescence emitted by the powders is identified by employing the colour chart of Ridgeway (1886). The following reagents were used to study the fluorescence property:

1. 1N NaOH
2. 1N Hcl

3. 1N NaOH in Methanol

4. 50% $H_2 SO_4$

Fluorescence analysis of the powder of Aegle marmelos Corr. and Catharanthus roseus Linn. were carried out and the results are presented in Table 4.

5. BIOCHEMICAL ANALYSIS

This includes :

a. Estimation of Total Proteins and

b. Estimation of Total Free Sugars

For the estimation of total proteins from the dried drug, Lowry et al., (1952) method was followed. This method is based on the principle that different proteins contain different amounts of aromatic residues, which react with Folin - Ciocalteau reagent giving a blue colour and this is read in colorimeter.

The estimation of total free sugars present in the experimental powders were done using Yemm and Willis (1954) method.

The results of protein and sugar estimation are presented in Tables 5 and 6 respectively.

6. DETERMINATION OF PHYSICAL CONSTANTS

ASH ANALYSIS

To determine the ash values, alcohol, water and acid soluble extractives, the procedure recommended by Anonymous (1966) and Indian Pharmacoepia were followed .

The results of the above tests are presented in Table 7.

7. PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of the powdered samples were carried out in order to find out the presence or absence of phenols, juglone, quinone, oils and fats, cellulose, flavonoids, lignan, syringaldehyde, saponin, sterols etc.

The results of the preliminary phytochemical screening of the two experimental plant powders are presented in Table 8.

Results

Taxonomy and Histology

TAXONOMY AND HISTOLOGY

AEGLE MARMELOS Corr.

Systematic Position

Class	:	Dicotyledonae
Subclas	:	Polypetalae
Series	:	Disciflorae
Order	:	Rutales
Family	:	Rutaceae

Hallier (1905) attributed particular significance to Rutaceae and included it under his order Terebinthales. He regarded it to have originated from some Ranalian ancestor. Engler and Bentham and Hooker included this family under order Geraniales. Benson (1957) included it under order Rutales. Rendle also included it under a separate order Rutales that was placed by him between Geraniales and Sapindales.

Distribution and habit of the plant:

Distribution

Wild in the sub-Himalayan tract, Central and South India; often planted all over India and Burma.

Habit (Photos 1 and 2)

A small or medium sized deciduous tree armed with straight sharp axillary thorns, 2.5 cm. long; leaves alternate, trifoliate, rarely five-foliate; petiole 2.5 to 6.3 cm. long, terete; Leaflets 5-10 by 2.5 - 6.3 cm., ovate -

lanceolate, crenate, acuminate, membranous, pellucid - punctate, the lateral opposite subsessile, the terminal long petioled; Flowers greenish white, sweet scented, about 2.5 cm.across, bisexual, in short axillary panicles; calyx flat, pubescent, four lobed; lobes rounded, sometimes obscure; petals 4, spreading, oblong, thick gland-dotted, much exceeding the sepals, imbricate; stamens numerous, anthers elongate, apiculate; filaments free or fascicled, inserted round an inconspicuous disc, ovary ovoid, cells 10-20; style terminal, short, deciduous; stigma capitate; ovules numerous, biseriate; fruit 5-18 cm.in diameter, globose, grey or yellowish, rind woody; seeds numerous, oblong, compressed with a wooly mucous testa, embedded in orange - coloured sweet pulp (Kirtikar and Basu, 1981).

Morphological nature of the leaf (Hickey, 1973)

I. Duration : deciduous

II. Orientation: apical (apex); convex (base)

III. Lamina :
1. Petiole: Petiolate, normal without noticeable thickenings or other processes
2. Nature : Stipules modified into thorns
3. Shape : Ovate
4. Venation: Pinnate and craspedodromous
5. Margin : Minutely serrate

6. Apex : Obtuse

7. Base : Normal

8. Texture : Coriaceous

Transverse section of the leaf (fig.1)

The T.S. of the leaf of Aegle marmelos Corr. reveals the following features:

EPIDERMIS

Both upper and lower epidermis is present. They are made up of uniseriate, compactly arranged rectangular parenchyma cells. Externally, both the epidermis are protected by cuticle.

Stomata is found to be present only on the lower epidermis.

MESOPHYLL

is differentiated into two portions:

a) Upper columnar, biseriate, compactly arranged cells with chloroplasts forming the palisade parenchyma; presence of oil gland in between the cells and

b) a multiayered loosely arranged parenchyma cells with starch grains forming the spongy parenchyma

VASCULAR REGION

The mid rib portion has two arc shaped vascular portions. The xylem cells are noted below the upper epidermis with the protoxylem facing towards the upper surface of the leaf. The phloem tissues are arranged with sieve tubes, companion cells and phloem parenchyma and are found below the xylem facing the lower epidermis. The two vascular bands are separated by transverse rows of rounded parenchyma cells.

In the midrib region, on either side of the vascular tissue are present rounded parenchyma cells. Termination of the vascular tissue occurs in the form of trachieds constituting the transfusion tissue.

CATHARANTHUS ROSEUS Linn.

Systematic position

Class	:	Dicotyledonae
Subclass	:	Gamopetalae
Series	:	Bicarpellatae
Order	:	Gentianales
Family	:	Apocynaceae

Bentham and Hooker have placed the family Apocynaceae under the order Gentianales. Engler included it under sub-order Gentianineae of the order Contortae. Hallier considered Apocycaceae along with Asclepediaceae and sought their origin from the Linaceae. Bessey accepted the

two families as distinct and treated both in his concept Gentianales which he derived from the Geraniaceae.

Distribution and habit of the plant :

Distribution

Probably a native of America. Very common in Indian gardens.

Habit (Photos 3 and 4)

A beautiful, perennial, herbaceous or somewhat suffruticose plant commonly grown in the gardens; 0.3 to 0.6 m. high; stem herbaceous, erect, cylindrical, green and branched; Leaves exstipulate, sub-sessile, opposite, simple, ovate; deep green polished leaves; Flowers pure white or deep rose-coloured; usually paired and sessile in the axis; base of petiole 2-glandular; corolla-5, fused, 2.5 to 5 cm. diam; fruit an etario of two follicles; 2.3 cm. long; seeds endospermic and unappendaged (Kirtikar and Basu, 1981)

Morphological nature of the root

Size, colour and behaviour:

Tap root; branched; 10 to 13. cms long; creamy white in colour; hard and tough.

Transverse section of the root (Fig.2)

The T.S. of the root of Catharanthus roseus Linn. shows the following regions:

EPIDERMIS

In older roots, the epidermis is replaced by the formation of periderm which consists of the phellum, phellogen and phelloderm.

CORTEX

Following the periderm is the secondary cortex formed of thin walled parenchyma cells with starch grains and intercellular spaces.

The endodermis and the pericycle are not visible in the mature root.

VASCULAR REGION

This mainly consists of the secondary xylem and phloem. In mature root, the secondary phloem occurs in the form of definite radial rows. They are formed of sieve tubes, companion cells and phloem parenchyma.

The primary xylem is recognisable towards the centre. The secondary xylem is made up of trachieds, vessels and xylem parenchyma.

PITH: is very little and becomes completely obliterated in older roots.

Morphological nature of the stem

Size, Colour and Behaviour:

60cms. in height; 1.4 cms. in diameter at the base and 6mms. diam.(average) for branches; green in colour; tough.

Transverse section of the stem (Fig.3)

The T.S. of the stem shows the following regions:

PRIMARY STRUCTURE

EPIDERMIS

Single layered; made up of regular, rectangular, compactly arranged parenchyma cells; outer wall cutinised.

CORTEX

Heterogenous; made up of two types of cells:

a) outer multilayered chlorenchymatous cortex and
b) inner loosely arranged multilayered parenchymatous cortex

ENDODERMIS

Inner most layer of the cortex; single layered; made up of compactly arranged barrel shaped cells with casparian strips.

STELE

Collateral, endarch, open.

SECONDARY STRUCTURE

After secondary thickening, the cortical portion of the stem shows the bark formed of phellum, phellogen and phelloderm.

In the stelar region, secondary xylem and secondary phloem are formed. The secondary phloem is formed of sieve tubes, companion cells and phloem parenchyma.

The pith is large, multilayered and parenchymatous.

Morphological nature of the leaf (Hickey, 1973)

I. Duration : Evergreen

II. Orientation: apical (apex); convex (base)

III. Lamina :
1. Petiole: Petiolate, normal without noticeable thickenings or other processes
2. Nature : Exstipulate
3. Shape : Ovate
4. Venation: Pinnate and Craspedodromous
5. Margin : Entire
6. Apex : Mucronate
7. Base : Normal
8. Texture : Smooth

Transverse section of the Leaf (Fig.4)

The T.S. of the leaf shows the following regions:

EPIDERMIS

Both upper and lower epidermis are present. They are provided with a single layer of rectangular parenchyma cells which are closely packed without intercellular spaces. Externally both the epidermis are protected by cuticle.

Stomata is found only on the lower epidermis.

MESOPHYLL

is differentiated into an upper columnar uniseriate, compactly aranged cells with chloroplasts forming the palisade parenchyma and a multilayered, loosely arranged parenchyma cells with starch grains forming the spongy parenchyma. Few laticiferous canals were found.

VASCULAR REGION

The midrib portion has a well developed vascular tissue with xylem and phloem. The metaxylem is found facing the lower epidermis and protoxylem facing the upper epidermis.

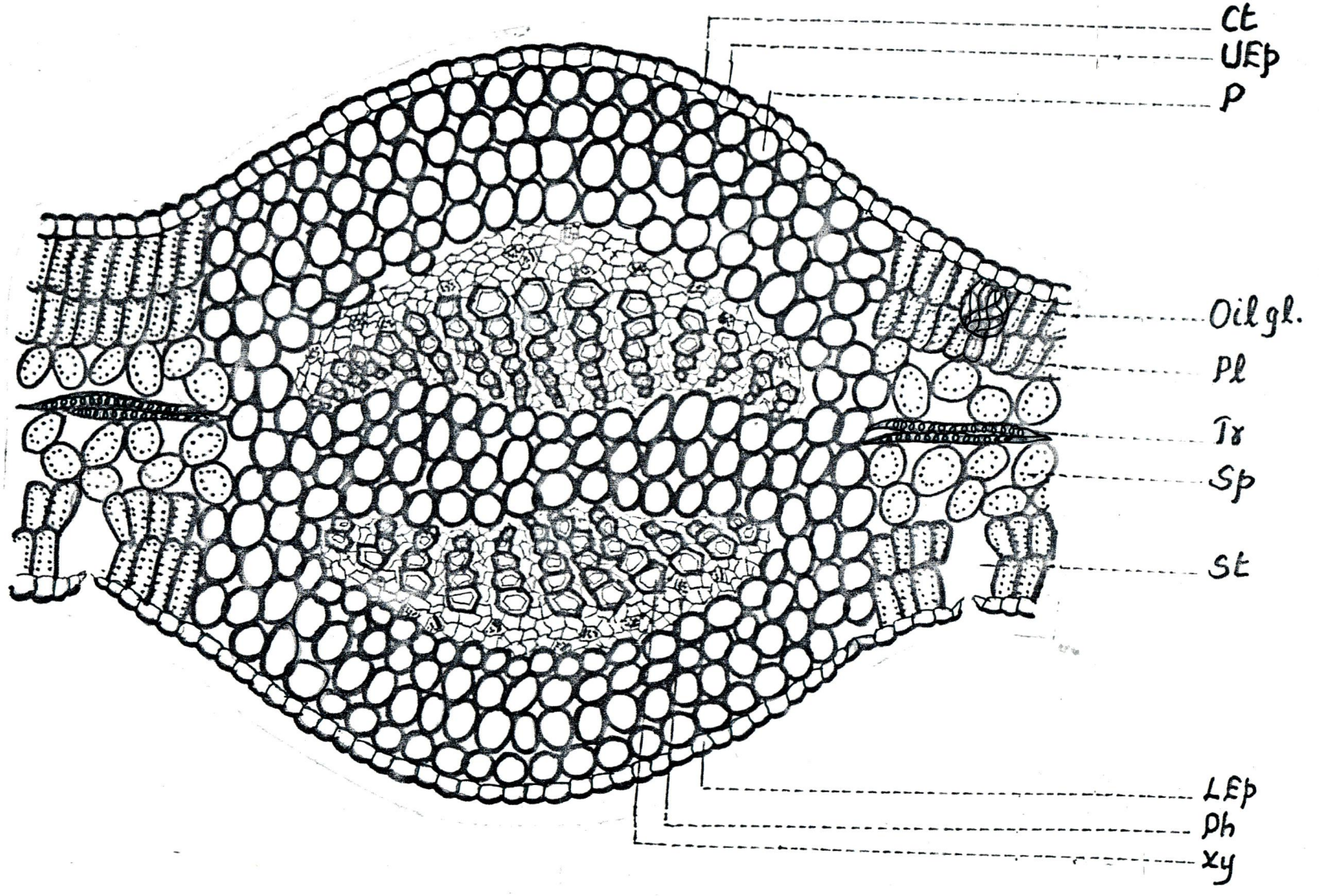
The phloem consists of phloem parenchyma, sieve tubes, companion cells and is present facing the lower epidermis below the xylem.

In the midrib portion, on either side of the vascular tissue are found rounded parenchyma cells. Termination of the vascular tissue occurs in the form of trachieds constituting the transfusion tissue.

Legend

Ct	-	Cuticle
UEP	-	Upper Epidermis
P	-	Parenchyma cells
Oil gl	-	Oil gland
Pl	-	Palisade parenchyma
Tr	-	Transfusion tissue
Sp	-	Spongy parenchyma
St	-	Stomatal pore
LEP	-	Lower Epidermis
Ph	-	Phloem
Xy	-	Xylem

Aegle marmelos Corr.
Leaf-T.S Fig.no.1



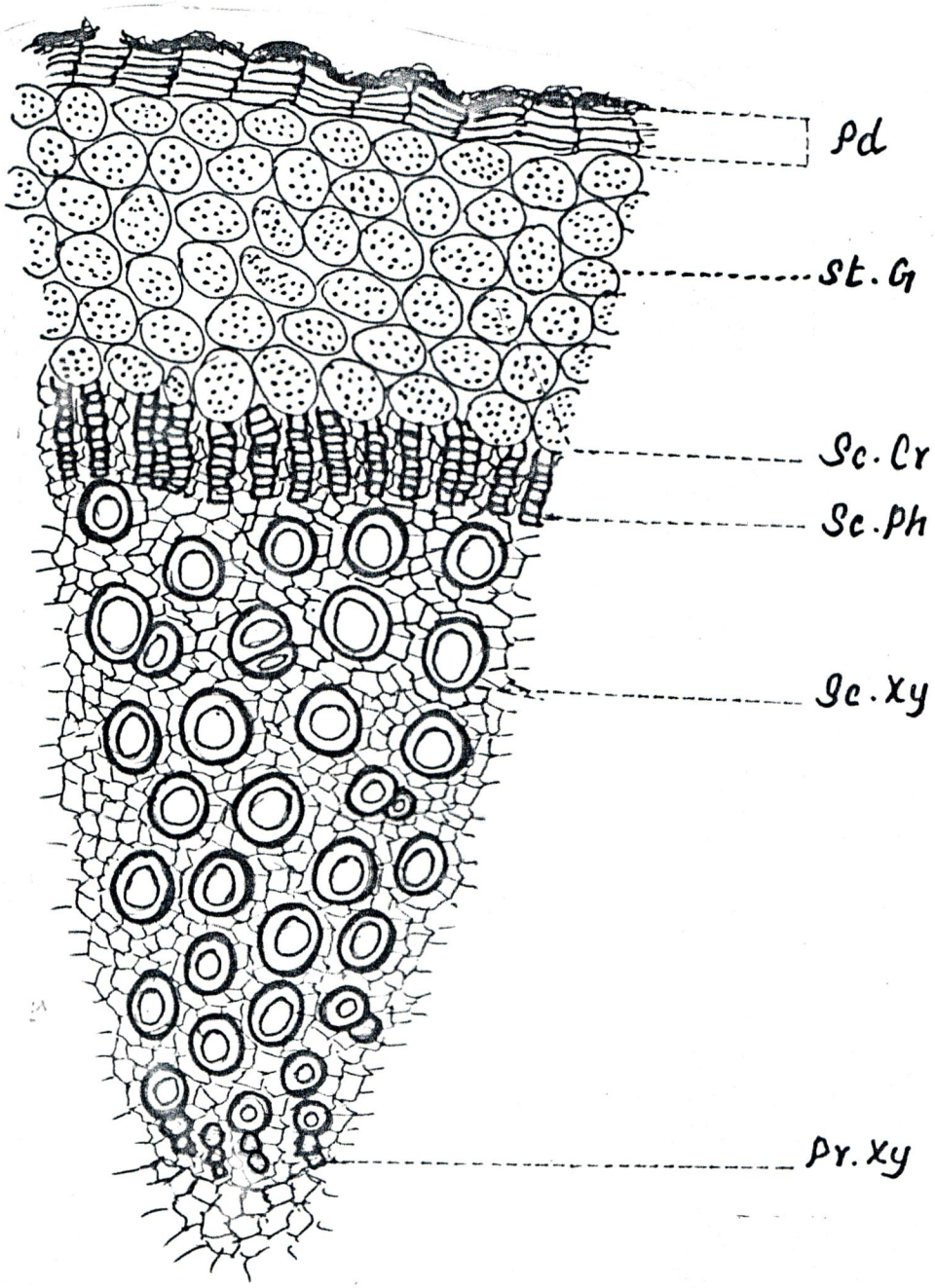
Legend

Pd	- Periderm
Sc.Cr	- Secondary cortex
Sc.Ph	- Secondary Phloem
Sc.Xy	- Secondary xylem
Pr.Xy	- Primary xylem
P	- Pith
St.G	- Starch Grains

Catharanthus roseus Linn.

Root - T.S

Fig. no. 2



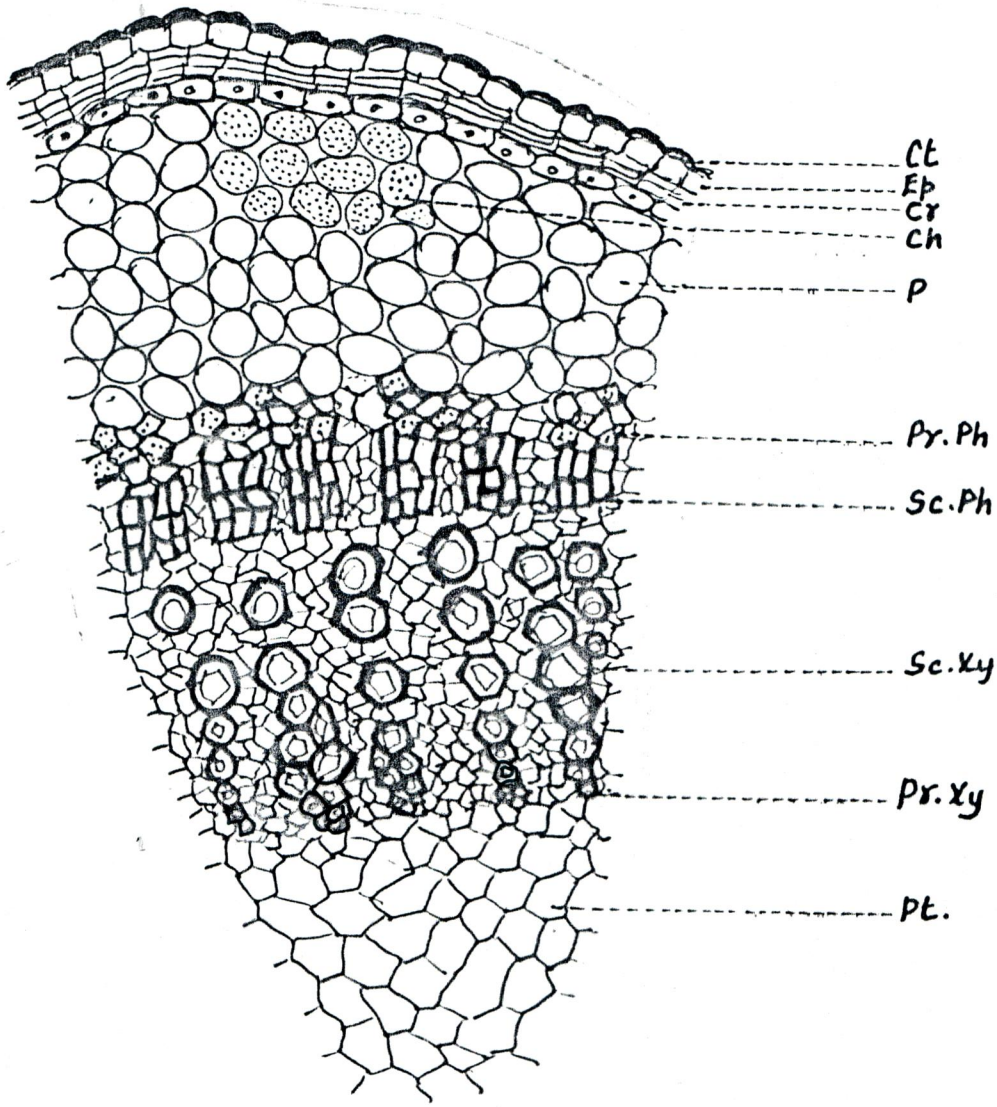
LEGEND

Ct	-	Cuticle
Ep	-	Epidermis
Cr	-	Cork cells
Ch	-	Chlorenchyma cells
P	-	Parenchyma cells
Pr. Ph	-	Primary phloem
Sc. Ph	-	Secondary phloem
Sc. Xy	-	Secondary Xylem
Pr. Xy	-	Primary Xylem
Pt	-	Pith

Catharanthus roseus Linn.

Stem - T.S

Fig. no. 3



LEGEND

Ct	-	Cuticle
UEP	-	Upper Epidermis
P	-	Parenchyma cells
Pl	-	Palisade parenchyma
Sp	-	Spongy parenchyma
Tr	-	Transfusion tissue
St	-	Stomatal pore
LEP	-	Lower Epidermis
Ph	-	Phloem
Xy	-	Xylem

Catharanthus roseus Linn.

Leaf-T.S
Fig.no.4

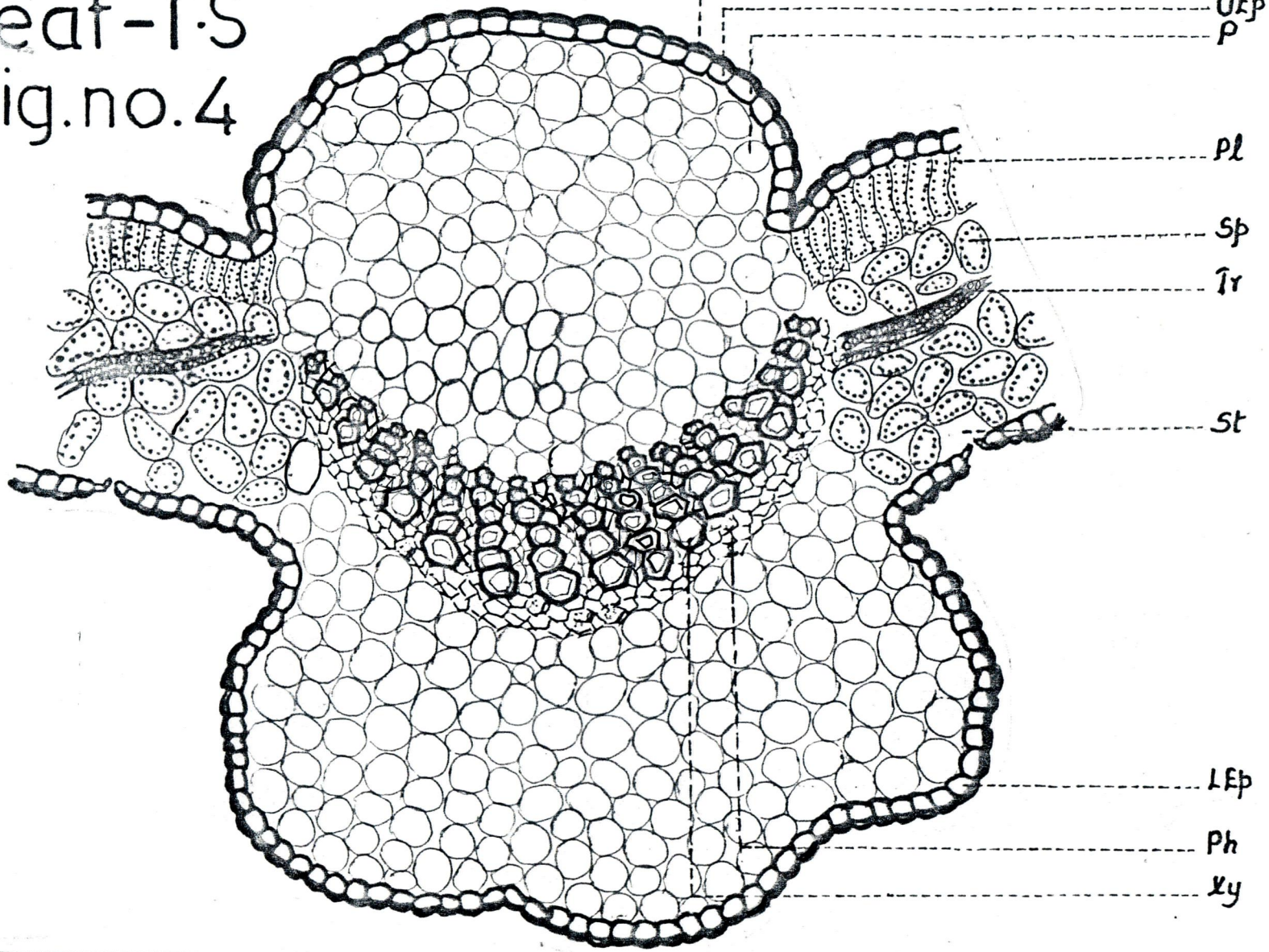


PHOTO 1

PHOTO 2

PHOTO 3

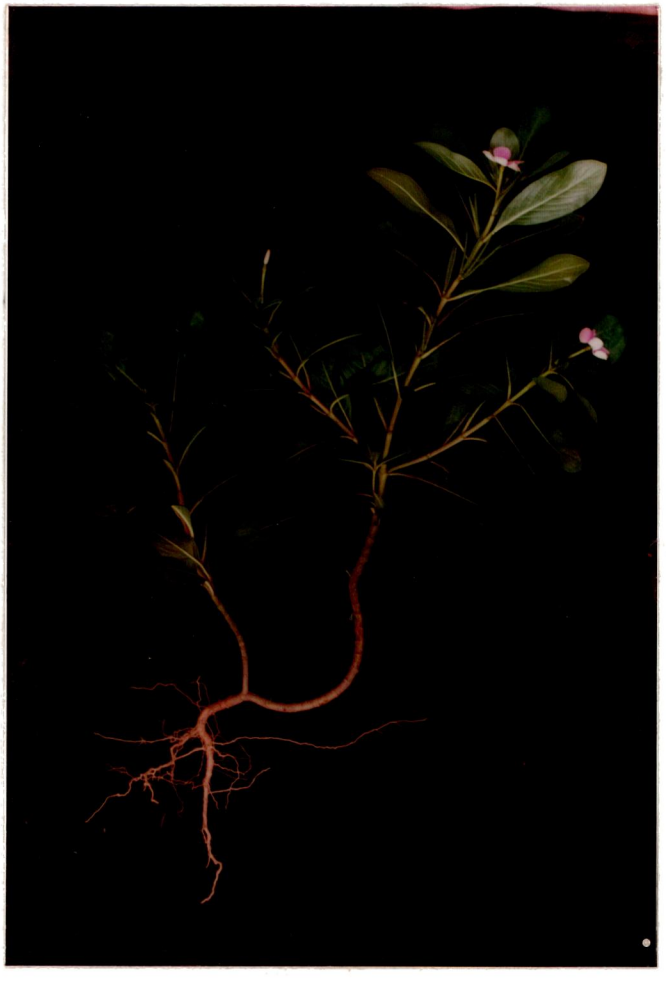


PHOTO 4

RESULTS

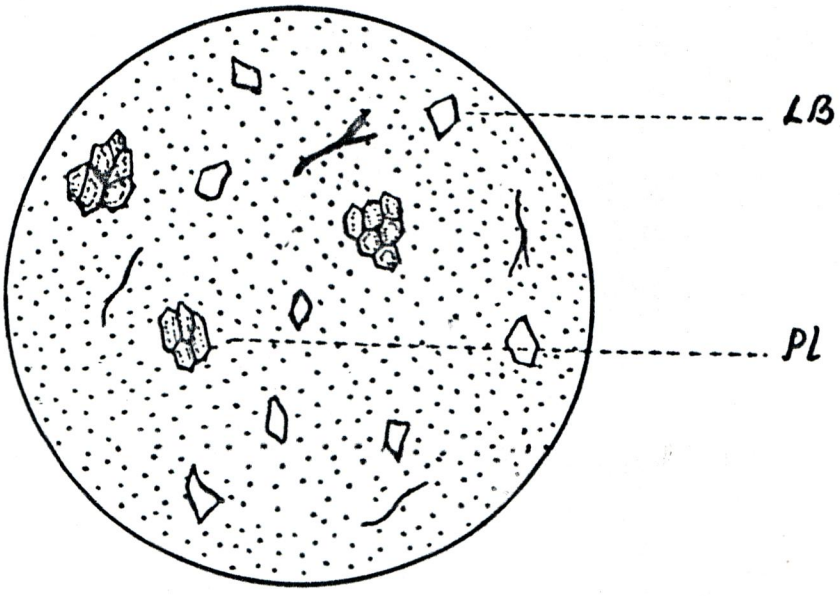
ORGANOLEPTIC STUDY OF THE POWDER

TABLE 2

S.No.	TESTS	AEGLE MARMELOS Corr.	CATHARANTHUS ROSEUS Linn.
1.	Colour	Green	Pale Green
2.	Texture	Coarse	Coarse
3.	Taste	Bitter	Bitter

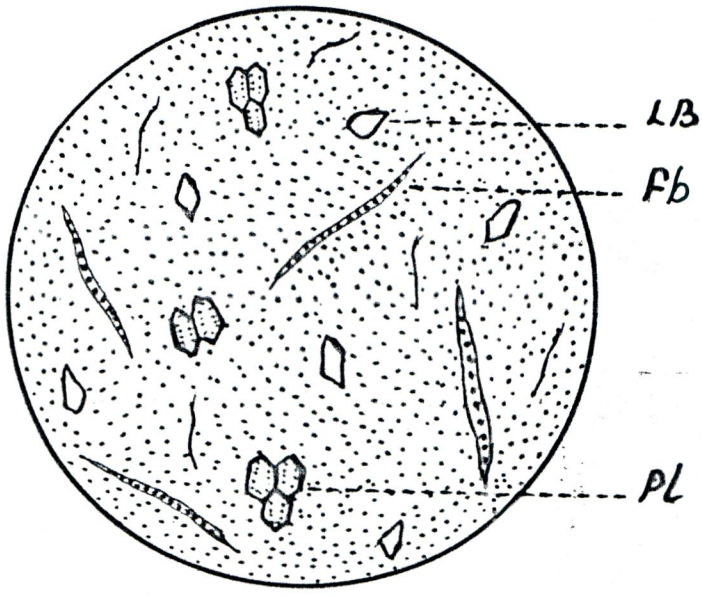
Aegle marmelos Corr.

Fig. 5



Catharanthus roseus Linn.

Fig. 6



THE BEHAVIOUR OF THE POWDER WITH DIFFERENT CHEMICALS

TABLE 3

S. No.	TREATMENT WITH CHEMICAL REAGENTS	OBSERVATIONS	
		AEGLE MARMELOS Corr.	CATHARANTHUS ROSEUS Linn.
1.	Powder as such	Green	Pale Green
2.	Powder + 1 N NaOH in Methanol	Greenish Yellow	Greenish Yellow
3.	Powder + Picric acid	Yellow	Yellow
4.	Powder + Acetic acid	Green	Greenish Yellow
5.	Powder + Conc.Hcl	Purple	Green
6.	Powder + 5% Iodine solution	Reddish Brown	Greenish Brown
7.	Powder + 5% Ferric Chloride	Blackish Green	Blackish Green
8.	Powder + Conc.HNO_3	Reddish Brown	Pale Brown
9.	Powder + Iodine	Brownish Green	Blackish Green

FLUORESCENCE ANALYSIS

TABLE 4

S. NO.	TREATMENT WITH CHEMICAL REAGENTS	OBSERVATION			
		AEGLE MARMELOS Corr.		CATHARANTHUS ROSEUS Linn.	
		VISIBLE LIGHT	U.V.LIGHT	VISIBLE LIGHT	U.V.LIGHT
1.	Powder as such	Green	Green	Pale Green	Pale Green
2.	Powder + 1N Hcl	Green	Brownish Green	Green	Brownish Green
3.	Powder + 1N NaOH	Pale Brown	Brown	Brownish Green	Brownish Green
4.	Powder + 1N NaOH in Methanol	Greenish Yellow	Dark Green	Greenish Yellow	Dark Brown
5.	Powder + 50% H_2SO_4	Purple	Black Green	Blackish Black	Greenish

BIOCHEMICAL STUDIES

ESTIMATION OF TOTAL PROTEINS (LOWRY *et al.*, 1952)

Table 5.

S.No.	PLANT NAME	% OF PROTEIN/GRAM
1.	*AEGLE MARMELOS* Corr.	* 55.2
2.	*CATHARANTHUS ROSEUS* Linn.	* 48.7

* Average value of 2 replicatives per gram of the dry plant powder

ESTIMATION OF TOTAL FREE SUGARS (YEMM and WILLIS 1954)

Table 6.

S.No.	PLANT NAME	% OF SUGAR/GRAM
1.	AEGLE MARMELOS Corr.	* 5.23
2.	CATHARANTHUS ROSEUS Linn.	* 10.8

* Average value of 2 replicatives per gram of the dry plant powder.

PHYSICAL CONSTANT DETERMINATION

ASH ANALYSIS

Table 7.

S.NO.	NAME OF THE PLANT	TOTAL CONTENT	ACID INSOLUBILITY	SOLUBILITY IN WATER	SOLUBILITY IN ALCOHOL
1.	AEGLE MARMELOS Corr.	* 11.5%	* 5.2%	* 4.2%	* 9.8%
2.	CATHARANTHUS ROSEUS Linn.	* 8.5%	* 4.8%	* 8.4%	* 12.7%

* Average value of 2 replicatives.
Dry powder 10 grams.

PHYTOCHEMICAL SCREENING

Table 8

PHYTOCHEMICAL TEST	PLANT NAME	OBSERVATION	RESULTS
1. HOT WATER TEST:			
Mature leaf is dipped part-way into hot water 85 °C steadily for 5 seconds.	*Aegle marmelos* Corr.	Blackish Brown band develops	Presence of phenols
	Catharanthus roseus Linn.	Blackish Brown band develops	
2. Hcl. METHANOL TEST:			
A small amount of the powder is put into a test tube and covered with Methanol and Conc. Hcl (4:1) and stoppered Then the tube is allowed to stand. Occasional shaking for 4 to 5 hours.	*Aegle marmelos* Corr.	Reddish Brown band develops	Presence of Tannin
	Catharanthus roseus Linn.	Blackish Green band develops	
3. TEST FOR JUGLONE			
2 gms of finely chopped powder is put in a test tube and chloroform is added till it covers. It is stoppered and kept for several hours. The Chloroform extract is filtered off, evaporated to dryness over a water bath and the residue is taken up in a few ml. of ether and equal volume of dil.ammonia (1 vol. of conc.ammonia + 9 vol. water) and the mixture is shaken gently.	*Aegle marmelos* Corr.	No characteristic reaction	Absence of Juglone
	Catharanthus roseus Linn.	Blackish Green band develops	presence of Juglone

Contd.. Table 8.

PHYTOCHEMICAL TEST	PLANT NAME	OBSERVATION	RESULTS
4. SYRINGIN TEST			
Freshly hand cut leaf sections are mounted in a drop of aqueous H_2SO_4 (50%)	*Aegle marmelos* Corr.	No characteristic reaction	Absence of syringaldehyde
	Catharanthus roseus Linn.	No characteristic reaction	
5. SAPONIN TEST			
A small amount of fresh leaves are finely chopped, placed in small glass stoppered test tube and 5 ml of water is added. The contents are then boiled for 2 minutes, cooled, shaken vigorously and set aside for 5 minutes	*Aegle marmelos* Corr.	No characteristic reaction	Absence of saponin
	Catharanthus roseus Linn.	No characteristic reaction	
6. LIBERMANN - BURCHARD TEST			
50% H_2SO_4 is added to a mixture of Methanolic extract and Acetic Anhydride	*Aegle marmelos* Corr.	Reddish Brown band develops	Presence of Triterpenoids
	Catharanthus roseus Linn.	Blackish Green band develops	
7. SALAKOWSKI REACTION			
Chloroform and conc. H_2SO_4 are added to the alcoholic plant extract	*Aegle marmelos* Corr.	Pinkish Black colour develops	Presence of steroidal nucleus
	Catharanthus roseus Linn.	Blakish Brown band develops	
8. PHENOLIC TEST			
Plant extract + Ferric chloride solution	*Aegle marmelos* Corr.	Blackish Green precipitate formed	presence of phenols
	Catharanthus roseus Linn.	Blackish Green precipitate formed	

Contd.. Table 8.

PHYTOCHEMICAL TEST	PLANT NAME	OBSERVATION	RESULTS
9. TEST FOR CELLULOSE			
To the powder was added Iodine solution followed by H_2SO_4	*Aegle marmelos* Corr.	Pinkish Black colour develops	Presence of cellulose
	Catharanthus roseus Linn.	Pale Brown colour develops	
10. TEST FOR FIXED OILS AND FATS			
To the powder was added Sudan III.	*Aegle marmelos* Corr.	Reddish Brown colour develops	Presence of Fixed oils and Fats
	Catharanthus roseus Linn.	Reddish Brown colour develops	
11. TEST FOR FLAVONOID			
To the powder was added 10% NaOH	*Aegle marmelos* Corr.	Yellow band develops	Presence of Flavanoids
	Catharanthus roseus Linn.	Yellow band develops	
12. TEST FOR QUINONE			
To the powder was added Conc.Hcl.	*Aegle marmelos* Corr.	Pink colour develops	Presence of quinone
	Catharanthus roseus Linn.	No characteristic reaction	Absence of quinone
13. TEST FOR SAPONIN			
a. Lead acetate solution was added to the powder	*Aegle marmelos* Corr.	No characteristic reaction	Absence of saponin
	Catharanthus roseus Linn.	No characteristic reaction	
b. The powder was shaken well with water	*Aegle marmelos* Corr.	No frothing	Absence of saponin
	Catharanthus roseus Linn.	No frothing	

Contd.. Table 8.

PHYTOCHEMICAL TEST	PLANT NAME	OBSERVATION	RESULTS
14. TEST FOR SUBERIN			
The powder was heated with Conc.H_2SO_4	*Aegle marmelos* Corr.	Pinkish Black precipitate formed	Presence of suberin
	Catharanthus roseus Linn.	Blackish Brown precipitate formed	
15. TEST FOR STARCH			
Iodine solution was added to the plant powder	*Aegle marmelos* Corr.	Reddish Brown band develops	Presence of starch
	Catharanthus roseus Linn.	Reddish Brown band develops	

Discussion

DISCUSSION

For centuries the Indians have perpetuated an empirical science of herbology in relation to health which has been essentially ignored during these days of great advances in biomedicine. The healing science programme, encompassing traditional, spiritual and mythical roles, is both elaborate and lengthy. Apprentices of Navojo medicine for men, for example, learn to use nearly 200 medicinal plants often applying them to both physical and mental afflictions. People of prehistoric times used plants intuitively for food and shel ter apart from their medicinal uses. (Lewis and Elvin-Lewis, 1977).

The plants are omnipresent and grow in large scale and their forms are distinct and peculiar. They are easily available without any difficulty and provide all types of minerals, chemicals, etc., to man to prepare drugs.

Many medicinal plants served as the sole remedy for human diseases. Different parts of the plants, such as leaves, flowers, seeds, fruits, bark and roots were used in the treatment of various diseases like bronchitis, asthma, kidney troubles, digestive disorders, ulcers and small-pox. (Mac Leod, 1981).

Thompson studied 660 cuneiform tablets and made up a list of 250 drugs used by the Assyrians, many of which were also found to be in Egyptian prescriptions. Among the drugs commonly used were poppy, Indian hemp, Mandrak, carrub, Myrrh, Thyme, Juniper, Henbane, Colocinth, Saffron, Alum, Sulphur, Copper etc. (Pasquale, 1984).

To escape from the side effects of Allopathic medicines many are turning their attention to medicines of Ayurvedic preparations. Furthermore, the modern science finds no way to cure permanently certain diseases like leprosy, cancer, asthma, diabetes, rheumatism etc. But man can save the generation from diseases by utilizing the plants.

The fact that, of the approximately 600,000 medicinal plant species on earth, only about 5 per cent have been specifically investigated chemically or pharmacognostically is a challenge to chemists specializing in natural substances and to pharmacologists. It is thus necessary in the future to concentrate specifically on projects leading to the development of new medicinal preparations. (Wagner and Wolff, 1977).

Since Aegle marmelos Corr. is used in the treatment of diabetes mellitus, asthma and jaundice and that of Catharanthus roseus Linn. in the treatment of diabetes mellitus and cancer, an attempt is made to characterize the

drugs pharmacognostically with the following parameters: taxonomy, anatonomy, powder study, biochemical estimation, physical constant determination and phytochemical screening.

Identification and naming of drugs based upon some fundamental characteristics will bring drugs into large groups, each having some particular feature in common. Moreover pharmacognosy arises out of classification proper. The identification of the source of the material forming the drug and to determine its morphological nature are the two main functions of the Pharmacognosist (Wallis, 1985). In the present study Aegle marmelos Corr. was identified to be a deciduous tree, armed with spines whereas, Catharanthus roseus Linn. is an evergreen perennial herb. The leaves of the former is aromatic, gland-dotted, glabrous and trifoliate and that of the latter is simple, opposite, highly polished with axillary glands. The flowers of the former are greenish white and scented and that of Catharanthus roseus Linn. is pink or white arranged in such a way giving a cart-wheel appearance.

Anatomical characters are most important to check adulterations and substitutions and have on occasion been instrumental to establish guilt or innocence of suspected criminals. Furthermore, these characteristics make the identification of the plant possible even without the flowers (Metcalfe and Chalk, 1972). Correct conclusions can be arrived at only when more than one parameter is studied.

Present anatomical investigation on the leaf of Aegle marmelos Corr. shows the presence of upper epidermis; mesophyll with oil gland; lower epidermis with stomata; and two arc shaped vascular strands separated by parenchyma cells (Fig.1).

Anatomical studies on the leaf of Catharanthus roseus Linn. shows the presence of epidermis; mesophyll; a single vascular strand; and stomata on the lower epidermis. As Metcalfe and Chalk (1972) have reported, laticiferous canals were present in the mesophyll. (Fig.4).

Studies on the stem shows secondary thickening with the formation of periderm, heterogenous cortex and vascular region with secondary xylem and phloem. According to Metcalfe and Chalk (1972) intraxylary phloem was present in the stem, but in the present study it was found absent. (Fig.3).

Anatomical studies on the root, showed the formation of periderm; secondary cortex with starch grains; secondary xylem and phloem; and a small pith. (Fig.2).

To investigate the potency, purity and freedom from admixture, the powders were evaluated microscopically. The following components were observed: leaf bits and palisade cells were found in both; fibres appeared only in Catharanthus roseus Linn. (Fig.5 and 6).

Pandey et al., (1984) and Gupta, (1985) treated the plant powders with different chemical reagents and observed their behaviour. The same method was carried out and the results are presented. (Table 3).

The fluorescence properties are found to be a valuable aid in the identification of the powdered drug. Many substances, both plant and animal origin exhibit fluorescence when exposed to UV radiation. It is characteristic both qualitatively and quantitatively. Since the solvent and the pH are capable of modifying the fluorecence of many substances, the powder is treated with different chemicals and then observed under ultra violet light. (Kokoshi et al., 1958 and Chase and Pratt, 1949). A similar study was made, brown and brownish green fluorescence was found predominant. (Table 4).

Most biochemicals formed in an organism are the result of the metabolic pathways. These biochemicals are formed as intermediary products. These chemicals can alter the activity of the drug. In the present study, the protein content was markedly greater than that of sugar in both the plants. (Tables 5 and 6).

The mineral composition in a drug is most important for enzymatic activities concerned with the metabolism of the organism. Hence the physical constant is determined to find out the insolubility of the ash, thereby to determine

the geochemical content. The total ash content was found to be 11.5% in Aegle marmelos Corr. and 8.5% in Catharanthus roseus Linn. (Table 7).

The active principles in a drug is most important in curing the diseases and the supreme task is to link the drugs according to the constituents to which the activity is due, in order to construct a pharmaco-chemical method that leads to pharmacognostical study (Pasquale, 1984). In the present study it was found that phenols, tannin, triterpenoids, steroids, cellulose, fixed oils, fats, flavonoids, suberin, starch were present in both the plants; quinone was present only in Aegle marmelos Corr. whereas juglone was present only in Catharanthus roseus Linn. (Table 8).

Apart from the above chemical properties the plant Aegle marmelos Corr. (Vyas et al., 1979; Lalithakumari, 1988 and Santhoshumari et al., 1990) and Catharanthus roseus Linn. (Morrison et al., 1982) shows hypoglycaemic activity and hence they act as prime anti-diabetic plants. However, its efficacy in the treatment of diabetes mellitus, will be confirmed after determining its lethal dose and toxicity by our pharmacological studies in the near future.

Summary and Conclusion

SUMMARY AND CONCLUSION

The present pharmacognostic studies on the anti-diabetic plants - Aegle marmelos Corr. and Catharanthus roseus Linn. are used as a tool to elucidate and evaluate the drugs originating from it.

To study the external and internal characters of the drug, taxonomic and histologic parameters are used respectively.

Powder study of the above is made by using the parameter like

i Powder analysis
- Organoleptic and Microscopic Evaluation.

ii Behaviour of the powder with different chemicals.

iii Reaction of the powder with chemical reagents under UV light.

iv Biochemical estimation of total proteins and total soluble sugars.

v. Determination of ash content and its solubility.

vi Phytochemical screening for active ingredients.

If the above parameters are used in the identification of the drug one can check its adulterants or substitution.

From the present study and previous findings the plants may be given for diabetes mellitus after finding its lethal dose and toxicity.

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