PHARMACOGNOSTIC AND BIOLOGICAL (ANALGESIC ACTIVITY) STUDIES OF *CARISSA EDULIS* VAHL. (FAMILY APOCYNACEAE)

BY

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FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY

ZARIA, NIGERIA.

1997

DECLARATION

I hereby certify that the work reported in this thesis was carried out by me in the Department of Pharmacognosy and Drug Developement, A.B.U. Zaria, Nigeria. The works of other investigators are acknowledged and referred to accordingly.

I solemnly declare that no part of this thesis has been accepted in substance or concurrently submitted in candidature for any other degree.



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CERTIFICATION

This thesis entitled PHARMACOGNOSTIC AND BIOLOGICAL (ANALGESIC ACTIVITY) STUDIES OF THE PLANT *CARISSA EDULIS* VAHL.(FAMILY APOCYNACEAE)*by HAJARA IBRAHIM meets the regulations governing the award* of the degree of PhD (Pharmacognosy) of AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To my Family.

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ABSTRACT

This study deals mainly with the pharmacognostic and biological (analgesic activity) studies of the roots, stem, leaves, fruits and seeds of the plant *Carissa edulis* Vahl.

Carissa edulis belongs to the family Apocynaceae it is a much branched straggling or climbing glabrous shrub up to 16ft (5m) high with paired 2inch (5cm) axillary spines. In northern Nigeria the plant is used with other plants in treating sickle cell anaemia and for treating stomach ailment especially hernia. The fruits are also edible.

A detailed macroscopical, microscopical, evaluation of the morphological parts of the plant and phytochemical studies were carried out. The microscopical studies showed the plant to contain laticifers characteristic of the family Apocynaceae but without a bicollateral (interxylary phloem) vascular bundle. Peculiar groups of epidermal cells were also seen in the upper epidermis of the leaves (a group of panenchyma cells formed a circle with the upper epidermal cells between the elongated palisade cells).

The following quantitative values were obtained from the evaluation of the crude vegetable products: moisture content, roots, 8.0% $^{w}/_{w}$, stem 10.4% w/w leaves *12.6%* $_{w}$/, P, fruits *6.1%* $_{w}$/, seeds 9.3%$^{w}/_{w}$; ash value, roots *13.5%* $_{w}$/ stem, 15.(?%$_{w}$/, leaves *8 .*^{w}*5%* /, fruits 3.7%$^{w}/_{w}$, seeds 7.5^{w}%/ acid insolubule ash value, roots 6.0%^{w}/w, stem 5.7%^{w}/w, leaves 2.0%^{w}/w, fruits 1.2%^{w}/w, seeds 3.2%^{w}/w, alchohol soluble extractive value,: roots, 13.3%^{w}/w, stem, 4.0%^{w}/w, leaves, 20.7%^{w}/w, friuts, 28.3^{w}% /w, seeds, 27^{w}.3% /w, and water soluble extractive value: roots, 12.0%^{w}/w, stem, lO.O%^{w}/w, leaves, 17.0%^{w}/w, fruits, 25%^{w}/w, seeds, 24.3%^{w}/w.

The phytochemical screening of different parts of the plant studied revealed the presence of carbohydrate;;, tannins, flavonoids, cardiac glycoside, cyanogenic glycoside;; (found present only in the Leaves), saponins, steroids and terpenes. Alkalojids,anthraquinones, Resins and balsams were, however absent. The presence of lipids was observed in chemo-microscopy.

Chromatographic analysis indicated the presence of salicylates in the water extracts. Co-chromatographic analyses on paper and thin Layer indicated the presence of methyl salicylates.

The analgesic activity studies showed the water extracts of all organs had activity. The fruit was found to be most active followed by the leaves, seeds, root and stem respectively. Significant different $P<0.05$ was obtained with 50mg/kg roots water extract at 30mins and $P<0.001$ with 50mg/kg seeds and lOOmg/kg fruits water extracts at 45 mins after intraperitonial administrations of the extracts. The doses used were 10, 50, 100 and 150mg per kg body weight of mice, and were prepared from a stock solution of O.lg in 5ml of water extracts of the different parts of the plant were used. 10,20 and 30mg/kg body weight of metamizol (Analgin$^{(R)}$) were used as the standard analgesic. (Ampoules of 20mg/5ml were purchased).

This would justify the use of the plant as analgesics.

;X

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CHAPTER ONE

INTRODUCTION

1. INTRODUCTION

The plant *Carissa edulis* Vahl belongs to the family Apocynaceae. It was formerly known as *C. pubescens* (Irvine, 1961; Hutchinson and Dalziel, 1963).

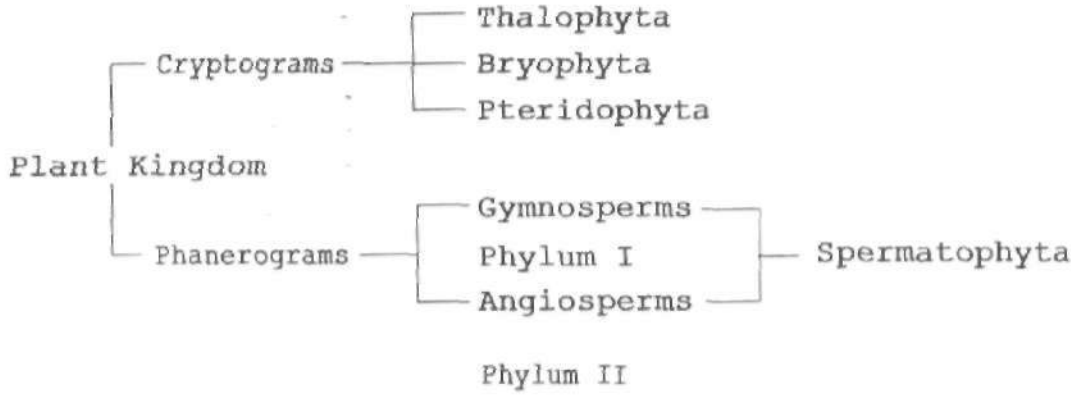
The family Apocynaceae is one of the largest families in the Wilcox Flora; other large families include Lauraceae, Fabaceae, Mimosoidaceae, Caesalpiniodae, Fabordae, Moraceae, Rhamnaceae, Sapindaceae, Sapotaceae, Anacarceae, Celastraceae, Polypodiaceae-L, Arecaceae, Rutaceae, and Meliaceae. (Datta, 1970; Takhtajan, 1969).

The word Apocynaceae was derived from the Greek word "ΑΠΟΚUVOV (Apkunon)" meaning "dog's bane" (Blatter and Millard, 1954; Plowden, 1972). The family is also known as Frangifani family. This name was obtained after the genus *Apocynum,* a name referring to the fruit of two separate carpels, which was thought to resemble two dog's tails (Nielsen, 1965).

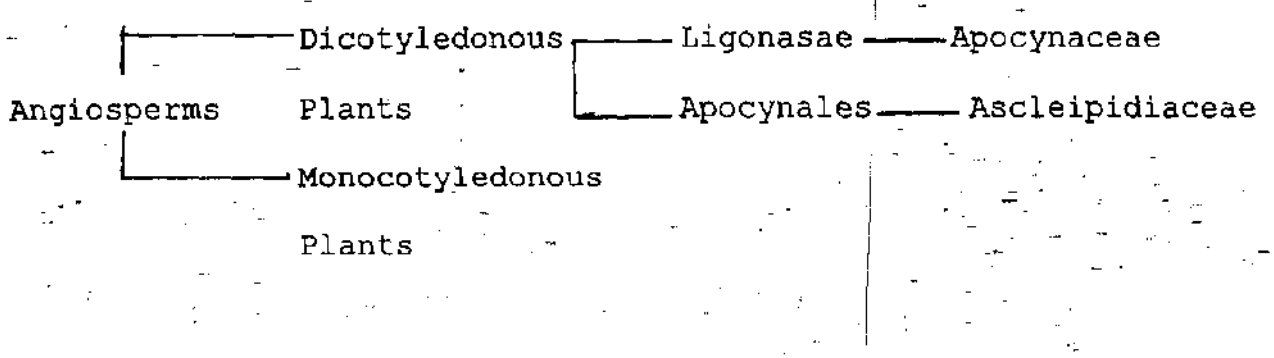
Principally, this family inhabits the intertropical zone of Old and New World especially Asia beyond the equator: Tropical Africa, extending southward to Rhodesia (now Zambia and Zinbabwe), Madagascar, Australia, Arabia, India and Indochina. They are comparatively rare in the hot and temperate regions (Blatter and Millard, 1954; Hooker and Jackson, 1875. Hutchinson and Dalziel 1963; Irvine, 1961). They are mainly found in the tropics and subtropics (Datta, 1973; Hutchinson, 1973; Metcalfe and Chalk, 1953).

Their major habitat is terrestrial. They are usually found in thick forest, forming 20 - 30% of the forest vegetation. They are most commonly found around the mountain or hilly areas.

Apocynaceae is dicotyledonous and belongs to the Phylum Angiosperms in the Vegetative Kingdom. The Vegetative Kingdom is divided into Cryptogram, in which there are no true flowers producing seeds, and Phenerograms in which the plants develop flowers or flower like structures in which seeds are produced. The Phenerograms include all higher plants and by this is meant all present day groups which exhibit the highest development and which are usually referred to as the Spermatophyta. This group is again sub-divided into two phyla, the Gymnosperms and the Angiosperms (Bor and Cumberlege, 1953; Purseglove, 1968).



The Angiosperms can be described further as follows:-



(Bor and Cummberlege, 1953; Mahal, 1979; Metcalfe and Chalk, 1950)

Apocynaeceae is divided into two sub families Echitoidae and Plumeroideae.

The Exhitoidae have corolla - lobes twisted to the right, and the stamen closely adhered to the stigma while plumeroidae have corolla lobes twisted to the left with stamens free or slightly attached to the stigma with anther - tails absent and the seeds are usually hairless.

Members of the Apocynaceae are very easy to identify. The milky latex, the twin pods or follicles and the overlapping lobes of the corolla just like a propeller (Bor and Cumberlege, 1953). This family is related to the Asclepiadceae in general habit, biocollateral vascular bondles, latex tubes, general flora and fruit characters. The two however, can be easily distinguished from each other by the characters of androecium and gynoecium. In Apocynaceae stamens distinct (not united with the stigma), corona (staminal) absent, pollen grains distinct (not in pollinia), and style one (not two) (Dutta, 1971).

The family Apocynaceae comprises of 180-300 genera with 1,800 species (Blatter, 1970, Hickey and King, 1981, Blatter and Millard, 1954).

The Family Apocynaceae has the following genera:

Adenium	Callichilia
Ancylobotrys	Carissa
Anthoditandra	Isonema
Aphanostylis	Malouetia
Catharanthus	Barssea
Clitandra	Strophanthus
Cycldropsis	Holarrhena
Cyclocotyla	Tabernaemontana
Dictyophleba	Vaocanga
Hunteria	Funtumia
Landolphia	Alstonia
Orthopichonia	Callichillia
Picralima	Mascarenhasia
Pleiocarpa	Alafia
Pleioceras	Oncinotis
Pycnobotrya	Motandara
Rauvolfia (Rauwolfia)	Barssea
Saba	Farquharia
Vahadenia	Hedranthera
Pterotaberna	

(Hutchinson and Dalziel, 1963)

Ervatamia

Conopharyngia

Plumeria

Nerium (Excell, 1944)

Haplophyton

Vinca Melodinus

Allamanda Echites

Parsonsia Cerbera

Dipladenia Aspidosperma

Akokanthera Alyxia

Mandevilla Prestonia

Forsteronia Apocynum

(Balfour and Berkeley, 1973; Hutchinson, 1973; Plowden, 1972)

Thevetia

Irachelospermum

Beaumontia

(Plowden, 1972)

Hancornia

(Balfour and Berkeley, 1973)

Cerbera

Kopsia

Pagiantha

Vallaris

Wrightia

Chenemorpha

Aganosma

Anodendron

Ichnocarpus

(Bor and Cumberlege, 1953)

Commonly occurring plants of the family are

Aganosma caryophyllata, (Roxb ex shims) G. Don

Allemanda cathertical

Willoughbeca edulis Roxb.

Beaumontia grandifolia Wall.

Ichnocarpus fructescens R.Br.

These are climbing shrubs

Nerium indicum Mill (Oleander),

Carissa congesta Will, (Bengal currants)

Ervatamia coronaria, Stapf

Thevetia peruviana K. Sçhum (Pears).

These four plants are shrubs

Alstonia scholaris R.Br. (Devil tree or Shatan wood)

Chenemorpha fragrans Alston (Moon)

Holarrhenaa anti-dysenteria Wall. ex A.Dc

Roupellia grata Wall

Plumeria rubra Forma *acuminata* santapau and Irani ex shahi

Wrightia coccinea sims,

Wrightia tinctoria R.Br

Wrightia tomentosa Roem & Schult

These are trees except that *Chenemorpha fragrans* is a giant climber.

(Datta, 1970)

1.1: DESCRIPTION OF THE FAMILY APOCYNACEAE

It is a mainly tropical and subtropical family of a very diverse habit including trees, shrubs, climbers and a few perennial herbs with latex (Metcalfe and Chalk, 1950; Irvine, 1961).

The leaves are opposite or verticillate, rarely alternate, simply entire, stipules usually absent. Flowers hermaphrodite, actinomorphic. Calyx often glandular inside, lobes 5 or rarely 4, imbricate. Corolla tubular, variously shaped, lobes contorted imbricate, very rarely valvate. Stamens 5 or rarely, inserted in the tube, filaments free or rarely united; anther often sagitate, free or connivent around the stigma, rarely adherent to the latter, 2 celled, opening lengthwise, connective often produced at the apex; pollen granular; disk usually present annular, cupular or of separate glands. Ovary superior, I celled with 2 parietal placentas adnate to the septa or capels 2 and free or connate only at the base with ventral placentas in each carpel; style 1, split at the base or entire, thickened and stigmatose below the apex; ovules 2 or more in each carpel. Fruit entire and indehiscent or of 2 separate carpels, baccate drupaceous or follicular. Seeds mostly with endosperm and large straight embryo, often winged or appendaged with long silky hairs (Datta, 1970; Irvine, 1961; Ghazanfar, 1989; Hickey and King, 1981).

The leaf is usually dorsiventral or isobilateral. The outstanding anatomical characters are the universal occurrence

of:

i. laticiferous canals with variously coloured contents

ii. intraxylary phloem or a bicollateral vascular bundle (Bor and Cumberlege, 1953; Dutta, 1971; Mclean, 1949; Mahal, 1979; Metcalfe and Chalk, 1950).

The phytochemical constituents of most plants containing laticiferous canals are present in these secretory cells than other types. Therefore the literature review of the laticifers is hereby undertaken which does not include only the phytochemical constituents that might be encountered but other properties of these laticifers.

1.2 LATICIFERS

A specialized cell or a row of such cells containing latex is termed laticifer. However, latex may also occur in unspecialized parenchyma cells. The term letex is used for a suspension or in certain cases an emulsion of many small particles in a liquid with a different refractive index (the refractive index of the suspension or emulsion is different from the liquid in which the small particles are suspend in) (Fahn 1979; Evans, 1989). The components which form the coarse dispersed phase of the latex can be divided into the following:-

1. Poly isoprene hydrocarbons
2. Triterpenols and sterols
3. Fatty acids and aromatic acids (esterified)

4. Carotenes

5. Phospholipids

6. Proteins

7. Inorganic constituents.

Poly isoprene hydrocarbons $(C_5H_8)_n$ have 2 isomers occurring in nature in which the methyl groups are cis (rubber) or trans (gutta percha) to the double bond. Also the polyisoprene of rubber contains 500 - 5000 residues while gutta - percha contains only about 100. (Fahn, 1979, Kretovich, 1966).

Some specific materials found in the latex of certain plants are sugars (Compositae), variously shaped starch grains *(Euphorbia* species), tannins (*Musa* species) alkaloids (*Papaver somniferum)*, and protein crystals (*Taraxacum bicorne)*.

A large amount of protein occurs in *Ficus callosa*.In *Allium cepa* crystals occur which show predominantly lipoidal properties, and in *Carica papaya* a proteolytic enzyme, papain is present. The latex of some *Euphoria* species was found to be rich in Vitamin B. Crystals of oxalate and malate are abundant in latex. The colour of latex varies in the different plant species - it may be white and milky (e.g. *Euphorbia)*, brown (e.g. *Cannabis)*, orange (e.g. *Chelidonium)*, or colourless (e.g. *Morus, Nerium)*.

Latex occurs in 12,500 species belonging to 900 genera and about 20 families, mostly of the Dicotyledonae however, a few families of the Monocotyledonae and a Pteriodohyte genus

Regnellidum of the Marsilliaceae are also included. Among the dicotyledons laticiferous plants are found in the Apocynaceae, Asclepiadaceae, Compositae, Euphorbiaceae, Papaveraceae, Sapotaceae, and in a few other families. Among the monocotyledons laticifers occur in species of the Areaceae, Liliaceae and Musaceae. (Fahn, 1979).

1.2.1 Classification of Laticifers

The laticifers are grouped in two main types, non-articulated and articulated. This classification has no relationship to taxonomic groups and thus different types of laticifers may be found in different species of one family. The non articulated laticifers are multinucleate. They develop from a single cell which greatly elongates with the growth of the plant. Such laticifers are also termed laticiferous cells. In some plants they develop into more or less straight tubes which are term non-articulated unbranched laticifers. In others they branch repeatedly forming immense systems. These laticifers are termed non-articulated branched laticifers.

Articulated laticifers, also termed laticiferous vessels, consist of simple or branched series of cells which are usually elongated. The end walls of such cells remain entire or become porous or disappear completely. In the latter case the final result is a large multinucleate structure as in the non-articulated laticifers. The simple articulated laticifers are termed non-anastomosing (unbranched) laticifers, the branched

articulated laticifers which form lateral anastomoses with neighbouring ones are termed articulated anastomosing (branched) laticifers. The following are examples of the various types:-

- Non-articulated unbranched laticifers: *Cannabis* (Cannabiuaceae); *Urtica* (Urticaceae), *Vinca* (Apocynaceae).
- Non-articulated branched laticifers: *Asclepias, Ceropegia,* Cryptostegia (Asclepiadaceae); *Bronssonetia, Ficus, Maclure* (Moraceae); *Nerium* (Apocynaceae); *Euphobia* (Euphorbiaceae).
- Articulated non-anastomosing laticifers, *Achras* (Sapotaceae); *Allium* (Liliaceae); *Chelidonium* (Papaveraceae); *Convolvulus, Ipomoea, Dichondia* (Convolvulaceae); *Musa* (Musaceae).
- Articulated anastomosing laticifers: Argemone, Papaver (papaveraceae); Carica (Caricaceae); Cichorium, Lactrice); Itawea, Manihot, (Euphorbiaceae); all or most genera of the Campanulaceae and Lobeliaceae.

(Fahn, 1979).

1.2.2 Latex Occurring in cells other than laticifers

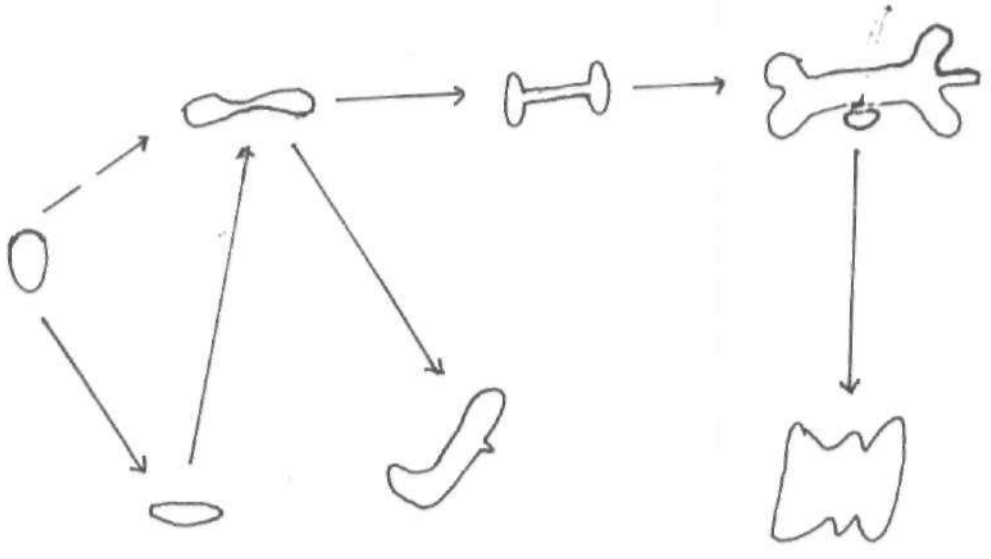
In the plant *Parthenium argentatum* (Guayula) of the compositae rubber occurs in parenchymatous cells which are not morphologically differentiated laticifers. The rubber containing cells occurs in most plants parts, especially in the stem and root. In the wood they are confined to the rays.

They are most abundant in the phloem rays. Rubber accumulates also in the epithelial cells of the resin ducts, but it is not secreted into the duct lumen. Twenty percent of the day weight of plants grown for four to five years in favoruable conditions consists of rubber. (Fahn, 1979).

1.2.3 Starch Grains in Laticifers

Starch grains occurs in the laticifers of some plants. The starch grains of the non-articulated *Euphobia* laticifer possess a specific morphology. The peculiar and variable shapes of the starch grains in laticifers of *Euphorbia* species has been as a result of the evolutionary conditions. The non-articulated laticifer, present in only a few families has been interpreted as being of recent phylogenetic origin. It was suggested that elongated laticifer starch grains were derived phylogenetically from the round or oblong grain present in the parenchymatous cells. It was further suggested that the specialization of the grains occurred by the deposition of additional starch at their tips resulting in osteoid grains. Further specialization led to the appearance of multiple lobes at the ends of the grain and to development of lobes along the mid-region of the grain. The discoid grains are regarded as the most complex forms (Fig. 1)

FIG.1: DIAGRAM SHOWING POSSIBLE TREND OF SPECIALISATION OF STARCH GRAINS OF EUPHORBIA LATICIFERS



Reproduced from Fahn, 1979

The fact that the laticifer starch grains differ from those of the other plant cells demonstrates that the laticifers are physically and cytologically isolated from the rest of the plant cells, although they differentiate from the same meristem (Fahn,1979).

1.2.4 Functions of Laticifers

a. Physiology

Different views have been expressed regarding the function of the laticifers since their discovery. Because of their distribution in the plant body and their liquid content that flows out promptly when the plant is cut, the early botanists called them "Lebenssaftgefasse" (vital sap vessels) and compared them with the blood vessels of animals. Their association with the vascular bundles, especially with the phloem (the pressure is high in both), brought the researchers later to the assumption that the laticifers take part in translocation of assimilates. This, however, could not be proved. As was mentioned earlier, the laticifer at the separation layer, of leaves of *Hevea* which have completed their development were found to be occluded by callose. This would prevent translocation of substances with phloem which may be an efficient supply of initial latex precursors from the assimilating tisues.

The laticifers have also been thought to take part in storage of food materials. The results of experiments have contradicted this assumption too. The food materials present

in the latex are not readily mobilized when the plant is grown under conditions unfavorable for carbohydrate synthesis.

The most common view is that the laticifers are secretory tissues. In most cases they apparently produce and store mainly substances which do not re-enter the plant metabolism.

Other functions ascribed to the laticifers were a role in regulating the water balance in the plant and in the transport of oxygen.

The most probable main function of the laticifers seems to be protection. The latex may play a role in wound healing as a defence against herbivores and also perhaps as a defence against microorganism. (Fahn, 1979)

b. Pharmacology

The latex of *Calotropis procera* (Asclepiadaceae), for example, was found to cause injury to human ocular tissues.

Some researchers did not detect antibacterial activity in Asdepias latex *in Vitro*, and suggested that this may be as a result of specific activity of the laticifer cells, in which the lysosomal system may be involved. (Fahn, 1979).

1.2.5 Rubber and Some Other Products of Latex

Plants which produce rubber-containing latex are numerous but only few contain rubber of appropriate quality and in sufficient amounts to be of economic importance. Within the same genus e.g. *Euphorbia*, many tropical species produce and

accumulate considerable amount of rubber, whereas the temperate species of the same genus may produce little or no rubber.

The most important rubber producing plant is para-rubber *Hevea brasiliensis* (Euphorbiacaea). Some other *Hevea* species e.g. *H. benthamia* and *H. guianensis,* also contribute to modern rubber cultivars.

Of lesser importance are: Panama rubber - *Castilla ulei* and *C. elastica* (Moracaee) ; Manicoba - *Manihot glaziovii; M. Heptaphyla M. piauhyensis* and *M. dichotoma* (Euphorbiaceae); Mangabeira - *Hancornia speciosa* (Apocynaceae); Landolphea - *Landolphia kikii, L. heudelotii* and *L. owariensis* (Apocynaceae); Palay Rubber - *Cryptostegia grandiflora* and *C. madagascariencis* (Asclepiadaceae); Russcan Dundelion - *Taraxacum koksaghyz,* Guayule - *Parthenium argentatum* (Compositae).

Several latax products which are neither elastic nor resilient upon coagulation are termed balatas. Such products are:- Chile (Chewing gum) obtained from *Achras sapota* (Sapotaceae), gutta-percha obtained from *Palaguium gutta* (Sapotaceae) and some other similar products obtained from a variety of the plant species. Opium is obtained from the latex of *Papaver somniferum* (Fahn, 1979).

The presence of latex in a plant does not guarantee the presence of rubber, and in fact the latex of many temperate *Euphobias* and *Compositae* contain triterpenol esters rather than rubber (Bonner and Verner, 1965).

1.3 MEDICINAL AND ECONOMIC USES OF THE FAMILY APOCYNACEAE

The members of the family Apocynaceae are mainly important as ornamentals where as some yield fruits, drugs, latex, and other articles.

The bark of *Alstonia scholaris* is astringent and forms the 'ditta bark' of commerce. *Aspidosperma quebracho-blanco* (Argentina) furnishes medicinal quebracho - blanco bark. The roots of *Evatamia coronaria* serve as antidote against scorpion sting. The bark of *Holarrhena antidysenterica* is used in amoebic dysentery. The cortex of *Hunterria zaylamica* (Malaya) is used for treating yaws. The bark and wood of *Nerium indicum* are useful for the manufacture of rat poison. The roots of *Rauwolfia serpentina, Rauwolfia nitida* are used as a wonder-drug for lowering blood pressure. The seeds of *Strophanthus komb'e, S. hispidus,* and others provide strophanthin a heart stimulant and precursor of cortisone which is specific for arthritis. *Strophanthus gratis* is used as arrow poison. Some other species of *Strophanthus* have been accepted as useful drugs in western medicine. The leaves of *Wrightia trictoria* provide wrappers for 'bidis', the seeds contain indigo-like blue dye called "goda-indrojoo" which is used in the treatment of snake bite, the seeds also yield a red oil which has medicinal applications. Some of the plants in this family are bitter and employed as a purgative or febrifuge or depurative, sometimes the taste is mild, scarcely bitter and simply used as laxative.

The economic uses are described as follows. Some species of *Agonosma, Allàmanda, Beaumontia, Ervatamia, Plumeria, Thevetia* are cultivated for their showy flowers. Decorative plants for warm region and green house cultivation in cooler climates include *Nerium oleander* and *Plumeria rubra* frangipani. *Aspidosperma cruentum* (Central America) supplies valuable timber. The bark of *Chonemorpha fragrant* yields fibre of good quality. Rubber is derived from *Dyera costulata, Ecertius acinthas, Funtumia elastica, Muconata,* (W. Africa), *Hancornia speciosa* (Brazil) and others. The wood of *Plumeria rubra* forma *acuminata* is used in Indian drum, tom-tom and other musical instruments. The dried fruits of *Rhazya stricta* are utilized as coagulant in cheese making. African or silk rubber is obtained from *Funtumia elastica* and *Funtumia africana* is used as a source of wood. *Wrightia dermartiniana* is used as perfume. The following species provide fruits that are edible.

(a) *Carissa macroptera* and *Carissa carandas* are grown for their edible fruits

(b) *Hancornia speciosa* Mangobeira also has edible fruits, but in addition, the sap is a source of rubber in South America.

(c) *Carissa bispinosa* "Amatungula" "Nun-num". Fruits are edible in South Africa.

(d) *Carissa brownii* bears edible fruits. It is of Australia origin but later introduced to India.

(e) *Carissa carandas* Linn "Karaunda" "Christ's thorn" fruits are edible, make very good jelly. Drinks can be made from them. In India, they are used as preservatives or in pickles.

(f) *Carissa ferox* is a hedge plant with edible fruits

(g) *Carissa grandiflora* (Natal plum). Berries are bright red, 2 to 3 cm long when half-ripe are made into jelly, while ripe ones are used for pies.

(h) *Carissa ovata* fruits are ovoid, as large as a small plum, consumed by the Japanese.

(i) *Carissa edulis* variety, *tomentosa* barriers are edible (Tanaka, 1976).

(j) *Carissa opaca* "Karaunda" ripe fruits are eaten

(k) *Carissa spinarum* Linn "Karaunda" fruits are eaten in tarts.

(l) Other plants with edible fruits are *Carissa arduina*, *Carissa inermis*, *Carissa macrophylla (Carissa lanceolata)*, *Carissa paucinervia* and *Carissa congesta*.

(Blatter and Millard 1954; Datta, 1970; Hickey & King, 1981; Omino and Kokwaro, 1993).

1.4 PHYTOCHEMICAL CONSTITUENTS OF THE FAMILY APOCYNACEAE

The reported constituent of the family Apocynaceae are:

- Alkaloids (e.g Indole alkaloids, Isoquinoline alkaloids and steroid alkaloids) (Alston and Turner, 1963; Omino and Kokwaro, 1993).
- Cadiac glycosides or cardenolidglycosides (Alston and Turner, 1963; Bor and Cumberlege, 1953).
- Phenolic compounds (Bentley *et al*, 1984)

- Sesquiterpenes (Achenbach *et al*, 1985; Sofowora, 1986)

Some of the reported constituents of the family Apocynaceae (Alston and Turner, 1963) are given in Table 1

Table 1: Chemical constituents of the Apocynaceae as related to the systematic treatment of the family. (Alston and Turner, 1963)

Plant		Content	
1. Sub-family: Plumieroidea			
Tribe	Arduinea		
Sub-tribe	Melodininae		
Genus	Acokanthera	Abyssinin = amorphes Ouabain = Carissin Acofriosid Acolongiflorosid Acovenosid A and C Ouabain = Strophantin Venenatin	
	Carissa	Carissin Carisson Odorosid	Cardenolidgly coside
Tribe	Landolphiinae		
Genus	Hancornia	Thevetin	
Tribe	Pleiocarpae	unknown	
Tribe	Plumiereae		
Sub-tribe	Alstoniinae		Bitter principles
Genus	Alstonia Plumeria	Plumierid = Agoniadin	
	Gonioma Holarrhena	Kamassin Conamin Conessidin Conessimin Conessin Conimin Conkurchin Conkurchinin Holarrhenin Holarhessimin Holarrhimin Holarrhin Isconessin Kurchin	Steroid alkaloids

	Plant	Content	
	Alstonia	Alstonamin Alstonidin Alstonin Ditamin Echitamin Echitamidin Echitenin Lacton C and S Macralstonidin Macralstonin	Alkaloid
	Aspidosperma	Villalstonin Aspidosamin Aspidospermatin Aspidospermicin Aspidospermin Hasslerin Hypoquebrachin Paytamin Paytin Quebrachin	Alkaloid
	Haplophytum	(Yohimbin?) Cimicidin	
	Lochnera	Haplophytin δ-Yohimbin	
	Vinca	Pubescin Reserpin Vincarosin Vincassin Vinin	
Sub-tribe	Tabernae-montaninae		
Genus	Tabernanthe	Ibogain Tabernaemontanin	
	Geissospermum	Geissospermin Pereirin Vellosin	
	Taberae-montana	Coronarin Tabernaemontanin	

	Plant	Content	
Sub-tribe	Rauwolfinae		
Genus	Vallesia	Aspidospermin	
	Alyxia		
	= Gynopogon	Ajmalicin	
	Rauwolfia	Ajamalin = Rauwolfin	
		Ajmalinin = Alkaloid C	Alkaloid
		Alstonin	
		Chalchupin A und B	
		Isorauhimbin	
		Raumitorin	
		Raupin	
		Rauwolsnin	
Genus	Rauwolfia	Rauwolscin	
		Reserpin	
		Sarpagin	
		Semperflorin	Alkaloid
		Seredin	
		Serpentin	
		Serpentinin	
		β-Yohimbin	
Sub-tribe	Cerberinae	Alkaloid	Alkaloid
Genus	Ochrosia	Alkaloid	
	Pseudochrosia	Alkaloid	
	Kopsia	Kopsin	
		Acetylneriifolin= Cerberin	
	Tanghinia	= Veneniferin	
		Desacetyltanghini	
	Cerbera	Tanghiferin	
		Tanghinin	
	Thevetia	Tanghinosid	Cardenolidglyc oside
		Cerberin = Acetylneriifolin	
		Acetylthevetin	
		Thevetin	

Plant	Content	
2. Sub-family: Echitoideae		
Tribe Echitideae		
Genus Adenium	Abobiosid Digitalum Verum Honghelin Honghelosid A,C,G = Somalin Odorosid B	
Urechitis	Urechitin Urechitonin Urechitoxin Androsin	Cardenolidglyc oside
Apocynum	Cymarin	
Nerium	Adynerin Desacetyloleandrin Digitalum verum Neriantin Odoroside Oleandrin (=Folinerin) Strophanthin - K	
Strophantus	Ambosid Ambostrosid 16-Anhydrostrospesid Boistrosid Caudosid Christyosid Cournmontoside Cymarin Cymarol	Cardenolidglyc oside.

PLANT		CONTENT	
		Digitalum verum Divaricosid Emicymarin Gracilosid Honghelin Honghelosid A,C,G. Inertosid Intermediosid Leptosid Millosid Musarosid Ouabain = Acokantherin = Strophatin - G Panstrosid Pauliosid Periplocymarin Pseudostrophantin Quilenglosid Sarmentocymarin Sarmentosid A und B Sarnovid Strobosid Strophantin-K Strospesid	
Tribe Genus	Parsonsiae Wrightia	Conessin	
	Parsonsia Malouetia Forsteria	Guachamacin = Curarin Forsteronin	Steroid alkaloid

1.5 DESCRIPTION OF THE GENUS CARISSA

Carissa occur as spiny shrub up to 5m high, branches glabrous or pubescent with exillary spines 1 - 3cm long; leaves ovate, usually acute at apex, shortly cuneate or rounded at base, 2 - 6cm long, 2 - 5cm broad with 3-5 pairs of lateral nerves, glabrous or puberscent, petiole short; inflorescence dense, shortly pedunculate terminal or axillary cymes. calyx 3-5mm long with subulate teeth, corolla tube 7-20mm long, lobes 3-10mm long always shorter than the tube (as in *Carissa edulis)* (Hutchinson and Dalziel, 1963).

In *Carissa,* the terminal bud is modified into a pair of thorns while in other plants that possess thorns, like lemon, duranta, pomegranate, vangueria, the exiallary bud is modified into a thorn (Dutta, 1971).

The genus *Carissa* L is known by the following names in different languages

ga - calac, clapalou [coromandel]

ge - carisse

An East - African arrow poison is made from the bark and wood of a species of *Carissa;* this poison is named: ouabaio, wabayo, wabie, wabaio.

ga - ouabaie

(Gerth and Wijk, 1962).

The genus *Carissa* has the following species

C. acuminata A.Dc

C. africana A.Dc

C. arduina L.am

C. *axillaris* Roxb

C. *bispinosa* Desf = *C.* *arduina*

C. *brownii* F.Muell = *C.* *lanceolata*

= *C.* *ovata*

= *C.* *et scrabra*

C. *condolleana* Jaub. & Spack

C. *carandas* Lian

C. *carandas* Lodd = *C.* *paucinervia*

C. *carandas* Lous = *C.* *africana*

C. *congesta* Wight = *C.* *carandas*

C. *coriacea* Wall

C. *cornifolia* Jaub & Spach

C. *cryptophlebia* Baker

C. *Dalzellii* Bedd

C. *diffusa* Wall = *C.* *paucinervia*

C. *dulis* Schum & Thonn = *C.* *edulis*

C. *edulis* Vahl

C. *erythrocarpa* A.Dc

C. *ferox* A.Dc

C. *grandiflora* A.Dc

C. *grandis* Bert ex A.Dc

C. *haematocarpa* A.Dc

C. *hirsuta* Roth

C. *inermis* Vahl

C. *lanceoata* Dalz = *C.* *macrophylla*

C. *macrocarpa* A.Dc

C.	*macrophylla* wall		
C.	*madagascariensis* Thou		
C.	*mitis* Heynh	=	*C. macrophylla*
C.	*mitis* Vahl	=	*Holarrhena mitis*
C.	*myrtoider* Desf		
C.	*oblongifolia* Hoclist	=	*Acokanthera oblongifolia*
C.	*ovate* R.Br		
C.	*paucinervia* A.Dc		
C.	*pubescens* A.Dc		
C.	*richardiana* Jaub & Spach	=	*C. edulis*
C.	*salicina* Lam	=	*C. spinaruim*
C.	*scabra* R.Br		
C.	*schimperi* A.Dc	=	*Acocanthera schimper*
C.	*sechellensis* Baker		
C.	*sepiaria* Royle	=	*C. spinarum*
C.	*spinarum* Linn		
C.	*spinarum* Lodd	=	*Damnacanthus indicus*
C.	*suavisima* Bedd		
C.	*tomentosa* A. Rich		
C.	*Villosa* Roxb	=	*C. spinarum*
C.	*xylopicron* Thou		

(Hooker and Jackson, 1875)

The chemical constitutents of some of the species of *Carissa* are as follows: *C. carandas* and *C. spinarum* contain cardiac glycosides, a minor component of the latter might be odoroside H. *C. edulis* did not contain any glycosides from this report (Bisset,

1957). *C. Carandas* was reported to contain cardiac glycosides A,B,C,D which gave positive Raymond's test, another Raymond negative substance P, carisson, β - sitosterol and an amorphous water soluble polyglyceride fraction. Odoroside H was absent (Rastogi *et al*, 1966). *C. carandas* contains new lignan carinol which was isolated and identified by UV, ir and NMR spectra (Raghwendra *et al*, 1975). *C. Carandas* also contains carindone. *C. bispinosa* contains tritriacontane ($C_{33}H_{68}$) and uroslic acid. The residue from a benzane extract of the leaves was chromatographed on AL_2O_3. From the pet-ether eluate after purification, tritriacontane, $C_{33}H_{68}$ was isolated. The solid residue obtained from the combined fractions of the ether - and ether alcohol mixture eluates was purified ursolic acid was identified (Motawi and Hammouda, 1964). *C. bispinosa* also contains allagochrome. *C. congests* containa carissone and D - glucoside of β - sitosterol (Joshi and Boyce, 1957). *C. opaca* contains tanning materials in the leaves. *C.grandiflora* contains a plant regulator (nonindolic citrus auxin). This anxin, originally found in the fruit of *Citrus sinensis*, was detected in fruits of lemon, date, fig peach and Natal plum (*C.grandiflora)*. It was not present in fruits of guava and strawberry which contained indoleacetic acid instead (Lewis *et al, 1965)*.

Pharmacological studies from the root of *C.carandas* as well as *C. spinarum* could lower the blood pressure in cats. The ethanol fraction was prepared by seperating other fractions by successive treatment with pat-ether (60-80%) and chloroform (Chatterjee and Roy, 1965).

A typical scotoactive stomatal behaviour was noticed in *C. spinarum*, and other plants (*Gymnosporia emerginata, Maba buxifolia, Flacourtia sepiarta, Dodonaea viscosa* and *Randia malabarica)*. This stomatal behaviour was reversed artificially by foliar application of paraquat at 100ppm or 2,4,5 - T at 2000 ppm. The size of the stomatal aperture, stomatal conductance and the rate of transpiration were enhanced by the herbicide treatment (Rao *et al*, 1977). *C. edulis* among other can be eradicated by 2,4,5, - I (Michel, 1957).

1.6 POLLINATION IN CARISSA

The pollination in *Carissa grandiflora* has been described Varieties either have short-styled flowers or sterile anthers. The latter type tended to give poor fruit yields particularly when planted in isolation, and artificial cross-pollination gave increased yields. However, few insects were observed to visit the flowers. Honey bees occasionally collected nectar from the base of the pistil, but usually only after the corolla tube had fallen off and even then very few entered the flower to do so. Many of the flowers contained thrips but their value as pollinators has not been ascertained (Free, 1976).

This type of pollination could serve as prototype for other species of *Carissa* as well.

1.7 *DESCRIPTION OF CARISSA EDULIS VAHL*

C. edulis Vahl forma *C. pubescens* subspecie - *continentalis* is an erect specie up to 16 ft (5m) high (Hutchinson, 1963). It is a much branched, straggling or climbing glabrous shrub with paired 2inch (5m) axillary spines. Flowers are white, pink or purplish, fragrant in dense cymes, calyx lobes subulate, corolla tube longer than the lobes nearly 1 inch (5m) long. Leaves 3 x 2 in ovate, ecute base rounded or broadly cuneate, with 3-5 pairs of faint internal nerves. Fruits are syncarpous berries, often paired, about ½ inch (1.25cm) diameter, red to black when ripe (Fig.2)(Irvine, 1961; Hutchinson and Dalziel 1963; Ghazanfar, 1989).

The plant flowers and fruits from March to August. It can be propagated by seeds and by cuttings which soon take root. It forms a dense, impenetrable hedge, which is nearly always in flowers (Irvine, 1961).

Habitat: Coastal thickets and dry deciduous forest. Often an termite mounds. Localities: Mostly in colony. Distribution: throughout Tropical Africa extending south wards to Rhodesia (now Zambia and Zimbabwe), and in Arabia (Irvine, 1961).

The plant is commonly known in Hausa in Northern Nigeria as cizaki (Gbile, 1980), in Malawi as Mpambala, Mkoloko (Sofowora, 1986).

Dried leaves of *Carissa edulis* is used in Egypt to control diabetes mellitus (Johns *et al*, 1996). Root of *Carissa edulis* is used in East Africa against malaria (El-Firy *et al*, 1996). In Kenya the root is used for indigestion, lower abdominal pains, malaria, Chronic chest pains (mixed with other plants) swollen glands, diarrhoea. The root is used as a health drink, and to strenghten

FIG.2:CARISSA EDULIS VAHL



A, Flower longitudinal section (Reproduced from Irvine 1961)

backbone. The root is also used as a snake repellant. The fruit is used as astringent and as food (Omino and Kokwaro, 1993).

The dried root in Ethiopia is used for psychiatric problems and gingivitis (treatment by smoke inhalation)(Arnold and Gulumian, 1984).

The root is macerated in cold water for several hours to 2 days and the liquid taken orally 3 times daily for treatment of tuberculosis (Arnold and Gulumian, 1984).

The dried leaf extract was reported to have antihyperglycemic activity at a dose of 2.0g/kg. 95% ethanolic extract was adminsitered to male rats by the intragastric route.

Results significance at $P<0.05$ level, versus streptozotocin induced hyperglycaemia (El-firy *et al*, 1996)

Ethanolic (95%) extract of the dried leaf has week mulluscidal activity. Concentration used was 200mg/l (El-firy *et al*, 1976).

The water extract of the stem at concentration of 1000ppm had antischistosomal activity against *schistosoma mansoni* (Elshelkh *et al*, 1990)

The dried leaf ethanolic (95%) extract does not have hypoglycaemic effect. The extract was adminsitered by intragastric route to male rats at dose of 2g/kg (EL-Firy *et al* 1996).

The dried stem ethanolic (95%) extract did not have cytotoxic activity. ED_{50}>30.0 MCG/ML (Hussain and Kingston, 1982). The

In Northern Nigeria (Kaduna and Katsina state, the fruit is edible, the root, stem and leaves are used in the treatment of sickle cell anaemia. The boiled water extract is given to the patients. The plant is used alone or in combination with either *Strychnos spinosa* or *Strychnos innocua, Securidaca longepedunculata,*

Combretum micranthum. The roots are usually given to adult patients while the stem and leaves are given to children (Yako, 1992). The stem bark is used as aphrodisiac and the leaves for treating hernia (Audu, 1994).

The water extracts either by maceration, decoction, concoction of infusion were used as the form of preparations of the medicaments for the treatment of various disease.

The above mentioned uses of *Carissa edulis* can be evaluated under the following headings: antipyretic and/or analgesic, anti-inflammatopry, antipsychotic, antibacterial, anthemintic, antisicling and antidiabetic. Others include bitters, tonic, purgative and abortifacient.

1.8 MEDICINAL AND ECONOMIC USES OF CARISSA EDULIS VAHL

The root is used in Ghana as a tonic, being cleaned, and chewed with the juice. The root is used as an aphrodisiac and was formerly used as bitters, macerated in rum, gin. The crushed roots smell like methyl salicylate. The grated roots are used in Fr. W. Africa for inflamed glands. The root-bark, with spices, is used as an enema for lumbago. In Guinea a poultice of boiled leaves is applied to toothache. The fruits are sometimes put in the food of sick persons. In Tanganyika (now Tanzania), the plant is used for worms in man and cattle. It is said to be semi-parasitic e.g. on Henna (Irvine, 1961).

The fruits are sweet, pleasant to eat, and thirst-quenching. Vinegar is sometimes made from them. The edible fruits are also made into jam in Kenya. The root imparts an agreeable flavour to food and drinks. The plant is browsed by goats and camels in the

dry parts of Sudan. *C. edulis* is also used as a dye (Bankar and Verma, 1987; Irvine, 1961; Omino and Kokwaro, 1993).

In East Africa the root is used for chest complaints and cough remedy; while the Massai take it with goat meat as a treatment for gastric ulcer.

A root decoction is taken in Senegal (to which 'pimento' is added) as an anthelminthic especially against *Taenia*. The root decoction is also used for venereal diseases (Burkill, 1985).

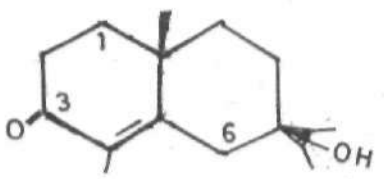
In Ghana the root is used locally as a tonic and expectorant (Oliver, 1960).

The leaves, stem and root barks are used for oedema, toothache, abortifacient and purgative (Sofowora, 1986).

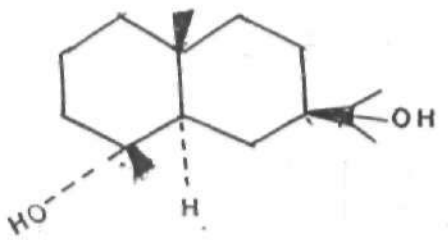
1.9 PHYTOCHEMICAL CONSTITUENTS OF CARISSA EDULIS

The methanolic extract from the root of *C. edulis* contains 5% lignans, among them are (-) nortra chelogenin, carbinol and unknown carissanol (Archenbach *et el*, 1983). Three types of sesquiterpenes were isolated from the root, carissone, carissonene and edulone (Sofowora, 1986). The methanolic extract from the roots (collected in Ghana) contained 5% sesquiterpenes. Seven sesquiterpenes (FIG 3) were isolated carissone[1] which had been isolated from other *Carissa* species was found to be the main sesquiterpene, as minor components were isolated cryptomeridiol[2] and β-eudesmol[3], along with three unknown hydroxylated carissones[4-6] and the novel germacrane type sesquiterpene[7] (Achenbach *et al*, 1985). A phenolic compound was found to be one of the constituent and 2-Hydroxy acetophenone was identified to be the principal root volatile constituents (Bentley *et al*, 1984). The root of *C. edulis* contains a cardiac glycoside carisson (Oliver, 1960).

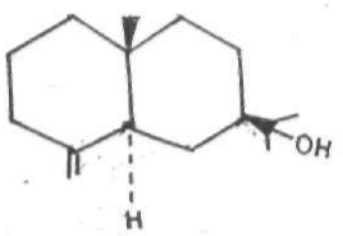
FIG·3: THE SESQUITERPENES OF THE ROOT OF CARISSA EDULIS



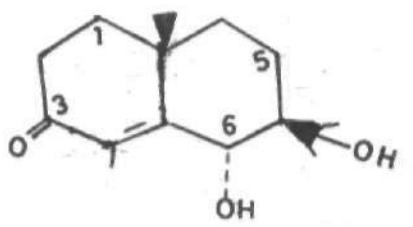
(1)



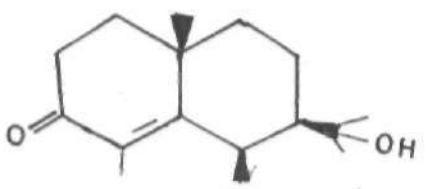
(2)



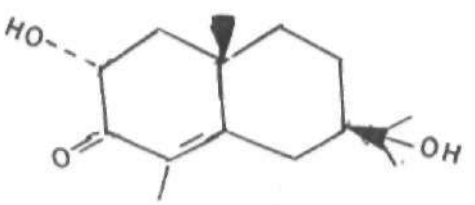
(3)



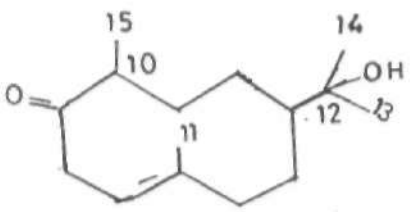
(4)



(5)



(6)



(7)

KEY

(1)= Carrisone
(2)= Cryptomeridiol
(3)= β-eudesmol
(4)-(6)= Unknown hydroxylated carissones
(7)= Novel germacrane type sesquiterpene

(Achenbach et al, 1985)

1.10 PHARMACOGNOSTIC EVALUATION

1.10.1 MICROSCOPY

THE MICROSCOPEC AND ITS SIGNIFICANCE IN PHARMACOGNOSY

The microscope is an optical instrument which is used to magnify smaller objects (particularly those which are invisible to the naked eye) in order to study the details of their structure, constitution and inclusions. It is also used to study the details of the internal structure and contents of larger objects, plant parts and animal tissues.

There are many types of microscope, for example the simple microscope, the compound microscope and the electron microscope.

The one normally used in the laboratory is the compound microscope. It consist of two convex lenses of short focal lengths for producing high magnifications.

The microscope in one of the most commonly used optical instruments in the study of crude plant drugs . Qualitative minute merhological and histological structures which are most frequently used to identify most natural drugs and to detect adulterants in them can only be studied under the microscope in this sections and in powders. The microscope is indispensable in the identification of powdered drugs and detection of adulterants in them as they passes very few macroscopical characters other than colour, odour and taste.

Quantitatively the various microscopical structures such as trichomas, calcium exalate crystals, starch grains etc can be measured under the microscope with the help of microscopic accessories like the micrometers. Physical constants like palisade ratio, stomatal number, stomatal index, vein islet number and vein termination number of leaf drugs determined by

quantitative microscopy only the microscope is also used for quantitative micro analysis of admixed or adulterated powdered drugs. The quantitative parameters are valuable in the identification and purity determination of crude plant drugs.

Drawing of internal structures of plant or animal organs and various other minute structures to scale, in their exact natural shape and arrangement can be done under the microscope.

METHODS OF MICROSCOPY

The following are the different methods of microscopy

- Bright Filed Microscopy
- Modified Microscopy which include:
  a. Dark field microscopy
  b. Phase contrast microscopy
  c. Polarised light microscopy
  d. Ultra microscopy
  e. Ultra violet microscopy
  f. Intra red microscopy
  g. Interference microscopy
  h. Electron microscopy

(Cosslett, 1966, Evans, 1989)

The bright field, polarised light and phase-contrast microscopy were used in this study.

Bright-Field Microscopy

When there are (at least) differences in contrast in the specimen, it is possible to carry out bright - field microscopy or examinations. With the bright field transmitted light method, the light coming from the condenser falls on the

specimen. Depending on the structure, the light on the specimen is diffracted and absorbed. The objective takes in more or fewer diffraction maxima, depending on the aperture. The diffracted rays are merged again in the image plane, the so-called secondary image and interfere. An image similar to the specimen is formed here by the interference The higher the aperture of the objective or by analogy the more diffraction maxima (higher orders) are received by the objective, the more true the reality does the image appear. However to obtain any image similar to the specimen, the objective must receive at least one diffraction maximum of first order as well as the diffraction maximum of zero order (Traber, 1971).

Polarised-Light Microscopy

The apparatus' consists of a polariser or Nicol Prism, fitting below the microscope stage and a similar prism forming the analyser fitting above the objective.

When both the polariser and analyser have their diagonal surfaces parallel the ray of plane polarised light is transmitted by the analyser. If now the polariser is revolved the light diminishes in intensity until at a position 90° from the first it is entirely extinguished, the polarised light being totally reflected by the analyser This position when the diagonal surfaces of the two nicols are at right angles is termed `crossed nicols'.

Isotropic substances are characterised by having the same physical properties in all directions, e./g. Gases, liquids aid isometric crystals. Such substances are monorfringent i.e they have only one refractive index. Isotropic substances are not visible, however they be oriented, when examined between

crossed Nicols. They in no way affect the polarised light passing through them from the polariser.

Anisotropic substances exhibit different physical properties according to the direction along which they are examined. Such substances show more than one refractive index. The great majority of crystalline materials show birefringence (Evans, 1989).

Phase Contrast Microscopy

An `amplitude specimen' is visible because it locally absorbs light.

`Phase Specimens' are those which are not normally visible to the human eye. Because of local differences in refracture index or thickness, they produce a phase displacement of light instead of selectively absorbing it. In order to make this phase displacement visible, the phase contrast microscopy developed (Traber, 1971).

What usually obtain is to put a phase-shifting plate in the undiffracted beam or the diffracted beam either as a disc at the axis in the direct beam or as a ring in the outer zone where the diffracted beam passes. The phase plate may be made to retard either the diffracted or the direct beam, giving a positive or negative phase contrast, respectively. Whether a particular specimen detail appears bright on a dark background, or vice versa, will depend on whether its index of refraction is greater or less than that of its surrounding, as well as on the type of phase contrast (Casslett, 1966).

It is only under these conditions that an image with high contrast is to be expected. Also there is no superimposition and therefore reduction in contrast of the diffraction maxima

due to direct light (Traber, 1971).

This phase contrast microscopy is particularly useful in the examination of living cells, the constituents of which show little differentiation.

Monochronic light is employed and there is no reduction in the resolving power of the microscope (Evans, 1989).

1.10.2 PHYTOCHEMISTRY

Phytochemistry is the study of chemical constituents present in plants. The plants constituents are usually classified into two groups, the primary metabolites such as carbohydrates, proteins and lipids and secondary metabolites such as glycosides, alkaloids tannin, terpenes, steroids, rasins and balsams. The primary metabolites do not have pharmacological activity but may be of pharmaceutical necessity while the secondary metabolites have pharmacological activity.

Carbohydrates:

The carbohydrates are compounds consisting of carbon, hydrogen and oxygen, the two elements being usually present in the same proportions as in water. They are among the most abundant constituents of both plants and animals. Many such as the sugars and starches, are important food reserves for the plant and foodstuffs. Cellulose and other polysaccharides are constituents of cell walls of plants. Of special pharmaceutical importance is the fact that sugars unite with wide variety of other components to form glycosides.

The carbohydrates are generally water soluble, the sugars have a sweet taste while the more complex ones are tasteless. The solubility decreases with increase in complexity.

The carbohydrates are usually classified into the monosaccharides, oligosaccharides and polysaccharides. The monosacchrides are made up of one sugar molecule of 3-9 carbon atoms. These of five and six carbon atoms (pentoses and hexoses respectively) are the most abundant in plants. The oligosaccharides are made up of two to nine sugar molecules. The polynaccharides consist of more than nine sugar molecules. (Evans, 1989)

Tannins

The term`tannin' denote substances present in plant extracts which are able to combine with protein of animal hides, prevent their putrefaction and convert them into leather. From the above definition, tannin is a substance which is detected qualitatively by a tanning test (the gold-beater's skin test) and is determined quantitatively by its absorption on standard hide powder. This definition excludes simpler phenolic substances, often present with tannins such as gallic acid, catechins and chlorogenic acid, although under certain conditions they may give precipitates with gelatin and be partly retained by hide powder. Such substances of relatively low molecular weight are called `psuedotannins'. Most true tannins have molecular weights of from 1000 to 5000. These true tannins are classified into hydrolyzable tannins and condensed tannins (Evans 1989).

Glycosides

Glycosides are complex compounds which yield upon hydrolysis one or more sugars (glycone) and one or more other products (aglycone) (Miller, 1957) Glycosides have many

functions. In plants they serve as sugar reserves, as waste products of metabolism, as a means of detoxification, to regulate osmosis and to stabilise labile substances of importance in metabolism (McIlory, 1951)

The glycosides are normally linked between the glycone and aglycone through oxygen. These form of glycosides are known as O-glycosides. They are easily hydrolysed to the parent sugars and the aglycone by either enzymes or acids. The sugar moiety can exist either in α or β forms, thereby yielding both α or β glycosides. The sugar oxygen ring is retained and the sugar can occur in the pyranose or furanose firm. The former is the most common.

If the reducing group of the sugar is linked to a thiol, S-glycosides are obtained.

Present in plants also are N-glycosides which involve linkage to an amino group such as the nucleosides from ribose and purines, vicine and crotonoside.

Several glycosides are known in which the sugar moiety is not a true sugar, but a sugar derivative such as uranic acid.

Included in the consideration of glycosides is a group of compounds known as C-glycosides, which resist normal acid hydrolysis but have infra red spectra, and yield alkaline degradation product which indicate the presence of a sugar-link chain (Miller, 1973).

The different types of glycosides include:

- Cyanogenic glycosides
- Anthraquinones.
- Flavonoids
- Saponins
- Cardiac glycosides

Cyanogenic glycosides are those glycosides that liberate hydrocyanic acid among the products of hydrolysis. Many of these glycosides, but not all, are derived from the nitrile of mandelic acid. Although they contain netrogen, their structure is that of 0 - and not N-glycosides (Evans, 1989)

Anthraquinones are glycosides consisting of anthraquinone nucleus or its derivative; the anthrone and anthranols, the oxanthrones or the dimers of these compounds. Bontrager's test is usually used for their identification (Evans, 1989).

Flavonoids which occur in both the free and as glycosides are the largest group of naturally occurring phenols. The flavonoids have a basic nucleus of the chromone (benzo-γ-pyrone). They may occurs in different forms such as the flavones, isoflavones, flavonols, flavanones, chalcones or xanthones Most are O-glycosides and may occur as dimeric compounds (biflavonyls). Flavonoids dissolve in alkalios giving yellow solutions which on the addition of acid became colourless (Evans, 1989). The flavonoids are important to man not only because they contribute to plant colour but because many member (e.g. coumestrol, phleridin, rotenone) are physiologically active (Harborne *et al*, 1975).

Saponins are glycosides characterised by their property of producing a frothing aqueous solution. They have haemolytic properties. The fact that a plant contains haemolyic substances is not a proof that it contains saponins. When taken by mouth saponins are comparatively harmless.

Saponins have a high molecular weight and their isolation in a state of purity present some difficulties. As glycosides they are hydrolysed by acids to give an aglycone (sapogenin) and various sugars and related uranic acids. According to the

structure of the aglycone two types of saponins are recognised, the steroidal and triterpenoidal. Both of these have a glycosidal linkage at C-3 and have a common biogenic origin via mevalonic acid and isoprenoid units (Evans, 1989).

Cardiac glycosides are those glycosides which therapeutically strengthen a weakened heart. The therapeutic efficiency depend both on the structure of the genin and the type and number of sugar units to which it is attached. There are two types of cardiac glycosides depending on whether the genin has five or six numbered lactone ring. These types are known as cardenolides and bafanolides or bufadienolides (Evans, 1989).

Generally, the glycosides are soluble in aqueoes solvent while the aglycones are soluble in non-polar solvents.

Resins and Balsams

The term resin is applied to more or less solid, amorphous products of complex mixtures of resin acid, resin alcohols (resinols), resin phenols (resinotannols), ester and chemically inert compounds known as resenes. Rasins are often associated with volatile oils and gum (Oleo-gum-resins).

Balsam is used to denote substances containing a high proportion of aromatic balsamic acids (Evans, 1989).

Alkaloids

A precise definition of the term alkaloid (alkali-like) is somewhat difficult because there is no clear cut boundary between alkaloids and naturally occurring complex amines. Typical alkaloids are derived from plant sources. They are basic, contain one or more nitrogen atoms (usually in

heterocyclic ring) and usually have a marked physiological action on man or other animals. (Evans, 1989)

1.10.3 QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS

The evaluation of a drug requires that it be identified both in its entire and powdered form by means of its morphological and cytomorphological characters. It is possible that faulty collection or extended storage may alter the constituents of the drug to a part where it is no longer acceptable. Therefore, drugs are subjected to evaluative procedures which will indicate their acceptability by criteria other than their morphology. (Brain and Turner, 1975).

The different determinations of quantitative analysis of crude vegetable drugs include the followings;

Determination of Moisture Content:

The determination of moisture content is important because the presence of moisture causes the following:-

- It decreases the percentage quantity of the drug by increasing the weight.
- Causes activation of enzymes which lead to hydrolysis of the constituents.
- Causes chemical reactions and
- Harbours the growth of micro-organisms and insects.

Therefore the moisture content of all drugs should be kept reasonably low (Shellard, 1958)

Determination of Acid Insoluble Ash Value

The determination of total ash is a method to measure the amount of residual substance not volatalised when the drug is

ignited (African pharmacopoeia, 1986). The total ash represent the `physiological ash' (i.e. ash that comes from the plant tissue itself) and non`physiological ash' (i.e. ash from the extraneous matter (African Pharmacopoeia, 1986). The physiological ash consist of the carbonates, phosphates, nitrates, sulphates, chlorides and silicates of the various metals present in the plant which it took up from the soil when growing. Example of elements taken up by the plant from the soil include sodium, potassium, calcium, magnesium, iron, copper, cobalt, manganese, carbon, phosphorous, nitrogen, sulphur, oxygen, chlorine and silicon (Shellard, 1958).

Determination of Extractives

The determination of extractive is a method designed to measure the amount of constituents which are extractable by the solvent under specified conditions. It is applied to those vegetable drugs for which there is no suitable method of chemical or biological assay for their active constituents (African Pharmacopoeia, 1986). The evaluation is important to guard against adulteration or substitution with drugs which have already been extracted (Shellard, 1958).

Before the studies of analgesic activity it would be necessary to have a literature review on pain and different forms of analgesia.

1.11 PAIN

The word pain is derived from the Greek for penalty. Pain cannot edequately be defined in words. To overcome this problem one asks operative questions e.g.

(1) What is the purpose of pain? It is a warning of tissue damage and its persistent nature has a protective function by ensuring that the subject cannot ignore it except with considerable voluntary effort.

(2) Is pain a universal experience? Complete congenital insensitivity to pain is very rare and apart from these few subjects with this condition, it is known from childhood what is meant by pain. However, the highly subjective nature of pain makes it difficult for one to communicate about it verbally; and then the words used reflect cultural, educational, emotional and other values.

(3) Does intense harmful stimulation always produce pain?. The answer is very firmly, no, one has only to witness the astonishing denial of pain in a significant proportion of major casualties, especially on the battle field, to realise that there can be on occasion no relation between trauma and pain. Whatever the explanation for this absence of pain it suggest that the body has its own pain controls which can be called upon to obtund pain in certain circumstances.

One can go on asking questions like these until a more complete mental picture of what is meant by pain is built up. (Hannington-Kiff, 1974).

Scientist from many disciplines have investigated pain and each group has used a definition suitable for that field. Terms used include pain, discomfort, suffering and nociception (the sense that damage is being doing done in some area of the body or the sense of something noxious). Pain is often listed as a sensation (Benjamin and Gorsky, 1981).

1.11.1 Nature of Pain

Acute pain usually warn one that something is wrong, that some body part has suffered or is suffering damage. Pain calls attention to the part since we can localize pain quite specifically. Localization of pain may be incorrect; that is pain which is caused at one place may seem to arise in another. Such pain is called referred pain. Patterns of referred pain are well known and are generally quite helpful to physicians in understanding clinical syndromes e.g. in Appendicitis there is referred pain around the umbilicus (navel).

Chronic pain however, exists for years, sometimes for decades, and is usually not associated with nociception.

Not only is there this functional difference between acute and chronic pain, there is some apparent differences in associated mood. One is afraid what the pain represents, afraid that the pain will become worse, and afraid that it may

be impossible to be rid of the pain. On the other hand, the chronic pain sufferer is typically depressed. His anxiety has in effect burned itself out and he is left with chronic depression. He is sad because of the continuing existence of pain, and of the changes which have occurred in his life due to the pain.

Some of the research dealing with pain usually deals with nociception. The research dealing with nociception infact provides information about the perception of pain. However, that is not really true. The information about nociception is useful and describes the sensory apparatus which is normally associated with the perception of acute pain. However, since the perception of pain is a high central nervous system function, pain perception is not adequately described if only the peripheral mechanisms are taken into account (Benjamin and Gorsky, 1981).

1.11.2 MECHANISM OF PAIN

There are broadly three theories underlying mechanisms of pain with parallel views about cutaneous sensation or nociception.

(1) Specificity Theory.

(2) Pattern Theory

(3) Dualistic Theories of Input Control or Gate Theory (Hannington- kiff, 1974; Benjamin and Gorsky, 1981).

The "Specificity theories" assume that there are particular nerve endings required for the perception of tissue

damage (ultimately to be interpreted as pain). These nerve endings are stimulated when damage is occurring in some body part. But, it is now known that when cells are damaged many substances are liberated from the damaged cells into the surrounding tissue e.g. histamine, bradykinin which may stimulate the nerve.

A nerve impulse originating on one side of the body is transmitted by a nerve into the spinal cord. There a synapse or connection is made with another nerve Which crosses to the other side of the body in the spinal cord and then rises to a structure on the brain called the thalamus. Pathways then exist from the thalamus to the cerebral cortex where conscious perception could occur. This pathway within the spinal cord is called spinothalamic tract and it was thought that this tract was responsible for most pain perception occurring in the body up to the neck. Pain perception in the head and neck is handled in an analogous fashion, but in a separate pathway. Thus, the specificity theories view the nervous system according to a rather simple wiring model. The neurosurgeon felt that any pain problem could be treated with an appropriate destruction along this pathway but since pain perception involves so much more than the peripheral sensory and transmission system, such procedures are often unsuccessful.

The neurosurgeon thought that an appropriate destruction along the spinothalamic tract could alleviate pain. This was not successful as there was continued pain after surgery. As a result other pathways were postulated which led to the modification of the specificity theory. These Theories

supposed that it is not the stimulation of a specific nerve pathway which is involved with nociception and therefore pain perception, but rather it is the pattern of stimulation in one of many pathway which ultimately leads to the perception of pain. These theories imagine that pain occurs when there is stimuli summation in one of two ways.

Pain could also be mixed with other sensation e.g. the perception of sound is not usually painful but the perception of a very loud sound or a continuing loud sound may become painful. Likewise vision is not painful but the perception of very, very bright light may be painful.

Besides the modification of the specificity theory by the discovery or postulating of other pathway, there also exists other group of theories called "pattern theories". These theories suppose that it is not the stimulation of a specific nerve pathway which is involved with nociception and therefore pain perception, but rather it is the pattern of stimulation in one of many pathways which ultimately leads to the perception of pain. These theories imagine that pain occurs when stimuli summate in one of two ways. Stimuli occurring in many locations in the body all at one time may so bombard the nervous system that the continuing presence of a given stimulus may lead to the perception of pain e.g. A perception of pressure When something is leaning on a small part of the body is usually not painful. But if that pressure is applied to one whole side of the body, to the entire chest or to the entire

abdomen, although, the pressure per unit area may still be the same, the perception may change from one of pressure to one of pain. Similarly, the perception of heat and cold may become painful if the Degree of duration of stimulus is sufficient. All these are perfectly normal and it is this normal experience which lead the theoreticians to believe that pattern, rather than specifics, might be responsible in producing pain.

Of course neither of these mechanism is exclusive. It is possible that one group of pathways may exists for nociception and that other sensory pathways may transmit information ultimately perceived as pain when those other pathways are stimulated in excess of their usual levels. This emphasizes again not only the difference between sensation and pain perception but also the role of prior experience in pain perception.

Besides this normal mixing of pain with other sensations, there is an abnormal circumstance known as dyesthesia (the perception of pain instead of the more normal sensation) which occur in many neurologic conditions e.g. when a nerve regrows, before normal sensations occur, there may be period of time during which many senses will be mistakenly interpreted and in particular, stimulation of many modalities may be perceived as pain. Therefore as nerve regrows; stimulation of the body part by light touch or very mild changes in temperature may produce an acute painful response. Very little can be done about this except wait until the nerves system itself sorts the impulse and changes various sensory modalities to their proper

perceptions.

Scientists proposed the "gate theory" which in effect comobines the specific and pattern theories. The gate theory assumes that there are interactions between various pathways so that When one pathway is stimulated, that stimulation may affect the transmission of impulses in another pathway. In the original formulation the scientists described stimulation coming from some body part along two ways. The first pathway made up of comparatively large nerve fibers which transmit information relatively quickly, would be used for sensations other than pain. The other pathway, involving very small fibres which transmit information relatively slowly, is typical of fibers which do carry nociceptive or pain producing information.

Melzack and Wall assumed that a specific group of cells known as T cells (or transmission cells) exists in the spinal cord and that typically these T cells are stimulated by nociceptive fibers. They in turn pass information to higher central nervous systems structures. However, there are also negative inputs on the T cells which prevent their functioning when large fiber pathways are being stimulated. The gate theory may be used to explain why rubbing a painful spot can soothe the pain, why the application of a poultice, a chemical irritant, my soothe deep pain and why the stimulation of accupunture or other electrical stimulation of the central nervous system (CNS) may work.

There is certainly no lack of theoretical formulations to explain the existance of pain. The clinical problem is one of

selecting which of the many explanations may be more near the truth and which may apply to any given individual at any particular point in time. In many cases none of the available theories will adequately explain why a given patient has pain.

All the mechanisms described for nociception may have importance in other animals as well as man. However, once one leaves the area of nociception and enters the area of pain, suffering and discomfort one comes to cognitive functions which are difficult to evaluate in other species. One other complication is that pain can occur as a memory. Much of the science of pain is really that of nociception (Benjamin and Gorsky, 1981).

1.11.3 CENTRAL INTEGRATION OF PAIN

(a) The Spinal Cord

In the past the knowledge of ascending path in the spinal cord was inevitably limited because degeneration studies showed only the long tracts. What was not seen was put out of mind despite the fact that major part of the spinal white matter was accounted for. It was until physiological experiments drew attention to the reticular system that this deficit in the knowledge was fully realised. Advance in histological staining made it possible to show up fine nerve fibers but the diffuse "unbundled" organisation of these fibers and their multi-synaptic course make it possible to trace them effectively. Similarly the knowledge of the organisation

of the spinal grey matter is not inadequate, particularly with regard to the small neurones. Consequently, anatomy of the spinal cord and much of the rest of the CNS must be relearned. The following account of the central integration of pain is intended to emphasize those broad anatomical aspects which are germane to the understanding of pain.

The spinal ascending system may be conveniently divided into two, Oligosynaptic and Multisynaptic. In this way a more rational explanation will be possible of many of the clinical findings in pain.

i. **Oligosynaptic Ascending System (O.A.S)**

This comprises two principal pathways in relation to pain, the lateral spinothalamic tract and the exteroceptive (as opposed to proprioceptive) fibers of the dorsal columns. Both are rapidly conducting and the dorsal column fibers in particular carry precise information about the localisation of a painful stimulus because of their organised mapping on to the higher composite tract, comprising at least spinotectal, spinoreticular, spinorubral and spinovestibular pathways, consequently few fibers in this tract reach the thalamus directly. Sometimes a separate anterior spinothalamic tract is shown in textbook drawing of pain tracts but it is doubtful whether this exists.

The neurones (T cells) which give rise to the so-called lateral spinothalamic tract are situated more deeply in the dorsal horn than was previously thought, and it is incorrect to show the cells of a substantia gelatinosa as the origin of this tract. Unfortunately little is known about the cytoarchitectonics of the dorsal horn, though the laminar scheme based on findings in the cat provides a useful model.

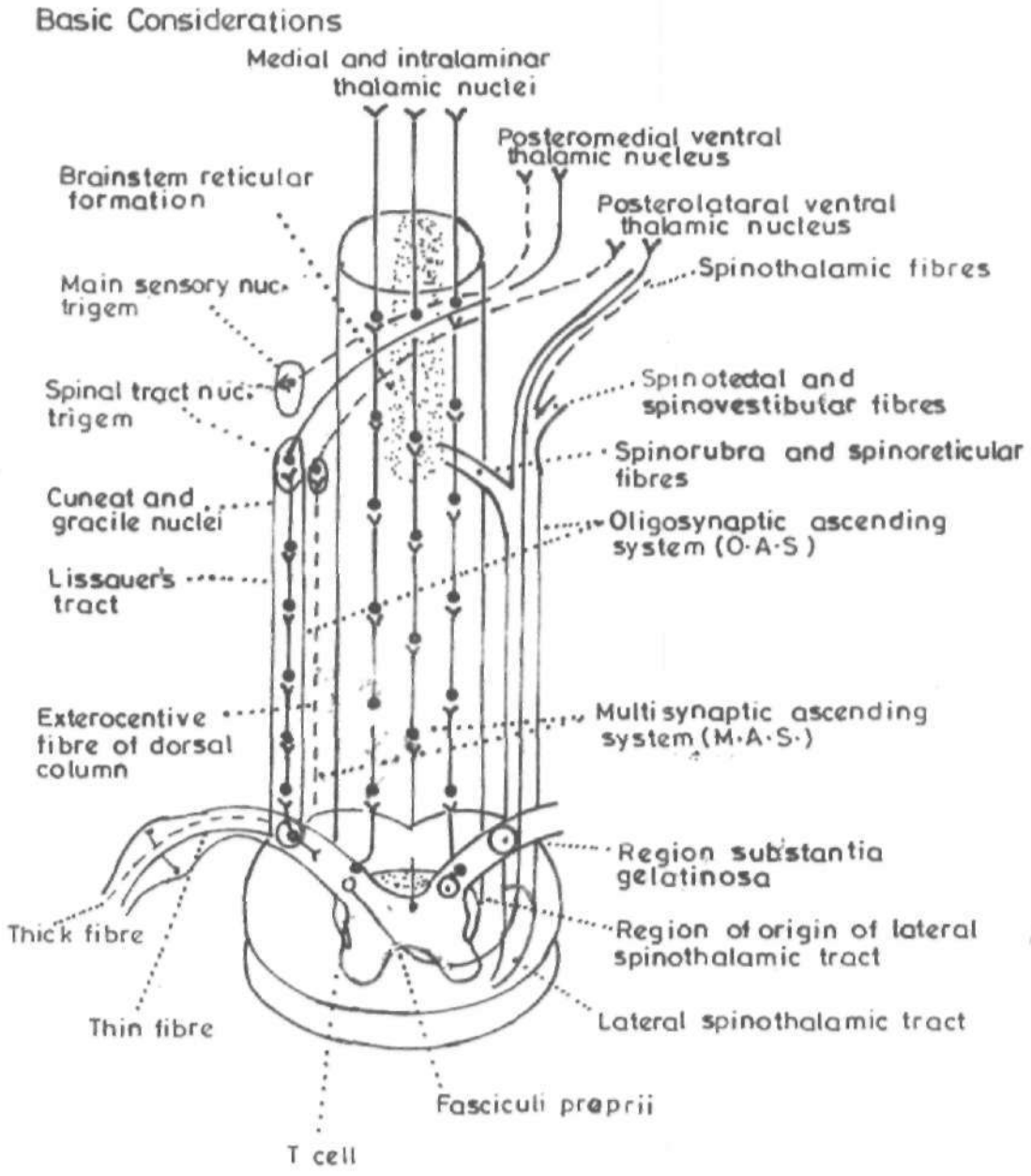
ii. <u>Multisynaptic Ascending System (M.A.S.)</u>

This comprises two pathways, the fasciculi proprii or ascending spinal reticular system and Lissauer's tract. Though they are both considered here as ascending system, each has descending fibers as well. The fasciculi proprii contain large numbers of fibers, which surely are indicative of the importance of this system. The main features of the fasciculi proprii have been reviewed. The origin of the fasciculi proprii is from the whole central grey matter of the spinal cord and not just from the posterior region. The upward extension of the fasciculi proprii to the brainstem reticular nuclei is suggested by the shaded central column in

(fig.3). The cell stations shown as an ascending chain in (Fig. 4) will of course lie in the central grey matter, and the axones will span a limited number of segments in either direction. Lissauer's tract may be considered as a special part of the main multisynaptic ascending system in the fasciculi proprii. Axones from the substantia gelatinosa cells run out into Lissauer's tract and travel over about one to three segments in either direction before re-entering the central grey matter to synapse with other substantia gelatinosa cells. In the medulla the substantia gelatinosa becomes continuous with the nucleus of the spinal tract of the trigeminal nerve, which is intimately associated with pain arising in most of the head region.

The M.A.S. lacks a somatotopic organisation and the passage of information to supraspinal levels is relatively slow. Whereas the O.A.S. is thought to localise the site of a pain and warn of its initiation, the M.A.S. is thought to underlie the diffuse persistent quality of the fully appreciated pain.

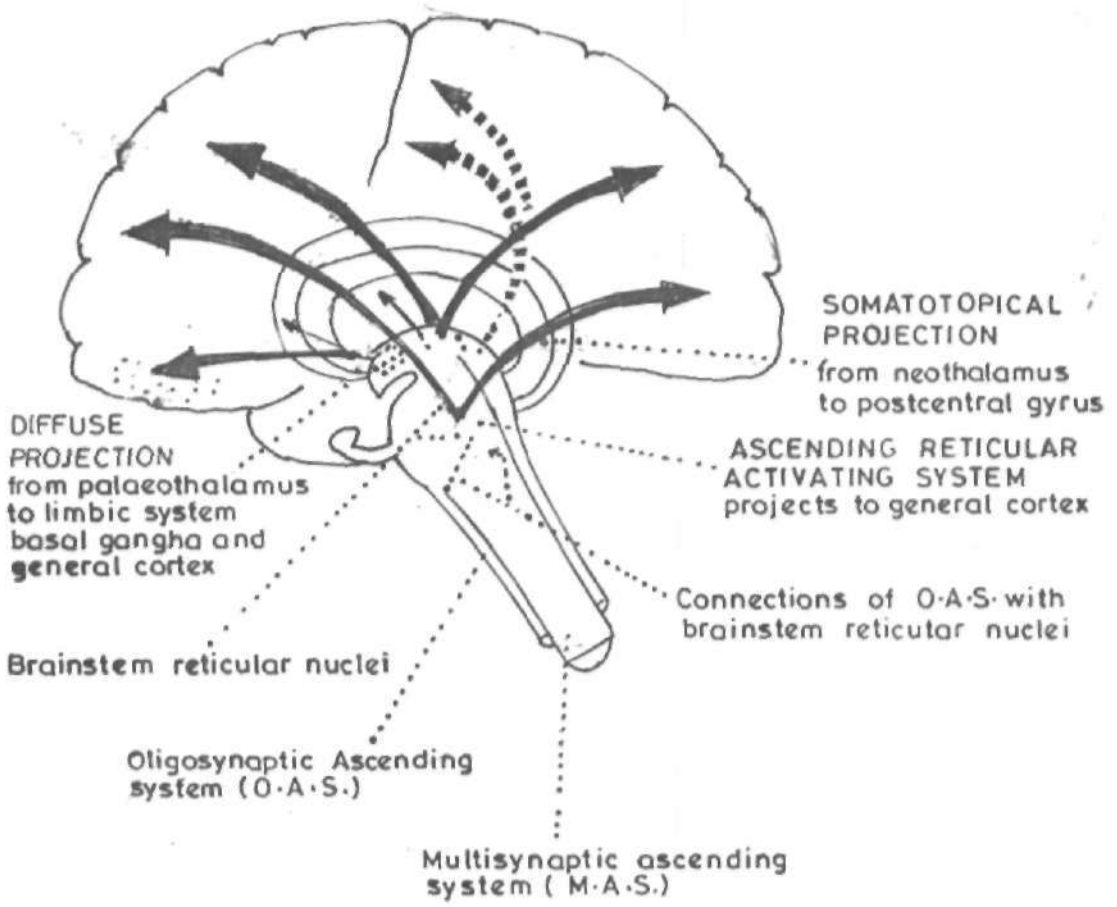
FIG. 4: THE PROBABLE ASCENDING SPINAL PATHS SUBSERVING PAIN. THE NEURONAL CONNECTIONS ACROSS THE GAP BETWEEN THE SUBSTANTIA GELATINOSA AND THE ORIGIN OF THE LATERAL SPINOTHALAMIC TRACT ARE DISPUTED.



(Hannington – Kiff, 1974)

FIG.5; THE HIGHER PROJECTIONS OF THE OLIGOSYNAPTIC AND MULTISYNAPTIC ASCENDING SYSTEMS

The widespread connections of the central reticular core should be noted.



(Hannington – Kiff, 1974)

(b) <u>The Brain</u>

The O.A.S. may be considered as a sheet around the M.A.S. which emphasizes the fundamental difference in the central connections of the two systems. The reticular core may be considered as the phylogenetically older system with the more recent descriminative system added to the outside. The central core begins below in the fasciculi proprii and widens above to include the brainstem reticular activating system from which information radiates out to the general cerebral cortex. This radiation is believed to subserve the general alerting effect of pain and other stimuli. The rostal projection of the central core is the palaeothalamus, comprising the medical and intralaminar nuclei, from which there is a diffuse radiation to the general cortex, the basal ganglia and the limbic system. The limbic system subserves the emotional attribute of pain and its hypothalamic connections account for the associated autonomic changes. The outer sheath, representing the O.A.S. sends connections to the brainstem reticular nuclei, and maps its contents of precise information on to the neothalamus (the posteroventral nuclei), from which it is somato-typically relayed to the post central gyrus. This pathway subserves the

definition of the pain in terms of space and time. It integrates present and post experience to decide the significance of the pain and the most appropriate, rational response. (Hannington-kiff, 1974)

(c) Manipulations of the Nervous Systems

The concepts presented for the Specificity theory of nociception led to the therapeutic practice of destroying portions of the nervous systems in order to achieve control of pain. There are many reasons why this may not be true as discussed, but in some situations it may be true and such destructive procedures do have a limited place in clinical practice. Unfortunately, these procedures have been applied indiscriminately in the past, when knowledge of pain mechanisms was even less precise than it is today.

When the clinician decides that a nerve should be destroyed, the procedure may be done in several ways, depending on the nerve's location. Perhaps the simplest procedure involves the injection of a substance which will destroy the nerve. In this case the injection must be done in a semi-blind fashion, since there is no precise way to localise nerves in the body . Since nerves do not show

directly on X-rays, various techniques must be employed in order to determine whether the needle is located sufficiently close to the nerve that the injection of a neurolytic (nerve destroying) substance will destroy the nerve rather than other structures. In some situations, x-ray can be used if it is known that the nerve lies very close to a bone landmark which can be seen on the X-ray. In other situations the needle which will be employed to inject the neurolytic substance may be used as an electrode, and electrical impulses may be transmitted through the needle to the nerve. These impulses may be such that sensations are felt by the patient when the needle is close to nerve or they may be impulses which will cause contraction of a muscle known to be supplied by that nerve. Employing those methods or less precise methods which involve the measurement of distances and angles from known landmarks which can be felt through the skin, a needle is placed as close as possible to the nerve to be destroyed and then the neurolytic substance is injected. While procedures like that are simple, they are only applicable to a few locations in the body where the anatomical criteria are met and placement of the needle adjacent to the nerve can be accomplished with

sufficient precision. In some situations, after satisfactory needle placement is achieved, a strong current is passed through the needle to generate sufficient heat to destroy the nerve in that way rather than by injecting a neurolytic substance. The choice between those two methods largely depends on a number of technical factors and typically makes no difference in the technical factors and typically makes no difference in the outcome. As an alternative to the destruction of a nerve with a needle, single sensory nerves can be cut surgically. Depending on the nerve's location this may be a simple or complicated surgical procedure.

Methods of Manipulations:-

(a) Neurolytic substances:-

1. Local anaesthetic agent
2. Alcohol (absolute alcohol)
3. Concentrated sodium chloride (Nacl)

Local anesthetic abolishes pain for some time but does not destroy nerve. Post alcohol neuritis usually occur after both injection due to Dyesthesia which often occur when the nerve regenerated after being damaged, i.e. after injection of 1 and 2 above pain usually re-occur. In fact many practitioners believe that neurolytic procedures should be

attempted only for patients with terminal diseases. Concentrated Nacl was explored for injection into the spinal fluid as a less noxious substance as compared to alcohol. To date no one knows quite how the injection of concentrated solutions work but sometimes the effects are beneficial.

(b) Cardotomy;- destruction of the spinothalamic tract.

(c) TENS:- transcutaneous electrical stimulation.

TENS has become a popular modality for treating pain. It may be that TENS works according to the gate theory by stimulating large diameter fibers and therefore turning off the impulses arriving at the nervous system from small diameter fibers. It may be that the TENS impulses arriving at some location in the CNS cause an inhibition of pain perception very high in the nervous system. At present, the actual mechanism is not known as a result it is difficult to predict which patient may benefit from TENS. However, since the procedure is so easily accomplished and so harmless, many patients can receive a trial of therapy with an electrical stimular and if it does not work very little has been lost. To use TENS a patient places electrodes on the skin and carries the pulse generator in a pocket. The nerves are stimulated continuously throughout the day.

Examples: (i) A tumour patient who continued to have pain after removal of tumour from chest benefited from injection of local anaesthetic in the intercostal muscle. But pain returned after a few hours when the local anaesthetic action had stopped. Another nerve block was done with absolute alcohol (a substance which destroys nervous tissue), the pain was completely gone. Alcohol injections are not without risk. Aside from the potential risks related to injections of alcohol in the wrong spot, there is a pain syndrome which sometimes occurs following its injection.

(ii) A patient had severe testicular tumor, all treatment had failed to arrest its progression. He was in great pain and he was not expected to live more than a few months. Investigations with local anaesthetic nerve blocks revealed that so many nerves were involved that single nerve injections could not be done. Furthermore, since he had pain on both sides of the body, injection of a neurolytic solution into the cerebro spinal fluid (CSF) could not be carried out. The probability of injecting concentrated salt was 50/50. The injection of the concentrated salt into the CFS was carried out and he had excellent relief for a few days, but the pain gradually returned. The injection was repeated and then pain

relief persisted until his death. There were no major complications but minor transient leg weakness occurred for a few days following the second injection.

iii A patient, 63 years having a tumour involving the rectum and the adjacent portion of large intestine benefited from a percutaneous cordotomy in the neck. The pain returned many months later. The patient was continued on opiates until his demise.

iv. Two patients suffering from chronic back ache got relief with spinal injection of local anaesthetic.

1.11.4 **Measurement of Pain Threshold**

There are three general methods used for assaying the effectiveness of an analgesic agent.

- testing the effects of the agent on the reaction of an animal to noxious stimulation (thermal, electrical, chemical or mechanical stimuli).
- measuring the pain-reducing effects in normal human subjects with experimentally induced pain.
- measuring the pain reducing effects in patients experiencing pain spontaneously.

In general it may be said that animal testing serves as a rough assay of analgesia, test on normal human subjects offer a more precise assay of strength of analgesia, and measurements in patients with pain evaluate the effectiveness of the agents

under circumstances most relevant to clinical use (Balla and Bhargava, 1980; Dipalma, 1971).

Methods for Investigations of New Narcotic Analgesics

1. Mouse Narcotic Dependence Model
2. Hot-plate Tests for Analgesia: Mouse
3. α - NOAA - Induced Jumping, mouse
4. Mouse Writhing Test for Analgesia
5. Rat Narcotic Dependence Model
6. Rat Self - Administration Model
7. Monkey Self - Administration Model

Hot - Plate Test

These modified versions of the method described by Eddy and Leimbach are used to detect narcotic agonist and antagonist activity of unknown compounds by their effect on the reaction time to a thermal (nociceptive) stimulus. In each case, groups of 10 male mice 18-25g are tested 3x at 20min interval to determine the time in secs to respond to being placed, individually in a restraining cylinder, on a hot plate (55 +0.3°c) either by licking a foot or jumping. Mice taking longer than 15 sec to respond are discarded. The median of the three pretreatment response time is used for later comparisms in all three versions of the test.

1. In the initial screening procedure, saline or the compound of interest is administered iP immediately following the 3rd pretrial. 30 mins later, the mice are retested on the hot plate to determine inherent analgesic activity of the test compound. This trial is followed by administration of morphine sulfate at a standard dose of 40mg/kg iP and by further hot plate trials 15, 30, 60 and 90 min later. If a mouse fails to respond within 30 sec after being placed on the hot plate, the trial is terminated and the analgesic effect is considered to be maximal.

2. To evaluate narcotic antagonist, Morphine sulfate at a standard dose of 40mg/kg ip is administered after 3rd hot plate trial. 15mins later, the mice are retested on the hot plate to determine the new response time for each mouse. Saline or the compound of interest is then administered ig or ip at a dose of 25mg/kg and the mice are retested on the hot plate at 15, 30, 60 and 90 min post injection.

3. To evaluate analgesic (antinociceptive activity), the standard hot-plate procedure is used. Following the 3rd hot-plate trial, saline or the compound of interest is administered ip or ig. Each mouse is then retested at 15, 30, 60, 90 and 120mins following drug administration.

In determining analgesic activity, a positive response is taken to be a reaction time at any of the succeeding trial which is double the partial median time. A dose of test compound is considered active if 50% or more of the treated group respond positively.

To determine actively in the antagonist test, the group mean response time from those animals treated with both morphine and the test compound is compared with the mean from the group receiving both morphine and saline. Statistically shoter mean response latencies at any three retest times are taken to indicate activity. The standard narcotic anatagonists naloxone and nalophine, both block and reverse morphine analgesia in these procedures (Ehrenpreis and Neidle, 1975).

1.11.5 Drugs Used in Pain Relief

(1) Analgesics

Certain drugs can alleviate pain by reducing the source of the pain: for e.g. in the pain of myocardial ischemia amyl nitrate may dilate the coronary arteries and reverse the chain of events which has caused the noxious stimuli. In distinction the analgesics prevent, without producing unconsciousness, the nervous integration of noxious stimuli which would otherwise be interpreted as pain by the central nervous system. These drugs are in two groups.

(a) Antipyretic Analgesics

These are weak analgesics, of which acetyl salicylic acid (ASA) is typical, and it is notable that much of their effect is peripheral in the nervous system so that behavioral changes do not usually occur. ASA produces analgesia by blocking the effects of bradykinin which is an important mediator of pain. It also interferes with the synthesis of prostaglandin which sensitises the nerve endings to beadykinin and this effect will also contribute to the analgesia. The antipyrexial and antinflammatory effects of ASA are also probably the results of its interference with the synthesis of prostaglandin since these play a key role in febrile and inflammatory processes.

The initial members of this group were produced as synthetic substitutes for quinine despite their lack of antimalarial activity, they have proved useful antipyretic, analgesic and antiinflammatory agents. More recently introduced compounds have been selected for their analgesic and anti-inflammatory properties rather than their antipyretic activity. Broadly this group now includes derivatives of the following chemical compounds: salicylic acid, aniline, pyrazole, indole and anthranilic acid.

(b) Narcotic Analgesics

Morphine is typical of the drugs in this group, which acts more centrally in the nervous system than the antipyretic analgesics and produces, narcosis (i.e. stupor) and other behavioral changes in addition to potent analgesia. Furthermore their repeated use is associated with tolerance, which results in the rapid escalation of the dosage required to control pain, and the very serious problems of psychic and physical dependence. Morphine owes much of its success to its calming effect on the emotional attribute of pain and the early judicious use of specific tranquilizers and other psychotropic drugs together with weaker non-addicting analgesics may prove an effective substitute for narcotic analgesics, at least for a time, in the management of chronic pain.

Opium, the dried juice obtained from the unripe capsules of *Papaver somniferum*, has been used from ancient times. It contains over twenty alkaloids but its analgesics effect is almost entirely due to morphine. Morphine still remains one of the most successful potent analgesic available and it is the standard by which the analgesic activity of any new synthetic potent analgesic is judged. It is also unhappily the standard by which addiction is assessed.

Narcotic analgesics may be conveniently divided into three main groups depending upon their chemical

relationship to morphine, pethidine or methadone. The morphine group, unlike the other two groups, comprises both natural derivatives and synthetic agents.

- Morphine group - Natural Derivatives

  morphine

  codeine

- Morphine group - synthetic agents

  Diamorphine hydrochloride (heroin)

  Levorphanol tartrate

  Phenazocine hydrobromide

  Pentazocine

  Dihydrocodeine tartrate

  Oxycodone pectinate

- Pethidine droup

  Pethidine hydrochloride

  phenoperidine hydrochloride

  Fentanyl citrate

  Ethoheptazine citrate

- Methadone group

  Methadone hydrochloride

  Dextromoramide tartrate

  Dipipanone hydrochloride

  Dextropropoxyphene hydrochloride

(2) <u>Other Analgesics</u>

a. Carbamazepine

b. General anaesthetic agents

nitrous oxide

trichloethylene

methoxyflurane

(3) **Psychotropic Drugs**

In those patients in whom there is hightened anxiety, pain can often be relieved by decreasing the emotional attribute with tranquilizers. When there is depression associated with chronic pain, changing the emotional attribute towards euphoria with an antipressant or psychostimulant may well ease suffering.

These drugs are divided into:- major tranquilizers minor tranquilizers, antidepressants and psychostimulants.

(a) **Major Tranquilizers:**

These have profound effects on the central nervous system, including its autonomic responses. So powerful are their effects that psychotic patient can be made manageable and even brought into remission. Included in this group are:- Rauwolfia alkoloids (reserpine and deserpidine), phenothiazine (chlorpromazine Hcl - P.O. or IM), butyrophenones (Droperidol, haloperidol and oxypertine).

(b) **Minor Tranquilizers**

These have only limited effects in the CNS compared with the major tranquilizers and are used to treat neurotic stages and anxiety reactions to

every-day stresses. Included in this group are benzodiazepines and carbamate.

i. **Benzodiazepines**

Most commonly used are: chlordiazepoxide, diazepam, medazepam and oxazepam.

ii. **Carbamate**

Meprobamate is the commonly used drug. Diazepam is rather prescribed in preference to this due to its less muscular relaxation.

(c) **Anti-depressant Drugs**

These fall into 2 groups

i. Monoamine oxidase inhibitors

Owing to the widespread inhibition of menoamine oxidase by these drugs, serious complications can occur should either sympathomimetic agents, or pethidine and other narcotic analgesics drugs be administered.

ii. Tricyclic (Dibenzazepine) Antidepressants

Imipramine HCl 25mg t.d.s. has ben found to be helpful in the treatment of chronic pain associated with rheumatoid anthrisis and osteoarthrisis. Side effects with tricyclic anti-depressants are not usually a serious problem as with monoamine oxidase inhibitors but 2 important reactions are the antagonism of

drugs like guanethidine and bethanidine used in the treatment of hypertension and the potentiation of nor-adrenaline and adrenaline either of which may be injected with local anaesthetic drugs during nerve blocks.

(d) **Psychostimulants**

These include: caffeine
amphetamine
cocaine

They stimulate the ceretral cotex and affect the mental state.

Caffeine in the amount contained in a strong cup of coffee or tea (about 150mg) can alart the cortex and produce mild degree of euphoria.

Amphetamine cannot be recommended in pain relief due to its serious side effects including addiction.

Cocaine - the intractable pain and general anguish of terminal malignancy can be greatly eased by cocktails of cocaine, diamorphine and morphine in sweetened spirits. These cocktails produce excellent euphoria and have the advantage of avoiding the need for injection to produce potent analgesia.

(4) **The Barbiturates**

These drugs are most commonly prescribed hynotics. They are also used as minor tranquilizer despite the development of more apt drugs. Barbiturates cause particular restlessness in the presence of pain. It has been suggested that they have an antianalgesic effect but more probably they increase the reaction to pain by removing natural inhibitions. It will be gathered then that barbiturates are not ideal hypnotic in the presence of pain and nitrazepam which is the hypnotic homologue of diazepam is a much better choice for night sedation. Nitrazepam is also much safer in over dose than a barbiturate, which is an in-patient consideration as prolonged pain can cause suicidal tendencies.

(5) **The Placebo Effect**

About 30 of patients may be expected to report symptomatic relief in any illness following administration of inert substances. This serves to emphasize the power of mind over body. It also illustrate the inter play of the rational, emotional and physical attributes of pain. The physician need to have no feelings of guilt when a placebo intentionally given produces improvement. He need only regret that most of these improvements are temporary. (Hannington -kiff, 1974)

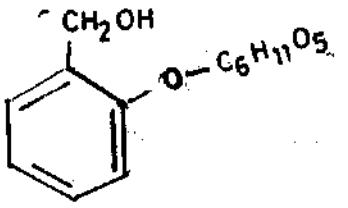
1.11.6 MEDICINAL CHEMISTRY OF SALICYLATES

Salicin (salicyl alcohol glucoside) was the first compound belonging to this category that exhibited medicinal value. It was employed as a substitute for guinine as a febrifuge. Salicin and methyl salicylate (Fig 6 &7) may be found in the leaves, barks and fruits of many trees and plants e.g willow (genus *Salix*). These botanical services were known to Hippocrates some 2,400 years ago and were used by Galen in the second century for the treatment of certain local diseases such as ulcers, fistulas and erusipelas. All through the middle ages salicylates were used in many medical treaties including diuresis. Willow bark was used to substitute cinchona bark as antipyretic because the later was more expensive (Dipalma, 1971; Kar, 1993).

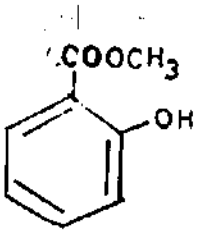
Salicin was first extracted early in the nineteenth century from willow bark, and a few years later it was isolated in its pure state. The first preparation of salicylic acid was from salicin. Although it had been prepared by oxidation of salicylaldehyde and later by hydrolysis of anthranilic acid (0 - aminobenzoic acid). In 1860, the commercial method of preparing salicylic acid from sodium phenate was introduced. Salicylic acid was not generally available until after its synthesis from phenol in 1874. Perhaps the relationship of salicylic acid to phenol led to its early use as an antiseptic. (Dipalma, 1971, Kar, 1993).

Salicylic acid (M.p. 159°) occurs mainly as esters and glycosides. It is found in small amounts in cherries, strawberries and raspberries (Kretorich, 1966). It occurs less frequently compared to the following phenolic acids (Fig. 8a & 8b)

FIG.6: SALICIN AND METHYL SALICYLATE



SALICIN
(Evans, 1989)



METHYL SALICYLATE
(Dipalma, 1971)

FIG.7: SALICYLIC ACID
(Dipalma, 1971)

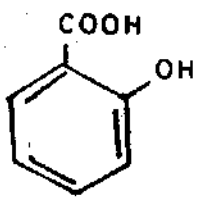
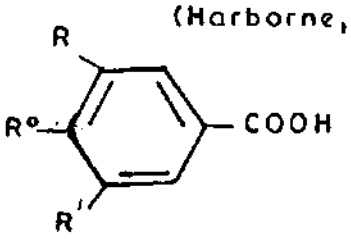


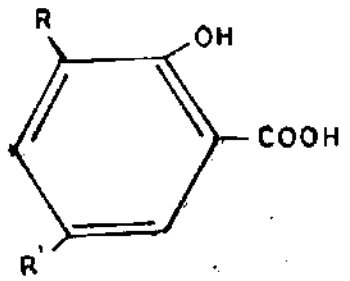
FIG.8: PHENOLIC ACIDS
(Harborne, 1964)

Fig.8a.



P- Hydroxybenzoic = (R = R' = H)
Protocatechuic (R = OH, R' = H)
Vanillic (R = OMe, R' = H)
Gallic (R = R' = OH)
Syringic (R = R' = OMe)

Fig-8b.

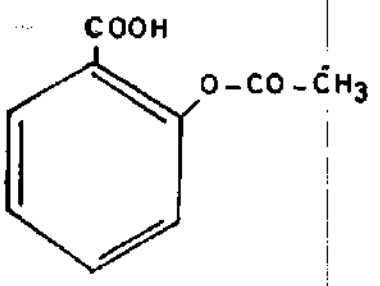


Salicylic ($R = R' = H$)

O-Pyrocatechuic ($R = OH$, $R' = H$)

Gentisic ($R' = OH$, $R = H$)

FIG·9; ACETYL SALICYLIC ACID (ASA)
(Dipalma, 1971)



It was originally isolated from *Gaultheria procumbens* as the methylester (the oil of wintergreen of the pharmacpoeia) it was later found in the bark of birch, *Betula lenta,* and occurs in the essential oils of numerous other plants. Salicylic acid and the other related o-pyrocatechnic acid seem to be characteristic constituents of Ericaceae (Harborne, 1964).

In 1876 salicylate were first used in rheumatic fever and about 20 years later aspirin (acetylsalicylic acid) (Fig. 9) was introduced into medical practice (Dipalma, 1971).

Acetylsalicylic acid (ASA) was first synthesized in 1852 but more or less remained obscure until its detailed pharmacodynamic properties were studied in 1899. The name 'aspirin' was derived from "a" of acetyl and adding to it "spirin", an old name of salicylic or spiric acid obtained from spirea plants.

ASA can be synthesised by acetylation of salicylic acid with acetic anhydride. The crude product may be recrystallized from benzene, mixture of acetic acid and water (1.1) or various other non-aqueous solvents (Kar, 1993).

The methyl and ethyl esters of salicylic or acetylsalicylic acid can be prepared by treating these acids with acidified (concentrated Hcl or H_2SO_4) methanol or ethanol respectively. The methyl esters of these salicylic acids can also be prepared by treating them with ethereal diazomethane. The products are prodrugs, since they yield the pharmacologically active acids by hydrolySis following their

absorption either from the gastrointestinal tract or through the skin (Rainsford, 1984).

ASA is used as an antipyretic, anti-inflammatory and an analgesic in a variety of condition ranging from headache, discomfort and fever associated with the common cold, and muscular pains or aches. Aspirin is regarded as the drug of choice in the reduction of fever because of its high degree of effectiveness and wide safety margin. As aspirin inhibits platelet function, it has been employed prophylactically to minimize the incidence of myocardial infarction and transient ischemic attacks.

The antipyretic and anti-inflammatory actions of the salicylate are due to their inhibitory effect on prostaglandin synthesis (Kar, 1993).

The above mentioned salicylates (salicin, methyl salicylate, salicylic acid and acetylsalylic acid) are principal members of this class.

1.11.7 NATURALLY OCCURRING SALICYLATES

Various salicylates are found in an immense variety of plant and bacterial species (Table 2). Saligenin and its glycoside, salicin, occur in the willow and poplar trees and the black haw. Methyl salicylate (oil of wintergreen or *Gaultheria)* occurs in various species, ranging from trees (e.g. birch, myrtle and beech) to grasses (e.g. wheat, rye, sugarcane), legumes (e.g peas, beans, clover) and exotic plants

(e.g Indian liquorice, tea berry and coffee). Even the content of methyl-salicylate present in conventional (black) tea has been found to be related to its flavour characteristics, the optimal salicylate content being 7-10 ug/kg. Salicyl-aldehyde is produced by members of the *spirea* genus, *Filipendula* spp. (e.g. meadowsweet, bridal wreath). Salicylic acid as well as its 3-hydroxy and 6 - methyl derivatives are produced in relative abundance by some mould, several marine organisms and bacteria.

Different types of 6-n-alkyl salicylic acid are present in the shell liquid of the cashew nut (*Anacardium occidentale*) as a mixture comprising various anacardic acid compounds [6-($C_{15}H_{31-x}$), where x = 0, 2, 4, or 6]. Other 6-n-substituted salicylic acids are found in various plants sources e.g. ginkgolic acid [6-($C_{15}H_{39}$) - salicylic acid] in *Ginkgo biloba*, pelandjauic acid in *Pentaspadon* spp., and others in *Chrysanthemum* spp. 6-n-pentadecylsalicylic acid and 6-n- tridecylsalicylic acid have also been isolated from various plants and marine organisms.

The reason why these salicylate are produced in such relatively high abundance by plants is not clear. However, salicylic acid and its 3-hydroxy derivative are apparently growth factors for certain bacterial being required for ion transport Salicylate is not synthesized by *Mycabaterium* when iron (Fe^{3t}) is present in abundance, there being feedback control by Fe^{3t} on salicylate synthesis. Other aromatic acids (e.g. citrate oxalate) cannot substitute for salicylate in its role on iron transport, but excess phosphate does cause suppression of salicylate - mediated iron transport. It ia also possible that free salicylate may serve in the transport of irons (e.g Fe^{3+}) in plants. The synthesis of the glycoside or derivatives of salicylate represent end or inactivated products.

Salicylate have been identified in beaver castor (i.e of scent glands) from where it is secreted instead of via the usual urinary route. The salicylate are metabolic transformation products from vegetable sources in the diet of the beaver. The methyl ester of 6-methyl salicylate is produced as a defence secretion in the ponerine ant. Otherwise, salicylate do not appear to be abundant in animal tissues or secretions.

In bacteria, the carbon atoms of salicylic acid and its 3-hydroxy derivative are synthesized from the glucose metabolites, phosphoenolpyruvate and erythrose - 4 - phosphate, via the shikimate-chorismate pathway.

In plants, another branch of this pathway yields phenylalanine which is metabolized either via cinnamate to salicylaldehyde or through benzoate to salicylate. O-Glucosylation of salicylaldehyde forms helicin. Alternatively, salicylaldehyde may be oxidized to salicylalcohol (saligenin) which subsequently combines with glucose to produce salicyl alcohol glucoside (Salicin).

Salicylic acid is also obtained from the microbial oxidation of naphthalene in a strain of *Pseudomonas*. An entirely different series of reactions are involved in the biosynthesis of 6-methylsalicylic acid mycobacteria. In this case acetyl - SCOA and malonyl-SCOA serve as precursors which enter the polyketide pathway to form 6-methylsalicylic acid, which then serve as an intermediate in the synthesis of antibiotics. (Rainsford, 1984).

1.12 FRUITS

Hulme (1970)

1.12.1 Introduction

Fruits have long been valued by man for their attractive flavour, appearance and texture, in more recent years, their vitamin content has become recognized as an important feature. In all these properties sugars either in free state or as derivatives play an important role. Flavour is fundamentally the balance between sugar and acid, in addition specific flavour constituents are often glycosides; the attractive colours of many fruits are due to sugar derivatives of

anthocyanidins; texture is governed by structural polysascharides and finally ascorbic acid (Vitamin C) commonly considered to be a sugar derivative is found widely and sometimes abundantly in fruits.

1.12.2 SUGARS FOUND IN FRUITS

From the flavours of different fruits it is apparent that sugar content vary widely - from traces 0.72% in lime to 61% of fresh weight of dates. (Determinations from ripe fruits or at normal harvest).

Lime ripe fruit

Date normal harvest

Avocado 0.4% normal harvest

The values were determined after inversions (invert sugar), i.e. sugars other than glucose, fructose and sucrose are absent or present in small amount.

The average total sugar contents of many fruits lie in the region of 5 - 10%. Fruits preserved by drying (date, fig, raisin grape), those used in the production of alcoholic drinks (grape, cider, apple, parry pear) and a few tropical fruits (pineapple, banana), contain higher amounts.

A Monosaccharides

Hexoses

In most fruits the glucose concentration exceeds that of fructose, occasionally the former is double the latter, while in cranberry, apricot and plum it is 3 to 5 times greater.

Grape black berry, gooseberry, raspberry, strawberry and oranges contain similar amounts of glucose and fructose. In the olive, fructose comprises 39 - 67% of reducing sugars. Apples and pears are the main fruits in which fructose is present in amounts up to 3 times greater than glucose, in cider apples the relative proportion may be even greater as glucose comprises only 9 - 20% and fructose 91 - 93. 5% of the reducing sugars.

Hexose other than glucose and fructose are rarely found in fruits and then usually in trace amount only.

Mannose - In apple, peach and orange, olive pear (some authors did not detect this in apple and peach).

Galactose - Pears, apples, peaches, grapes, olives, sapodilla (5.4% of the total sugars)

<u>Pentoses</u>

Arabinose - has been detected in a number of fruits apples, quince, fig, pomegrante, lime, grape fruit, tangerine, grape, date, mango, guava.

Xylose - is well authenticated in fruits of the family Rosaceae, namely strawberry, cherry, peach, apricot, pear, apple, plum, damson, quince, olives.

$C_7C_8C_9$ SUGARS

The first longer-chain sugar to be found in fruits was n-mannoheptulose (perseulose) in the avocado. 2 others seven carbon sugars have been isolated from avocado:- D -talo - heptulose and D-glycero - D - galacto - heptulose. In addition 2 octuloses and 2 nonyloses have been isolated and identified -

D-glycero-D-manno octulose

D - glycero-L-galacto-octulose,

D - erythro-L-gluco-nonulose and

D - erythro-L-galacto-nonulose.

Heptuloses have also been found in cantaloupe melon, kadota fig., orange, strawberry, tomato and in the peel of grape fruit and orange.

B. Oligosaccharides

Disaccharides

Socrose is the main disaccharide present in fruits. It's concentration in fruits is often determined by the difference between the reducing sugar content before and after inversion. Disaccharides other than sucrose have rarely been reported, the sapodilla contains lactose and maltose which contribute 0.1% and 0.5% respectively of the total sugars.

Trace amounts of maltose and melibiose have been detected in the grape.

Tri and Higher Oligosacharides

Although many oligoseccharides occur in fruits usually in small amounts, raffinose, stachyose, manninotriose, (a

trisaccharide consisting of a glucose unit and 2 fructose were found in apricots, others were of glucose only and fructose with hexitol).

C. **Sugar Derivatives**

Sugar Acids

D-galacturonic acid is rarely found in the free state in sound fruit, but like D-xylose it does occur in some fruits of the family Rosacaee. Where microbial attact has occured galacturonic acid is accompanied by di, tri and higher oligogalacturonic acid. Sugar acids are found in cider apples, apples, tomatoes, grape, strawberry,

D-Glucuronic acid (mucic acid) have been isolated from ripe peaches and pears, cider apples, grapes.

D-glucuronic acid has been stated to occur together with D-galacturonic acid in grape juices. D-glucuronic acid is rare in the free state, it is found as a constituent of many polysaccharide e.g. plum fruit gum.

L-Ascobic acids together with dehydroascorbic acid is found in a wide range of fruits.

Polyols

(a) **Straight Chain Polyols** - There are few reports of the presence of the shorter chain members of this group in plant tissues. The olive is noteworthy in that it contains free glycerol. Free pentitols may be more wide spread than apparent. Ribitol probably occurs universally in fruits in the form of its derivatives riboflavin,

flavin mononucleotide and flavin adenine dinucleotide. Of the hexitols, D-glucitol (Sorbitol) is found in many fruits, notable those of the family Rosaceae. Although D-mannitol is found in many plant tissues it rarely occurs in fruits (seeds of melon 0.666%, unripe olives may contain more mannitol than total sugar but the proportion is as low as 23% at ripeness, a decrease from 3.8 to 0.34% of fresh weight).

The longer chain polyols D-glycerol-D-galacto-heptitol (perseitol) and D-erythro-D-galacto octitol have been isolated from avocado.

(b) **Cyclic Polyols** - Of this group of compounds only myo-inositol (formerly known as meso-inositol) has been found in fruits. It is probably ubiquitous in plant tissues and often occurs as its hexaphosphoric acid ester (phytate) in seeds. Although myo-inositol has frequently been tentatively identified by paper chromatographic means in fruits e.g. in apricot, peach, pear, and apple, it has rarely been identified chemically. Microbiological assay has been used for some fruits, particularly juices

Sugar Phosphates

These esters, active intermediates in sugar metabolism, are usually present in mature tissues in low concentrations only. Enzymatic method are used for their determination provided the extraction procedures employed rapidly inactivate posphatases. D-glucose-6-po_4 (F-6-P) is the ester found in the largest amounts, several times greater than that of D- fructose

6-PO4 (F-6-P); fructose-1,6-diphosphate (F-1,6 dip) is usually found in even lower concentrations Contents of sugar phosphates have been determined only in a few fruits (Typical value for Apple (Bramley's seedling) are:-G-6-P 2:2, F-6-P 0.5 and F-1, 6-dip 0.1 umoles/100g fresh weight).

Glycosides

Phenolic glycosides occur widely in fruits and many contain sugar not otherwise found in plant tissues.

Cyanogenic glycosides in particular mandelonitrile the cyanohydrin of a benzaldehyde are found in a number of fruits. Amygdalin (D(-)-mandelonitrile-β-gentiobioside occurs in the seeds of apple, apricot, plum, peach, cheery, quince and damson. Sambunigrin, L(+) - mandelonitrile-glucoside is a constituent of immature elder barries to which it imparts a bitter flavour.

One sterol glycoside has been identified in fruits β-sitosteryl-D-glucoside obtained in a yield of 0.00037% from the juice of Fluorida Valencia orange and of 0.0058% from commercial grape fruit pulp.

Sugar Nucleotides

The range of nucleoside found in plant sugar nucleotides include uridine, guanosine, cytidine, adenosine, thymidine, and 2-deoxyuridine; the commonest sugars found as nucleotide derivatives are glucose and galactose. They are present in fruits in trace amounts (as they play important part in sugar inter-conversions and synthesis of polysaccharide).

Other Sugar Derivatives

L-Rhamnose (6-deoxy-L-mannose) is a frequent constituent of glycosides but is rarely found free. It has been reported

after fermentation of grape juice but many have been released by glycosidase action. 2-deoxy-D-ribose was reported in the same wine. D-Glycosamine (2-amino-2-deoxy-D-glucose) has been identified in grape juice. Both glycosamine and galactosamine have been obtained from plant tissues as their uridine diphosphate-N-acetyl derivatives but as yet these have not been found in fruits. *Unripe guava fruits contain* 0.1% *of an* unusual sugar ester which completely disappears as the fruits ripen. The two cis hydroxyl groups attached to C's 3 and 4 of the sugar molecule. L-arabinose, are estrified by the 2-COOH groups of hexahydroxy-diphenic acid.

Olives contain 2 unusual sugar derivatives. The first is an ester of sucrose with oleuropeic acid (a monoterpenoid-carboxylic acid) and the *second oleuropein*, the bitter principle which is a complex glucoside.

1.12.3 CHANGES IN SUGARS DURING THE LIFE OF THE FRUIT

A. *Changes during growth*

During the early stages of fruit growth the developing ovules are nurtured in part at least by the ovary and its ancillary parts, sepals, bracts and the receptacle. Theses are normally green and capable of photosynthesis. While the fruit is small these organs play an important part in its nutrition, but as the fruits grow although the outer layer of cells may photosynthesize until maturity, the main source of nutrition is the leaf. Cell division in the young fruit may be completely in as little as 3 or 4 weeks for the apple or may proceed up to maturity as in avacado. Cell extension continues up to maturity in all fruits.

The main sugar transported from the leaves to the fruits is sucrose but other sugars of their derivative are important for translocation in some plants; these include sugar such as raffinose, heptulose and D-glucitol. In the apple it has been shown that ^{14}C labelled D-glucitol is translocated from the leaves at a greater rate than sucrose, and the labelling rapidly appear in sugars, aminoacids and phloridzin. While part of the sugar translocated to the young fruits is used for synthesis to pectic substances and other cell-wall materials, part is converted to the usual storage product, starch.

1. **Starch**

Starch is commonly found in the outermost cells of the fruit probably as the product of photosynthesis of these cells. Many young fruits e.g. apple, pear, tomato, citrus fruits,mango and banana contain starch; in some fruits an initial increase in concentration is followed by a decrease while in others the concentration may increase up to maturity. Among the latter, climacteric fruits are prominent e.g. banana, mango, and passion fruit, but in the plum, another climacteric fruit, very little starch is present from the immature to the mature stage where it may be completely absent.

2. **Sucrose**

No consistent patterns emerge from changes in sucrose concentration during growth and maturation with respect to the distinction between climacteric and non climacteric fruits. Some climacteric fruits e.g. apricot, peach, mango and passion fruit show a high sucrose content at maturity. But while in apricot and peach sucrose increases in concentration during

maturation, in passion fruits it shows a slight decrease. Other climacteric fruits, however, show very little or no sucrose during growth and maturation e.g. tomato, while plum has none at the immature stage but shows a rapidly increasing content from the yellow to ripe stage. Apple and pear show an increasing sucrose concentration up to normal harvest.

Among non-climacteric fruits immature oranges have fairly high sucrose concentration but during maturity this decreases slightly. A similar trend occurs in grape fruit. The sucrose content of pineapples increases rapidly during the last two months of maturation to a high value. In the cherry, sucrose is absent throughout growth and maturation. Throughout the whole age of the grape, both sucrose and raffinose concentrations are very low but reach their maxima at maturity.

(3) <u>Reducing sugars</u>

Reducing sugars often increase steadily throughout growth and maturation in both climacteric e.g. tomato, passion fruit and non-climacteric fruit e.g. cherry and grape. In cherry the proportion of glucose to fructose remains 1:1 to 1:0 throughout maturation. Unripe grapes contain slightly more glucose than fructose but at over-ripeness the proportions are reversed. In certain climacteric fruits considerable changes in relative proportions may occur. In apples and pears, the glucose exceeds the fructose concentration during the early weeks of growth, then the glucose concentration remains constant and the fructose concentration increases up to and just after normal harvest.

(4) Sugar derivatives

D-Glucitol shows a steady concentration of 5% on a dry weight basis throughout growth and maturation in victoria plum but in the kelsey plum after an intial accumulation of 1% of the fresh weight no further accumulation occurs until stone growth is complete and the reducing sugar concentration has become constant. Perceitol in the avocado reaches its maximum content at normal harvest. Free galacturonic acid remains at a steady concentration from the unripe to the ripe stage in delicious apples, while in the tomato the concentration increases ten-fold from unripe to ripe condition.

B Changes During Storage:

In fruits of the non-climacteric type, harvest is normally either at ripeness or may be later. Such fruits show only slow steady changes in sugar content, hence harvest may be spread over a long period without loss of quality. Citrus fruits are of this type and the sugar content may show an initial increase on storage as a result of metabolism of cell-wall polysaccharide. In the orange, the concentrations of sugars in both the juice and the rind decreases. During storage of grape fruit similar changes occur.

Climacteric fruits on the other hand are picked prior to the onset of ripening and ripeness is attained only during or just after, the climacteric rise in respiration. Changes in fruit colour and texture occur notably in the banana, mango, and avocado. Almost complete hydrolysis of starch occurs in

the banana and mango during ripening. In the former, approximately equal concentrations of glucose and fructose, together with a litter sucrose appear. After prolonged storage all 3 sugars decrease. The mango on the other hand, shows a large increase in sucrose concentration and a smaller proportion of reducing sugars as starch is hydrolysed. Later in storage, sucrose tends to disappear to be replaced by an equal amount of reducing sugars.

Initial hydrolysis of starch with the concomitant appearance of sucrose is seen in apples and pears, in apples, however, the increase in sucrose content is much greater than could be accounted for by hydrolysis of starch. Reducing sugars also increase initially and then decrease in apples, while in pears reducing sugars show less change. The sucrose content of apricots and peaches show little change on storage, reducing sugars in apricot decreases while in peach a decrease is followed by an increase.

D-glucitol in both plums and pears is rapidly metabolized on storage; in pears, loss of D-glucitol is accompanied by an increase in fructose content. In the apple, however, it seems likely that during low temperature storage fructose is reduced to D-glucitol.

The 7 - C compounds D-manno-heptulose and perseitol are both metabolized almost completely on storage.

Galacturonic acid increases very considerably from ripeness to over ripeness in apple, but tomatoes during storage show a small decrease in this constituent.

Sugar phosphates show variations in some fruits between the immature state and the Climacteric state. During the climacteric phase of banana the fructose 1,6 -diphosphate content increased twenty times, while glucose 6 - P04, and fructose - P04 increased only 2 or 3 times. Apples, however, showed no such rise and pre-climacteric and climacteric fruits showed similar glucose-6-P04 and fructose-1,6-diP04 contents.

1.12.4 **Organic Acids**

Extraction and Identification

Water or boiling water are generally used for extraction of organic acids from plant tissues. The solutions obtained in this way are passed down cation exchanger which remove cations and amino acids but not free acids or neutral components. It is possible to measure the total acidity with organic anions but not with neutral substances which pass through the column. After washing, elution of acids is carried out and separation is achieved by the use of paper or column chromatography if the material used for on exchange is too basic, a part of the sugar may be changed into acids, in some conditions, chlorogenic acid is hydrolysed and gives quinic acid.

For the isolation of acids from their salts, a cationic exchanger in the H^+ form may be used, it is easy to eliminate the resin after the reaction.

Oxo acids are very fragile and readily decarboxylate . For analysis they should be converted into 2,4 - dinitrophenylhdrazenes which may then be isolated by paper chromatography.

Acids may also be isolated from extracts by silica gel chromatography.

Ion exchange was also applied. Gas chromatography was used after esterification using trimethyl silated derivatives. Identification of acids is generally obtained by using paper chromatography , together with information given by implementary reactions such as sublimation, colour, reactions, fluorescence etc.

In particular cases, other methods have been used, e.g. biological and spectrophotometric methods for quinic, shikimic and chlorogenic acids.

Many of the acids found in fruits are present in traces and these have been discovered as a result of modern methods of analysis. The most common and abundant acids of fruits are citric and malic. The presence of lactic acids in fruits may be due to the reaction of micro-organisms or to reaction with ion-exchange resins used in the separation of the acids.

1.12.5 <u>Volatile Compounds</u>

<u>The Aroma of Fruits</u>

Aroma is only one component of flavour. Taste is the other major one but appearance, behaviours on manipulation, feel in the mouth and even the sounds emitted on chewing all

play a part. Aroma is the subjective sensation produced by smelling.

Nature of Volatile Constituents

The aroma-bearing constituents can be isolated from foods usually in yields of less than 100 ppm. Fruits as might be guessed from their immediate impact of their aromas occupying the upper end of this range. For example:

Valeny bananas contains 65 - 338 ppm of volatile components other than H_2O.

Gross Michael Bananas 12 to 8 ppm

Black currants 1 - 22 ppm

Cucumber - 17ppm

Strawberries 5 - 10 ppm

Tomatoes 2 - 5 ppm

Raspberries 2 - 3 ppm

American cranberries 1 - 1 ppm

Mountain cranberries 0.8 ppm

The classes of compound which have been found among fruit volatiles are not only esters, long characterised by their fruity aromas, but also lactone, alcohols, acids, aldehydes, ketones, acetal and hydrocarbons, together with examples of phenols, ethers and heterocyclic oxygen compounds. Such a classification is to some extent arbitrary and often it can be more informative to draw together related compounds belonging to different classes such as terpenoid hydrocarbons, alcohols and aldehydes or compounds containing an aromatic ring

particularly when biogenetic origin comes into consideration.

It cannot be emphasized too strongly that, in order to have an odour a compound must be volatile, odour is by no means a necessary consequence of volatility and indeed, the detection threshold concentrations to substances vary widely, (quoting values ranging upwards from 0.007kg/litre of aqueous solutions of β-corione). Gas chromatography detectors vary in sensitivity according to design and to the nature of the substances detected. They can far exceed the human nose in sensitivity with compound with little or no olfactory effect, but they can be up to 10,000 time less efficient than the nose for those compounds which the nose most readily senses

1.12.6 Fruit Phenolics

Introduction

Phenolic compounds enjoy a wide distribution in the plant kingdom, and they are particularly prominent in fruit where they are important in determining colour and flavour. It has been suggested that they were originally by products of the metabolism of aromatic amino acids. There exist elaborate biosythetic pathways for their production in plants, and perhaps because they are secondary products and apparently play no vital role in metabolism, these pathways lead in different fruits and in the same fruits to wide variety of compounds. For instance the lowbush blue berry *(Vaccinium angustifolium)* has at lease fifteen anthocyanins. Such diversity has the

development of improved methods of isolation and identification, primarily through chromatographic procedures, which has stimulated a tremendous amount of work in this field in recent years.

The accumulation of phenols in fruits may be higher or lower than in other parts of the plant, such as bark, leaves or heart wood. The concentration of phenolic decreases as a fruit matures, but usually the total amount per fruit increases (Hulme, 1970) [For example, the monomeric flavans are continuously synthesiced in the living tissue and polymerized as the organ ages. Thus, in unripe fruit there is greater percentage of low-molecular weight flavolan than in the ripe fruit, and it has been suggested that this change is the cause of the loss of astringency (Bonner and Varner, 1965)]. Fruits and flowers often contain considerable amount of some types of phenolic such as anthocyanin, while other parts of the same plants that is, leaves or bark, have very little or none. Other phenolic, such as phloridzin of apples and arbutin of pears, are present in significant amounts in other organs but are virtually absent in the fruit (Hulme, 1970).

The levels of phenolic in fruits vary widely from species to species, variety to variety, season to season , and location to location. Disease also influences concentration. The class making up the largest contribution is normally either the cinnamic acid derivatives or the monomeric-polymeric flavans. These two classes are also more uniformly distributed

throughout the tissues of the fruits than the other classes such as anthocyanin and flavonol glycosides.

The Common Phenolic Compounds of Fruits

Cinnamic Acid Derivatives

The hydroxy cinnamic acids combine the characteristics of almost universal distribution and high concentration in higher plants. This may be due, in part to the key role they play in the formation of other, more complicated phenolic compounds. Despite their widespread presence in Angiosperms, those found consists almost entirely of the four trans acids, (p-coumaric, caffeic, ferulic and sinapic acids) (Fig. 10a). They are rarely present in an uncombined form, but usually as esters by far the most common combination is with quinic acid. Of the quinic esters the most frequently observed form involves the 3 position hydroxyl group of the quinic acid.

Accordingly, chlorogenic acid is the most important cinnamic acid derivative found in fruits and is found in large enough concentrations in apples to permit its isolation and characterisation. A second ester of importance is p-coumaryl quinic acid which also involves the 3 position of quinic acid. Esterification of caffeic acid at other positions on quinic acid gives rise to a whole series of isomeric esters.

In some fruits, such as dates, shikimic acid esters, dactyliferic acids, are formed in much the same pattern as seen for quinic acid with the caffeic acid derivatives being presummably the 3 position ester. Malic and tartaric acids

have also been found in which one or, more rarely, two cinnamic acids are esterified on the sugar. Anthocyanin and flavonoid glycosides are also found acylated by cinnamic acids through their sugar hydroxyl groups. Here p-coumaric acid is most commonly the acylating agent. Besides forming esters, the hydroxylated cinnamic acids also form, to a lesser extent, O-glycosides (fig. 10b) with sugars. While depsides predominate in fruits, derived fermentation products often contain considerable amounts of free cinnamic acid and quinic acid due to hydrolysis during fermentation and storage.

Chlorogenic acid is sometimes the predominant single phenolic compound in fruits, its concentration being as high as 0.25% in the bilberry *(Vaccinium myrtillus)*. Isomers are also prominent, and often occur together, as in the peach *(Prunus persica)* and apple *(Malus pumila)*. In the sweet cherry, *(Prunus arium)*, and other *Prunus* species, the most prominent depside is neochlorogenic acid. Several dicaffeoylquinic acids are found in coffee.

The concentration of cinnamic acid derivatives declines as fruit matures, however, the amount per fruit increases as the fruits enlarge during the growing season. An exception is found in the date where the darkening accompanying ripening results from a condensation of dactyliferic acids to give insoluble polymeric products. As is the case for most fruit phenolic, the concentration of cinnamic acid derivatives is highest in the skin and peel.

Flavans

These phenolics are widely distributed, they and their derivatives are present in particularly large amounts in higher plants. While there are a large number of types found in nature, those detected in fruits are rather restricted in composition. The presence of two or three asymmetric centres makes possible a great many isomers (+). Catechin and (-) epicatechin (Fig. 10c)are the most common forms but even though they often appear together in fruits, varieties of the same fruit can differ in their relative content of these compounds. An extreme case is the pear (*Pyrus communis)* where (+) catchin and (-) epicatechin are present in desert types such as the Bartlett variety, but are not present in detectable amounts in parry pears. In all cases of fruits where the flavans have not been found as monomers, polymeric materials are present which are unquestionably derived from them. Flavan-2, 4-diols have not been found in fruits and only rarely in other plants. They are, however, suspected to be precursors of the very common polymeric proanthocyanidins.

Flavan monomers are not found in fruits as glycosides nor are they esterified or methylated or in any other heterogeneous combinations except for the identification of (-) epicatchin -3 - gallate in grapes. In tea *(Thea sinensis)*, where large amounts of gallic acid accumulates, galloyl esters of (-) epicatechin and (-) epigallocatechin are present.

The flavans are more prominent in the skin and peel of fruits, although they are also distributed throughout the flesh. The concentration is three times as high in apples peel as in apple flesh. Their concentration in immature fruits is higher than in mature fruits.

Anthocyanidins and Anthocyanins

These compounds (Fig. 10d), largely because they are coloured and easily detected have received considerable attention.

The normal form of these in fruits are as the glycosides with sugars at the 3 and 5 positions only very rarely do the other anthocyanidin hydroxyls take part in a glycosidic bond. The sugars are normally present in the pyranose form. While a great variety of sugars have been found, the most frequently occurring mono-and disaccharides are D-glucose, D-galactose, L-rhamnose, L-arabisone, rutinose, sophorose and sambubiose. Branched tri-saccharide have also been reported. The 3 position, with one exception, is always glycoslated. Di-saccharide and tri-saccharide examined so far contain at least one glucose. The linkages found are β1-2, β1-6, or α1-6. At the position, the substituent is always glucose.

The sugars, in turn may be acylated with cinnamic acids, most commonly with p-coumaric acid followed in frequency by caffeic and ferulic acids. This acylation occurs on the sugar attached to the 3 position. Various positions of the sugar can take part in the formation of the ester. As reported for

different anthocyanin, acylation occur on the 4 position of rhamnose, the 6 position of glucose and the 2 position of the terminal glucose of sophorose. There can be considerable quantitative variations in pigment content with variety.

There is evidence that anthocyanin may be combined with other cell constituents. Metal complexes are described and a metal-flavonoid complex was isolated from cornflower (*Centaurea cyanus*). Such combinations may explain some of the colour effects in fruits.

The anthocyanin are typically located in the epidermal layers of a large number of fruits, such as apples *(Malus pumila)*, plums *(Prunus domestics)* and pears (*Pyrus cummunis)*, although with some apples, such as the berry variety anthocyanin is also seen in the flesh. With sweet cherries (*P. avium)*, if the epidermis contains significant amounts of anthocyanin, the flesh will generally have somewhat smaller concentrations of the same compound(s), but with the sour cherry (*P. cerasus)* there are some varieties, such as *Montmorency*, with anthocyanin only in the skin and others, such as *Morello* having the pigment in the flesh as well. Berries have a more uniform distribution of anthocyanins than other fruits. In contrast to the cinnamic acids and the flavans, the concentration of anthocynins increases rapidly in fruits as they near maturity.

Many fruits possess an enzyme that is capable of decolourising anthocyanins. Some of these systems require

trace amounts of catechol, catechin or other orthodihydroxyl phenols as well as oxygen. The system in egg plant *(Solanum melongena)* differs in that a second, catachol-like phenol is not required. Still another variant, this time found in the apple, is that sugar is first removed and then brings about an oxidative ring opening of the aglycone, but since activity increased on storage there is the possibility that micro-organisms may have been responsible for the enzymes observed. Peroxidase has also been associated with anthocyanin destruction.

Flavonols and Flavonol Glycosides

The flavonols enjoy a wider distribution than the anthocyanin, although they are not so readily detected because of their faint or absent colour. The structure of the common flavonols bears some resemblance to that of the anthocyanidins, and like the anthocyanidins, they are usually found as glycosides. Position 3 is almost always occupied, and when two positions are occupied the 7 position is the second one to become glycosylated. There are no known 5-glycosides in fruits. Perhaps the formation of a glycoside at this position is hindered by the strong hydrogen bond type interaction of this hydroxyl group with the carbonyl function at the 4 position. The sugar forming glycosides are usually D-glucose or L-rhamnose, with rutinose being the most common disaccharide. The types of flavonol glycosides in fruits such as kaemferol, quercetin, isorhamnetin, myricetin, (Fig. 10e), show much less variety than was seen with the anthocyanin.

The amount of flavonol present in a fruit can vary considerably between varieties. In apple peel they range from 0.47 to 18.8 mg/g. fresh weight while almost none is found in the flesh. Since the concentration in the peel decreases slightly as the fruit matures, it follows that the formation of flavonol glycosides is not governed by the same factors as the synthesis of the anthocyanin. It appears that light is of some importance to flavonol production in as much as the concentration of flavonol glycosides on the sunny side of individual fruits is twice that on the shady side.

The relative amounts of the different types of flavonol glycosides are related to variety. For instance, in the grape, where there are wide differences with regard to anthocyanin, there are also differences in flavonol glycosides.

Condensed Polyphenols

These are extremely widespread compounds, both with regard to the type of fruit and the location within the fruit. The condensed materials (Fig.10f) are, like the monomeric flavans, more uniformly distributed in fruit tissue than the anthocyanins and the flavonol glycosides, but, even so, they are concentrated in the peel than in the flesh. Flavans appear to be the precursors of these covalently linked polymers. The exact nature of the bonds involved in the condensation reaction is not known with certainty, but the mass of evidence points to a carbon-carbon bond between the 4 position of one flavan moiety and the 6 or 8 position of another. Such a linkage would not reduce the number of phenolic hydroxyl groups per flavan unit. Some of these

condensed flavans yield anthocyanidins when heated with dilute mineral acid. This type of condensed flavans have been termed leucoanthocyanidin or proanthocyanidin and is present in all fruits.

Condensed flavans having two flavan units have been isolated in pure form from strawberry leaves, avocados, apples and a number of other fruits while the presence of such dimers is suspected in additional cases. Those studied thus far have yielded cyanidin on treatment with dilute acids. Some also produce (-) epicatechin. More highly condensed, less well characterised materials also form cyanidin and catechin and others yield pelargonidin in addition. Still more highly condensed materials have been found, verging on colloidal size, in parry pears. The most striking change occurring with fruit maturation is an increase in the degree of condensation.

Less Common Polyphenols

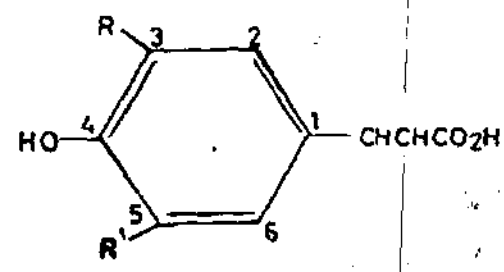
Flavones:

These compounds are widely distributed in higher plants, but they are not prominent in fruits other than citrus, where they usually occur as glycosides with sugar attached at the 7 position or occasionally at the 5 position, e.g. Apigenin, luteolin and tricin. (Fig. 10g).

Flavonones

These are also prominent only in citrus fruits where they are present as the 7 glycosides. Some of these are responsible for a bitter taste. An extensive presentation of the citrus flavonoids has been given, e.g. Pinocembrian, naringenin, eriodictyol. (Fig. 10h)

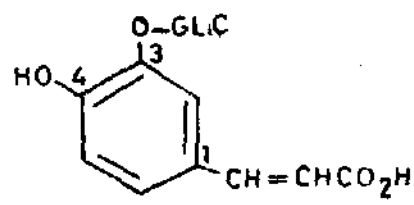
FIG.10 : STRUCTURES OF SOME COMMON PHENOLIC COMPOUNDS OF FRUITS



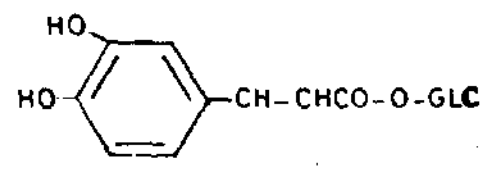
p- COUMARIC (R=R'= H) FERULIC (R= OME, R'= H)

CAFFEIC (R=OH, R'= H) SINAPIG (R= R'= OME)

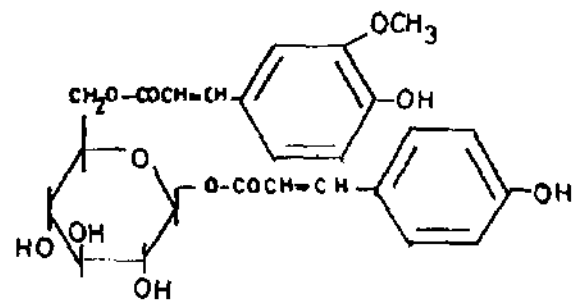
FIG.10a. Cinnamic acids



CAFFEIC ACID 3-GLUCOSIDE



1- CAFFEOYLGLUCOSE

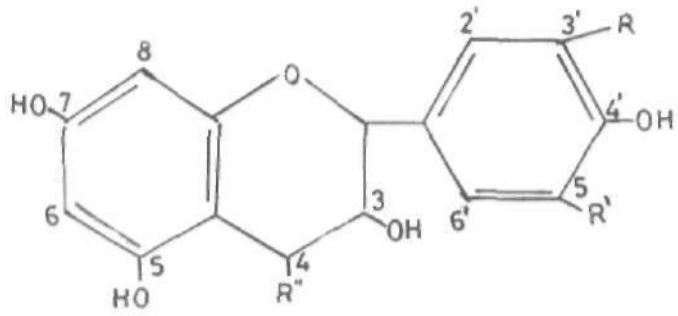


SHAKUCHIRIN

FIG.10b. Cinnamic glycosides

(Hulme, 1970)

FIG.10. CONTD.

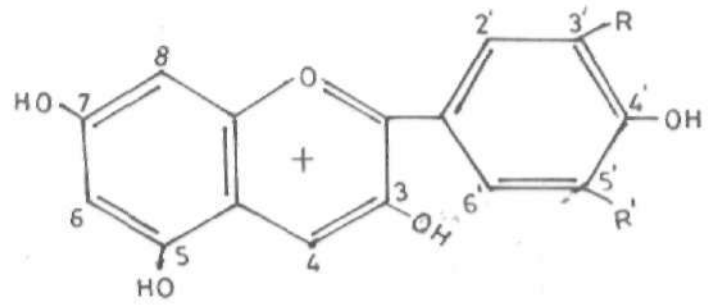


CATECHIN OR EPI CATECHIN ($R = OH_2, R' = R'' = H$)

GALLOCATECHIN OR EPI GALLOCATECHIN ($R = R' = OH_2, R'' = H$)

FLAVAN = 3,4 - DIOL ($R = R' = OH$, $R'' = H$)

FIG.10c: FLAVANS

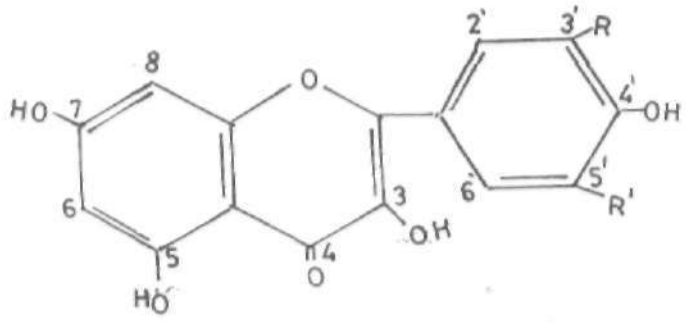


PELARGONIDIN ($R = R' = H$) DELPHINIDIN ($R = R' = OH$)

CYANIDIN ($R = OH$, $R' = H$) PETUNIDIN ($R = OH$, $R' = OME$)

PEONIDIN ($R = OME$, $R' = H$) MALVIDIN ($R = R' = OME$)

FIG.10d: Anthocyanidins



KAEMPFEROL ($R = R' = H$)

QUERCETIN ($R = OH$, $R' = H$)

ISORHAMNETIN ($R = OME$, $R' = H$)

MYRICETIN ($R = R' = OH$)

FIG.10e: Flavonols

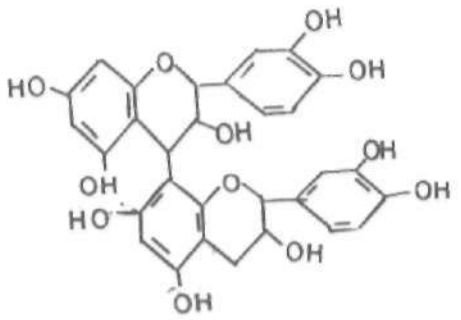
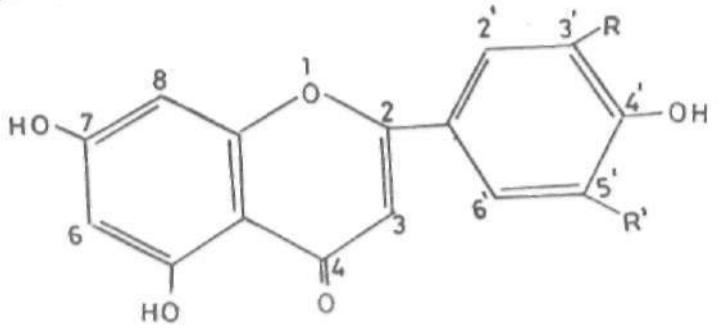


FIG.10f : Condensed flavans.

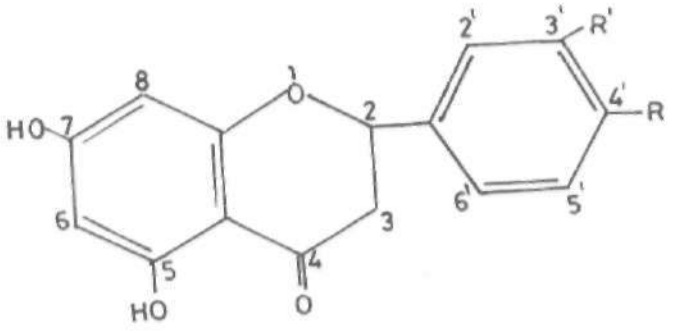


APIGENIN (R=R'=H)

LUTEOLIN (R=OH R'=H)

TRICIN (R=R'=OME)

FIG.10g : Flavones.

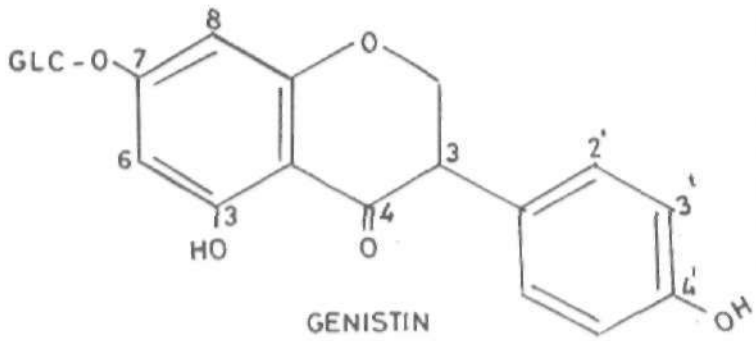


PINOCEMBRIN (R=R'=H)

NARINGENIN (R=R'=OH)

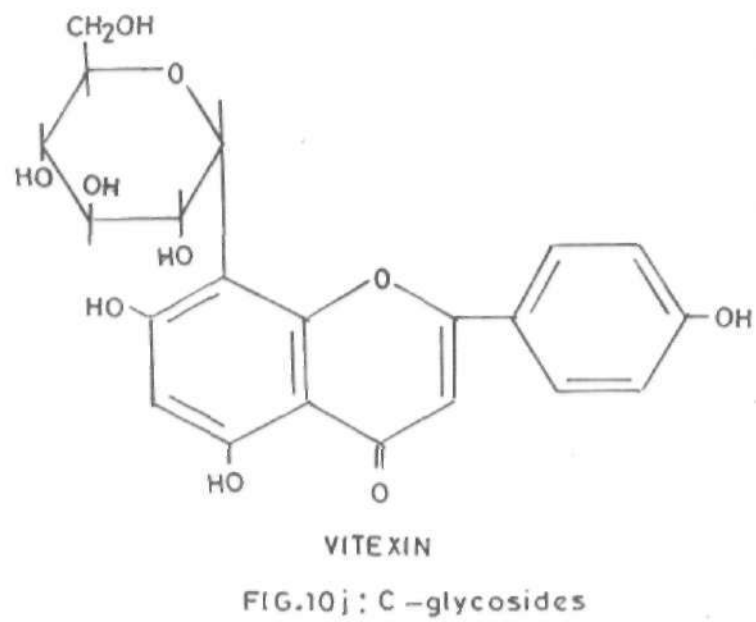
ERIODICTYOL (R=R'=OH)

FIG.10h: Flavanones



GENISTIN

FIG.10i ; Isoflavonces



VITEXIN

FIG.10j ; C-glycosides

Isoflavones

The isoflavones comprise a class 4 compounds showing wide diversity in structure, but as yet they have not been found to be significant in fruits. They frequently occur as the 7-glycoside, e.g. genistin. (Fig. 10i)

C-glycosides

This group is made up of those flavonoid, usually flavone, where sugar is attached by a C-C bond at the 6 or 8 position. (Fig.10j) While they have not been reported in commercial fruits, they have been found in other parts of fruit-bearing plants, such as in the leaves of grapes.

Taste Characteristics of Phenolics

The great majority of the phenolic compounds found in fruits have no particular taste characteristics when tested at low concentrations in the pure form. The exceptions to this general rule are the sources associated with phenolic acids, the astringency of condensed flavans and the bitterness associated with some of the citrus flavonoid.

Astringency

For several decades there has been steadily mounting evidence that astringency is due to phenolics of a certain degree of polymerization, and this has come to mean molecular weights roughly between 500 and 3000. simple phenolics, such

as hydroxy cinnamic acids, catechin and anthocyanins, are not astringent, but dimers of the flavans shows this character, as do other carefully purified condensed fruit phenolics. Varieties of fruits high in phenolic are more astringent than varieties low in phenolic, for example, parry pears compared with desert pears and red grapes compared with white grapes. During maturation the condensation of phenolics continually increases, and at the same time the astringency decreases, perhaps because the highly condensed flavans are less soluble and tightly bound to other cell components. Furthermore, there may be an optimum size of the polymer for maximum astringency per unit weight of phenolic. The question of the relation between astringency and phenolic has been comprehensively reviewed. Astringency in closely connected to the tanning reaction of condensed tannins and the ability of tannins to inhibit enzymes. Thus it is dependent upon the number of phenolic hydroxyl groups per molecule.

The astringency of fruit juices may be reduced by removing tannins through adsorption on substances containing peptide groups such as gelatin. Other proteins, or artificial peptide polymers such as nylon. In some cases as with apple juice, the addition of pectolytic enzyme decreases the stabilising effect of pectin and thus allows the precipitation of pectin-tannin-protein complexes.

Bitterness

The pronounced bitterness of some citrus flavonoid should be mentioned, despite their somewhat restricted distribution. One of them, naringin, is the chief flavonoid constituent of the grape fruit *(Citrus paradise)* and the pummelo *(C. grandis)* and also occurs is several types of oranges. It is exceedingly bitter and is easily detectable in 10^{-5} to 10^{-4} molar solutions. It is composed of the aglycone naringenin and the disaccharide neohesperidose (2-O-α-L-rhamnopyranosyl - D-glucopyranose). Another flavonoid glycoside with about one-tenth the bitterness of naringin is neohesperidin. It also conatains neohesperidose in the free form. The type of sugar at the 7 position is crucial since neosperidose imparts strong bitterness, glucose gives much less, while rutinose imparts no bitterness at all. A derivative of neohesperidin, its dihydro-chalcone, readily formed by ring splitting in alkali followed by hydrogenation, has 20 times the sweet taste, on a molar basis, of saccharin.

1.12.7 **Pharmacology**

Fruits are particularly rich in polyphenolic compounds that posses considerable reactivity towards body constituents and might altar metabolic processes. The ability of condensed phenolics to complex with proteins, the metal complexing ability of many flavonoid, the antioxidant properties of phenolics, all suggest possible pharmacological effects. Despite this, fruit phenolics have very little effect on the animal body, largely because of permeability barriers to many

of the plant phenolics in the alimentary tract and detoxification mechanisms in other body organs such as the liver.

Such barriers are seen to be particularly effective against those plant phenolics having the greatest potential effect on the animal body. 5-Hydroxy-tryptamine, a powerful vasoconstrictor present in many fruits is totally excluded from the blood stream when taken by ingestion. Some undesirable plant phenolics, such as the glucosuria-inducing phloridzin, are present in appreciable amounts in other parts of the plant.

Much interest has been given to rutin, quercetin, hesperidin and eriodictyol because their action in decreasing the fragility of guinea pig capillaries suggested a therapeutic value for theses flavonoid. However, such a possibility has been fully established. These flavonoids, in large doses, act as antioxidants, protecting adrenaline and ascorbic acid, and help to relax smooth muscle. The relatively mild action of large amounts of these phenolics had led to considerable dispute about the practical value of taking advantage, in medicine of the metabolic activity of the flavonoids.

There are a number of phenolic materials that can pass into the blood stream, among these are flavonol, their glycosides and hydroxy cinnamic acid esters. Quercetin is metabolized to give 3, 4-dihydroxyphenylacetic acid and benzoic acid derivatives which are then excreted. Caffeic acid undergoes a variety of transformations including dehydration

and the formation of the side chain, ß-oxidation and the formation of conjugates.

The only really important pharmacological active group of flavonoid are the isoflavones, some of which have weak oestrogen-like activity and can be converted in the animal to much more active substances such as equol. These isoflavones are wide spread in legumes but as yet have not been found to be important in fruits.

Finally, plant phenolics have long been suspected of playing a role in the disease resistance of plants, and this function has been accounted for in a number of ways. While there are correlations between levels of phenolic and disease resistance in fruits such as the apple, only a few cases have been presented where performed phenolic are definitely identified as protective substances. Perhaps more important than normal phenols is the rapid rise in polyphenol concentration that takes place after infection. Together with this rise there is often a considerable amount of phenolic oxidation mediated by both host and parasite polyphenol oxidase. The oxidized phenolics are more potent anti-fungal agents than the non-oxidized materials and they may also play a role in the hypersensitivity reaction which consists of the rapid death of a few cells resulting in the confinement of the pathogen to a restricted area of high polyphenols oxidase and oxidized phenol content. Oxidized polyphenols are potent inhibitors of pectolytic enzymes commonly necessary for the invasive ability of fungal pathogens. It has also been

suggested that the susceptibility of plant tissues to pathogens is dependent upon the oxidation reduction balance of the tissue and the action of polyphenol oxidase upsets this balances, creating a less favourable environment for the pathogen. It appears that, although phenolics are important in disease resistance, specific diseases and hosts must be individually evaluated for significance and mechanism.
(Hulme, 1970).

1.13 AIMS AND OBJECTIVES

The plant *Carissa edulis* grows wildly in Nigeria. It is commonly used for the treatment of many ailments in northern Nigeria. Herbal medicine is part of the socio-economic life of the people in Nigeria. Therefore, there is the need to evaluate these plants to justify their uses and to document them.

From the literature review, the pharmacognostic, phytochemical and therapeutic actions of the plant have not been studied, except for some chemical constituents of the roots which have been reported.

Therefore, this project aims at the following:

- Macroscopical studies of the different parts of the plant, roots, stem, leaves, fruits and seeds.
- Microscroscopical and chemomicroscopical studies of the various parts of the plant
- Evaluative studies of the crude vegetable drugs (powered roots, stem, leaves, fruits and seeds), such as Moisture content, Ash values and Extractive values.
- Phytochemical screening of the various parts of the plant
- Study of the Analgesic properties of the plant
- Attempt at identification of the chemical constituent responsible for the analgesic activity.

CHAPTER TWO

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1 PLANTS COLLECTION AND IDENTIFICATION

Samples of *Carissa edulis* were collected in July, 1994 from the bushes of Zango, a place between Aviation and Ahmadu Bello University (A.B.U) Samaru Zaria. The roots, stem, leaves, fruits and seeds were collected.

The plant (Plate 1 and 2) was identified in the fields using keys and descriptions given in the "Flora of West Tropical Africa" (Hutchinson and Dalziel, 1963) and "Woody Plants of Ghana" (Irvine, 1961). Later, confirmation of identify was obtained by comparism with the original herbarium specimen placed in the Herbarium of Biological Sciences Department, A.B.U., Zaria, and the voucher specimen number was collected 900132.

2.2 DRYING

The barks of the roots and stem were stripped off from the sample collected and piled separatly. The leaves were removed from their branches. The barks (roots and stem), leaves and fruits were dried both under the shade and in an oven at 50°C. When the samples were dried, the fruit pericarp was separated from the seeds.

The different parts of the plant were dried under controlled condition, at low temperature, in order to prevent chemical changes occuring due to enzymatic degradation and microbial infection during storage.

PLATE 1 CARISSA EDULIS IN ITS NATURAL HABITAT



PLATE 2 LEAVES AND FLOWERS OF CARRISA EDULIS



The dried samples were powdered with mortar and pestle and sieved using sieve of 20 mesh size. The powdered samples are easy to store and provide a greater surface area for penetration of solvents during pharmacognostic and biological studies. The powdered samples were stored in air tight container until required for use.

Some fresh samples were used for studies where applicable.

2.3 REAGENTS

The reagents used were all of labouratory grade. In all cases where water was to be used, distilled water was used.

The reagents used were :-

- Acetic Anhydride
- Acetone
- Alcohol
- Ammonia Solution
- Barfoed's Reagent
- Chlor-zinc-iodine
- Chloroform
- Chloroform Water 0.25%
- Copper Acetate
- 3, 5, Dinitro benzoic acid in 95% alcohol
- Dragendorff's Reagent (Bismuth potassium iodine)
- Fehling's solution I (A-copper II oxide) and II (B-sodium hydroxide).
- Ferric Chloride
- Formaldehyde
- Glacial acetic acid

- Hager's reagent (a saturated solution of picric acid)
- Hydrochloric acid
- N/50 iodine
- Lead acetate (10% w/v in carbon dioxie free water) (B.P.C 1963)
- Lime water
- Mayers reagent (potassium mercuric iodide)
- Magnesium fillings
- Molisch's reagent (10% solution of alpha-naphthol in alcohol).
- Phloroglucinol
- Ruthenium red
- Silica gel (for thin layer chromatography)
- Sodium acetate
- Sodium chloride (0.9%)

2.4 MACROSCOPICAL EXAMINATION OF THE DIFFERENT PARTS OF CARISSA EDULIS

2.4.1 ROOT BARK

The following features used for characterisation of whole bark samples were observed and noted:-

- origin and preparation
- size
- surface
- colour
- fracture

- fractured surface
- odour and taste (of powdered bark)

2.4.2 STEM BARK

- The features used in characterisation of whole bark as applied to root samples were employed.

2.4.3 LEAVES

The following features were used for the macroscopical identification of the leaves.

- duration
- leaf base
- petiole
- lamina
- incision
- shape
- venation
- margin
- apex
- base
- surface
- texture
- colour
- odour and taste (powdered sample)

2.4.4. FRUITS

The fruit sample was characterised using the following headings:-

- class
- shape and dimensions
- adhesion
- dehiscence
- pericarp (colour, textile, marking, number of sutures and whether uniform or differentiated into epicarp, mesocarp and endocarp).
- placentation
- seed (number)
- colour
- odour, taste, (of powdered sample)

2.4.5 SEEDS

The following headings were used in characterising the seeds sample

- size
- shape
- colour
- hilum and micropyle (size and position)
- seed coats
- perisperm
- endorsperm
- embryo
- funicle
- raphe
- aril

2.5 MICROSCOPICAL AND CHEMONICROSCOPICAL EXAMINATIONS OF THE DIFFERENT PARTS OF CARISSA EDULIS

2.5.1 MICROSCOPICAL EXAMINATION

The powdered or macerated samples and the transverse, longitudinal and tangential sections of the roots, stem, leaves, fruits and seed of *Carissa edulis* were used for this study (Evans, 1989). Both qualitative and quantitative studies were carried out.

A small quantity of the powdered sample or a thin anatomical or macerated section was placed on a clean slide. A few drops of chloral hydrate solution was added and covered with a clean coverslip. The slide was heated over a bunsen burner flame for about 30 seconds or more. Overheating was avoided as this would cause formation of chloral hydrate crystals leading to false observation. When the tissue was sufficiently cleared, air bubbles if any were removed. A few drops of dilute glycerol were then added, and the slides observed under a compound microscope.

The various anatomical sections of the roots and stem were also examined under polarised light microscope. This was to enable the study of the structures not clearly seen with the compound microscope like the cambium and the layers of cell differentiation (i.e. to confirm if the plant has a bicolateral vascular bundle).

(c) Stomatal Index

Pieces of leaf other than extreme margin or midrib were suitably cleared with chloral hydrate, mounted in glycerol and viewed using a 4mm (x 40) objective. Counts were made of the number of epidermal cells and of stomata (the two guard cells and ostiole being considered as one unit) within the square grid. Successive adjacent fields were examined until about 400 cells have been counted and the stomatal index value was calculated (Trease and Evans, 1989).

$$\text{Stomal index } I = \frac{S}{E + S} \times 100$$

S = number of stomata per unit area

E = number of ordinary epidermal cells in same unit area.

(d) Vein Islet Number

Pieces of leaf were cleared with chloral-hydrate solution and mounted in glycerol then viewed using a 16mm (x 10) objective. Using a camera lucida, the veins were traced in four contiguous square in a square of 1mm x 1mm. The vein-islet number was counted excluding those which were incomplete or cut by the top and left hand sides of the square but included if cut by the other two sides.

(e) Veinlet Termination Numbers

Using the same method as for vein-islet number, the number of vein-let termination per mm of the leaf surface was determined.

PHASE CONTRAST MICROSCOPY (P.C.M.) HISTOLOGICAL TECHNIQUES

The following were the techniques employed to study the various sections of the root and stem of *C. edulis*.

Fixation

The plant materials were collected and cut into pieces not more than 3mm long and fixed in Formalin - Acetic - Alcohol (F.A.A) or 70% alcohol making sure damage is minimised when cutting them. This was left overnight.

F.A.A has the following composition:-

70% alcohol	-	90ml
40% formeldehyde	-	5ml
Glacial acetic acid	-	5ml

Dehydration

i. The materials were transferred into 70% alcohol to dehydrate for 2 hours.

ii. Transferred to absolute alcohol for 2 hours.

iii. Transferred to 25% chloroform/alcohol for 2 hours.

iv. Transferred to 50% chloroform/alcohol for 2 hours.

v. Transferred to 70% chloroform/alcohol for 2 hours.

vi. Transferred to pure chloroform - two changes for 2 hours.

Infiltration

i. Infiltrated by adding chips of 58 - 60°C paraffin wax and kept adding until the wax started to gel in the chloroform. Left over-night to allow infiltration to take place properly.

ii Evaporated chloroform by pouring material with gelled paraffin wax into a shallow dish and left on top of embedding oven at 60 C for one hour.

iii. Transferred to embedding oven to allow evaporation to take place better for half an hour, making sure there was no more smell of chloroform in the wax.

iv. Transferred material into pure molten wax in embedding oven at 60 C for half an hour.

v. Transferred to another pure molten wax for half an hour.

Embedding (Preparation of Paraffin block)

i. L angle mould, an embedding mould was used.

ii. The inner surface of the embedding mould was smeared with dilute glycerine.

iii. Molten wax, slightly heated above 60 C, was poured into the mould to about half its volume.

iv. Transferred specimen into the wax with the aid of warm forceps and oriented them into their cutting angle.

v. Allowed a skin film to form on top of wax after orientation by blowing air on it. Warm spatular was used to transfer them into mould and oriented with warm fine forceps.

vi. Submerged in cold water when the thin film formed on top of wax. Left under running water and allowed the block to harden. Removed the mould from the block and placed in a cool dry and dust free atmosphere ready for sectioning.

Sectioning

i. Trimmed a cut piece of block with specimen in equal sides, so as to get a straight ribbon when sectioning, i.e. top or bottom edges straight and parallel. The sides at right angles and seen that the object was carefully placed in the centre of the block to be sectioned.

ii. Mounted trimmed block on a microtome specimen holder by using warm blunt scalpel to melt the wax on the surface of object holder, submerged in water to stick the block firmly before sectioning.

iii. Sections were cut with a sharp microtome knife to enable sections to ribbon properly.

Mounting of Ribbon on Slide

i. A clean slide was smeared with a drop of Mayers glycerine or egg albumen

ii. A portion of ribbon was cut and mounted on the albumenised slide with some few drops of distilled water on the slide.

iii. Section was stretched on top spirit lamp flame making sure that not to melt the section

iv. Drained the water carefully after stretching and pressed the section to stick on the slide with aid of several thickness of filter papers using the side of the hand as pressure. Sections were left to dry in a dust free atmosphere for three days before staining.

Staining Method

i. Dewaxed slides in xylene - two changes - 5 minutes each.

ii. Treated section with absolute alcohol for 2 minutes .

iii. Treated section with 95% alcohol for 2 minutes.

iv. Treated section with 70% alcohol for 2 minutes.

v. Treated in safranin for 20 minutes.

vi. Stained in 50% alcohol for 1 minute.

vii Washed in 70% alcohol for 1 minute.

vii. Washed in 95% alcohol for 1 minute.

ix. Washed in absolute alcohol for 1 minute

x. Counter stained in fast green prepared in 95% alcohol for 1 minute

xi Differentiated in 95% alcohol for 1/2 minute

xii. Transferred to absolute alcohol for 1/2 minute

xiii Cleared in Xylene I and II

xiv. Mounted in D.P. X using a clean coverslip.

xv. Dried on a warm hot plate

xvi. Labeled and kept in slide cabinet for observation under the phase contrast microscope.

2.5.2 CHEMOMICROSCOPICAL EXAMINATION

The powdered samples and the different anatomical sections were treated separately on microscope slides and observed under the compound microscope for the presence of chemical substances such as cellulose, tennins, gums and mucilages, fats and oils, starch, protein, cutin, suberin, lignin, and calcium oxalate crystals.

(a) **Test for lignin**

The powdered samples and anatomical sections of roots, stem, leaves, fruits and seeds were mounted in phloroglucinol and conc. hydrochloric acid. A red colour indicates the pressure of the lignin. The intensity of red colour indicates the extend of lignification (Evans, 1989).

(b) **Test for Cellulose**

The various samples were mounted in chlor-zinc-iodine; or N/50 iodine followed by 66% sulphuric acid. A blue colour indicates the presence of cellulose (Evans, 1989)

(c) **Test for Tennins**

Each of the various samples was mounted in ferric chloride solution. A bluish or greenish colour indicates the presence of tannins (Evans, 1989).

(d) **Test for Mucilages**

The different samples were mounted separately in Ruthenium red. A red or dark pink colour indicates the presence of mucilages (Evans, 1989).

(e) **Test for Starch**

The different samples were mounted separately in N/50 iodine. A blue colour indicates the presence of starch (Evans, 1989).

(f) Test for Calcium Oxalate crystals

The samples were separately cleared in chloralhydrate solution. If calcium oxalate crystals are present, they are seen as bright structures of definite shapes and sizes. After addition of a few drops of conc. sulphuric acid (80%) and reviewed under the microscope the disappearance of calcium oxalate crystals confirms their presence (Evans, 1989).

(g) Test for Oils

The samples were separately mounted in Sudan IV reagent. A pink colour in any of the structures indicates the presence of oils.

(h) Test for Cutin and Suberin

When the different samples are stained with tincture of alkanna, a red colour indicate the presence of cutin and suberin.

(i) Test For Callose

The different samples were stained with corallin soda. Red colour indicates the presence of callose.

(j) Test for Protein

1% Picric acid and Million's reagent were used. When the different samples were stained separately with picric acid a yellow stain on the microscopic structures indicates the presence of protein. A red stain with Million's reagent indicates the presence of protein.

2.6: PHYTOCHEMICAL SCREENING OF POWDERED ROOTS, STEM, LEAVES, FRUITS AND SEEDS

2.6.1: TEST FOR CARBOHYDRATES

(a) Extraction

3g of the powdered samples were boiled separately in 50ml of distilled water on a hot plate for three minutes. Each mixture was filtered while hot and the resulting filtrate cooled and used for the following carbohydrate tests.

(b) General Test - Molisch's Test

A few drops of Molisch's reagent was added to 2ml of each of the water extract obtained above, then a small quantity of conc. Sulphuric and was allowed to form a lower layer. A purple ring at the interface of the liquids indicates the presence of carbohydrates.

Each mixture was then shaken and allowed to stand for 2 minutes and diluted with 5ml of water a purple precipitate also indicates the presence of carbohydrates (Evans, 1989).

(c) Test for sugars

Barfoed's Test - (General Test for Monosaccharide)

1ml of dilute solution of each sample of the water extract and 1ml of Barfoed's reagent were taken in a test tube and heated in a water bath for 2 minutes. A red precipitate indicates the presence of monosaccharide (Evans, 1989).

Fehling's Test

i. Standard Test for Free Reducing Sugars

To 2mls of each water extract was added 5mls of a mixture of equal volumes of Fehling's solutions I (A) and II (B) and boiled in a water bath for 2 minutes. A brick red colour indicates the presence of free reducing sugars (Evans, 1989).

ii. Standard Test for Combined Reducing Sugars

1ml. of each of the water extract was hydrolysed by boiling with 5ml of dilute hydrochloric acid. This was neutralised with solium hydroxide solution. The Fehling's test was repeated. A brick red colour indicates the presence of combined reducing sugars (Evans, 1989).

Resorcinol or Salivanoff's Test (Standard Test for Ketoses).

To 2ml of each of the water extract was added a few crystals of resorcinol and an equal volume of conc. Hydrochloric acid, then heated over a bunsen burner flame. A rose colour indicates the presence of Ketoses (e.g. Fructose) (Evans, 1989).

Test for Pentoses

To 2ml of each of the water extract was added an equal volume of hydrochloric acid containing little phloroglucinol and heated over a bunsen flame. A rose colour indicate the presence of pentoses (e.g. xylose) (Evans, 1989).

2.6.2: Test for Tannins

(a) Extraction

3g of each of the powdered samples was boiled in 50ml of distilled water for 3 minutes on a hot plate. The mixture was filtered and the resulting filtrate was used to carry out the following tests for tannins.

(b) Ferric Chloride Test

A portion of each of the water extracts was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A blue or green colour indicates the presence of tannins (Evans, 1989).

(c) Lead Sub-acetate Test

To 1ml of each of the water extract was added 0.25% ferric ammonium citrate solution. To the mixture was added sufficient solid sodium acetate to adjust the solution to pH 8 using pH indicator paper. This was boiled in a water bath and filtered. A coloured precipitate indicates the presence of tannins (Evans, 1989).

(e) Bromine Water Test

A few drops of bromine water was added to 1ml of each of the water extract. A precipitate indicates the presence of condensed tannins.

(f) Formaldehyde Test

To a small portion of each of the water extract was added a drop of formaldehyde solution and 3 drops of 10%

hydrochloric acid. A precipitate indicates the presence of hydrolysable tannins.

(g) Test for Chlorogenic Acid

To 1ml of each of the water extract was added two drops of 10% ammonia solution. The mixture was heated over a flame and then exposed to air. A green colour indicates the presence of chlorogenic acid (Evans, 1989).

(h) Lime-Water Test

To a small portion of each of the water extract was added 1ml of lime-water. A precipitate indicates the presence of psuedotannins.

2.6.3 Test for Glycosides

(a) Test for Cyanogenic Glycosides

A small amount of each of the powdered samples (Roots, stem, leaves, fruits and seeds) was placed in a test tube and sufficient distilled water was added to cover the samples. A prepared moist sodium picrate paper was suspended in the neck of the tube by means of a cork. The tube was placed in a water bath for one hour. A brick-red colour on the paper indicates the presence of cyanogenic glycosides (Evans, 1989).

(b) Test for Anthraquinone Derivatives
Borntrager's Tests

i To Show the Presence of Free Anthraquinones

0.5g of the powdered samples (Roots, stem, leaves, fruits and seeds) was taken in separate dry test tubes and 10ml of chloroform was added and shaken for 5mins. The extract was filtered and equal volume of ammonia was added to the filtrate and shaken. A bright pink colour in the upper aqueous layer indicates the presence of free anthraquinone (Evans, 1989).

ii To Show the Presence of Free and/or Combined Anthraquinones

0.5g of each of the powdered samples (Roots, stem, leaves, fruits and seeds) was boiled with 10ml. of 10% hydrochloric acid for 2min. The extract was filtered. To the filtrate was added equal volume of chloroform. The tube was inverted a couple of times avoiding vigorous shaking. The solution was transferred into a separating funnel and the two layers allowed to separate. The lower chloroform layer was poured into a clean test tube and 10% ammonia solution was added and shaken. The two layers were again allowed to separate. A bright pink colour in the upper equeous layer indicates the presence of free and/or combined anthraquinones (Evans, 1989).

iii <u>To Show the Presence of Anthraquinone Derivatives in a Reduced Form which are not easily Hydrolysed.</u>

0.5g of each of the powdered samples (Roots, stem, leaves, fruits and seeds) was boiled with 10ml of 10% feCl, and 5ml of 10% HCl for 5min. The mixture was filtered and to the filtrate was added equal volume of chloroform. The layers were allowed to separate in a separating funnel. The chloroform layer was transferred into another tube containing 5ml of 10% ammonia solution. A bright pink colour in the upper aqueous layer indicates the presence anthraquinones (the C-glycosides) (Evans, 1989).

c. <u>Test for Saponins</u>

i. **<u>Froth Test</u>**

To a small quantity of each of the powdered samples was added 95% ethanol, and boiled. The mixture was filtered and 2.5ml. of the filtrate was added to 10ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 sec. then it was allowed to stand for over half an hour. A honey-comb froth is indicative of the presence of saponins (Sofowora, 1993).

ii. <u>Haemolytic Test for Saponins (Steroidal and Triterpenoid)</u>

0.2g of the chemical substance from powdered roots, stem, leaves, fruits and seeds was extracted with 10ml of warm water, filtered and the filtrate retained. 2ml of 1.8% sodium chloride solution was added to six test tubes

(5,14,2). To five of the test tubes (t_1) 2ml of each of the extracts was added. The concentration of NaCl in each case is now isotonic with blood serum. The sixth tube (t_2) acts as control. Blood was obtained by pricking the thumb at the base of the nail and the blood was drawn into a small pipette. Five drops of the blood was added to each of the test tubes labelled (t_1 and t_2). The tubes were stoppered and inverted gently to mix the contents. Haemolysis in the test tube containing extract (t_1) and not in the control (t_2) indicates presence of saponins (Brain and Turner, 1975).

d. <u>Tests for Cardiac Glycosides</u>

i. <u>Extraction</u>

0.5g. of each of the powdered sample was boiled with 10ml of 95% alcohol for 2 min. The resulting mixture was filtered and cooled. The filtrate was diluted with water and three drops of a strong solution of lead sub-acetate was added. This was mixed thoroughly and filtered. The filtrate was divided into two portions. One portion of the filtrate was kept for the test (diii) described below. The other portion of the filtrate was extracted with 5ml chloroform in a separating funnel. The lower chloroform layer was divided into two small evaporating dishes and evaporated to dryness.

ii. Keller - Killiani Test for Digitoxose

One of the chloroform residues from (d(i)) above was dissolved in 1ml of glacial acetic acid containing a trace of ferric chloride solution.

This solution was carefully poured on the surface of 1ml. sulphuric acid already contained in a test tube to form a separate layer. A reddish-brown colour at the interface of the liquids indicates the presence of Digitoxose. (Evans, 1989).

iii. Kedde Test (for Free or Combined Cardenolide Aglycone)

The reserved filtrate (from di above) was treated with 1ml of 2% solution of 3, 5-dinitrobenzoic acid in 95% alcohol. The solution was made alkaline with 5% sodium hydroxide. A purple-blue colour indicates the presence of free or combined cardenolide aglycone (Sofowora, 1993).

iv. Legal Test (for Cardenolide Aglycone)

The second reserved residue (from di) was dissolved in a few drops of pyridine and a few drops of 2% sodium nitroprusside was added then, a drop of 20% NaOH was added. A deep red colour indicates the presence of a cardenolide aglycone (Sofowora, 1993).

2.6.4 Test for Terpenes and Sterols

(a) Extraction

5g of each of the powdered samples was extracted by maceration with 50ml of ethyl alcohol (95°), filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10ml. of anhydrous chloroform and then

filtered. The filtrate was divided into two equal portions and the following tests were carried out.

(b) Liebermann - Burchard Test

The first portion of the chloroform solution (from [a] above) was mixed with 1ml of acetic anhydride, followed by the addition of 1ml of conc. sulphuric acid down the wall of the test tube to form a layer underneath. The formation of a reddish violet colour at the junction of the two liquids and a green colour in the chloroform layer indicates the presence of terpenes (Sofowora, 1993).

(c) Salkowski's Test

The second portion of the solution was mixed with 2ml of conc. sulphuric acid carefully so that the sulphuric acid formed a lower layer. A reddish brown colour at he interface indicates the presence of a steroidal ring (Sofowora, 1993).

2.6.5. Test for Flavonoids

(a) Extraction

5g of each of the powdered samples was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered and the filtrate was used for the following tests.

(b) Lead Acetate Test

To 5ml of the detanned water extract was added 10% lead acetate solution. A coloured precipate indicates the presence of flavonoids.

(c) Sodium Hydroxide Test

5ml of 10% NaOH was added to an equal volume of the detanned water extract. A yellow solution indicates the presence of flavonoids (Evans, 1989).

(d) Ferric Chloride Test

2ml of the detanned water extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% $FeCl_3$ solution was added. A green or blue solution indicates the presence of phenolic nucleus.

(e) Shinoda Test

0.5g. of each of the powdered samples was extracted in ethanol by boiling in a water bath for 5 min. filtered and cooled. To the filtrate was added four pieces of magnesium fillings followed by few drops of conc. hydrochloric acid. A pink on red colour indicates the presence of flavonoids (Geissman, 1962).

(f) Amyl Alcohol Test

3g of each of the powdered samples was macerated in 50ml of 1% HCl and filtered.

i. 4ml of the filtrate was shaken with 5ml of amyl alcohol and the colour produced was observed. Production of a yellow colour indicates the presence of free flavonoid aglycones.

ii. 10ml of the filtrate was shaken with 7ml of amyl alcohol and the mixture transferred to a separating funnel. The amyl alcohol layer was discarded and

the aqueous layer boiled with 10ml of 10% HCl for 2 mins. The acidic solution was cooled and divided into two portions.

The first portion was shaken with amyl alcohol. Production of a yellow colour indicates the presence of combined flavonoid.

To the second portion magnesium turnings were added and the colour of the solution observed (Abdurahman, 1986; Hilal, 1976); Production of a red colour indicates the presence of flavanone and flavonol glycosides

2.6.6 TEST FOR RESINS AND BALSAMS

(a) Test for Resins

i. 15ml of petroleum ether extract was made from 0.1g, of each of the powdered samples (Roots, stem, leaves, fruits and seeds) and filtered into a test tube. An equal volume of copper acetate solution was added and shaken vigorously then allowed to separate. A green colour indicates the presence of resins.

ii. 0.5g. of each of the powdered samples was dissolved in acetic anhydride and 1 drop of concentrated sulphuric acid was added. A purple or violet colour indicates the presence of resins.

(b) Test for Oleo-Gum-Resins

0.1g of each of the powdered samples was triturated in 0.5g. of sand. The mixture was transferred into a test tube containning 3ml. of ether and shaken. This was filtered into an evaporating dish and the filtrate allowed to evaporate to a thin film. In the fumecupboard bromine vapour was passed over the residue. A violet colour indicates the presence of oleo-gum-resins.

(c) Test for Balsams

2 drops of alcoholic ferric chloride solution was added to 5ml. of 90% ethanol extract of each of the powdered samples, (Roots, stem, leaves, fruits and seeds) A dark green colour indicates the presence of balsams.

2.6.7: TEST FOR ALKALOIDS

(a) Extraction

10g. of each of the powdered samples was taken in a small beaker and a strong solution of ammonia solution was added in a quantity sufficient to just moisten it and allowed to stand for 10min. after thorough mixing of the contents.

Sufficient quantity of a mixture of chloroform and ethanol solution (1:1) was added just to soak and suspened the powder. The mixture was allowed to stand for 20 min. with occasional stirring with a rod.

The mixture was filtered through a plug of cotton wool. The marc was washed twice with 2ml of chloroform and the washings were combined with the filtrate. The bulked filtrate was concentrated to dryness without overheating. The residue was cooled and dissolved in 5ml. of chloroform only.

The chloroform solution was transfered to a small separating funnel and shaken with 3ml of dilute sulphuric acid.

The two layers were allowed to separate, the chloroform lower layer was drained off and discarded. 3ml of chloroform was further added and shaken, drained off and discarded until upper acid layer was colourless.

The acid layer was made completely alkaline with strong ammonia solution (tested with indicator paper). The extraction with 3ml of chloroform extracts were retained and evaporated to dryness (Brain and Turner, 1975).

The residue was dissolved in 3ml of ethanol and the following tests were carried out after neutralising with dilute sulphuric acid.

(b) **General Tests**

A set of five test tubes were taken for each of the different drugs samples. To each little amount of the ethanolic solution from above was added drop wise a few drops of the following reagents:-

Mayer's reagent - (Potassium mercuric iodine solution)

Dragendorff's reagent - (Potassium bismuth iodine solution)

Wagner's reagent - (Solution of iodine in potassium iodine)

Hager's reagent - (A saturated solution of picric acid)

10% tannic acid solution

The presence of precipitate in at least 3 or all of the above reagents indicates the presence of alkaloids (Evans, 1989).

2.7 QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS (POWDERED ROOTS, STEM, LEAVES, FRUITS AND SEEDS)

2.7.1 Determination of Moisture Content by the "Loss on Drying Method"

An evaporating dish was heated to a constant weight in an oven at 105°C and its constant weight was noted. 3g. of the powdered stem bark was accurately weighed into the dish. The evaporating dish with its content was put in the oven at 105°C and the content dried to a constant weight at 30min. interval after initial drying of one hour. Two consecutive same weights confirm a constant weight. The total loss in weight (i.e. the weight of the moisture) was determined by substracting the constant wight of the dish and powdered sample after heating from the weight of the dish and content before heating. The

percentage of the initial weight of the powdered sample used was then calculated. This was done by dividing the weight of the moisture by the weight of the drug taken multiplied by a hundred. Three different determinations were done and the average of these was taken as the moisture content of the drug (Evans, 1989). This was carried out for each of the different five examples.

2.7.2 DETERMINATION OF ASH VALUE

(a) Determination of Total Ash

A nickel crucible was heated at 105C to a constant weight and it's accurate weight was noted after cooling in the desiccator. 2g. of the powdered sample was accurately weighed into the crucible. The crucible and its content were gently heated over a microbunsen flame until it was moisture free and completely charred. The microbunsen flame was slightly increased until most of the carbon had volatalised and inorganic ash could be seen. The ash was cooled, weight and the weight noted. The heating and cooling was continued until the weight of the ash was constant. The weight of the ash was calculated by subtracting the weight of the crucible from the final weight of the crucible and ash (B.P., 1980). This was done to each of the different five samples.

(b) Determination of Acid Insoluble Ash Value

The crucible with the ash from the experiment (a) above was transferred into a beaker containing 25ml of 10% hydrochloric acid. The beaker and its content were boiled for

5 mins. and filtered through an ashless filter paper. The crucible and the beaker were washed with distilled water and the washing were repeated three times, every time the washings were passed through the filter paper in a manner as to allow the collection of the residue at the tip of the cone of the filter paper. The funnel along with the filter paper were dried in the oven at 105 C. The weight of a clean and heated nickel crucible was accurately determined using a sensitive balance. The filer paper with its residue was folded into a small cone and transferred into a crucible. The crucible was gently heated until the filter paper was completely ashed, then heated strongly for a few minutes. The crucible and its content were cooled,weighed and the final weight weight was noted. The weight of the residue (ash) was then calculated. This was done by substracting the constant weight of the crucible from the constant weight of the crucible and its content (ash). Also, the acid insoluble ash value with reference to the initial weight of the powdered sample was calculated and expressed in percentage. The weight of ash divided by the initial weight of the drug multiplied by hundred was taken as the acid insoluble ash value (B.P., 1980). The five different samples were treated separately.

2.7.3: *DETERMINATION OF EXTRACTIVES*

(a) Alcohol - Soluble Extractive Value

5g of each of the powdered samples was accurately weighed into a 250ml. stopped conical flask. 100ml of 90% ethanol was added and the stopper was replaced firmly. The flask was shaken on a mechanical shaker for six hours and then allowed to stand for eighteen hours. The extract was filtered by suction filtration. The weight of a heated, cooled flat bottom evaporating dish was accurately determined. 20ml of the filtrate was taken into the weighed evaporating dish. The filtrate was dried to a constant weight at 105°C in an oven and the final weight was noted. The weight of the residue obtained from 20ml extract was determined by subtracting the weight of the evaporating dish from the total wight of evaporating dish and the residue. The alcohol extractive value was then calculated with reference to the initial weight of the powdered drug and expressed in percentage (Brain and Turner, 1975).

b. Water-Soluble Extractive Value

The method (a) above was repeated using 0.25% chloroform water instead of 90% ethanol.

2.8 CHROMATOGRAPHIC ANALYSIS

Chromatographic analysis was carried out to check and confirm the presence of salicylates in the plant samples which might be responsible for the analgesic and other activities.

A Preparation of Thin Layer Plates

Microplates made of slides were used to minimize use of solvent and to reduce time of analysis. These plates were prepared by dipping the slides in a slurry of silica gel G for

thin layer chromatography (50%w/w). The slurry was prepared by mixing the silica gel G in distilled water. The plates were dried in atmospheric air. When dried the silica gel was scraped off one side.

Some of the plates were activated in the oven at 105°C for 30 mins in order to remove moisture.

The plates were stored in desiccator until required for use.

b Preparation of Samples

The following samples were used

- Water extracts
- Salicylic acid in chloroform (1% $^{w}/_{v}$)
- Methyl salicylate

0.1g of the powdered samples of the roots, stem, leaves, fruits and seeds were dissolved separately in 5ml of distilled water and warmed in a water bath to enhance dissolution. Another tube was made in which few drops of 10% HCl was added to hydrolyse the water extract of the fruit. This was also heated in the water bath, cooled and neutralised with few drops of 5% NaOH solution.

The methyl salicylate was synthesized from salicylic acid by adding 1ml of methanol and 1ml of conc. H_2SO_4 to 0.1g of the salicylic acid in a dry test tube. The mixture was heated in a water bath, cooled and added to 2.5ml of distilled water. When cooled precipitates were formed. The precipitate was filtered and dried by placing the funnel with the filter paper in an oven for drying glassware at 40°C. The crystals were

dissolved by adding chloroform to the funnel and collected the solution in a sample tube (Rainsford, 1984; Vishnoi, 1979)

C. Test for Salicylic Acid

To 0.1g of the salicylic acid was added 1ml of methanol and 1ml of conc. H_2SO_4 in a dry test tube. The mixture was heated in water bath, cooled then porred onto a 2.5ml distilled water contained in another test tube. A characteristic smell of oil of wintergreen (methyl salicylate)(or robb) indicates the presence of salicylic acid (Vishnoi, 1979).

The water extracts of the different samples was chromatographed using the following systems. Hydrolysed water extract and salicylic acid were also used.

D. Chromatographic Systems

System I

Adsrobent : Silica gel G (0.25mm), activated

Solvent System : n-Butanol-acetic acid-water (27:1:1)

Visualization : 1% ferric chloride in ethanol

Technique : Ascending

System II

Adsorbent : Silica gel G. (0.25mm), activated.

Solvent System : Ethylacetate-n-Butanol-acetic acid - water (50:20:15:15)

Visualization : 1% ferric chloride in ethanol

Technique : Ascending

System III

Adsorbent	:	Silica gel G., air dried.
Solvent System	:	Toluene-acetone (50:50)
Visualization	:	1' ferric chloride in ethanol
Technique	:	Ascending

<u>System IV:</u>

Chromatography paper		What man No. 1 (20cm x 20xm)
Solvent system	:	n-butanol-acetic acid-water
Visualization	:	1' ferric chloride in ethanol
Technique	:	Ascending

System V:

Chromatography paper::		Whatman No. 1 (20cm x 20cm)
Solvent system	:	Ethylacetate-n-butanol-acetic acid - water (50:20:15:15)
Visualization	:	1' ferric chloride in ethanol
Technique	:	Ascending

<u>System VI:</u>

Adsorbent	:	Silica gel G, air dried
Solvent system	:	Pet ether - Methanol (20:1)
Visualization	:	1' Ferric chloride in ethanol
Technique	:	Ascending.

(B.P., 1980; Giesemann, 1962; Stahl, 1969)

2.9 ANALGESIC ACTIVITY

2.9.1 Preparation of Samples

The water extracts of the different parts (roots, stem, leaves, fruits and seeds) of the plant *C. edulis* were used for the study to emulate the practice of the traditional medical practitioners and the local populace.

The powdered samples were extracted in distilled water by boiling over a water bath for three hours. The mixture was filtered while hot using a muslin cloth. It was difficult to filter a cold water extract by the simple method of extraction using a funnel and filter paper. Another alternative for filtration is by using suction device.

The water extracts were separately dried in an evaporating dish over a water bath. The dried samples were powdered and transferred into sample bottles.

When the samples were required for the experiments, a stock solution of 0.1g in 5mls of distilled water was manally prepared. The solution was prepared in a testtube and placed in a water bath for the powdered samples to be dissolved (the samples were more soluble in hot water than cold water). The samples were cooled before use.

The mice were obtained from the animal house of the department of pharmacognosy and Drug Development, Ahmadu Bello University Zaria. They were fed on grower's mash. The feed was normally prepared by placing the mash in a container and water was added just enough to moisten it. Small balls are then made and dried in the oven to obtain had balls.

To obtain the required doses some calculations were made. For example 10mg/kg of root extract to be injected to a mouse weighing 21g.

Stock solution = 0.1g in 5ml

= 100mg in 5ml

= 20mg in 1ml

Dose

10mg/kg = 0.01mg/g

for 21g mouse = 0.01 x 21

= 0.21mg

.. Number of mls (volume) to be taken from stock solution =

$$\frac{0.21 \times 1}{20}$$

= 0.0105

= 0.01 mls

For metamizol (Analgin (R), ampules of 20mg / 5ml each were obtained. To inject a dose of 10mg/kg body weight to 21g mouse for example the following calculations was done:

10mg/kg = 0.01mg/g

for 21g mouse = 0.01 x 21

= 0.21mg

20mg/5ml of metamizol = 4mg in 1ml

.. 0.21mg = $\frac{0.21 \times 1}{4}$

= 0.0525

= 0.05ml

2.9.2 Measurement of Pain Threshold - Mechanical Method (Tail-Clip Method):

The Ugo Basile analygesy -meter has been designed to screen analgesic drugs on animal according to the Randal-Selitto test (Tuner and Hebboin, 1971).

The instrument is a device which exerts a force that increases at a constant rate (a certain number of grams per second). This force is continuously monitored by a pointer moving along a linear scale.

The force was applied on the tail of the mice about an inch from the end. The tail was placed on a small plinth under a cone-shaped pusher with a rounded tip. The plinth is made of teflon which is biologically inert and has a very low friction co-efficient. Thus if the animal suddenly withdraws its tail it slips out easily without being injured.

A pedal switch was pressed to start the mechanism which exerts the force. When the mouse struggles indicating pain, the pedal was immediately released and reading on the scale was recorded.

A trial experiment was first carried out to test for analgesic activity in the plant extracts. Six groups of 2 albino mice per group were used. The animals were kept off their feed for 24 hours before the experiment but water was maintained. The animals were weight and placed in cages, one animal per cage and labelled. The weight of each animal and the sample injected was labelled on the cages. The test was

carried out as described above. The first readings were taken before any sample was injected to the animals. 3 readings each were taken for each animal, the average of which was later calculated. 10mg/kg and 50mg/kg of the roots and stem water extracts were administered intraperitoneously to four groups. A different dose or extract for each group. 10mg/kg metamizol (Analgin[R]) was injected (ip) as the standard and the sixth group was administered (ip) 0.2mls of normal saline 10.9% NaCl to serve as the control. Then readings were taken at 15 minutes interval for 45 mins. The 10mg/kg of the water extracts did not show much activity, indicating higher doses were to be used.

Eleven groups of six albino mice per group were used for the main study. The animals were treated as in the test experiment. 50mg/kg of the water extracts of the roots, stem, leaves, fruits and seeds, 100mg/kg and 150mg/kg of the fruits water extracts were administered (ip) to seven groups. 10mg/kg, 20mg/kg and 30mg/kg of metamizol were adminsitered to three groups. The eleventh group was administered (ip) 0.2mls of normal saline. The test was carried out and readings were was taken at 15 minutes intervals for 75 minutes.

The mean and standard deviations fore ach group was calculated. The significant difference using the student test was also determined.

CHAPTER THREE

RESULTS AND DISCUSSION

3. RESULTS AND DISCUSSION

3.1 MACROSCOPICAL EXAMINATION OF THE DIFFERENT PARTS OF CARISSA EDULIS

3.1.1 Root Bark

The following features were observed for identification of the whole bark.

Origin and preparation - The root of *C. edulis* was derived from the root and was prepared whole (i.e. it was a whole bark consisting of all parts outside the cambium).

Size - length 10 - 20cm

width 0.5 - 2cm

thickness 0.1 - 0.2cm

Shape - curved (0.5 -1.0cm in width) to channel (1.0 - 2cm in width).

Surface - (i) inner surface - light brown

(ii) outer surface - dark brown with striations

Colour - light brown

Fracture - short

Fractured surface - smooth.

Odour - pungent

Taste - salty

3.1.2 Stem Bark

The following features were observed for identification of whole back.

Origin and preparation. The stem bark of *C.edulis* was derived from the stem and was prepared whole.

Size - length 10 - 20cm
width 1 - 2cm
thickness 0.1 - 0.15cm

Colour - brown

Fracture - short

Fractured surface - fibrous

Shape - curved (0.5 - 1.0cm in width) to channel (1.0 - 2cm in width). Bigger piece were double quilled.

Surface inner surface - brown with striations
outer surface - Dark brown with light brown patches
Has striations, furrows and lenticels

Odour - spicy

Taste - salty

3.1.3 **Leaves**

The features of the leaves are as follows:-

Duration - ever green

Arrangement - opposite decussate

Petiole - Petiolate, short (1 -2cm)

Lamina

i) Composition - Simple and pinnate

ii) Incision - None

iii) Shape - ovate

iv) Venenation- reticulate

v) Margin - entire

vi) Apex - acute

vii) Base - cordate

viii Surface - green, upper surface darker green than lower surface, glabrous with the naked eye.

ix) Texture - smooth and soft.

Odour - aromatic

Taste - slightly bitter

3.1.4 **Fruits**

The fruit samples had the following features:-

Class - simple, berry

Shape and Dimensions - round 2-3cm in size

Adhesion - Superior

Dehiscence - indehiscent

Pericarp - green when unripe, red partially ripe and black when completely ripe. Modified into epicarp, mesocarp and endocarp.

Placentation - parietal

Seeds - 4

Colour - reddish brown

Odour - aromatic

Taste - salty

3.1.5 **Seeds**

The seeds were found to have the following features:-

Size	-	length 0.5 - 1cm, width 0.2 - 0.3cm 0.1cm thickness
Shape	-	oblong
Colour	-	dark brown
Hilum	-	present
Micropyle	-	present
Seed coat	-	one, thickness about 0.5cm smooth with ridges and markings
Perisperm	-	present
Endosperm	-	present
Ridge	-	present
Odour	-	adourless
Taste	-	Salty

3.2 MICROSCOPICAL AND CHEMOMICROSOPICAL EXAMINATIONS OF THE DIFFERENT PARTS OF CARISSA EDULIS

3.2.1 Microscopical Examination:

(a) Root:

The microscopical examinations of the powdered root bark revealed the following features: cork cells, lignified and non lignified parenchyma cells, phellogen (cork cambrium), phloem fibers, sclereids, sieve tubes, trichomes, starch grains secretory tissues (cells and laticifers) and calcium oxalate crystals.

All these features observed were typical of a powdered bark sample; except the laticifiers which are expected in latex containing plants only, as in the family Apocynacaea, Euphorbiaceae, Convolvullaceae and Compositee (Fahn, 1979). Xylem elements were absent which conforms with the theory that bark is that part of the stem or root outside the cambium (Evans, 1989).

There is no specific characteristic microscopical feature that can be attributed to *C.edulis* root bark but the features can help in the identification of the bark.

The various microscopical features of the powdered root bark of *C. edulis* are illustrated in fig. 11 and may be summarized as follows:-

<u>Cork Cells</u>

Shape	-	elongated and polygonal
Anticlinal walls		straight
Thickening	-	present

<u>Phloem Fibres:</u>

Size	-	length, 150 - 275µ
	-	width 3 - 20µ
Frequency	-	moderate
Apex	-	tapering

<u>Secretory Cells:</u>

Type	-	tannin and oil cells
Size	-	20 - 30µ
Shape	-	circular
Frequency	-	few

Laticifers:

Type - non-articulated and branched

Frequency - few

Schereids (stone cells)

Size - length 10 - 30μ

width 5 - 10μ

Shape - round, oblong, angular

Type - mostly singly, compound

Frequency - moderate

Trichomes

Type - uniseriate, unicellular covering trichomes

Size - length; 10 - 113μ

width, 2.5 - 5μ

Frequency - few.

Starch Grains

Shape - round

Size - 2.5 - 5μ

Hilum - central

Aggregation - simple and compound

Frequency - numerous

Maltese cross - obtained with polarizers

Sieve Tubes

Size - length, 25 - 70μ

width, 10 - 15μ

Frequency - moderate

Companion cells - present

Calcium Oxalate Crystals

Type - Prism

Size - length, 20 - 30μ

width, 15 - 25μ

The various anatomical sections (Transverse, radial longitudinal and tangential longitudinal) revealed the arrangements of the different tissues seen in the powdered sample and other features not usually seen in the bark samples such as cambium, xylem tissues and type of vascular bundle.

The transverse section of the root showed eight (8) different layers of tissues: the cork, phellogen, phelloderm, cortex, phloem, cambium, xylem tissues and pith. The cork layer consists of 1 - 3 rows with or without lenticel. The phellogen was of 2 rows while the phelloderm had 3 rows. The cortex had irregular sizes of parenchyma cells with air spaces and secretory cells. It consisted of about nine rows of cells. This layer was more transparent or lighter coloured than the layers above, confirming the periderm above the cortex which is usually more thickened than the cortex. While the cork had thicker walls than the phellogen and phelloderm as expected.

The next layer was the phloem consisting of phloem fibres and phloem parenchyma with intermittent secretory cells. The cambium was not seen with the compound microscope. This could have been due to the fact that the wood was separating from the upper part of the transverse section. The Xylem tissues were seen to consist of fibres, vessels and a single row of parenchyma cells as the medullary ray crossing intermittently between the xylem tissues. The last layer was the pith very much smaller than that of the stem consisting of circular parenchyma cells with air spaces in between.

FIG. 11a: MICROSCOPICAL FEATURES OF THE POWDERED ROOT BARK OF C. EDULIS

x 400

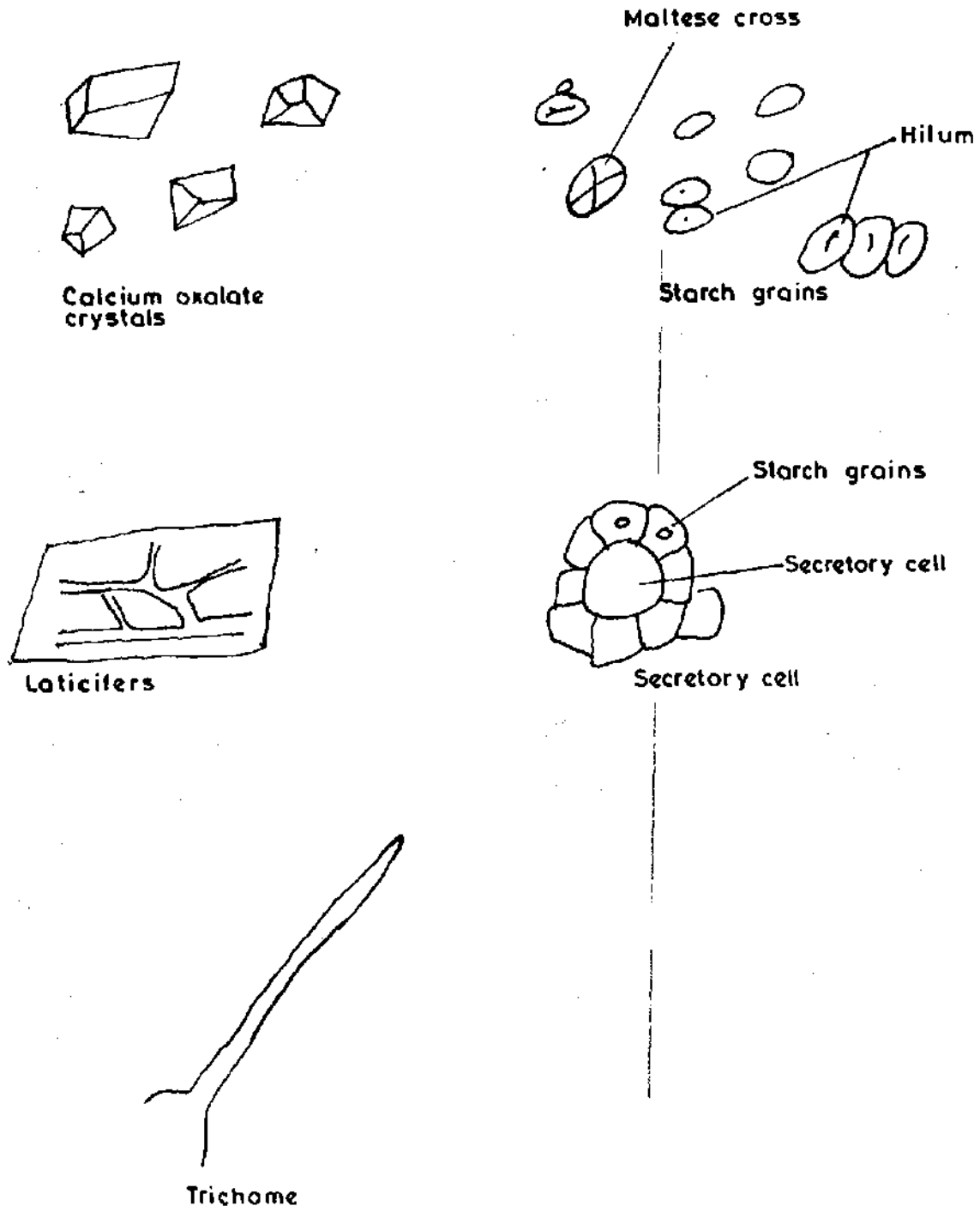
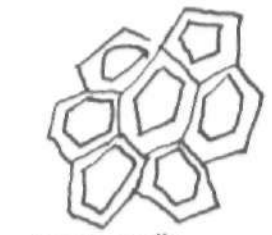
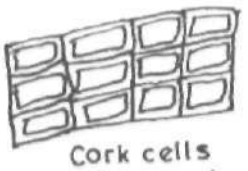


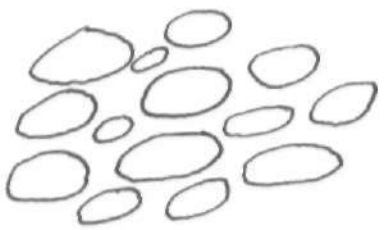
FIG. 11a : CONTD.



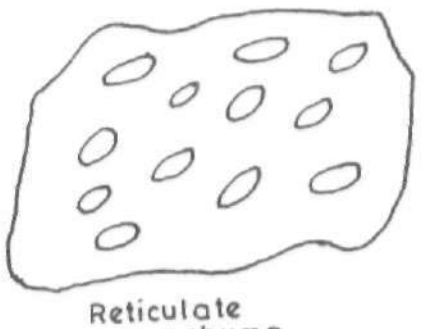
Cork cells
(Surface view)



Cork cells
(Transverse view)



Cortex



Reticulate
parenchyma





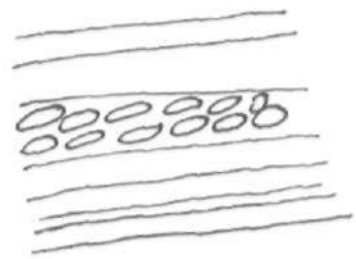


Sclereids

FIG. 11a : CONTD.



Phloem fibres



Group of phloem fibres



Phloem fibre with parenchyma cells

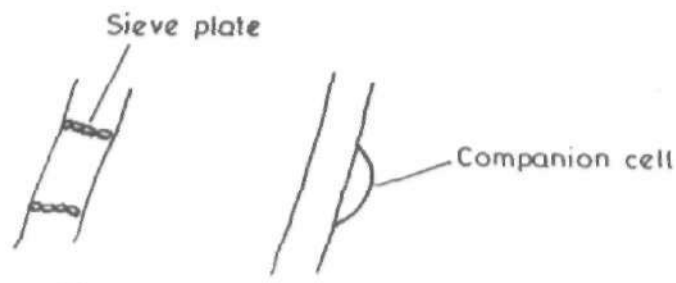


FIG.11b: TRANSVERSE SECTION OF THE ROOT OF C. EDULIS
X 400

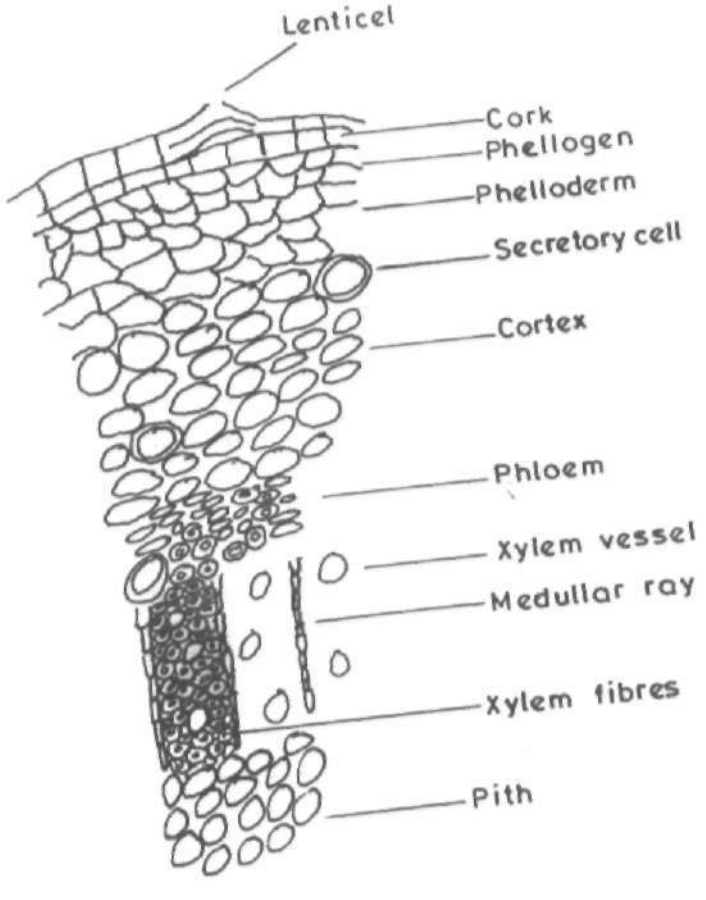


FIG.11c : RADIAL LONGITUDINAL SECTION OF THE ROOT OF C. EDULIS
X 400

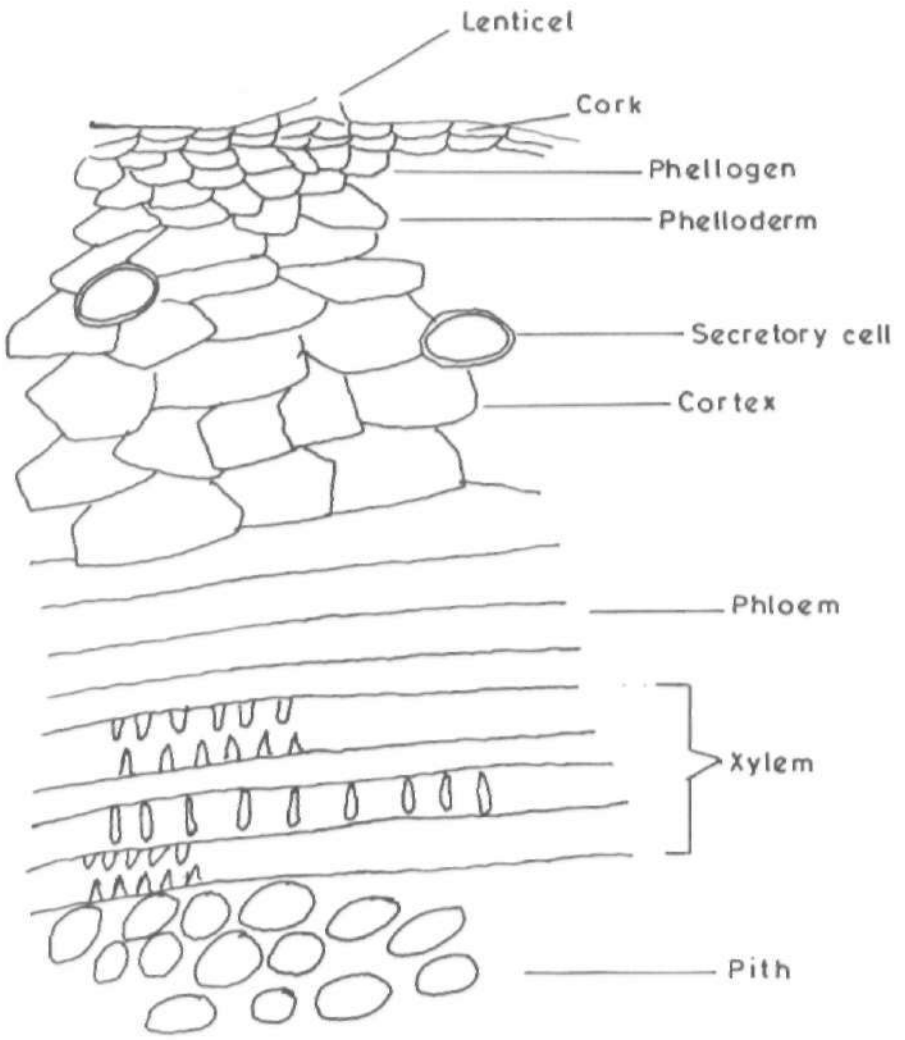


FIG. 11d : TANGENTIAL LONGITUDINAL SECTION OF THE ROOT OF C. EDULIS

X 400

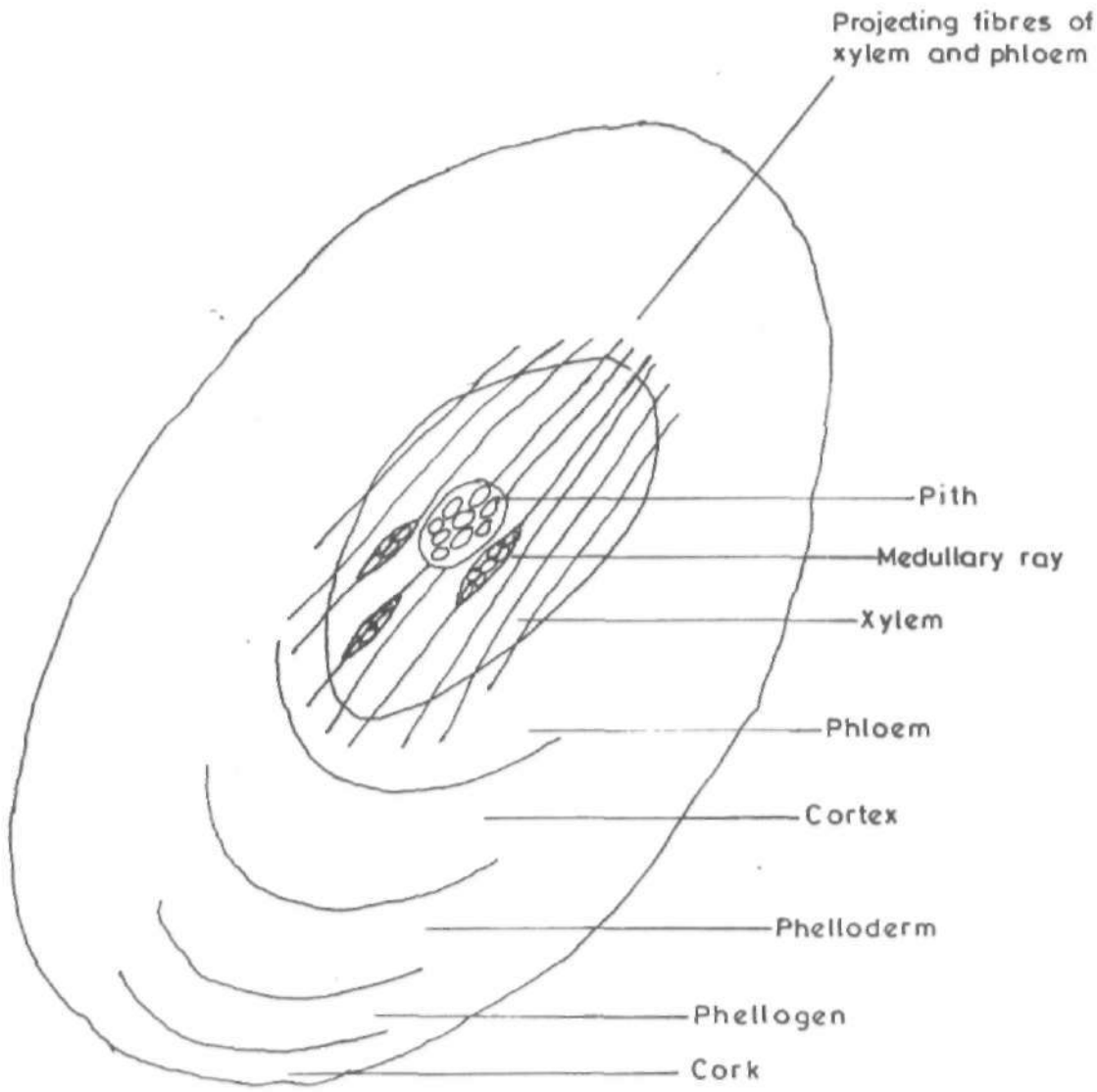


FIG.11e: TRANSVERSE SECTION OF THE ROOT OF C. EDULIS UNDER P. C. M.

X 40

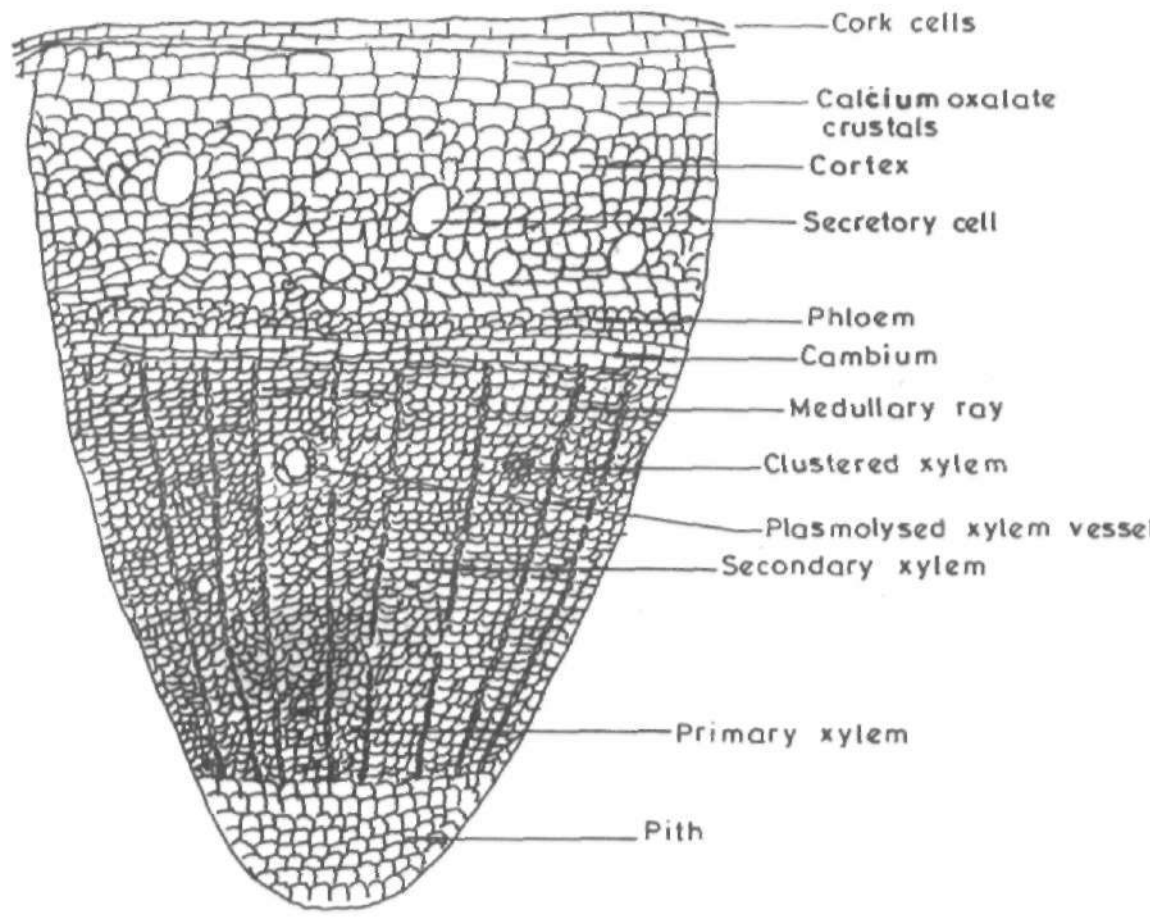


FIG.11f: RADIAL SECTION OF THE ROOT OF C. EDULIS UNDER P.C.M. X 40

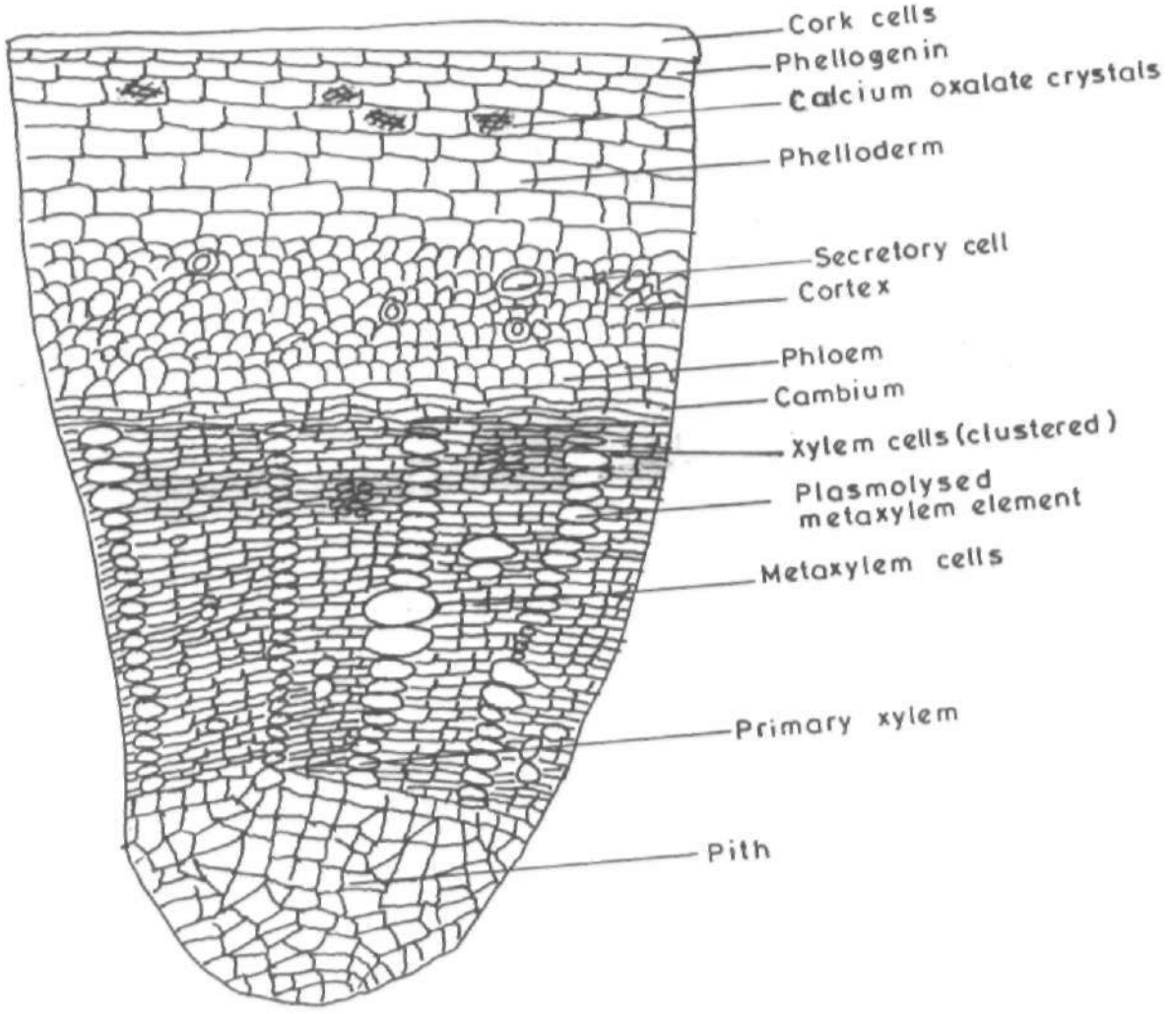


FIG.11g: LONGITUDINAL SECTION OF THE ROOT OF C. EDULIS UNDER P.C.M.
X 40

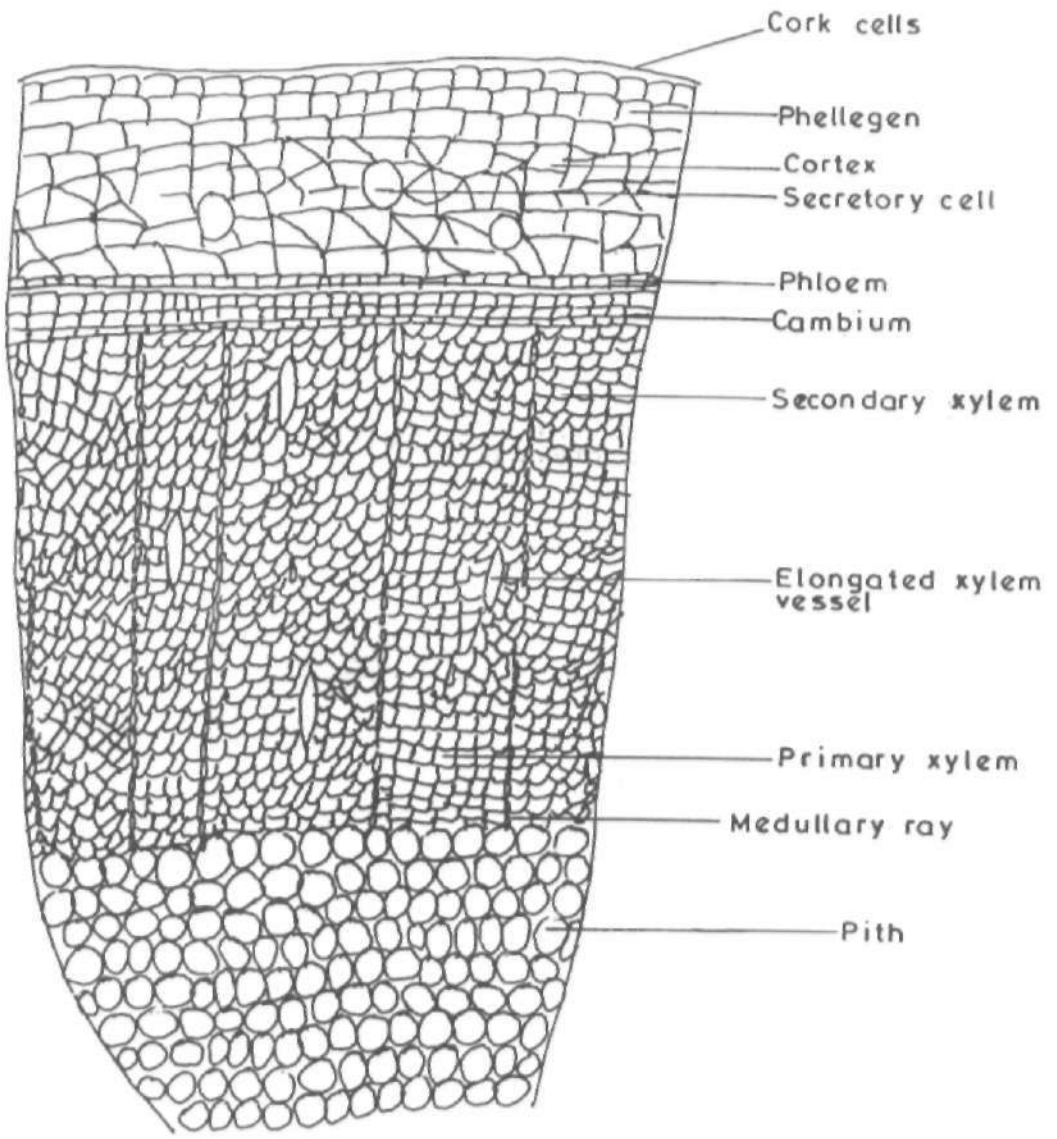
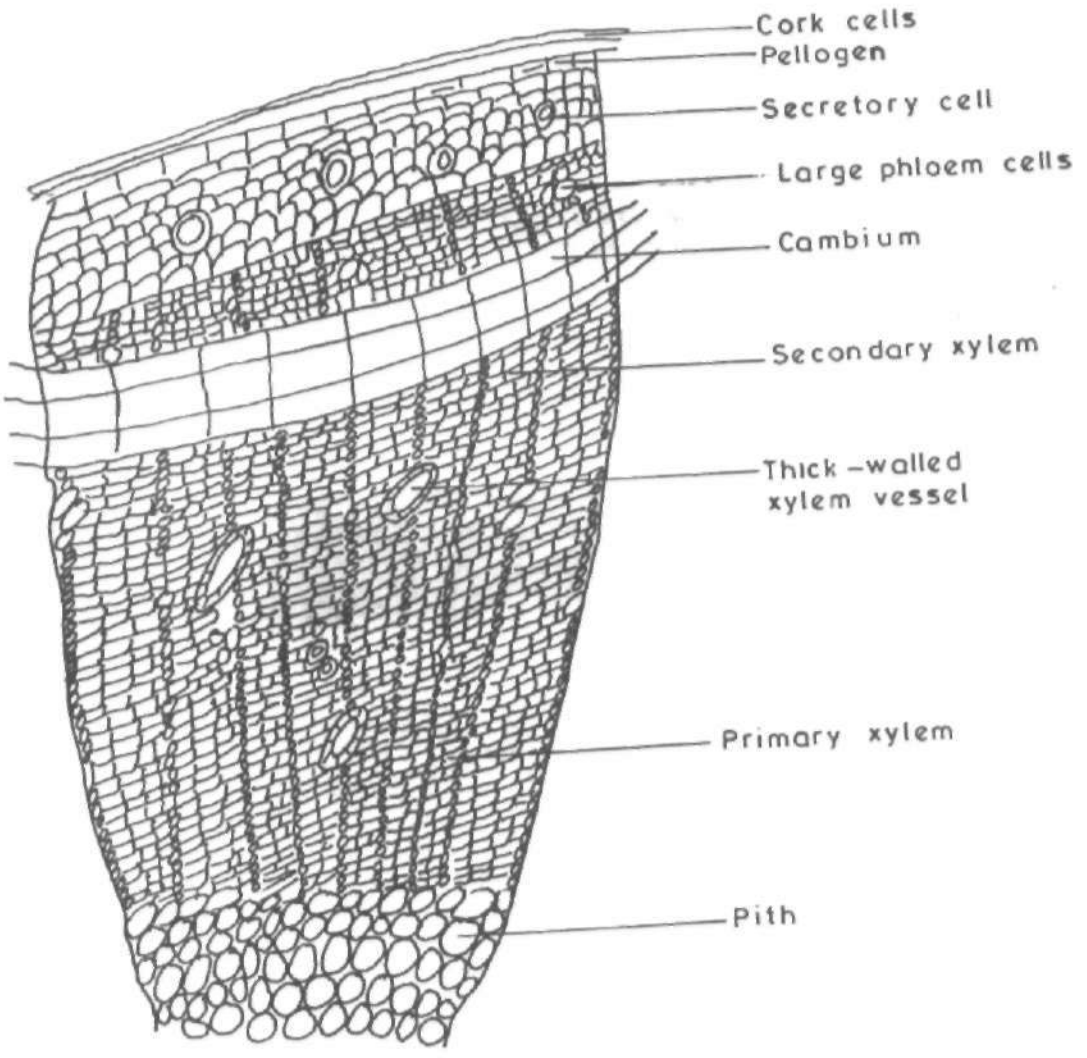


FIG.11h: TANGENTIAL SECTION OF THE ROOT OF C. EDULIS UNDER P.C.M.

X 40



b. Stem

The microscopical features of the powdered stem bark were as follows; cork cells, phallogen, lignified and non-lignified parenchyma cells, phloem fibres, sclereids, sieve tubes, trichomes, secretory tissues (cells and laticifiers) starch grains and calcium oxalate crystals. These features were typical of powdered bark sample and similar to the root sample except that dimensions and frequency might differ.

The various microscopical features of the powdered stem bark of *C. edulis* are illustrated in Fig.12 and may be summarized below:-

Cork Cells

Shape	-	elongated and polygonal
Anticlinal walls	-	straight
Thickening	-	present

Phloem fibres

Size	-	length 450-10470µ; width 20 - 130µ
Frequency	-	numerous
Apex	-	tapering

Sieve tubes

Size	-	length 15 - 20µ
		width 2 - 3µ
Frequency		moderate
Companion cells		present

Solereids

Shape	-	circular and polygonal
Size	-	80 -130µ
Frequency	-	moderate

Secretary cells

Type	-	tannin and oil cells
Shape	-	circular
Size	-	15 -25µ
Frequency	-	very few

Laticifers

Types	-	non articulated and branched
Frequency	-	moderate

Trichomes

Type	-	uniseriete of 1 - 4 cells covering trichome
Size	-	length 340 - 500µ
	-	width, 85 - 90µ
Frequency	-	few

Starch grains

Shape	-	round
Size	-	5 - 15µ
Hilum	-	central
Maltese cross	-	present
Frequency	-	few
Aggregation	-	simple and compound

Calcium oxalate crystals

Type	-	prisms, needle

Size - length, 30 - 170μ

width, 10 - 100μ

The macerated stem and various anatomical sections (transverse longitudinal, redial and tangential) revealed additional features to the ones seen in the powdered bark. The arrangement of the features seen in powdered sample was also significantly observed in sections.

Additional features observed were as follows cambium, xylem tissues (xylem vessels and tracheids), medullary rays, pith, stomata and type of vascular bundle. The trichomes were observed in powdered bark sample but more clearly seen in the fresh samples. They were longer and more frequent on the younger, terminal part of the shoot than the matured stem. Stomata which were not seen in the powdered sample was observed.

These microscopical features can be described as follows:-

<u>Stomata</u>

Type - actinocytic

Size - 40 - 50μ

Frequency - moderate

<u>Calcium Oxalate Crystals</u>

Type - Rosette

Size - 15 - 30μ

Frequency - scanty

Xylem Tissues:

Type - reticulate and annular thickened vessels; tracheids

Size - length 100 - 150μ

width 20 - 90μ

Frequency - numerous

The transverse section (Fig. 12) was seen to consist of 8 to 9 rows of differentiated tissues. The outermost layer consisted of polygonal cells, dark, thick and usually of 2 rows of cells. This layer was the thinnest with trichomes and lenticels about to form. It had a lot of deposit inside and on the cells. The second layer was yellowish brown in colour, polygonal cells and usually of 1-3 rows of cells. The third layer was consisting of 4 -5 rows of parenchyma cells. The fourth layer, was about 4 rows, very dark layer of polygonal cells with intermittent circular secretary cells (yellowish brown less thickened cells). Apparently the size of cells or first layer was less than that of second which was less than that of third layer, that of fourth layer was less than that of the third layer. These layers may be the cork, phellegen, phelloderm and cortex respectively from the outer most layer.

The fifth layer of cells consisted of phloem tissue (phloem fibres, phloem parenchyma) and secretary cells. This was made of 2-3 irregular rows. Then follows the cambium which was very difficult to observe even when numerous sections were made until the phase contrast microscopy was used and later the terminal tissue of a twig was cut and observed. These appeared with compound microscope as parenchyma cells of about 3 to 4 irregularly arranged row of cells. Next, the most conspicuous rows of tissues were the xylem elements, because of the xylem vessels. Though the different

elements were difficult to obtain but observed to consist of xylem vessels, xylem fibres with medullary rays of one or two rows of elongated parenchyma cells and wood parenchyma making a separate row of small cells closely packed without air spaces below the xylem vessels and fibres. Finally was the pith consisting of large parenchyma cells with air spaces between them. The apparent size of layers of tissues was 8th < 9th < 7th < 4th < 3rd < 5th ≤ 2nd < 1st < 6th (i.e wood parenchyma < pith < xylem cortex < phelloderm < phloem ≤ Phellogen < cork < cambium).

All these features were also observed in the tangential longitudinal and radial longitudinal sections (Fig. 12) except that the cambium was not easily observed with compound microscope but with phase contract microcopy (fig. 12). These features appeared differently in the different sections as illustrated in the diagrams. Rosette type of calcium oxalate was observed only in the anatomical sections.

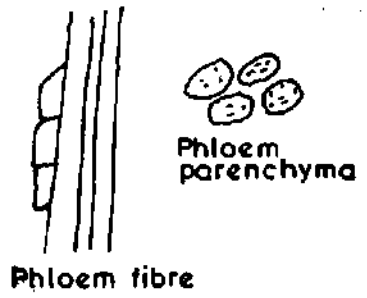
The tracheids were more clearly observed in the macerated sample.

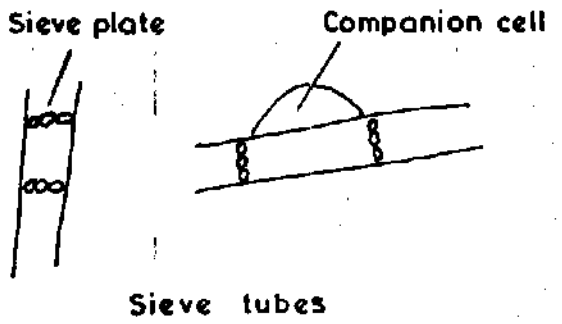
The features demonstrated in the different sections show that secondary growth has just started as lentical were not completely formed, cork layer was small and trichome were still present.

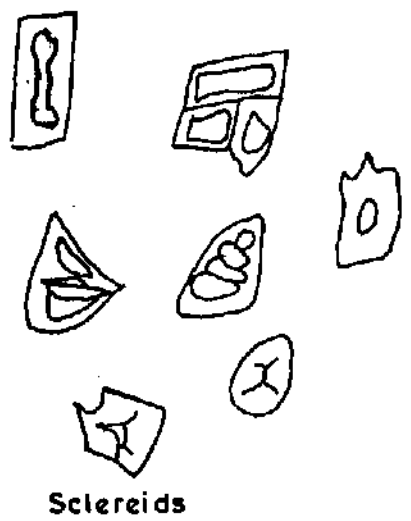
The pith was bigger than in the root and the wood parenchyma as a distinct layer was not observed in the root. The stem can be said to consist of more strengthening tissues (fibres and wood parenchyma) than the root.

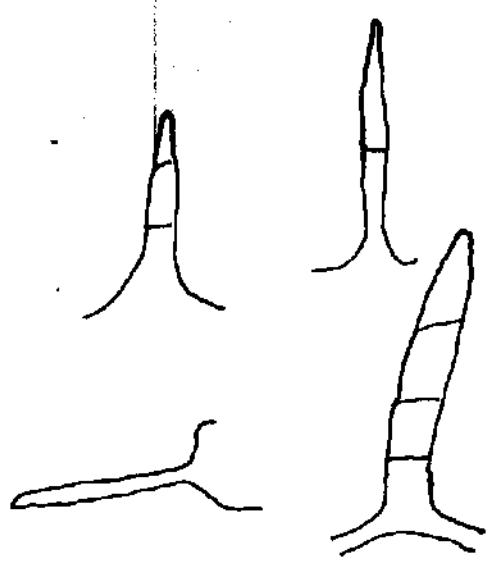
FIG. 12a : MICROSCOPICAL FEATURES OF THE POWDERED STEM BARK OF C · EDULIS

X 400

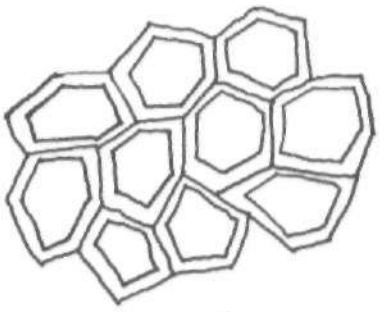




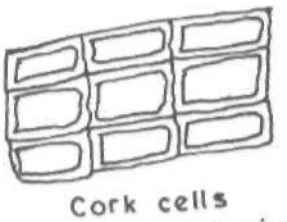




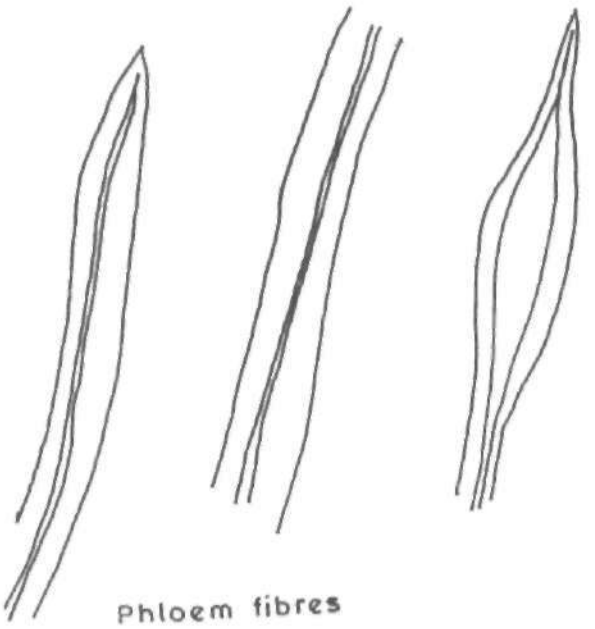
x 400



Cork cells
(Surface view)



Cork cells
(Transverse view)



Phloem fibres



Phloem fibres
(Surface view)

FIG. 12a ; CONTD.

X 400

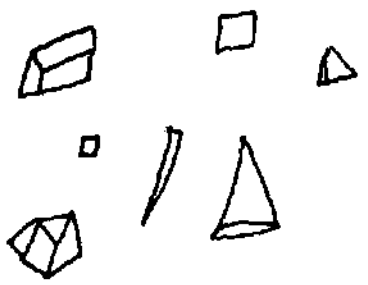




Laticifers



Starch grains



Calcium oxalate crystals

FIG. 12b: TRANSVERSE SECTION OF THE STEM OF C. EDULIS

X 400

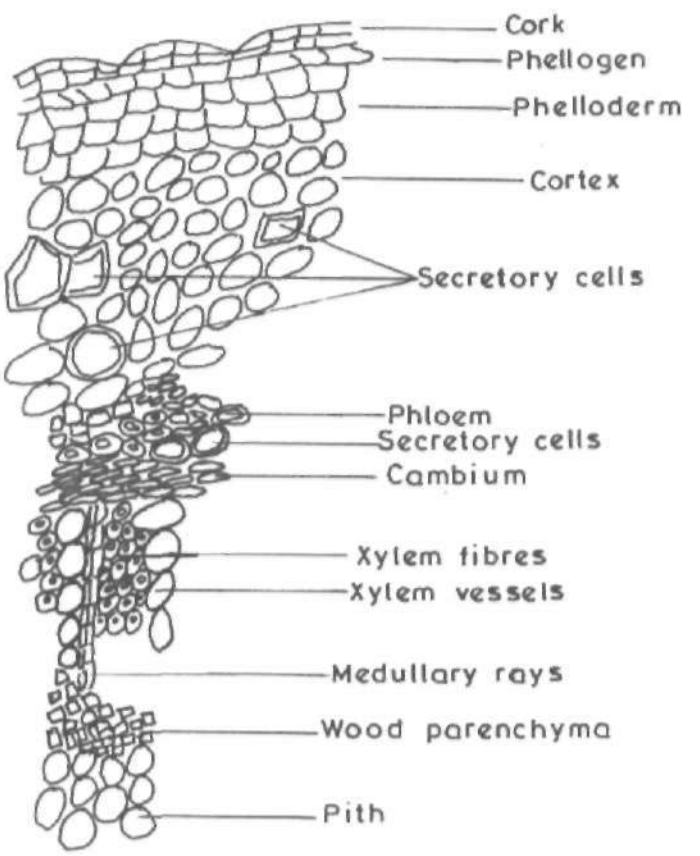


FIG.12c;RADIAL LONGITUDINAL SECTION OF THE STEM OF C. EDULIS

X 400

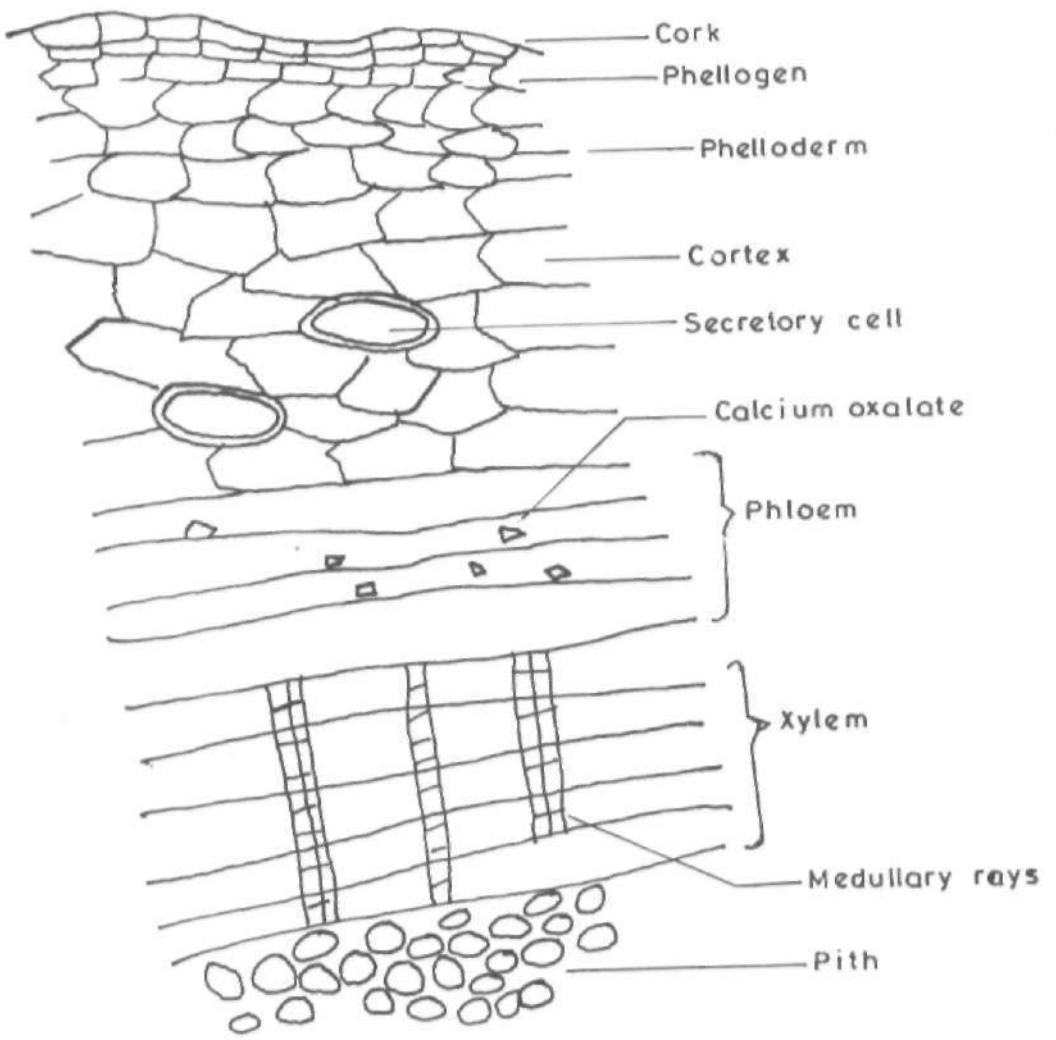
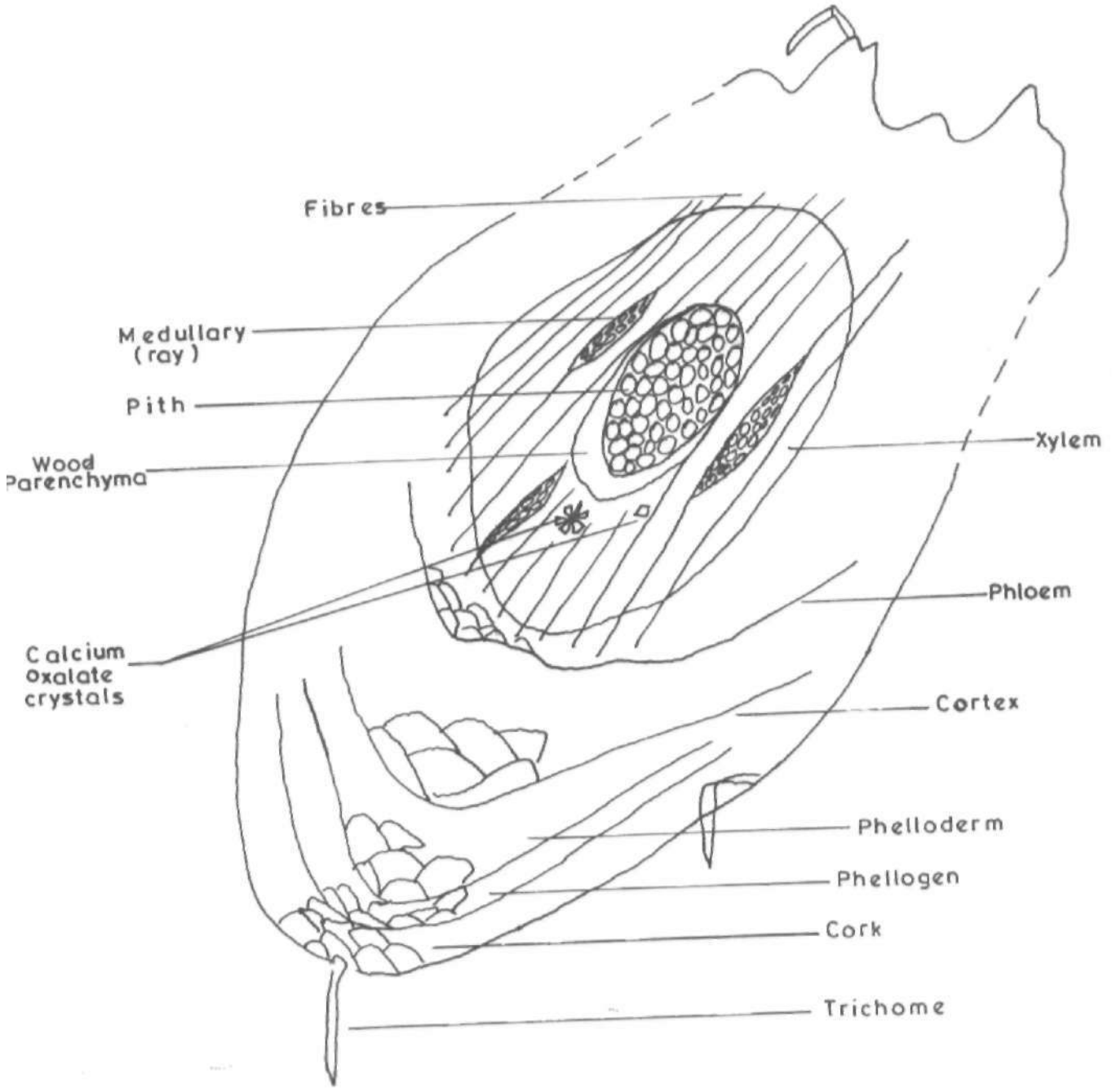


FIG. 12d: TANGENTIAL LONGITUDINAL SECTION OF STEM OF C. EDULIS

X 400



Vessels projecting towards the pith, fibres projecting towards opposite direction

FIG.12e: A TRANSVERSE SECTION OF THE STEM SHOWING LATICIFERS AFTER STAINING WITH SUDAN IV

X 400

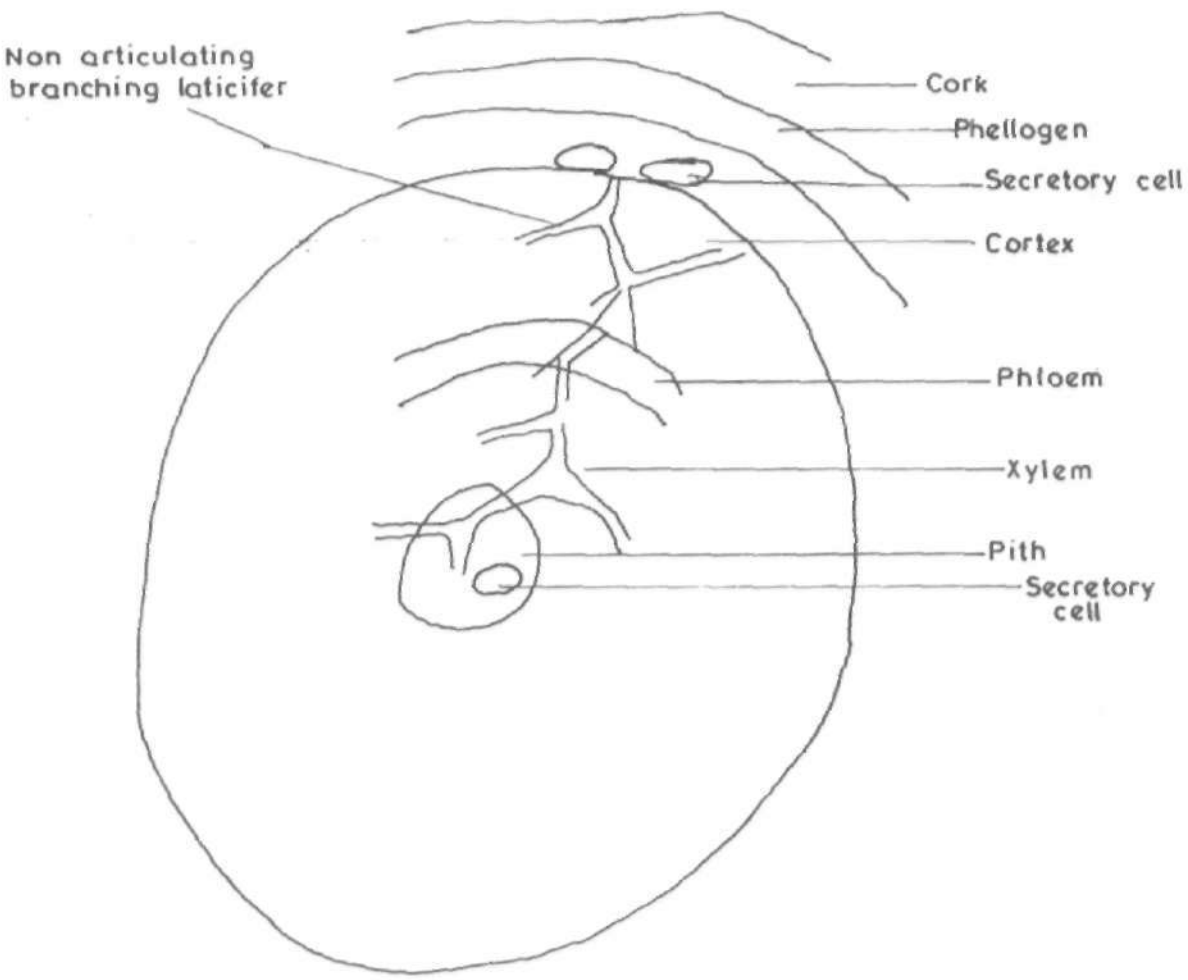


FIG.12f: TRANSVERSE SECTION OF THE STEM OF C. EDULIS UNDER P.C.M.

X 40

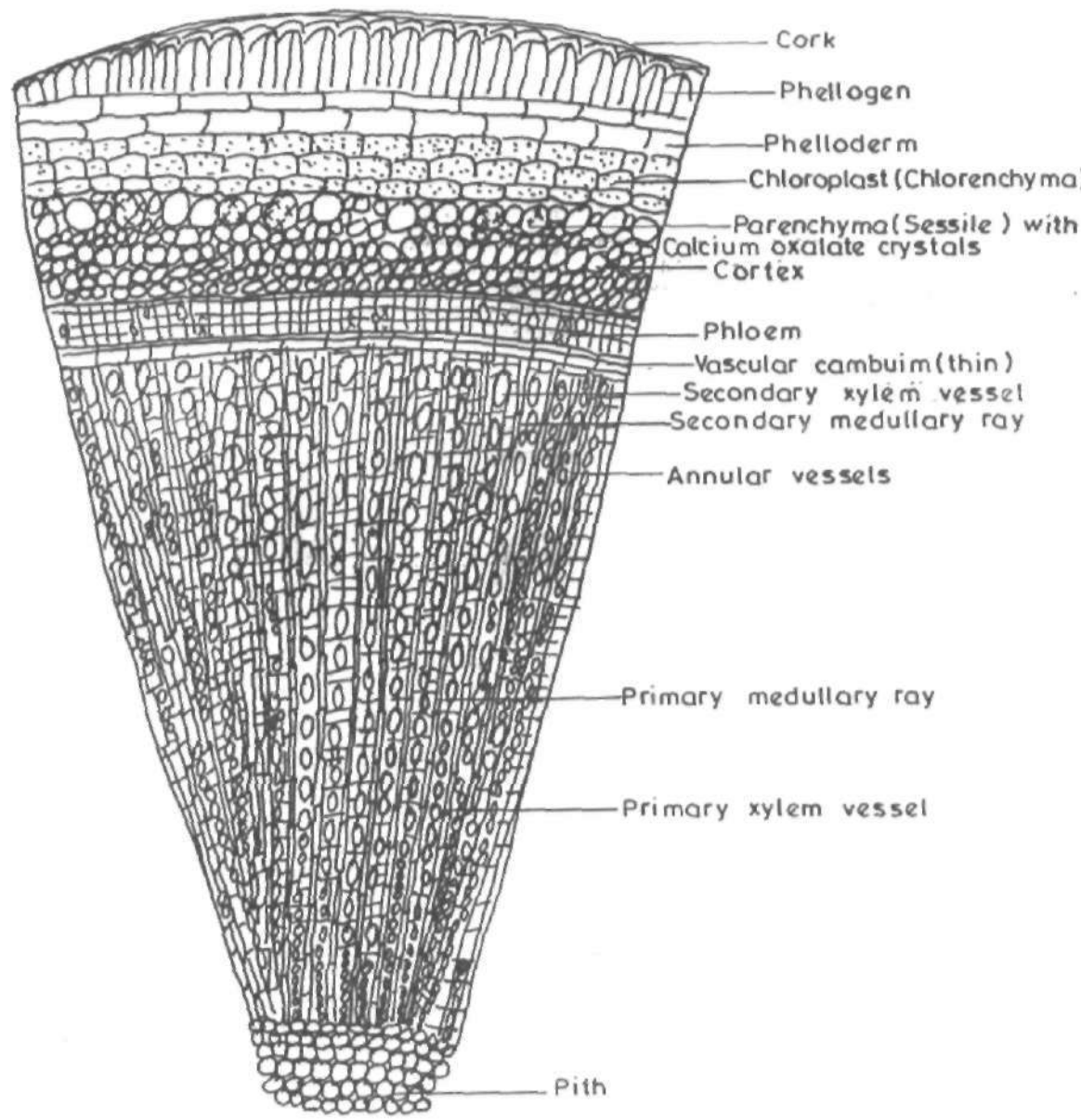


FIG.12g : RADIAL SECTION OF THE STEM OF C. EDULIS
UNDER P.C.M.

X 40

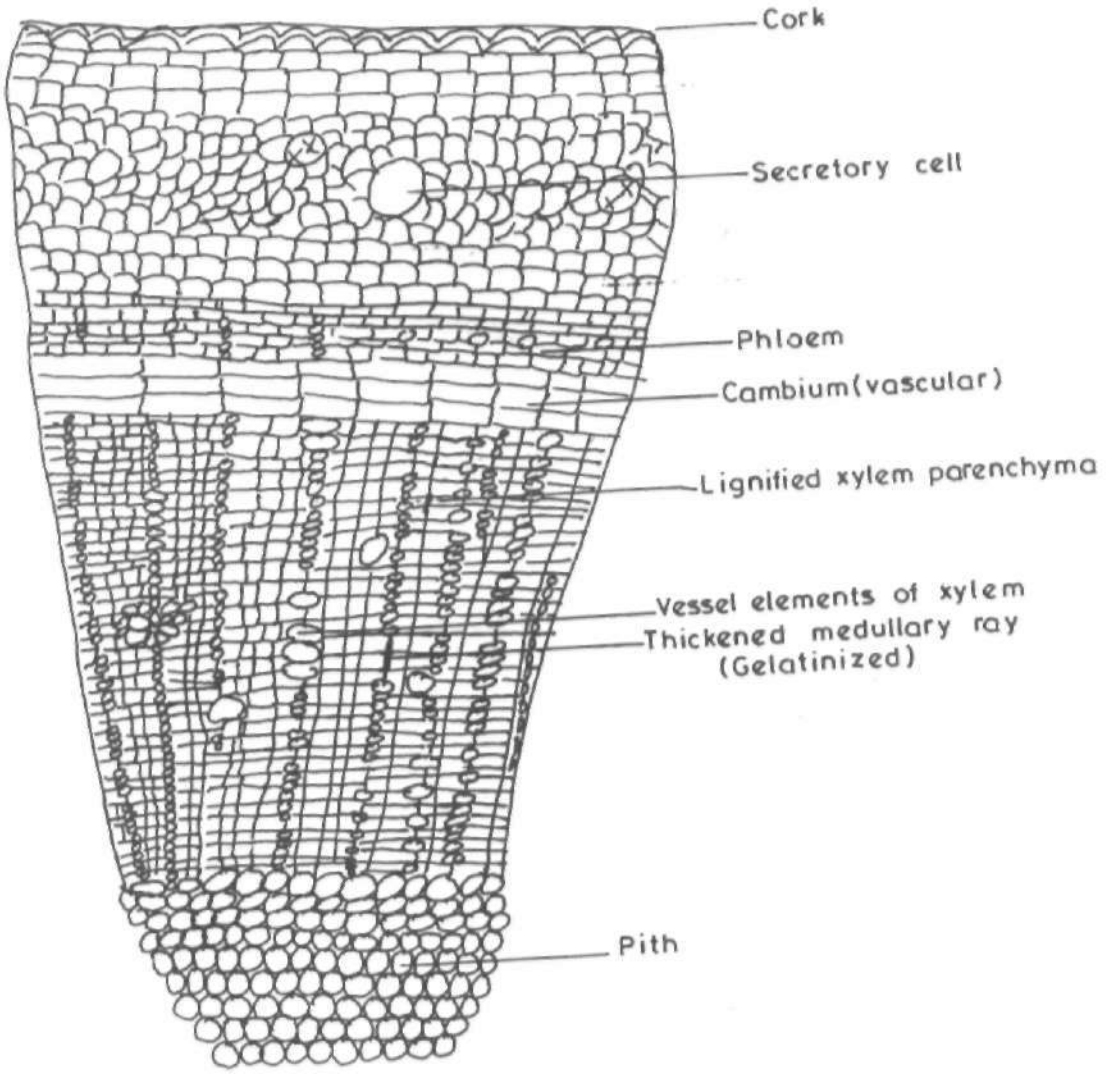


FIG. 12h: LONGITUDINAL SECTION OF THE STEM OF C. EDULIS UNDER P.C.M.

X 40

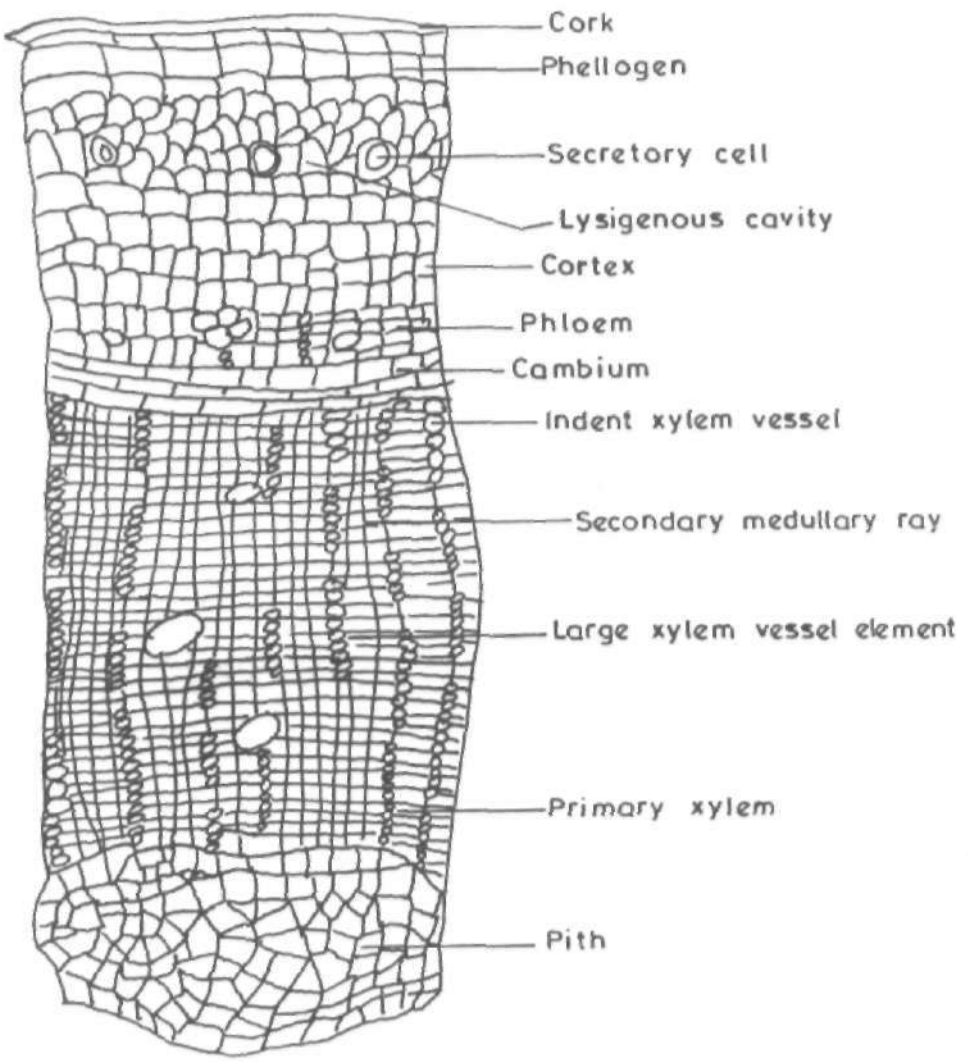
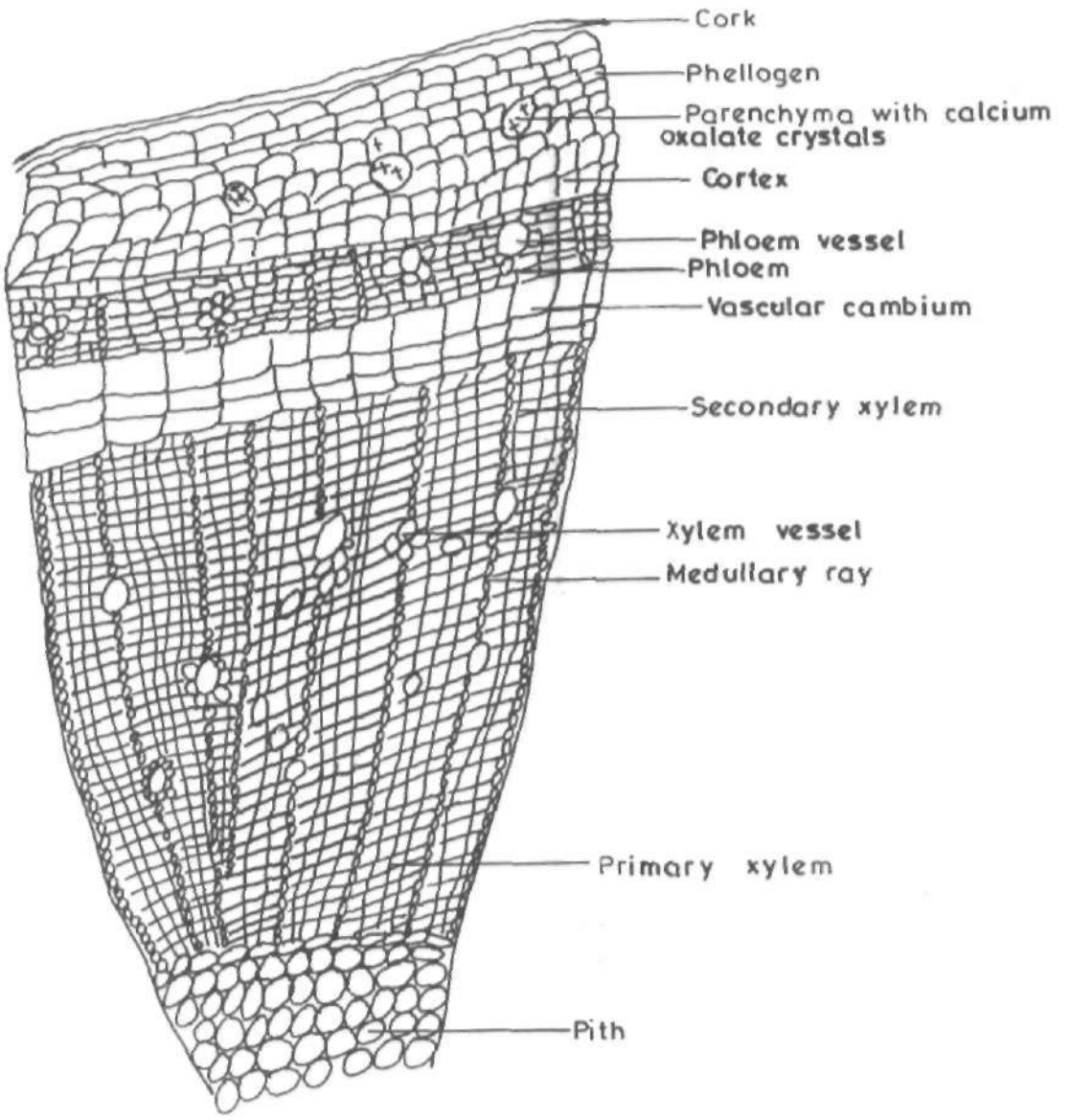


FIG.12 i: TANGENTIAL SECTION OF THE STEM OF C · EDULIS
UNDER P.C.M.

X 40



c. **Leaves**

The microscopical features of the powdered leaves sample were as follows:- epidermis with stomata, fragments of lamina with palisade cells, vascular bundle and spongy mesophyll intact; trichomes, laticifers, sclereids, phloem fibres, xylem vessels, calcium oxalate crystals, starch grains and secretory cells.

These features are illustrated in Fig.13 and can be described as follows:-

Stomata

Type - actinocytic

Size - 25µ

Frequency - moderate

Phloem fibres:

Size - length 8 - 45µ

- width 2 - 5µ

Frequency - moderate

Apex - tapering

Sclereids:

Size - 3µ - 5µ

Type - oval

Frequency - few

Trichome:

Type - Unisereate, 1-4 cells, covering trichomes

Size - length 5 - 15µ

width, 2 - 3µ

Frequency - moderate

Starch Grains

Shape - oval

Size - 2 -5μ

Hilum - central

Maltese cross present

Aggregation Simple

Frequency - few

Calcium Oxalate Crystals

Type - Prism, rosette

Size - (i) Prism:

length, 5 - 20μ

width, 5 - 5μ

(ii) rosette 10μ

Frequency - numerous

Secretary cells

Type - tannin, oils

Shape - round, irregular

Size - 20μ

Frequency - few

The various transverse sections of the lamina, midrib and petiole (Fig. 13)revealed the exact positions and arrangement of the different tissues found in the leaf of *C. edulis*. The leaf was found to be dorsoventral i.e the palisade cells occurring on one side of the two epidermis. This leaf had its palisade cells below the upper epidermis only. This conforms with the report that Apocynacese leaves are dorsicventral or isobilatral (Tanaka, 1976).

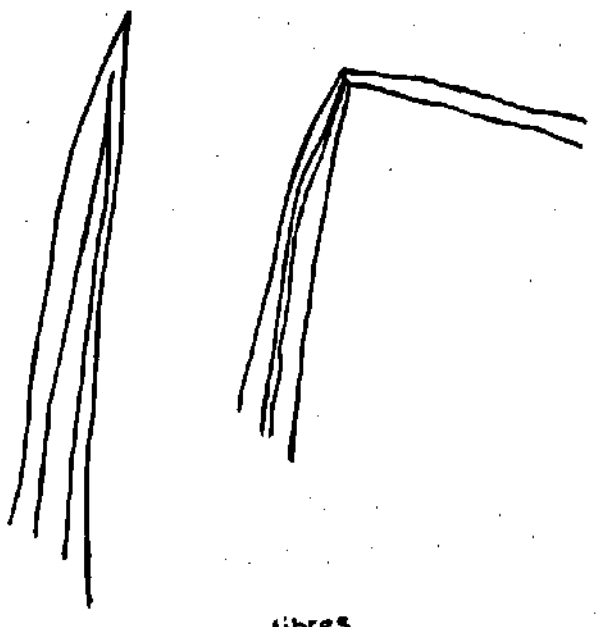
The lamina consisted of a row of epidermal cells with a layer of cuticle on top of it.. A specialized group of cells were seen to have formed with the upper epidermis with a circular arrangement into the palisade cells below, as illustrated in the diagram Fig. 13. This could be a peculiar feature to the leaf of this plant. The upper epidermis was followed by a row of closely packed palisade cells. Next was the spongy mesophyll made up of parenchyma cells with small air spaces in between them. These cells constituted about 7 - 9 rows of irregularly arranged cells. In this layer were found the vascular bundle in groups. Also secretory cells and laticifiers were observed. It was noticed that not only non-articulating branching laticifers exist but non-articulating non-branching laticifiers as well. Rosette crystals were quite obvious in the lamina. They were few but more than was seen in the tangential section of the stem. Below the spongy masophyll was the epidermal cells with stomatal openings. Trichames were also seen on the lower epidermis. These were not present on the upper epidermis.

The midrib was found to consist of upper epidermis with cuticle without palisade cells below it. The midrib had parenchyma cells below the upper epidermis than the vascular bundle. Next to the vascular bundle were some secretory cells containing oil droplets and other contents. Below this was the collenchyma cells and the lower epidermis. Some sections had collenchyma below the upper epidermis.

The petiole had a single layer of epidermis, parenchyma cells with vascular bundle in the middle having a crescent shape of xylem tissues. Usually a single trichome was observed.

FIG.13a: MICROSCOPICAL FEATURES OF THE POWDERED LEAVES OF C. EDULIS

X 400



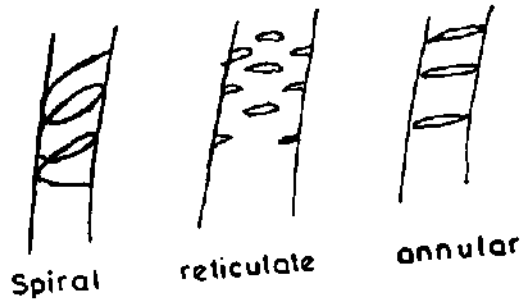
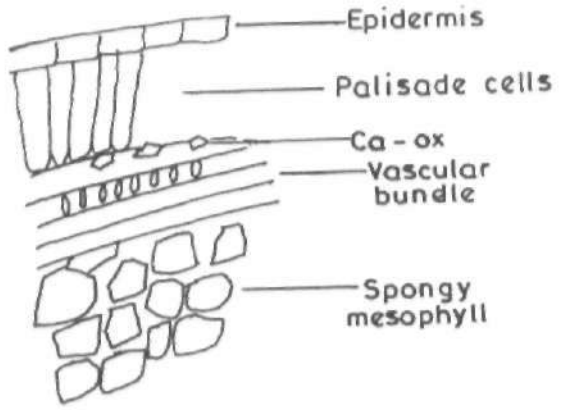
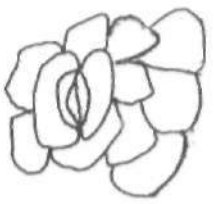


FIG.13a : CONTD.

x400



Fragment of leaf



Actinocytic stomata



Trichomes



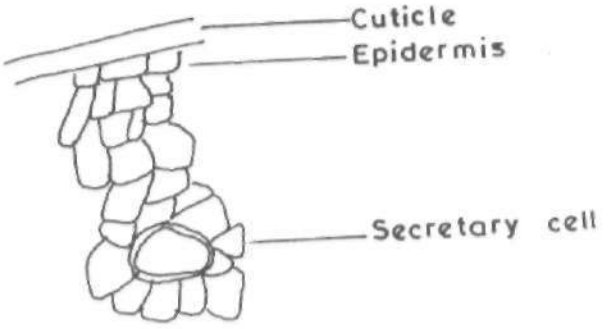
Sclereid

FIG. 13a ; CONTD.

X 400



Laticifers



Fragment of leaf

FIG.13b: TRANSVERSE SECTION OF THE LEAF OF C · EDULIS SHOWING MIDRIB AND LAMINA

X 400

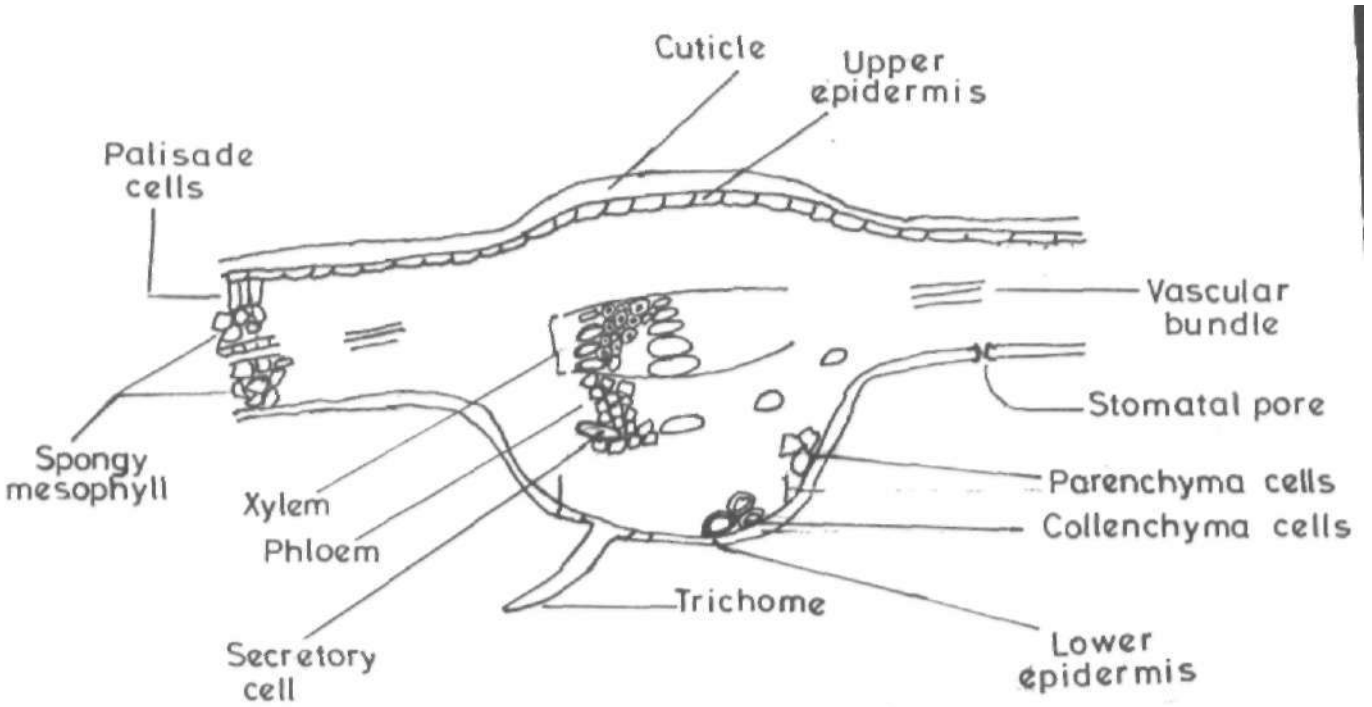
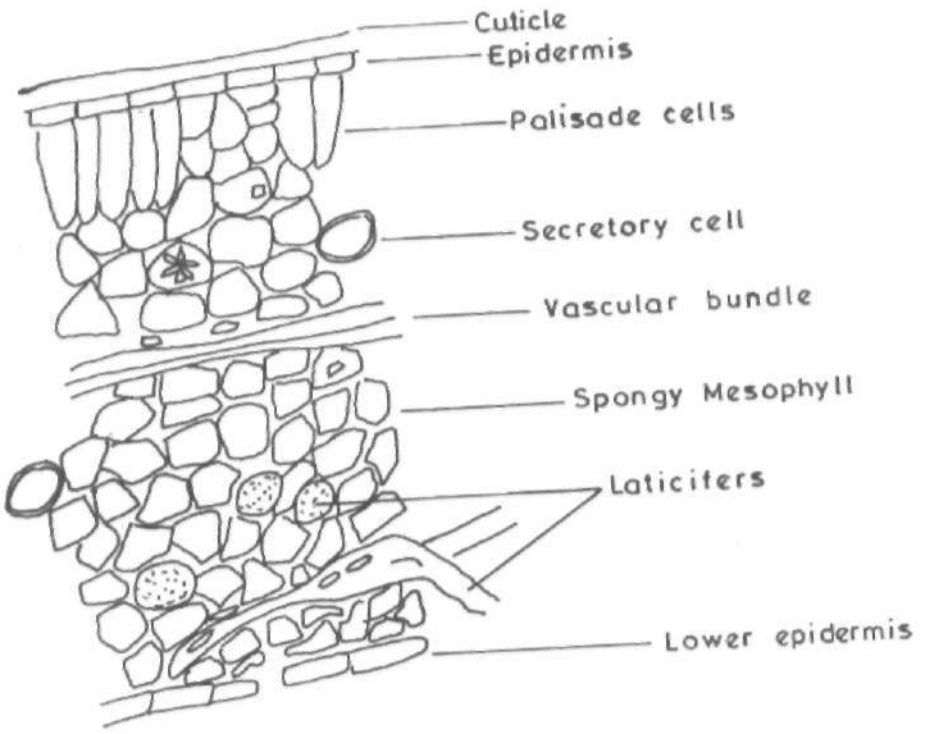
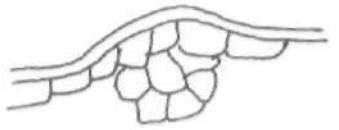


FIG.13c: TRANSVERSE SECTION OF THE LEAF OF C. EDULIS THROUGT THE LAMINA

X 400





Specialised group of cells below the upper epidermis

FIG.13d: TRANSVERSE SECTION OF THE LEAF OF C. EDULIS THROUGH THE MIDRIB

X 400

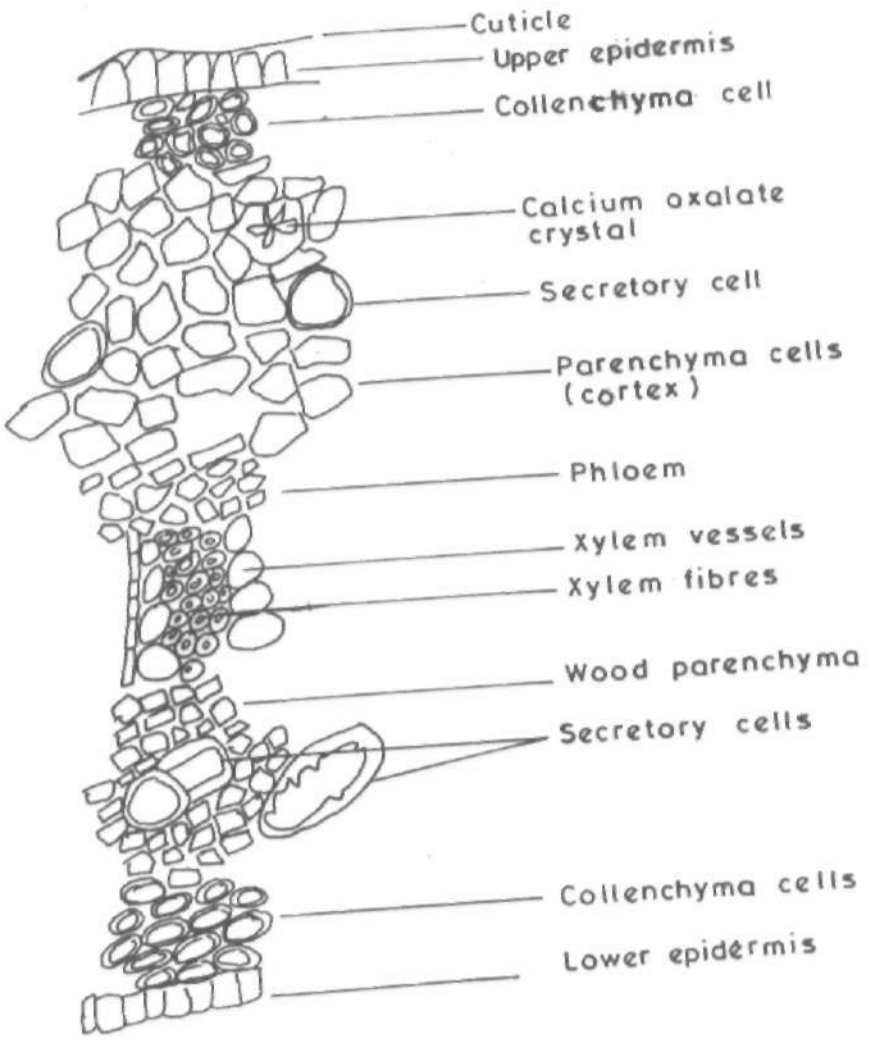


FIG. 13e : TRANSVERSE SECTION OF THE PETIOLE OF C · EDULIS

X400

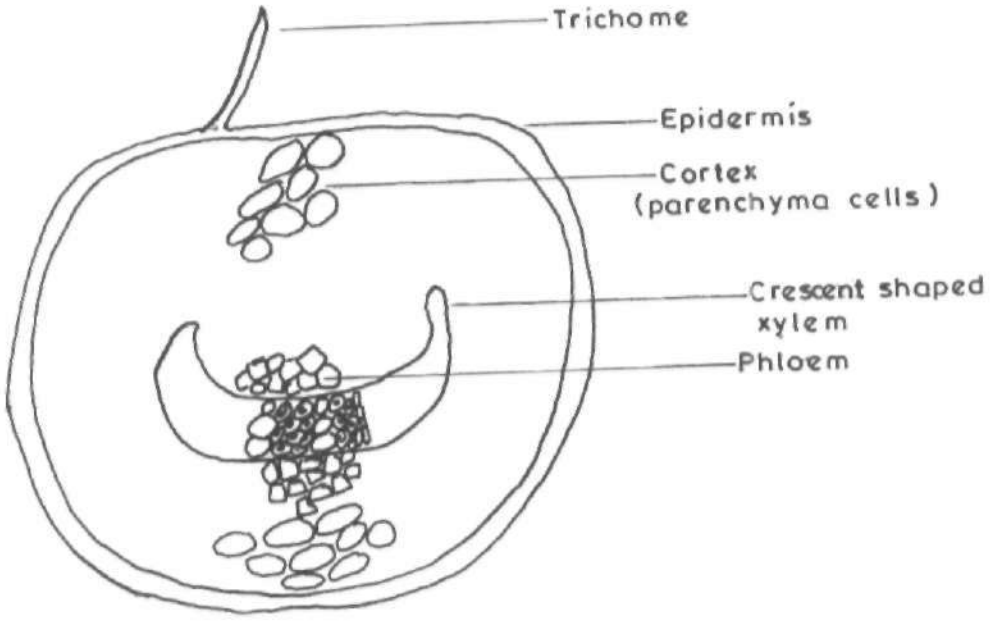
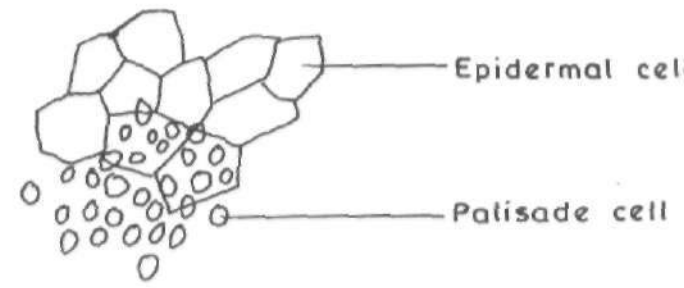
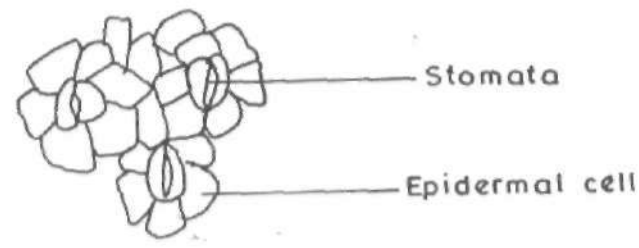


FIG.13f : THE EPIDERMAL LAYERS OF THE LEAF OF C . EDULIS



Upper epidermis



Lower epidermis

Generally very few trichomes were observed on the leaf sections.

The upper and lower epidermises were peeled off and observed to have polygonal parenchyma cells with cell contents. The upper epidermis had no stomata but palisade cells could be observed to be numerous of various sizes. The lower epidermis on the other hand had numerous closely packed stomata.

The results of the quantitative leaf microscopy of *C. edulis*is given in table 3. The results were obtained from at least 4 observations.

TABLE 3 QUANTITATIVE LEAF MICROSCOPY

PARAMETERS	VALUES	
	RANGE	MEAN
Palisade Ratio	6.2-6.5	--
Stomatal Number	342 - 362	359
Stomatal Index	14.2 - 14.5	14.4
Vein-islet number	18-20	19
Vein termination number	28-33	--

d **Fruit**

The powdered samples of the fruit was found to contain epidermal cells, laticifers, sclereids, xylem vessels, phloem fibres and calcium oxalate crystals.

These are illustrated in Fig.14 and are described below:-

Laticifers

Type (1) Smooth, non-articulating branching Laticifer.

(2) articulating - branching laticifer

Frequency - Numerous, Type (1) more frequent than (2)

Calcium oxalate crystals

Type - Prism

Size - 3 - 10μ

Frequency - Few

Xylem vessels

Type - annular

Size - length 80-100μ, width - 5- 10μ

Frequency - few

Pholoem fibres

Size - length - 50-70μ, width 5-8μ

Frequency - few

The anatomical features (fig.14) of the fruit sample was as follows. The fruit is differentiated into three, epicarp, mesocarp and endocarp. These were easier to observe separately.

A peel of the epicarp (fig. 14)revealed polygonal parenchyma cells with numerous oil droplets and very few calcium oxalate crystals. The sizes and shapes of these cells veried with straight double anticlinal walls.

The mesocarp (fig.14) had numerous laticifers as seen in the powdered samples. The vascular bundle was seen crossing

these laticifers in branches also. These structures were on a ground tissue of parenchyma cells which looked like interconnecting strands. A few sccereids and secretary cells were observed. There were many prism shape calcium oxalate crystals. There were about 5 - 6 rows of xylem and about 5 rows of phloem fibres, annular and spiral Xylem vessels were observed.

The endocarp (fig.14) was similar to the mesocarp with interconnecting parenchyma cells With no vascular bundle or sclerenchyma. But, laticifers were seen including non-branching types not seen in the mesophyll. Few calcium oxalate crystals were seen.

FIG.14a: MICROSCOPICAL FEATURES OF THE POWDERED FRUITS OF C . EDULIS

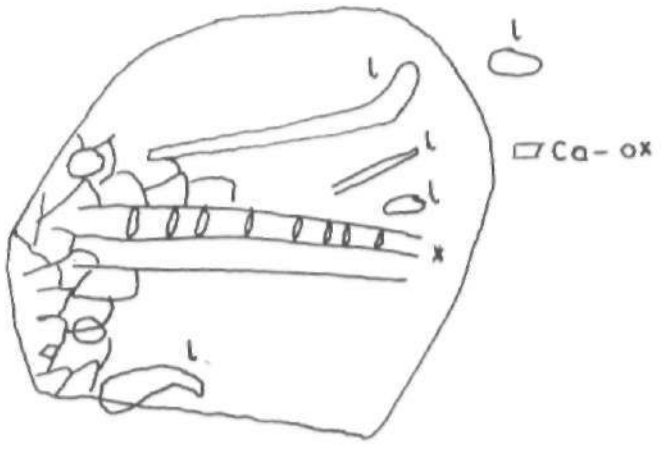
X 400





FIG.14a: CONTD.

x400



Cellular(mesocarp) fragment with laticifers (l) and vascular bundle (x)



Starch grains



Calcium oxalate crystals

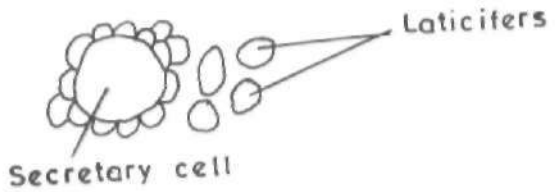


FIG.14b: TRANSVERSE SECTION OF FRUITS OF C. EDULIS

X 400

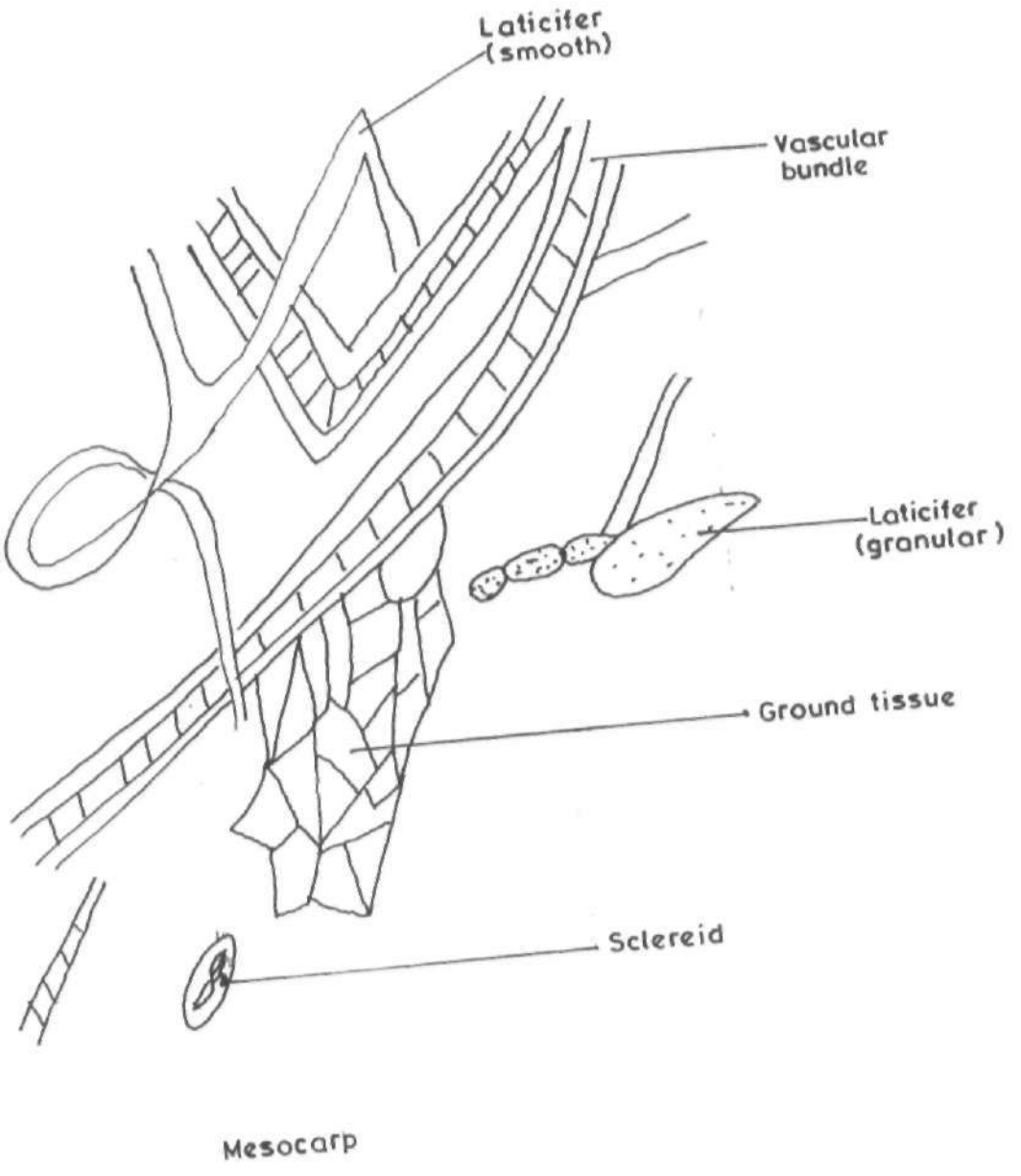
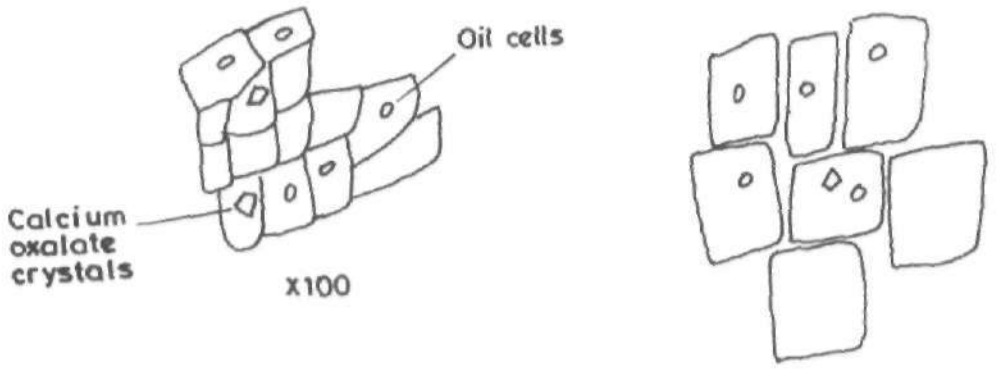
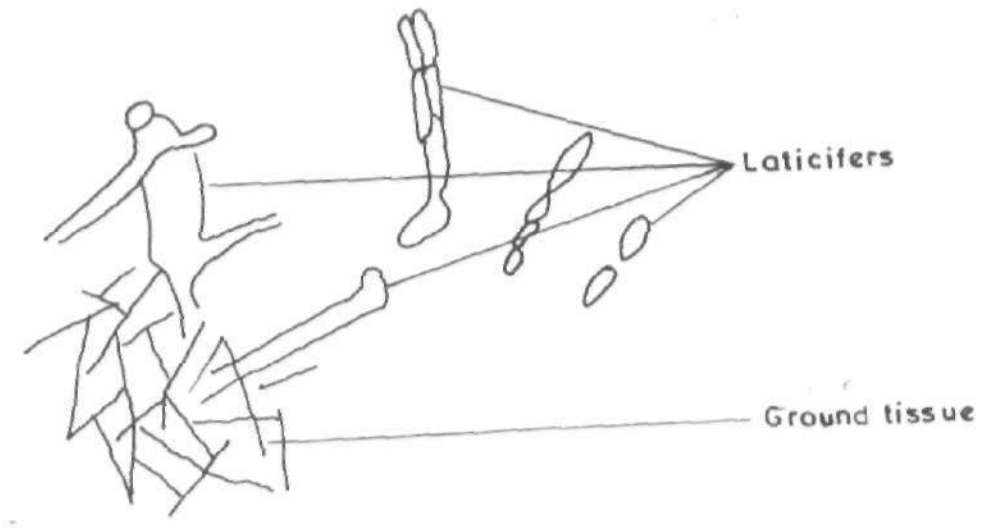


FIG.14b : CONTD.

x400



Epicarp



Endocarp

e **Seeds:**

The microscopical features of the powdered seeds were as follows:- cellular structures, phloem fibers, sieve tubes with companion cells, laticifers, calcium oxalate crystals, aleurone grains and starch gains. These features are illustrated in fig.14 and can be described as follows:

Phloem fibres

Size	-	length : 300 - 400u
	-	width : 2 - 4 u
frequency	-	few
Apex	-	tapering

Xylem vessels

Type	-	annular
frequency	-	few
Apex	-	blunk

Sieve tubes

frequency	-	few
sieve plate	-	present
companion cell	-	Present
Apex	-	blunt

Laticifers

Type	-	non articulated and branched
Frequency	-	few

Aleurone grains

Frequency	-	Moderate
Type	-	grains
Shape	-	round
Size	-	3 - 5µ

Starch grains

Shape	-	round
Size	-	2 - 5μ
Hilum	-	Central
Maltese cross	-	present
Frequency	-	numerous
Aggregation	-	Simple and compound.

Calcium oxalte crystals

Type	-	prisms
Size	-	2-20μ

The anatomical sections (fig.14) of the seed revealed 3 layers of differentiated tissues with the first layer separated from others by a pigmented line. Vascular bundle and laticifers could be seen from the transverse section. But the laticifer was more clearly seen when the endosperm was squeezed out from the testa, after a longitudinal section. Secretory cells, starch, grains, calcium oxalate crystals and protein were found to be present.

3.2.2 CHEMOMICROSCOPICAL EXAMINATION

(a) **Test for Lignin**

A red colour was observed indicating the presence of lignin. The fibres, xylem vessels, sclereids, and some parenchyma cells of bark samples were found to be lignified. The xylem vessels were highly lignified while the phloem fibres and parachyma cells were lightly lignified. Sclereids were also lignified.

FIG.15a : MICROSCOPICAL FEATURES OF THE POWDERED SEEDS OF C . EDULIS

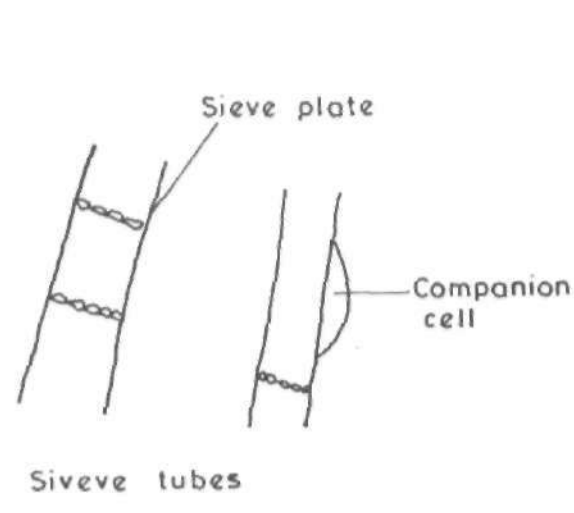
X 400



Many cellular structures

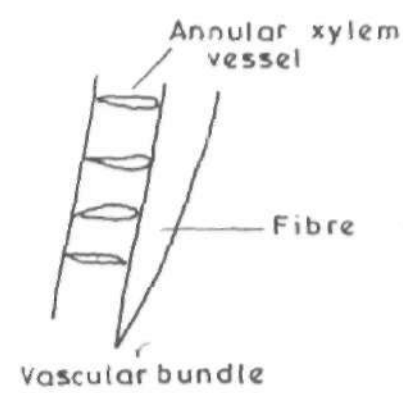
Stained dark completely with N/50 iodine

FIG. 15a : CONTD.

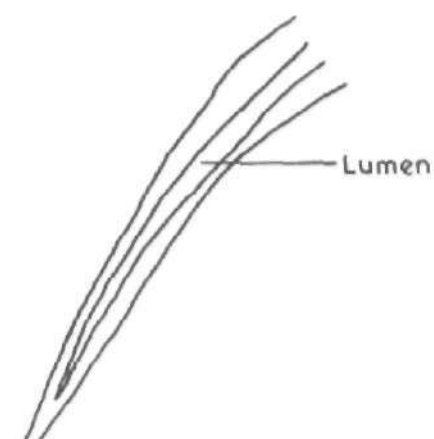


Siveve tubes

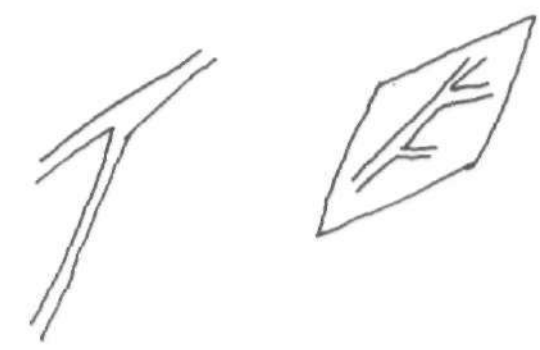
x 400



Vascular bundle



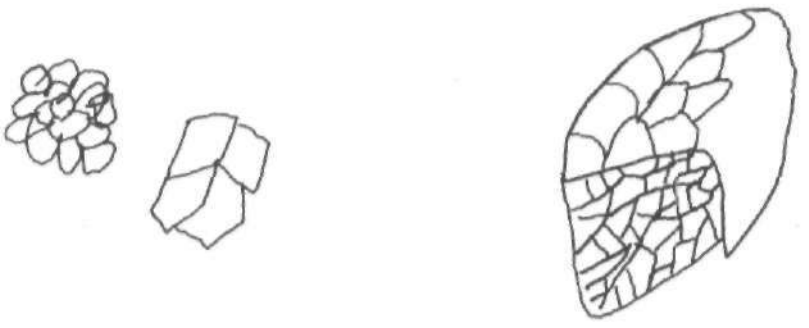
Phloem fibre



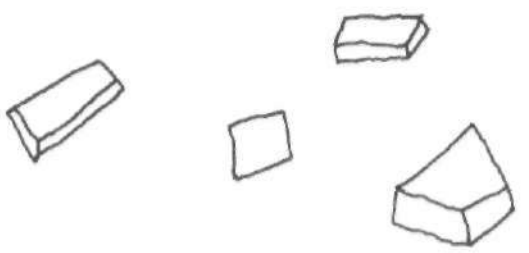
Laticifers

FIG.15a : CONTD.

X 400



Cellular structures



Calcium oxalate crystals

FIG.15b : MACROSCOPY OF FRESH SEED OF C. EDULIS

X 400



FIG.15c: MICROSCOPICAL EXAMINATION OF THE WHOLE SEED OF C. EDULIS

X 400

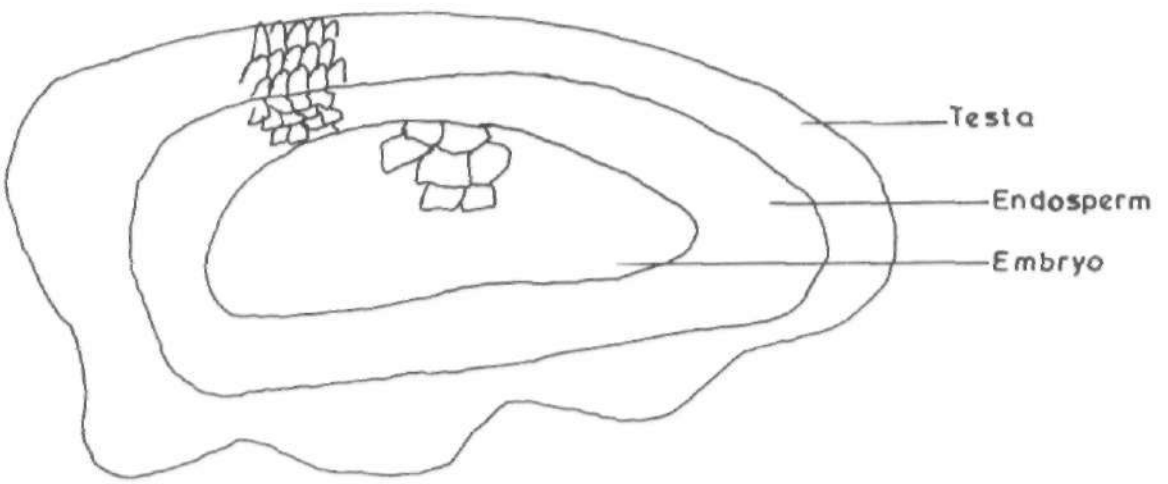
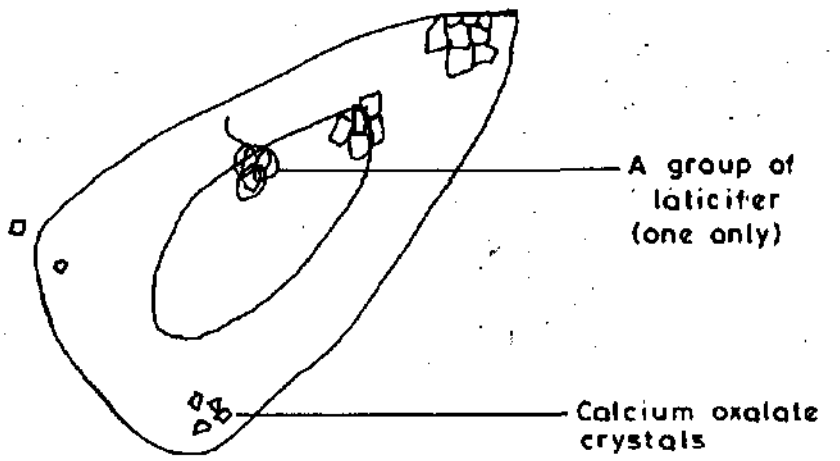
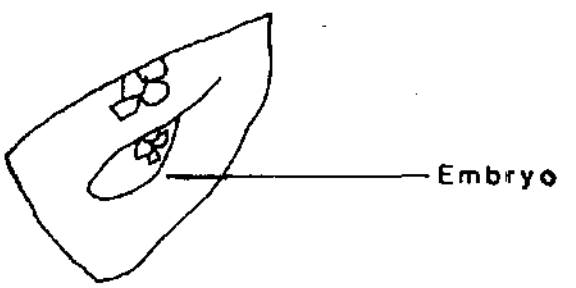


FIG.15d: MICROSCOPICAL EXAMINATION OF THE ENDOSPERM OF SEED OF C. EDULIS

X400



First layer of endosperm



Second layer of endosperm

FIG. 15e: LONGITODINAL SECTION OF THE SEED OF C. EDULIS

X 400

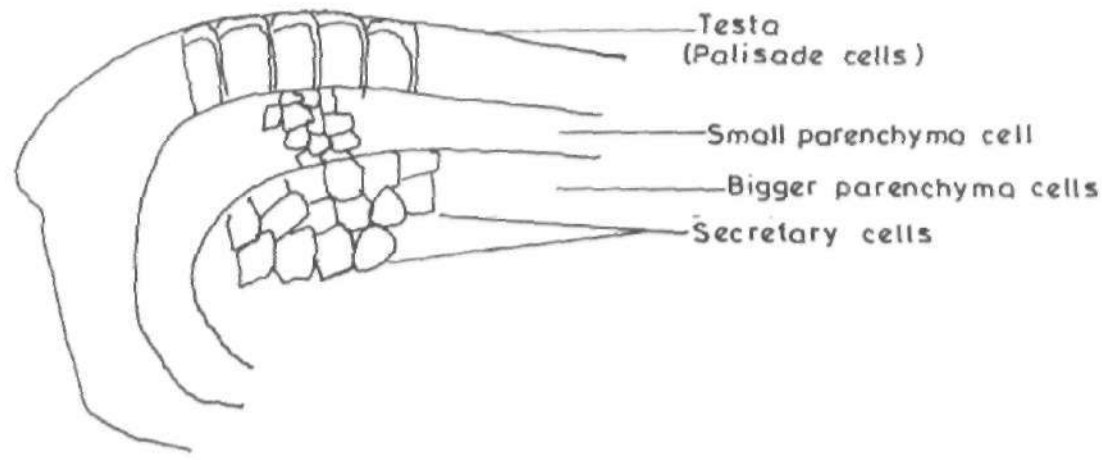
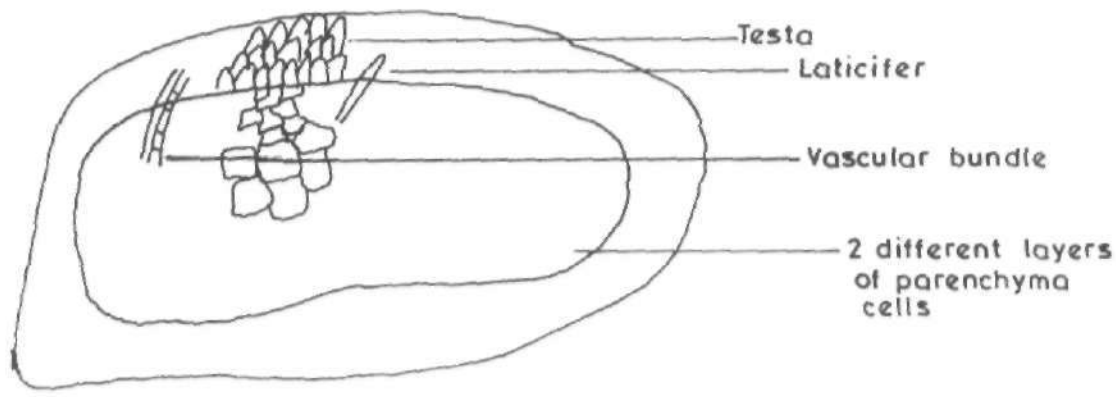


FIG. 15f: TRANSVERSE SECTION OF THE SEED OF C. EDULIS

X 400



(b) **Test for Cellulose**

Cellulose was found to be present as blue to black colour was observed with chlor-zinc-iodine and N/50 iodine plus 66% sulphuric acid. Epidermal Cells, parachyma cells, cell wall of sieve tubes, cell walls of secretory cells and laticifers were stained blue to black. The cork cells were stained reddish brown in colour probably due to presence of suberin and other deposits. The phloem fibres and calcium oxalate crystals were not stained. The test for cellulose was positive as expected.

(c) **Test For Tannins**

Some cells had brown or yellowish brown contents which might indicate presence of tannins. This was confirmed by the appearance of greenish black and green colour when mounted in ferric chloride solution

(d) **Test for Mucilages**

No secretory tissue was found to contain mucilage but the cell wall of the stem and fruit gave positive results (a red and pink colour respectively indicating the presence of mucilage).

(e) **Test for Starch**

A blue - black coloured grains were observed indicating presence of starch. The seed had the largest amount of starch followed by the fruit. Patches of black stains were observed on the laticifers of the mesocarp and most part of the tissues appeared black. With the seed when N/50 iodine was added the whole endosperm stained black, the edges of the testa only was

staind black. These tests were on the fresh samples of fruits and seed. Very few starch grains were observed in the stem and leaves. Moderate amount in the root. Similar results were obtained with powdered samples.

f **Test for Calcium Oxalate Crystals:**

The bright structures observed disappeared on the addition of 80% H_2SO_4. This confirmed the presence of calcium oxalate crystals.

g **Test for Oils**

Some cells (secretory cells) and laticifers stained red with sudan IV this indicated the presence of oils or lipids.

h **Test for Cutin and Suberin**

The cork cells were found to test positive (red colouration) with tincture of alkanna, indicating the presence of suberin, Cell wall of the leaves was stained red indicating the presence of cutin.

(i) **Test for Callose**

The sieve plates of sieve tube stained red with corallin soda indicating the presence of callose.

(j) **Test for Protein**

Red stains with Million's reagent and yellow stains with picric acid indicated the presence of protein. This was observed in the fruits, seeds and root of the plant. It was easier to detect proteins with Millions reagent as compared to picric acid as picric acid also gives a yellow background stain.

The result of the chemomicroscopical examinations is given in table 4.

TABLE 4 CHEMOMICROSCOPICAL EXAMINATIONS OF THE ROOTS, STEM, LEAVES, FRUITS AND SEEDS OF C.EDULIS

CONSTITUENTS	ROOTS	STEM	LEAVES	FRUITS	SEEDS
Lignin	+	+	+	+	+
Cellulose	+	+	+	+	+
Fannins	+	+	+	+	+
Mucilages	-	+	-	+	-
Starch	++	+	+	+++	++++
Calcium Oxalate Crystals	+	+	+	+	+
Oils	+	+	+	+	+
Cutin	-	-	+	+	+
Suberin	+	+	-	-	-
Callose	+	+	-	-	-
Protein	+	-	-	+	+

Key

(+) - Present

(-) - absent

3.3 PHYTOCHEMICAL SCREENING OF POWDERED ROOTS, STEM, LEAVES, FRUIT AND SEEDS

The results obtained from the various phytochemical test are described below:

3.3.1: Test for Carbohydrates

(a) Extraction

Most carbohydrates are wholly or partially soluble in water. Therefore the water extracts were used for conducting the various tests for different classes of the carbohydrates (Monosaccharides, Oligosaccarides and Polysaccharides).

(b) **Molisch's Test**

This is a general test for carbohydrates. A purple ring at the common surface of the liquids (Molisch's reagent and water extract) and a dull violet precipate when the mixture of the liquids was shaken indicated the presence of carbohydrates. The roots, stem, leaves, fruits and seeds extracts gave this positive result.

Carbohydrates are normally present in all parts of the plant. This positive result is as expected.

(c) **Test for Sugars**

A positive test (a brick red precipitate) with Barfoed's reagent was obtained with the leaves only. This indicated the presence of monosaccharides in the leaves and their absence in the fruits, seeds, stem, and roots.

The Fehling's test for free reducing sugars (a brick red precipitate) was positive for fruits, seeds, leaves and root except for the stem. This indicated the presence of free reducing sugars. This might be oligonaccharides in the fruits, seeds and roots as the monosaccharide tests were negative.

The Fehling's test for combined reducing sugars was positive for fruits, leaves and roots but negative for seeds and stem. This test indicates the presence of Oligosaccharides and Polysaccharides which give positive result to this test after hydrolysis. This test is also applicable to glycosides whereby he hydrolysed glycone from the aglycone gives a positive reaction.

Ketoses and pentoses were found to be present in the leaves.

3.3.2: **Test for Tannins**

Aqueous extracts of different parts of the plant responded to different tannin test as follows:-

(a) **Ferric Chloride**

Tannins give blue or green coloured precipitates with ferric chloride solutions (Evans, 1989). The water extracts of the different samples gave green to greenish black colour indicating the presence of condensed tannins.

(b) **Lead Sub-acetate Test**

The water extracts of the different samples gave brown precipitate of different shade indicating the presence of tannins. Tannins are precipitated by heavy metals, in this case precipitated by lead.

© **Ferric Ammonium Citrate Test**

The water extract of each of the different samples gave precipitate with this reagent. This test also indicated presence of tannins which are precipitated by the heavy metal iron.

(d) **Bromine Water Test**

The water extract of each of the different samples gave precipitate with this test. This indicated the presence of condensed tannins.

(e) **Formaldehyde Test**

No precipitate was obtained with any of the different samples with this reagent indicating the absence of hydrolyzable tannins.

(f) **Test for Chlorogenic Acid**

None of the different sample of *C. edulis* gave a positive test (a green colour). This indicated the absence of chlorogenic acid.

(g) **Lime - Water Test**

Precipitate was observed in each of the deferent water extracts of the five parts of *C. edulis*. This indicated the presence of psuedotannins.

From these results it might be concluded that *C. edulis* contains condensed tannins and psuedotannins.

The precipitating quality of tannins is used in the laboratories to detect gelatin, proteins and alkaloids (Tyler, 1981).

Therapeutically tannins have antiseptic properties. (Tyler, 1981). Tannins are affective against worms (Evans 1989). This justifies its used as anthelmintic.

3.3.3 Test for Glycosides

(a) **Test for Cyanogenic Glycosides:-**

A faint red colour on picrate paper was observed only with the leaves sample. This indicated the presence of cyanogenic glycosides. The picrate paper was decolourised

from its yellow colour by the roots, stem, fruits and seeds samples indicating the absence of cyanogenic glycosides in these parts of *C. edulis* plant.

(b) **Test for Anthraquinone Derivatives (Bontrager's Tests).**

No bright pink colour was observed before or after hydrolysis of the different samples. This indicated the absence of anthraquinones.

(c) **Test for Saponins**

I. Froth Test

Froth was observed which lasted for more than 30 mins with the leaves and fruits samples. This indicated the presence of saponin in the leaves and fruit samples. Negative results were obtained with the roots, stem and seeds samples (the Froth lasted less than 15 secs).

II. Haemolytic Test

Haemolytic test for saponins gave precipitates with the root, leaves, fruits and seeds while the stem did not, indicating the presence of saponins in the different parts of the plant except the stem.

But, the Froth test was positive for leaves and fruits only. This confirms the presence of saponins in these parts of the plant.

(d) **Test for Cardiac Glycosides.**

Postive results were obtained for the different parts of the plant with Keller - Killiani, Kedde and Legal tests. These indicated the presence of cardiac glycosides in each of the different parts of *C. edulis*

3.3.4: **Test for Terpenes and Sterols**

A reddish or reddish violet ring and green upper solution was obtained for the Liebermann - Buchnard Test. This indicated the presence of terpenes in all the five samples. A positive result (a reddish brown or brownish ring) was obtained with salkowski's test indicating the presence of sterols in the different samples of *C. edulis*. The colour intensity and heights of the interfacial rings varied with the different samples indicating different amounts present in same quantity of samples taken. In the Lieberman Buchard's test, the leaves, fruits and seeds had more terpenes than that of the roots while the stem had the least, In the Salkowki's test the stem showed less amount of sterols companred to the other parts of the plant (Table 7). The presence of cardiac glycosides which are steroidal or saponins which are terpenoidal or steroidal are further confirmed.

3.3.5: **Test for Flavonoids**

The result of various tests carried out for this

class of compounds were as follows:-

(a) **Lead Acetate Test**

The five different parts of the plant gave precipitate to this test, thus indicating presence of flavonoids.

(b) **Sodium Hydroxide Test**

A yellow colour indicated the presence of flavonoids. This was observed with fruits, seeds, root and leaves while stem gave negative result. It has been reported that flavonoids dissolve in alkalis giving yellow solution which on the addition of acid become colourless (Trease and Evans, 1989).

The flavonoids dissolved in alkali gave yellow solution which on the addition of acid became colourless.

(c) **Ferric Chloride Test**

A greenish colour was obtained with all the samples, indicating presence of phenolic nucleus. This test is applicable to both tests for tannins and flavonoids.

(d) **Shinoda Test**

Fruits, seeds and leaves gave positive test (a red colour) which confirmed the presence of flavonoids.

(e) Amyl Alcohol Test

Amyl alcohol was used to improve the extraction of flavonoids so as to aid their identification. The root which did not give positive result with sodium hydroxide reagents or Shinoda test gave positive result (yellow colour). This conforms with the literature.

Conclusively, fruits, seeds, leaves and roots can be said to contain flavonoids. The stem does not contain flavonoids but probably other phenolic compounds such as tannins.

3.3.6: **Test for Resins and Balsams**

Negative results were obtained for these classes of compound. Thus *C. edulis* does not contain resins and balsams.

3.3.7: **Test for Alkaloids**

Various tests for alkaloids were carried out but all gave negative results, no precipitates were obtained.

From the literature review alkaloids have not been reported in the genus Carissa but indole alkaloids are said to be present in the family Apocynaceae (Ting, 1986).

The results of all the phytochemical tests are given in Table 5 and 6.

TABLE 5 PHYTOCHEMICAL TESTS OF ROOTS, STEM, LEAVES, FRUITS AND SEEDS OF C. EDULIS

CHEMICAL TESTS	Roots	Stem	Leaves	Fruits	Seeds
Carbohydrate Test					
- Molisch's test	+ve	+ve	+ve	+ve	+ve
- Barfoed's test	-ve	-ve	+ve	-ve	-ve
- Fehling's test for reducing sugars	+ve	-ve	+ve	+ve	-ve
- Fehling's test for combined reducing sugars	+ve	-ve	+ve	+ve	-ve
- Ketose test	-ve	-ve	+ve	-ve	-ve
- Pentose test	-ve	-ve	+ve	-ve	-ve
Tannin Test					
- Pd subacetate	+ve	+ve	+ve	+ve	+ve
- $FeCl_3$	+ve	+ve	+ve	+ve	+ve
- Formaldehyde/HCl	-ve	-ve	-ve	-ve	-ve
- Bromine H_2O	+ve	+ve	+ve	+ve	+ve
- Chlorogenic acid	-ve	-ve	-ve	-ve	-ve
- Ferric Ammonium Citrate	+ve	+ve	+ve	+ve	-ve
- Lime water	+ve	+ve	+ve	+ve	+ve
- Phlobaphenes	-ve	-ve	-ve	-ve	-ve
Glycosides					
1. Sodium Picrate	-ve	-ve	+ve	-ve	-ve
2. Bontrager's	-ve	-ve	-ve	-ve	-ve
- Free	-ve	-ve	-ve	-ve	-ve
- Combined	-ve	-ve	-ve	-ve	-ve
- C-glycoside	-ve	-ve	+ve	+ve	+ve
3. Froth	+ve	+ve	+ve	+ve	+ve
4. Haemolytic	+ve	-ve	+ve	+ve	+ve
5. Keller Killiani	+ve	+ve	+ve	+ve	+ve
6. Kedde	+ve	+ve	+ve	+ve	+ve
7. Legal					
Terpenes/Sterols					
- Labermann-Buchards test	+ve	+ve	+ve	+ve	+ve
- Salkowski's test	+ve	+ve	+ve	+ve	+ve

TABLE 5 CONTD.

Chemical Tests	Roots	Stem	Leaves	Fruits	Seeds
Flavonoids					
- Pb acetate	+ve	+ve	+ve	+ve	+ve
- NaOH (20%)	+ve	-ve	-ve	+ve	+ve
- Shinoda test	-ve	-ve	+ve	+ve	+ve
- Amyl alcohol					
Free state	+ve	-ve	-ve	-ve	-ve
Combined State	-ve	-ve	-ve	-ve	-ve
Resins/Balsams					
- Cu-acetate	-ve	-ve	-ve	-ve	-ve
- Acetic anhydride and conc H_2SO_4	-ve	-ve	-ve	-ve	-ve
- Alcoholic $FeCl_3$	-ve	-ve	-ve	-ve	-ve
Alkaloids					
- Mayer's	-ve	-ve	-ve	-ve	-ve
- Dragendorff's	-ve	-ve	-ve	-ve	-ve
- Hager's	-ve	-ve	-ve	-ve	-ve
- Wagner's	-ve	-ve	-ve	-ve	-ve
- Tannic acid (10%)	-ve	-ve	-ve	-ve	-ve

TABLE 6: PHYTOCHEMICAL CONSTITUENTS OF THE ROOTS, STEM, LEAVES, FRUITS AND SEEDS OF *CARISSA EDULIS*

Constituents Groups	Roots	Stem	Leaves	Fruits	Seeds
Carbohydrates	+	+	+	+	+
- Monosaccharides	–	–	+	–	–
- Free reducing sugars	+	–	+	+	+
- Combined reucing sugars	+	–	+	+	–
- Ketoses	–	–	+	–	–
- Pentoses	–	–	+	–	–
Tannins	+	+	+	+	+
- Condensed	+	+	+	+	+
- Hydrolyzable	–	–	–	–	–
- Psuedotannins	+	+	+	+	+
Glycosides					
- Cyanogenic	–	–	+	–	–
- Anthraquinone	–	–	–	–	–
- Snponins	–	–	++	+	–
- Cardiac	+	+	+	+	+
Terpenes	++	+	+++	+++	+++
Sterols	++	+	++	++	++
Flavonoids	+	–	+	+	+
Resins/Balsams	–	–	–	–	–

3.4 QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS (POWDERED ROOTS, STEM, LEAVES, FRUITS AND SEEDS)

The following evaluative parameters (Table 7) were determined on the powdered roots and stem barks, leaves, fruits and seeds. All the values represent average of three separate determinations (Appendix 1).

TABLE 7: QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS

Evaluation Parameters	Roots	Stem	Leaves	Fruits	Seeds
Moisture Content (%w/w)	8.0	10.4	12.6	6.7	9.3
Ash Value (%w/w)	13.5	15.0	8.5	3.7	7.5
Acid insoluble ash value (%w/w)	6.0	5.7	2.0	1.2	3.2
Alcohol Soluble extractive value (%w/w)	13.3	4.0	20.7	28.3	27.3
Water Soluble extractive value (%w/w)	12.0	10.0	17.0	25.0	24.3

Determination of Moisture Content

The moisture content of the leaves was the highest followed by stem, seeds, roots then the fruits, which are fairly low.

The moisture content of some drugs from the literature is as follows:- Digitalis leaf 6.0%, Acacia 15% w/w (B.P., 1980). It can be seen that the moisture content of *C. edulis* is comparable with these values, therefore, can be said to be within acceptable standards

Determination of Ash Value

The root and stem were found to have high values of total ash. This was followed by the leaves, seeds and the fruits respectively. One would expect the root to have the highest value instead of the stem, considering it is an underground organ.

The total ash value of some official drugs include: Cardamon seeds 6.0% and wild cherry bark 4.0% From this it can be seen that the ash value is variable and diagnostic.

Determination of Acid Insoluble Ash Value

The acid insoluble ash is a method intended to measure the amount of silica, especially sand and siliceous earth, present in the drug (African Pharmacopoeia, 1986). In the acid insoluble ash determination it is the `physiological ash' that gets dissolved in the dilute acid (Shellard, 1958).

From the results the extraneous matter taken from the soil is highest in the root. But the total ash of the stem was higher than that of the root indicating that the `physiological ash' is higher in the stem.

Some reported official acid insoluble ash values include: cardamon seeds 3.5%, senna leaves 2%, Alexandrian Senna fruit (Pods) 6%, belladonna leaf or herb 4%, caraway fruits 1.5%, coriander fruit 1.5% and digitalis leaf 5.0%. The result from *C. edulis* can also serve as a differentiating and identifying characteristic.

Determination of Extractives

From the results the alcohol extractive values was more than that of the water extractive values. Also the values of the fruits was more than that of the seeds followed by the leaves, roots and stem respectively. It can be said that the biological activity of the fruit is expected to be the highest. It can also be said that the use of the leaves and roots by the traditional healers is justified; the stem having low extractive values and the fruits being seasonal.

The water extractive value of wild cherry bark is not more than 10% w/w (B.P., 1980). This is comparable with the barks of *C. edulis*, 12% w/w and 10%w/w for the root bark and stem bark respectively. For the stem of *C. edulis* the water extractive value alone cannot be used for it's identification as this value is the same with that of wild cherry bark.

3.5 CHROMATOGRAPHIC ANALYSIS

This was carried out to find out if salicylates are actually present since the root had a smell of methylsalicylates and phenolic compounds were found to be present in all parts of the plant.

Out of the various solvent systems used, systems II (Silica gel G and Ethylacetate-n-butanol-acetic acid-water) was the most effective in seperating the mixtures.

Chromatography of the unhydrolised and hydrolised water extracts of the fruits showed the presence of both salicylic acid and methyl salicylate in the sample (Table 8 and 9). The fruit

sample was used because it gave the highest analgesic activity. The colour of the spots obtained were violet and purple the spot for methyl salicylate was a compact spot while that of salicylic acid was tailing (characteristic spot of organic acids). The water extracts did not show clearly separated spots but various spots could be identified. The spots were observed from the origin to the solvent front. There were more violet or purple spots other than that corresponding to the salicylic acid and methyl salicylate.

TABLE 8 PAPER CHROMATOGRAPHY RESULTS:

SAMPLES	hR_f (%)	COLOUR
Unhydrolised water extract of fruit	98	Purple
	95.1	Violet
Hydrolised water extract of fruit	95	Violet
Standard salicylic acid	94	Violet
Standard Methyl salicylate	97.1	Purple

TABLE 9: THIN LAYER CHROMATOGRAPHY RESULTS

	SAMPLES	hR_f (%)
(a)	Unhydrolised water extract of fruit	50%, 73.3, 95
	Standard Methyl Salicylate	95
(b)	Hydrolised water extract of fruit	72, 56
	Standard Methyl Salicylate	96
C	Unhydrolised water extract of fruit Standard salicylic acid Standard methyl salicylate	52.8, 73.6, 86, 94.3 94.3 88.7

Colour of spots all violet

These results confirm the presence of salicylates in the plant *C. edulis*. Both free salicylic acid and the methylated derivative methyl salicylates have been indicated. This adds to the reported salicylate derivate 2-hydroxy acetophenone in the root (Bentley *et al*, 1984)

No spots were observed with the other systems but with Watman No. 1 and similar developing system V. Generally it was easier to obtain result with silica gel than with the paper.

3.6 ANALGESIC ACTIVITY

The water extracts were used as this was the form usually employed by the traditional medical practitioners and patients.

The three different doses of the water extracts used (50mg, 100mg and 150mg per kg) showed analgesic activity which was dose related (Figs 17a to 17g). Significant difference P taking $P<0.05$ as significant. $P<0.05$ was obtained for the roots 50mg/kg at 30min after injection and $P<0.001$ for seeds 50mg/kg and fruits 100mg/kg at 45 minutes after injection of the plant extracts (Appendix IV, table II)

The fruit sample was found to be the most active, followed by the leaves, seeds, roots and stem respectively (Fig.17f) Thee analgesic activity of the fruits sample (50mg, 100mg and 150mg per kg) was found to be mere than that of 30mg/kg metamizol (Fig.17g).

The results of the analgesic activity are given in Fig. 16,17; Appendix II, III, IV

Fig 16 Test for Analgesic Activity of 10, 50 mg/kg root and stem water extracts with 10 mg/kg metamizol (Tail Clip Method)

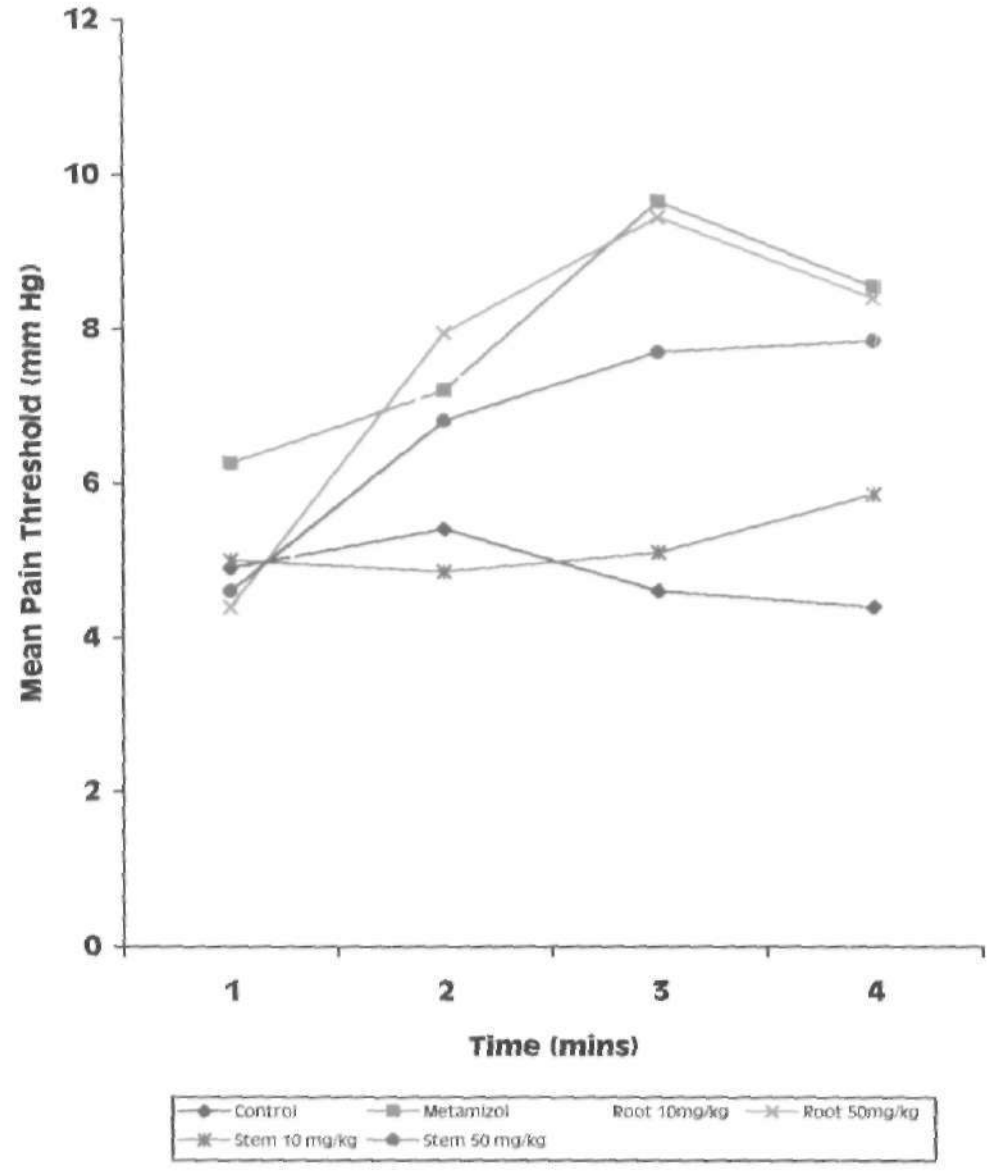


Fig 17a Analgesic Activity of 50 mg/kg root water extract and 10, 20, 30, mg/kg metamizol

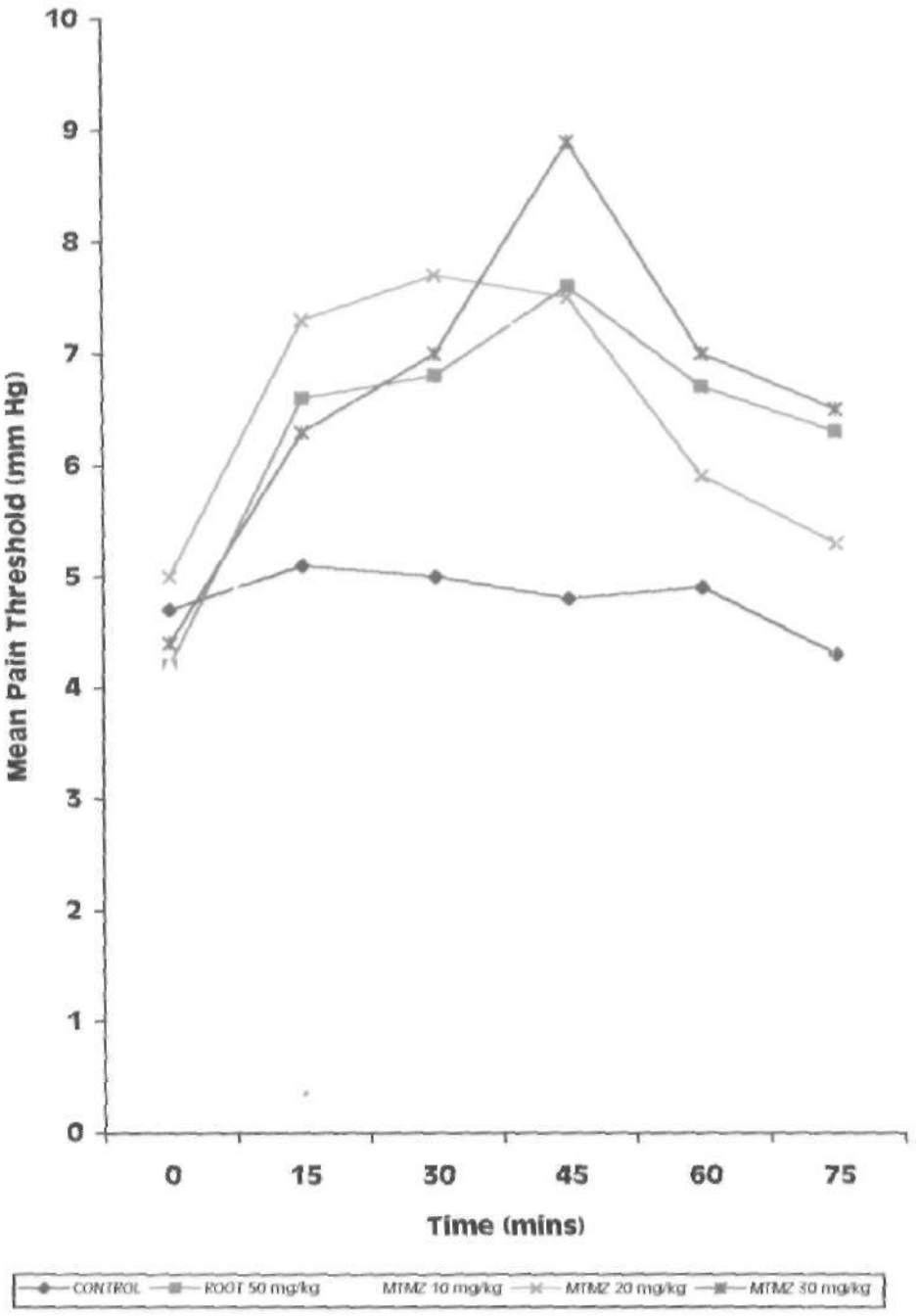


Fig 17b Analgesic Activity of 50 mg/kg stem water extract and 10, 20, 30 mg/kg metamizol

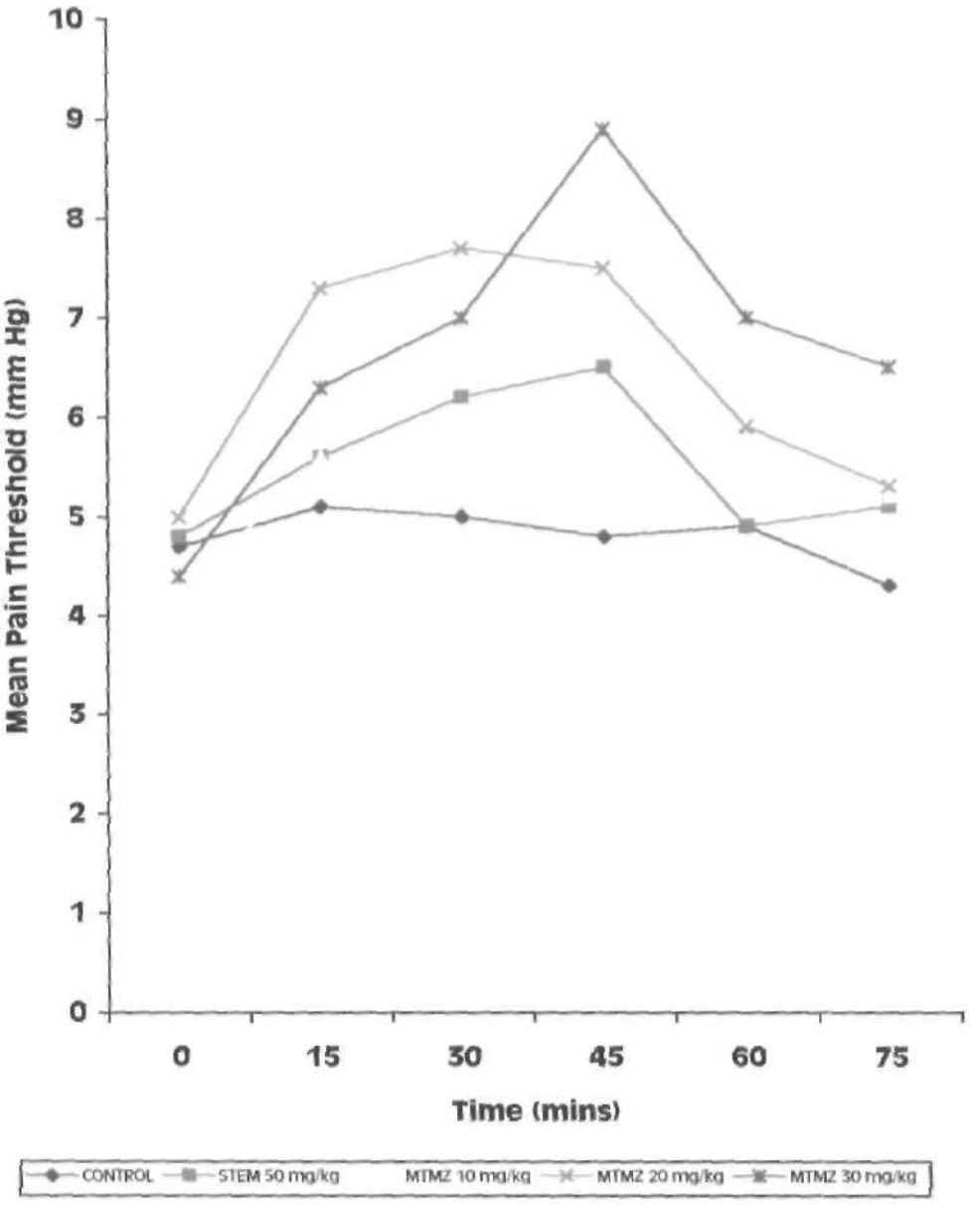


Fig 17c Analgesic Activity of 50 mg/kg leaves water extract and 10, 20, 30 mg/kg metamizol

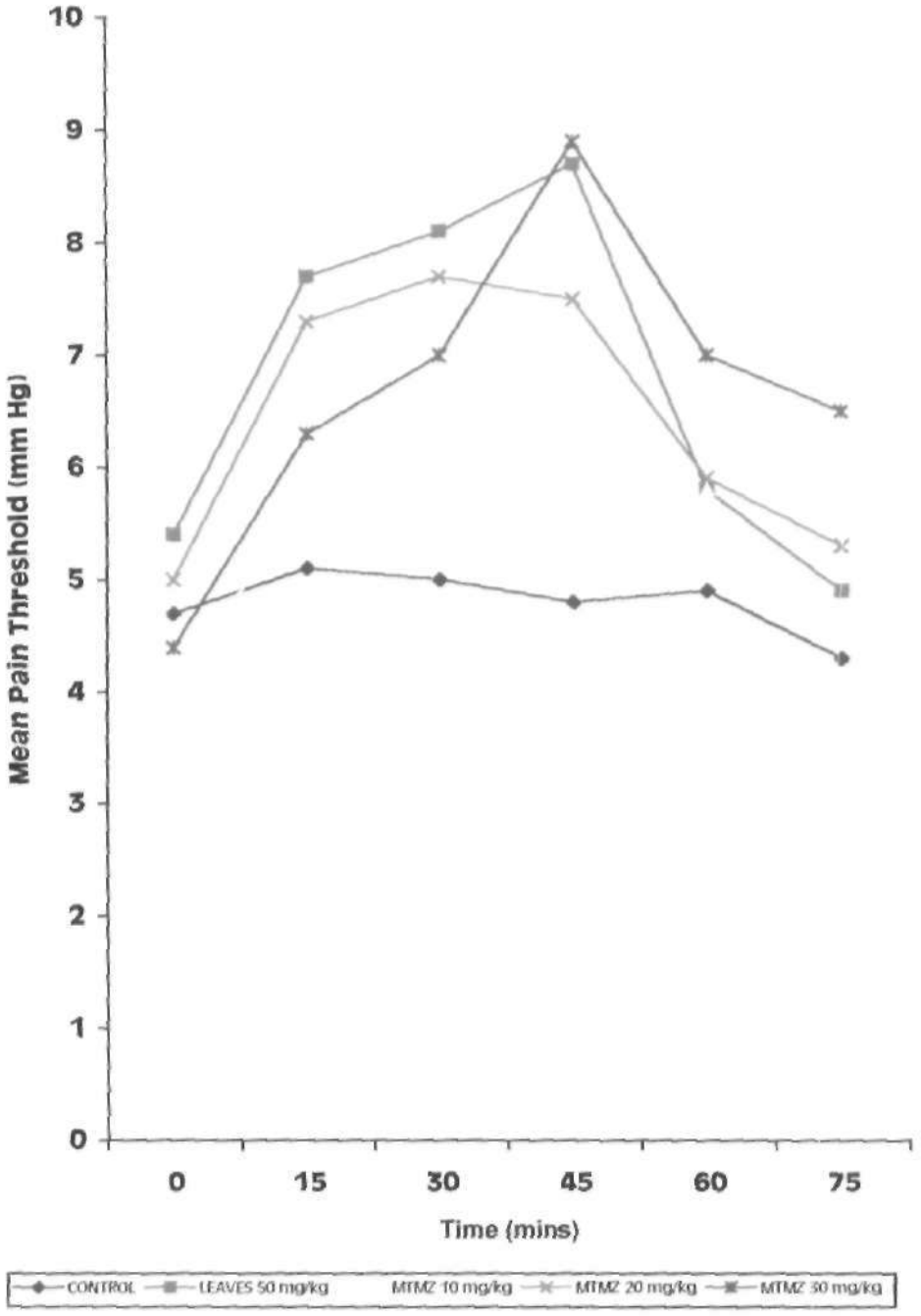


Fig 17d Analgesic Activity of 50 mg/kg fruit water extract and 10,20,30 mg/kg metamizol

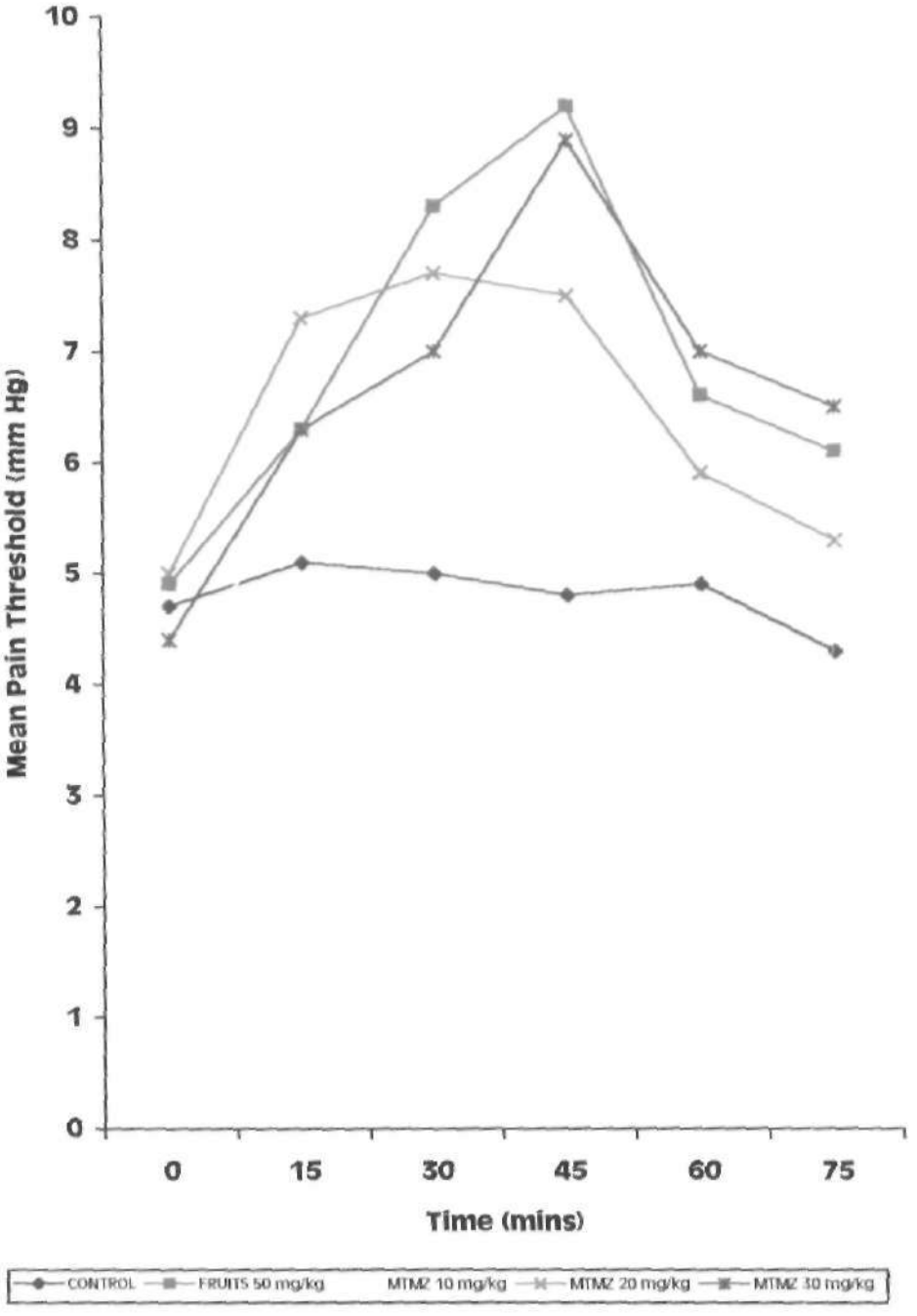


Fig 17e Analgesic Activity of 50 mg/kg seed water extract and 10, 20, 30 mg/kg metamizol

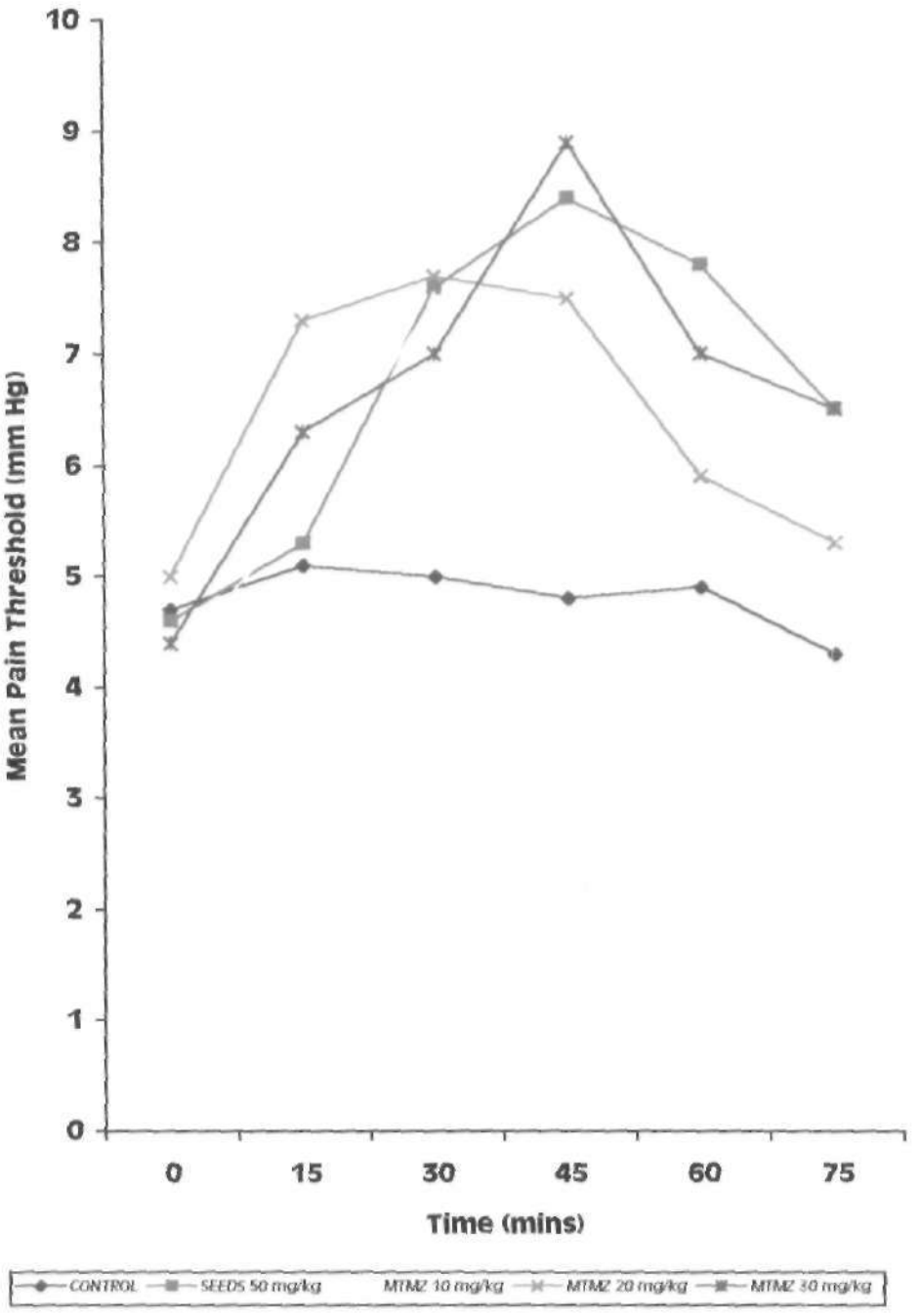


Fig 17f Analgesic Activity of 50 mg/kg water extract of roots, stem, leaves, fruits and seeds

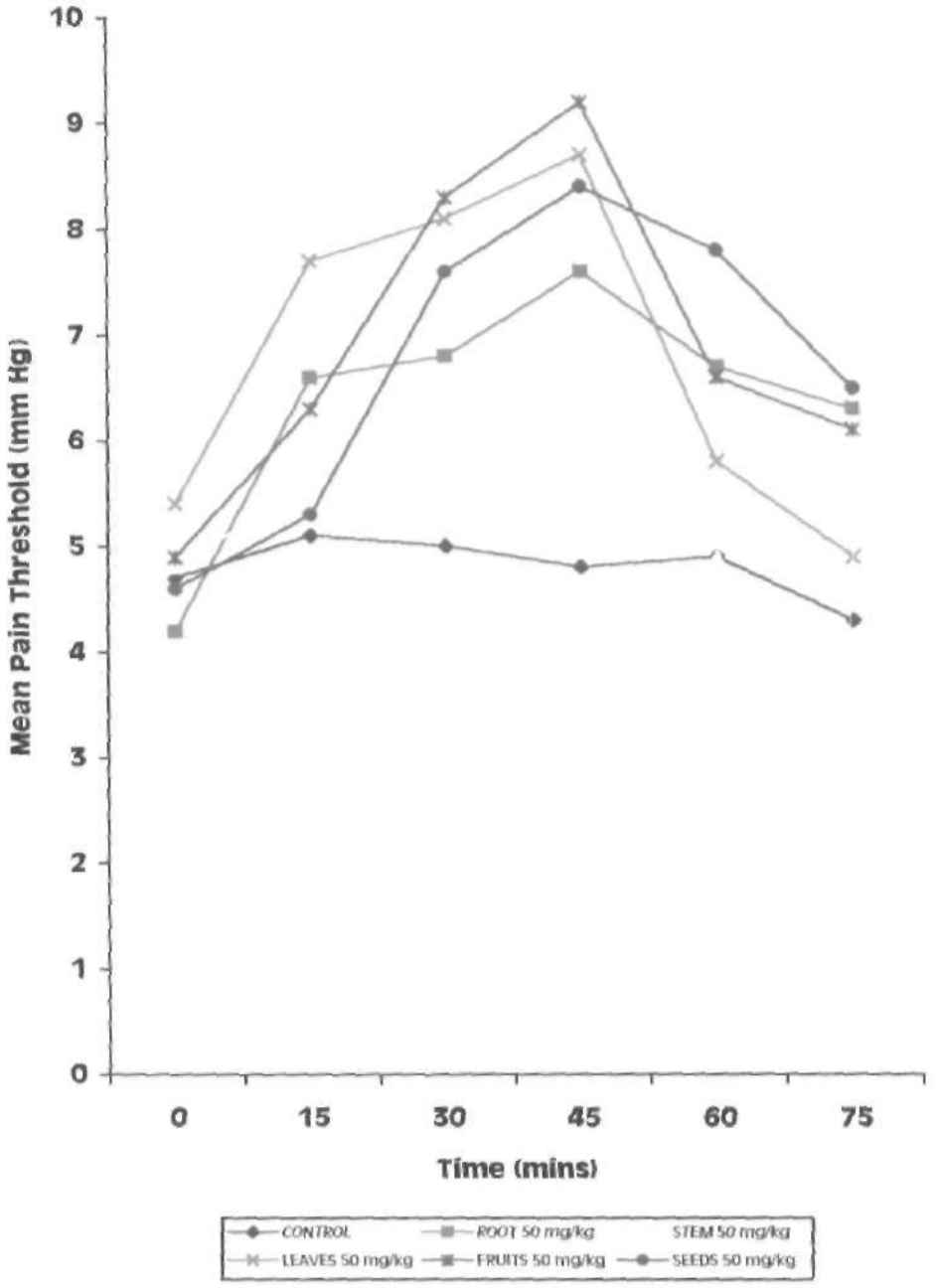
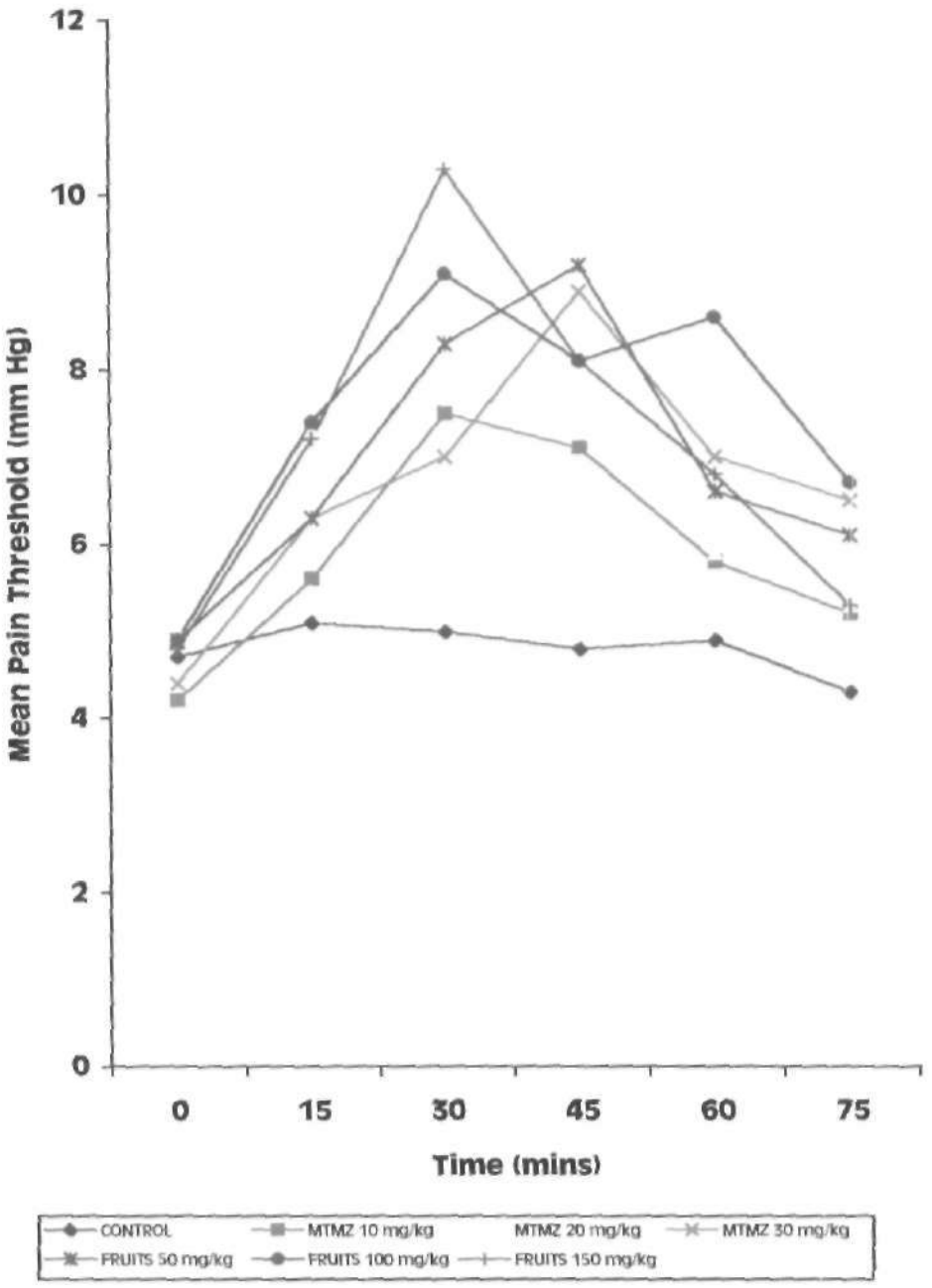


Fig 17g Analgesic Activity of 50, 100, 150 mg/kg fruit water extract and 10, 20, 30 mg/kg metamizol



CONCLUSION

The pharmacognostic studies and analgesic activity studies of the plant *C. edulis* have been carried out for the first time.

The macroscopical and microscopical studies showed some characteristic of the family Apocynacea as regards laticifers but without interxylary phloem or a bicolateral vascular bundle. The leaves showed a peculiar group of parenchyma cells at the upper epidermis which could be used as a differentiating character from other plants (A group of parenchyma cells formed a circle with the upper epidermal cells between the elongated palisade cells)

The phytochemical constituents of the plant include carbohydrates, tannins, flavanoids, sapanins and cardiac glycosides. Alkaloids, anthraqiunones, resins and balsams were however absent.

Analgesic studies showed the plant to possess analgesic activity. Chromatographic analysis showed the presence of salicylate which might be responsible for this activity.

Further work can be carried out on the plant such as isolation and identification of the different constituents by chromatographic and spectroscopic means. Microbiological and anti-inflammatory studies can also be carried out to confirm the claim of the traditional medical practioners.

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APPENDIX I

QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS

A. POWDERED ROOT BARK

(a) Determination of Moistre Content

3g of the Powdered Root Back was used.

		1	2	3
1	Constant weight of crucible(g)	28.64	28.44	25.50
2	Weight of crucible and content before heating (g)	31.64	31.44	28.50
3	Weight of crucible and content after heating (g)	31.42	31.26	28.30
		31.40	31.19	28.27
		31.40	31.19	28.27
4	Moisture content (1% w/w)	8.00	8.33	7.67

5 Average $= \frac{8 + 8.33 + 7.67}{3}$

$= 8\%$ w/w

6 Example of calculations

Moisture content $= \frac{\text{Step 2 - Step 3}}{\text{initial weight of drug}} \times 100$

$= \frac{31.64 - 31.40}{3} \times 100$

$= 8.00\%$ w/w

(b) Determination of Ash Value
2g of the Powdered Root Bark was used

		1	2	3
1	Constant weight of crucible(g)	24.63	26.50	24.56
2	Weight of crucible and content (g)	26.63	28.50	26.56
3	Weight of crucible and ash(g)	24.90	26.78	24.78
		24.90	26.76	24.78
			26.76	
4	Weight of ash(g)	0.27	0.26	0.28
5	Ash Value (1% w/w)	13.5	13.0	14.0

6 Average (Mean) $= \frac{13.5 + 13 + 14}{3}$

$=$ 13.5% w/w

Example of Calculations

Weight of ash $=$ Step 3 - Step 1

$=$ 24.90 - 24.63

$=$ 0.27g

Ash value $= \frac{\text{weight of ash}}{\text{initial weight of drug}} \times 100$

$= \frac{0.27}{2} \times 100$

$=$ 13.5% w/w

(c) Determination of Acid Insoluble Ash (Continuation of b)

		1	2	3
1	Constant weight of crucible(g)	24.58	24.53	30.25
2	Weight of crucible and ash (g) after acid treatment	24.70	24.68	30.37
		24.70	24.65	30.37
			24.65	
3	Weight of ash(g) after acid treatment	0.12	0.12	0.12
4	Acid insoluble sh value (% w/w)	6.00	6.00	6.00

5 Average (Mean) $= \frac{6 + 6 + 6}{3}$

$= 6\%$

6 Example of calculations

Weight of ash = Step 2 - Step 1
= 24.70 - 24.58
= 0.12g.

Acid insoluble ash value = $\frac{\text{weight of ash}}{\text{weight of drug}} \times 100$

$= \frac{0.12}{2} \times 100$

$= 6.0\%$ w/w

d. Determination of Extractive Values

(i) Alcohol - Soluble Extractive Value

5g. of the powdered root bark was used in 100ml of 90% ethanol

		1	2	3
1	Constant weight of crucible(g)	51.10	30.74	40.27
2	Weight of crucible and content after heating (g)			
		51.26	30.92	40.7
		51.23	30.88	40.4
		51.23	30.88	40.4
3	Alcohol soluble extractive value (% w/w)	13.00	14.00	13.00

4 Average (Mean) $= \frac{13 + 14 + 13}{3}$

$= 13.3\%$ w/w

5 Example of calculations

Alcohol soluble extractive value $= \frac{\text{Step 2 - Step 1}}{\text{initial weight of drug}} \times 5 \times 100$

$= \frac{51.23 - 51.10}{5} \times 5 \times 100$

$= 13\%$ w/w

(ii) Water - Soluble Extractive Value

5g of the powdered root bark was used in 100ml of 0.25% chloroform water

		1	2	3
1	Constant weight of crucible(g)	25.92	30.32	26.05
2	Weight of crucible and content after heating (g)			
		26.05	30.48	26.21
		26.04	30.44	26.17
		26.04	30.44	26.17
3	Water soluble extractive value (% w/w)	12.00	12.00	12.00

4 Average (Mean) $= \frac{12 + 12 + 12}{3}$

$= 12\%$ w/w

5 Example of calculations

Water soluble extractive value $= \frac{\text{Step 2 - Step 1}}{\text{Initial weight of drug}} \times 5 \times 100$

$= \frac{26.04 - 25.92}{5} \times 5 \times 100$

$= 12\%\ {}^{w}/_{w}$

B. **POWDERED STEM BARK**

(a) Determination of Moisture Content
3g of the powdered stem bark was used.

		1	2	3
1	Constant weight of crucible(g)	28.63	56.39	43.17
2	Weight of crucible and content before heating (g)	31.63	59.39	46.17
3	Weight of crucible and content after heating (g)	31.35	59.15	45.90
		31.32	59.06	45.87
		31.32	59.06	45.87
4	Moisture content (% w/w)	10.33	11.00	10.00

5. Average (Mean) $= \frac{10.33 + 11 + 10}{3}$

$= 10.4\%$ w/w

(b) Determination of Ash Value
2g of powdered stem bark was used

		1	2	3
1	Constant weight of crucible(g)	24.50	26.10	25.80
2	Weight of crucible and content (g)	26.50	28.10	27.80
3	Weight of crucible and content ash(g)	24.80	26.40	26.10
		24.80	26.40	26.10
4	Weight of ash(g)	0.30	0.30	0.30
5	Ash value (% w/w)	15	15	15

6. Average (Mean) $= \frac{15 + 15 + 15}{3}$

$= 15\%$ w/w

(c) Determination of Acid Insoluble Ash (Continuation of b)

		1	2	3
1	Constant weight of crucible(g)	24.53	26.50	24.52
2	Weight of crucible and ash(g) after acid treatment	24.63	26.65	24.64
		24.63	26.62	24.64
			26.62	
3	Weight of ash(g) after acid treatment	0.10	0.12	0.12
4	Acid insoluble ash value (% w/w)	5	6	6

5. Average (Mean) $= \frac{5 + 6 + 6}{3}$

$= 5.67\%$ w/w

$\approx 5.7\%$ w/w

d. *Determination of Extractive Values*

(i) Alcohol - Soluble Extractive Value

5g of the powdered stem bark was used in 100ml of 90% ethanol.

		1	2	3
1	Constant weight of crucible(g)	28.69	29.08	30.28
2	Weight of crucible and content after heating (g)	28.73	29.12	30.32
3	Alcohol soluble extractive value (% w/w)	4.0	4.0	4.0

4. Average (Mean) $= \frac{4 + 4 + 4}{3}$

$= 4\%$ w/w

(ii) Water - Soluble Extractive Value

5g of the powdered stem bark was used in 100ml of 0.25% chloroform water.

		1	2	3
1	Constant weight of crucible(g)	56.39	44.91	30.81
2	Weight of crucible and content after heating (g)	56.49	45.01	30.91
3	Water soluble extractive value (% w/w)	10.0	10.0	10.0

4. Average (Mean) $= \frac{10 + 10 + 10}{3}$

$= 10\%$ w/w

(C) **POWDERED LEAVES**

(a) *Determination of Moisture Content*

3g of the powdered leaves was used.

		1	2	3
1	Constant weight of crucible(g)	24.39	26.32	24.50
2	Weight of crucible and content before heating(g)	27.39	29.32	27.50
3	Weight of crucible and content after heating (g)	27.04	28.97	27.95
		27.01	28.94	27.87
		27.01	28.94	27.87
4	Moisture content (% w/w)	12.67	12.67	12.33

5 Average (Mean) $= \frac{12.67 + 12.67 + 12.33}{3}$

$= 12.56\%$ w/w

$\approx 12.6\%$ w/w

(b) Determination of Ash Value
2g of the powdered leaves was used

		1	2	3
1	Constant weight of crucible(g)	24.53	27.50	38.00
2	Weight of crucible and content (g)	26.53	29.50	40.00
3	Weight of crucible and ash(g)	24.82	27.69	38.25
		24.70	27.67	38.17
		24.70	27.67	38.17
4	Weight of ash (g)	0.17	0.17	0.17
5	Ash vale (% w/w)	8.5	8.5	8.5

6. Average (Mean) $= \frac{8.5 + 8.5 + 8.5}{3}$

$= 8.5\%$ w/w

(c) Determination of Acid Insoluble Ash (continuation of b)

		1	2	3
1	Constant weight of crucible(g)	24.52	27.50	38.00
2	Weight of crucible and ash(g) after acid treatment	24.58	27.46	37.96
		24.56	27.46	37.96
		24.56	27.46	
3	Weight of ash (g) after acid treatment	0.04	0.04	0.04
4	Acid insoluble ash value (% w/w)	2.0	2.0	2.0

5 Average (Mean) $= \frac{2.0 + 2.0 + 2.0}{3}$

$= 2.0\%$ w/w

d. Determination of Extractive Values:

i) Alcohol - Soluble Extractive Value
5g of the powdered leaves was used in 100ml of 90% ethanol.

		1	2	3
1	Constant weight of crucible(g)	24.39	56.39	24.40
2	Weight of crucible and content after heating (g)	24.59	56.60	24.61
3	Alcohol Soluble Extractive value (% w/w)	20.0	21.0	21.0

4 Average (Mean) $= \frac{20 + 21 + 21}{3}$

$= 20.67\%$ w/w

$\approx 20.7\%$ w/w

(ii) Water - Soluble Extractive Value
5g of the powdered leaves was used in 100ml of 0.25% chloroform water.

		1	2	3
1	Constant weight of crucible(g)	51.10	27.50	38.00
2	Weight of crucible and content after heating (g)	51.27	27.68	38.16
3	Water soluble extractive	17	18	16

4 Average (Mean) $= \frac{17 + 18 + 16}{3}$

$= 17\%$ w/w

D **POWDERED FRUITS**

(a) Determination of Moisture Content.
3g of the powdered fruits was used

		1	2	3
1	Constant weight of crucible(g)	24.39	24.48	28.63
2	Weight of crucible and content before heating (g)	27.39	27.48	31.63
3	Weight of crucible and content after heating (g)	27.18	27.32	31.45
		27.18	27.29	31.43
			27.29	31.43
4	Moisture content (% w/w)	7.0	6.33	6.66

5 Average (Mean) $= \frac{7.0 + 6.33 + 6.66}{3}$

$= 6.65\%$ w/w

$\approx 6.7\%$ w/w

(b) Determination of Ash Value
2g of the powdered fruits was used

		1	2	3
1	Constant weight of crucible(g)	24.71	24.56	28.65
2	Weight of crucible and content (g)	26.71	26.56	30.65
3	Weight of crucible and ash (g)	24.79	24.64	28.71
		24.79	24.64	28.71
4	Weight of ash (g)	0.08	0.08	0.06
5	Ash Value (% w/w)	4.0	4.0	3.0

6 Average (Mean) $= \frac{4.0 + 4.0 + 3.0}{3}$

$= 3.67\%$ w/w

$\approx 3.7\%$ w/w

(c) Determination of Acid Insoluble Ash (Continuation of b)

		1	2	3
1	Constant weight of crucible(g)	28.78	24.69	28.85
2	Weight of crucible and ash (g)	28.81	24.71	28.87
		28.81	24.71	28.87
3	Weight of ash (g) after acid treatment	0.03	0.02	0.02
4	Acid insoluble ash value (% w/w)	1.5	1.0	1.0

5 Average (Mean) $= \frac{1.5 + 1.0 + 1.0}{3}$

$= 1.16\%$ w/w

$\approx 1.2\%$ w/w

(d) Determination of Extractive Values

(i) Alcohol - Soluble Extractive Value
5g of the powdered fruits was used in 100ml of 90% ethanol.

		1	2	3
1	Constant weight of crucible(g)	43.17	28.95	24.40
2	Weight of crucible and content after heating (g)	43.45	29.23	24.69
3	Alcohol soluble extractive value (% w/w)	28	28	29

4 Average (Mean) $= \frac{28 + 28 + 29}{3}$

$= 28.33\%$ w/w

≈ 28.3 w/w

(ii) Water - Soluble Extractive Value
5g of the powdered fruits was used in 100ml of 0.25% chloroform water.

		1	2	3
1	Constant weight of crucible(g)	28.70	26.41	24.42
2	Weight of crucible and content after heating (g)	28.95	26.66	24.67
3	Water - soluble extractive value (% w/w)	25	25	25

4 Average (Mean) $= \frac{25 + 25 + 25}{3}$

$= 25\%$ w/w

E. **POWDERED SEEDS**

(a) *Determination of Moisture Content*

3g of powdered seeds was used.

		1	2	3
1	Constant weight of crucible(g)	26.49	24.48	28.63
2	Weight of crucible and content before healing	29.49	27.48	31.63
3	Weight of crucible and content after heating (g)	29.19	27.28	31.40
		29.15	27.24	31.37
		29.15	27.24	31.37
4	Moisture content (% w/w)	11.3	8	8.7

5 Average (Mean) $= \frac{11.3 + 8.0 + 8.7}{3}$

$= 9.3\%$ w/w

(b) Determination of Ash value

2 of the powdered seeds was used

		1	2	3
1	Constant weight of crucible(g)	24.58	28.50	24.60
2	Weight of crucible and content (g)	26.58	30.50	26.60
3	Weight of crucible and ash (g)	24.85	28.66	24.77
		24.73	28.65	24.75
		24.73	28.65	24.75
4	Weight of ash (g)	0.15	0.15	0.15
5	Ash value (% w/w)	7.5	7.5	7.5

6 Average (Mean) $= \frac{7.5 + 7.5 + 7.5}{3}$

$= 7.5\%$ w/w

(c). Determination of Acid Insoluble Ash (Continuation of b)

		1	2	3
1	Constant weight of crucible(g)	24.57	24.40	32.20
2	Weight of crucible and ash (g) after acid treatment	24.63	24.47	32.26
		24.63	24.47	32.26
3	Weight of ash (g) after acid treatment	0.06	0,07	0,06
4	Acid insoluble ash value (% w/w)	3	3.5	3

5 Average (Mean) $= \frac{3 + 3.5 + 3}{3}$

$= 3.17\%$ w/w

$\approx 3.2\%$ w/w

(d) Determination of Extractive Values

i. Alcohol - Soluble Extractive Value
5g of the powdered seeds was used in 100m of 90% ethanol

		1	2	3
1	Constant weight of crucible(g)	43.17	52.30	30.36
2	Weight of crucible and content after heating (g)	43.17	52.58	30.60
		43.17	52.58	30.60
3	Alcohol -soluble extractive value (% w/w)	30	28	24

5 Average (Mean) $= \frac{30 + 28 + 24}{3}$

$= 27.33\%$ w/w

$\approx 27.3\%$ w/w

(ii) Water-Soluble Extractive Value

5g of then powdered seeds was used in 100ml of 0.25% chloroform water.

		1	2	3
1	Constant weight of crucible(g)	30.40	28.70	55.30
2	Weight of crucible and content after heating (g)	30.65	28.96	55.54
		30.65	28.94	55.54
			28.94	
3	Water-soluble extractive value (% w/w)	25	24	24

5 Average (Mean) $= \frac{25 + 24 + 24}{3}$

$= 24.3\%$ w/w

APPENDIX II

TEST FOR ANALGESIC ACTIVITY
W CONTROL (NORMAL SALINE)

		PAIN THRESHOLD (mmHg)			
Wt (g) Time (Min)		0	15	30	45
TAIL CLIP METHOD					
23		4.0	4.3	3.7	3.8
26		5.8	6.5	5.5	5.0
	ΣW	9.8	10.8	9.2	8.8
	$\hat{W}$	4.9	5.4	4.6	4.4
	$\Sigma(W-\hat{W})$	0	0	0	0
	$\Sigma(W-\hat{W})^2$	1.62	2.42	1.62	0.72
	S^2	1.62	2.42	1.62	0.72
	S	1.3	1.6	1.3	0.9

KEY

W	=	individual reading
ΣW	=	Total of in-dividual readings
$\hat{W}$	=	Mean
S^2	=	Variance
S	=	Standard deviation
Wt	=	weight of mice

Each value at a given time is an average of three determination.

X ANALGIN(R) (METAMIZOL) 10MG/KG

		PAIN THRESHOLD (mmHg)			
Wt (g)	Time (Min)	0	15	30	45
25	TAIL CLIP METHOD	5.0	4.7	9.0	7.8
20		7.5	9.7	10.3	9.3
	ΣX	12.5	14.4	19.3	17.0
	$\bar{X}$	6.25	7.2	9.65	8.55
	$\Sigma(X-\bar{X})$	0	0	0	0
	$\Sigma(X-\bar{X})^2$	3.126	12.50	0.846	1.126
	S^2	3.126	12.50	0.846	1.126
	S	1.8	3.5	0.9	1.1

Y_1 ROOT WATER EXTRACT 10MG/KG

Wt (g)	TIME (MIN)	PAIN THRESHOLD (mmHg) 0	15	30	45
	TAIL CLIP METHOD				
22		5.3	9.7	7.3	6.5
16		2.8	5.3	4.3	3.7
	ΣY_1	8.1	15.0	11.6	10.2
	Y_1	4.05	7.5	5.8	5.1
	$\Sigma(Y_1 - Y_1)$	0	0	0	0
	$\Sigma(Y_1 - Y_1)^2$	3.12	9.68	4.50	3.92
	S^2	3.12	9.68	4.50	3.92
	S	1.8	3.1	2.1	2.0

Y_2 ROOT WATER EXTRACT 50MG/KG

Wt (g)	TIME (MIN)	PAIN THRESHOLD (mmHg) 0	15	30	45
	TAIL CLIP METHOD				
23		4.0	8.2	8.2	5.5
24		4.8	7.7	10.7	11.3
	ΣY_2	8.8	15.9	18.9	16.8
	Y_2	4.4	7.95	9.45	8.4
	$\Sigma(Y_2 - Y_2)^2$	0	0	0	0
	$\Sigma(Y_2 - Y_2)^2$	0.32	0.125	3.125	16.82
	S^2	0.32	0.125	3.125	16.82
	S	0.6	0.4	1.8	4.1

Z_1 STEM WATER EXTRACT 10MG/KG

		PAIN THRESHOLD (mmHg)			
Wt (g) TIME (MIN)		**0**	**15**	**30**	**45**
	TAIL CLIP METHOD				
25		4.7	5.7	4.0	6.0
23		5.3	4.0	6.2	5.7
	ΣZ_1	10.0	9.7	10.2	11.7
	Z_1	5	4.85	5.1	5.85
	$\Sigma(Z_1 - Z_1)$	0	0	0	0
	$\Sigma(Z_1 - Z_1)^2$	0.18	1.44	2.42	0.04
	S^2	0.18	1.44	2.42	0.04
	S	0.42	1.2	1.6	0.2

Z_2 STEM WATER EXTRACT 50MG/KG

		PAIN THRESHOLD (mmHg)			
Wt (g) TIME (MIN)	**0**	**15**	**30**	**45**	
	TAIL CLIP METHOD				
21		**4.7**	5.3	6.7	7.0
25		**4.5**	8.3	8.7	8.7
	ΣZ_2	**9.2**	13.6	15.4	15.7
	Z_2	**4.6**	6.8	7.7	7.85
	$\Sigma(Z_2 - Z_2)$	**0**	0	0	0
	$\Sigma(Z_2 - Z_2)^2$	0.02	4.5	2.0	1.44
	S^2	0.02	4.5	2.0	1.44
	S	0.14	2.1	1.4	1.2

TEST FOR ANALGESIC ACTIVITY (CALCULATIONS)

W CONTROL - TAIL CLIP METHOD

Zero Min

Wt.(g) W	W - Ŵ	(W - Ŵ)²
23	4.0	-0.9	0.81
26	5.8	0.9	0.81

$$S^2 = \frac{1}{N-1}\left[\sum_{i=1} (W-W)^2\right]$$

$$= \frac{1}{2-1} \times 1.62$$

$$= 1.62$$

$$\hat{W} = \frac{9.8}{2} = 4.9$$

$$S = S^2$$
$$= 1.273$$
$$\approx 1.3$$

15 Minutes

Wt.(g) W	W - Ŵ	(W - Ŵ)²
23	4.3	-1.1	1.21
26	6.5	1.1	1.21
	10.8	0	2.42

$S^2 = 2.42$

$S = 1.556$

≈ 1.6

$$\hat{W} = \frac{10.8}{2} = 5.4$$

30 Minutes

Wt.(g) W	W - Ŵ	(W - Ŵ)²
23	3.7	-0.9	0.81
26	5.5	0.9	0.81
	9.2	0	1.62

$S^2 = 1.62$

$S = 1.3$

$$\hat{W} = \frac{9.2}{2} = 4.6$$

45 Minutes

Wt.(g) W	W - Ŵ	(W - Ŵ)²
23	3.8	-0.6	0.36
26	5.0	0.6	0.36
	8.8	0	0.72

$S^2 = 0.72$

$S = 0.849$

$$\hat{W} = \frac{8.8}{2} = 4.4$$

X **METAMIZOL 10MG/KG - TAIL CLIP METHOD**

Zero Min.

Wt.(g)	X	X - X	$(X - X)^2$				
23		5.0	-1.25	1.563	S^2	=	3.126
20		7.5	1.25	1.563	S	=	1.768
		12.5	0	3.126		≈	1.8

$$X = \frac{12.5}{2} = 6.25$$

15 Mins.

Wt.(g)	X	X - X	$(X - X)^2$				
25		4.7	-2.5	6.25	S^2	=	12.5
20		9.7	2.5	6.25	S	=	3.536
		14.4	0	12.50		≈	3.5

$$X = \frac{14.4}{2} = 7.2$$

30 Mins

Wt(g)	X	X - X	$(X - X)^2$				
25		9.0	-0.65	0.423	S^2	=	0.846
20		10.3	0.65	0.423	S	=	0.92
		19.3	0.00	0.846		≈	0.9

$$X = \frac{19.3}{2} = 9.65$$

45 Mins

Wt(g)	X	X - X	$(X - X)^2$				
25		7.8	-0.75	0.563	S^2	=	1.126
20		9.3	0.75	0.563	S	=	1.061
		17.1	0	1.126		≈	1.1

$$X = \frac{17.1}{2} = 8.55$$

Y_1 ROOT WATER EXTRACT 10MG/KG - TAIL CLIP METHOD

Zero Mins

	Y	$Y_1 - Y_1$	$(Y'_1 - Y'_1)^2$			
22	5.3	1.25	1.56	S^2	=	3.12
16	2.8	-1.25	1.56	S	=	1.77
	8.1	0	3.12		≈	1.8

$Y_1 = \frac{8.1}{2} = 4.05$

15 Mins

	Y_1	$Y_1 - Y_1$	$(Y_1 - Y_1)^2$			
22	9.7	2.2	4.84	S^2	=	9.68
16	5.3	-2.2	4.84	S	=	3.11
	15.0	0	9.68		≈	3.1

$Y_1 = \frac{15}{2} = 7.5$

30 Mins

	Y_1	$Y_1 - Y_1$	$(Y_1 - Y_1)^2$			
22	7.3	1.5	2.25	S^2	=	9.68
16	4.3	-1.5	2.25	S	=	2.1
	11.6	0	4.50			

$Y_1 = \frac{11.6}{2} = 5.8$

45 Mins

	Y_1	$Y_1 - Y_1$	$(Y_1 - Y_1)^2$			
22	6.5	1.4	1.96	S^2 =		3.92
16	3.7	1.4	1.96	S =		1.98
	10.2	0	3.92			2.0

$Y_1 = \frac{10.2}{2} = 5.1$

Y_2 ROOT WATER EXTRACT 50MG/KG - TAIL CLIP METHOD

Zero Min.

	Y_2	$Y'_2 - Y'_2$	$(Y'_2 - Y'_2)^2$			
22	4.0	-0.4	0.16	S^2	=	0.32
16	4.8	0.4	0.16	S	=	0.566
	8.8	0.0	0.32		≈	0.6

$Y'_1 = \frac{8.8}{2} = 4.4$

15 Mins

	Y'_2	$Y'_2 - Y'_2$	$(Y'_2 - Y'_2)^2$			
23	8.2	0.25	0.0625	S^2	=	0.125
24	7.7	-0.25	0.0625	S	=	0.354
	15.9	0.00	0.125		=	0.4

$Y'_2 = \frac{15.9}{2} = 7.95$

30 Mins

	Y'_2	$Y'_2 - Y'_2$	$(Y'_2 - Y'_2)^2$			
23	8.2	-1.25	1.5625	S^2	=	3.125
24	10.7	1.25	1.5625	S	=	1.768
	18.9)	0.00	3.1250		=	1.8

$Y'_2 = \frac{18.9}{2} = 9.45$

45 Mins

	Y'_2	$Y'_2 - Y'_2$	$(Y'_2 - Y'_2)^2$			
23	5.5	-2.9	8.41	S^2	=	16.82
24	11.3	2.9	8.41	S	=	4.1
	16.8	0.0	16.82			

$Y'_2 = \frac{16.8}{2} = 8.4$

Z_1 STEM WATER EXTRACT 50.MG/KG TAILS CLIP METHOD

Zero Min.

Wt(g)	Z'_2	$Z'_2 - Z'_2$	$(Z'_2 - Z'_2)^2$			
21	4.7	0.1	0.01	S^2	=	0.02
25	4.5	-0.1	0.01	S	=	0.14
	9.2	0	0.02		=	

$Z_2 = \frac{9.2}{2} = 6.8$

15 Mins.

Wt(g)	Z'_2	$Z'_2 - Z'_2$	$(Z'_2 - Z'_2)^2$			
21	5.3	1.5	2.25	S^2	=	4.5
25	8.3	-1.5	2.25	S	=	2.12
	13.6	0	4.50		=	2.1

$Z_2 = \frac{13.6}{2} = 6.8$

45 Mins.

Wt(g)	Z'_2	$Z'_2 - Z'_2$	$(Z'_2 - Z'_2)^2$			
21	7.0	0.85 0.72		S^2	=	1.44
25	8.7	-0.85	0.72	S	=	1.2
	15.7	0	1.44			

$Z_2 = \frac{15.7}{2} = 7.85$

30 Mins

Wt(g)	Z'_2	$Z'_2 - Z'_2$	$(Z'_2 - Z'_2)^2$			
21	6.7	1.0	1.0	S^2	=	2.0
25	8.7	-1.0	1.0	S	=	1.4
	15.4	0.0	2.0			

$Z_2 = \frac{15.4}{2} = 7.7$

APPENDIX III

ANALGESIC ACTIVITY

A CONTROL (NORMAL SALINE)

		PAIN THRESHOLD (mm/Hg)					
Wt(g)	TIME (MINS)	0	15	30	45	60	75
23		4.6	4.3	3.7	4.8	5.6	5.0
22		5.0	6.4	5.5	5.2	5.7	4.4
31		4.2	4.5	4.7	5.8	4.2	4.2
22		4.5	5.0	5.1	4.5	3.5	3.5
26		5.4	4.7	4.5	4.5	4.7	4.0
21		4.5	5.7	6.5	6.0	5.7	4.7
	ΣA	28.2	30.6	30	28.8	29.4	4.7
	$\bar{A}$	4.7	5.1	5.0	4.8	4.9	4.3
	$\Sigma(A - \bar{A})$	0	0	0	0	0	0
	$\Sigma(A - \bar{A})^2$	0.92	3.22	4.54	2.74	4.26	1.40
	S^2	0.184	0.644	0.908	0.548	1.065	0.289
	S	0.4	0.8	1.0	0.7	1.0	0.5

KEY

A	=	Individual reading for control
ΣA	=	Total of individual readings
$\bar{A}$	=	Mean
S^2	=	Variance
S	=	Standard deviation
WT	=	Weight of mice

B. *METAMIZOL*

B_1 METAMIZOL 10MG/KG

		PAIN THRESHOLD (mm/Hg)					
Wt(g)	TIME (MINS)	0	15	30	45	60	75
25		4	4.7	8.0	7.8	5.9	4.7
20		4.5	6.8	9.3	8.5	6.3	5.1
17		4.0	5.8	7.3	8.2	5.8	7.3
21		3.6	5.4	6.9	7.3	5.8	4.6
29		5.0	6.2	7.2	5.6	5.6	4.5
23		4.1	5.2	6.3	5.2	5.4	5.0
	ΣB_1	25.2	33.6	45.0	42.6	34.8	31.2
	B_1	4.2	5.6	7.5	7.1	5.8	5.2
	$\Sigma(B_1 - B_1)$	0	0	0	0	0	0
	$\Sigma(B - B_1)^2$	1.18	3.34	5.43	9.56	0.46	5.58
	S^2	0.236	0.668	1.086	1.912	0.092	1.116
	S	0.5	0.9	1.0	1.4	0.3	1.1

B_2 METAMIZOL 20MG/KG

		PAIN THRESHOLD (mm/Hg)					
Wt(g)	TIME (MINS)	0	15	30	45	60	75
28		4.6	6.8	7.0	7.5	6.2	5.7
23		5.7	8.5	8.1	8.7	7.5	6.7
19		3.7	5.6	7.8	7.8	5.7	4.8
22		5.5	8.8	8.8	8.3	6.8	4.5
20		4.7	5.8	6.2	5.9	4.2	3.8
25		58	8.3	8.3	6.8	5.0	6.3
	ΣB_2	30	43.8	46.2	45.0	35.4	31.0
	B_2	5	7.3	7.7	7.5	5.9	5.3
	$\Sigma(B_2 - B_2)$	0	0	0	0	0	0
	$\Sigma(B_2 - B_2)^2$	3.32	10.08	4.48	5.22	4.64	6.26
	S^2	0.664	0.016	0.896	0.044	0.928	1.252
	S	0.8	1.4	1.0	1.0	1.0	1.1

B_3 METAMIZOL 30MG/KG

	PAIN THRESHOLD (mm/Hg)					
Wt(g) TIME (MINS)	0	15	30	45	60	75
31	4.2	6.5	7.2	9.7	7.2	6.8
28	4.5	6.2	6.8	9.0	7.8	6.5
29	4.7	6.4	7.0	8.9	7.0	6.8
24	5.2	5.8	6.7	8.2	6.3	6.0
25	3.8	7.0	7.0	8.0	7.0	6.6
21	4.0	5.9	7.3	9.6	6.7	6.3
ΣB_3	26.4	37.8	42.0	53.4	4.2	39.00
B_3	4.4	6.3	7.0	8.9	7.0	6.5
$\Sigma(B_3 - B_3)$	0	0	0	0	0	0
$\Sigma(B_3 - B_3)^2$	1.30	0.96	0.26	2.44	1.26	0.48
S^2	0.26	0.192	0.052	0.488	0.252	0.096
S	1.1	0.4	0.2	0.7	0.5	0.3

C ROOT WATER EXTRACT 50MG/KG

	PAIN THRESHOLD (mm/Hg)					
Wt(g) TIME (MINS)	0	15	30	45	60	75
23	4.3	6.3	6.2	6.8	6.5	6.0
24	4.7	6.6	5.0	6.5	6.9	6.0
23	4.0	7.3	8.2	5.5	5.8	5.8
20	4.8	7.7	9.3	11.3	7.8	5.0
19	3.6	5.2	6.3	6.2	5.5	6.0
24	3.8	6.5	5.8	9.3	7.7	7.0
ΣC	25.2	31.6	40.8	45.6	40.2	37.80
C	4.2	6.6	6.8	7.6	6.7	6.3
$\Sigma(C - C)$	0	0	0	0	0	0
$\Sigma(B - B)^2$	1.18	3.76	13.06	24.80	4.54	1.42
S^2	0.236	0.752	2.612	4.96	0.908	0.284
S	0.5	0.2	0.2	2.2	1.0	0.1

D STEM WATER EXTRACT 50MG/KG

	PAIN THRESHOLD (mm/Hg)					
Wt(g) TIME (MINS)	0	15	30	45	60	75
21	4.7	5.3	7.7	7.0	5.8	5.5
25	4.9	6.3	8.7	8.7	6.5	6.3
16	4.7	6.2	5.2	6.3	3.8	4.8
21	4.8	4.8	5.3	4.0	4.8	5.5
24	4.8	5.1	5.5	7.3	4.7	4.7
20	4.9	5.9	5.8	5.7	3.8	3.8
ΣD	28.8	33.6	37.2	3.9	29.4	30.6
D	4.8	5.6	6.2	6.5	4.9	5.1
$\Sigma(D - D)$	0	0	0	0	0	0
$\Sigma(D - D)^2$	0.07	1.92	8.95	12.66	5.84	3.70
S^2	0.014	0.384	1.79	2.532	1.168	0.74
S	0	0.1	1.3	1.6	1.0	0.9

E LEAVES WATER EXTRACT 50MG/KG

	PAIN THRESHOLD (mm/Hg)					
Wt(g) TIME (MINS)	0	15	30	45	60	75
19	8.5	9.0	10.3	9.5	6.8	5.8
23	6.7	10.4	10.0	8.8	5.8	5.8
17	2.8	4.2	5.2	8.3	5.5	4.7
27	3.5	7.3	6.8	8.3	5.8	3.2
220	7.6	9.6	10.0	8.7	5.7	5.2
27	3.3	5.7	6.3	8.4	5.2	4.7
ΣE	32.4	46.2	48.6	52.2	34.8	29.4
E	5.4	7.7	8.1	8.7	5.8	4.9
$\Sigma(E - E)$	0	0	0	0	0	0
$\Sigma(D - D)^2$	32.76	29.0	25.4	0.94	1.46	4.65
S^2	6.552	5.8	5.08	0.188	0.292	0.93
S	2.6	2.4	2.3	0.4	0.5	0.4

F_1 LEAVES WATER EXTRACT 50MG/KG

	PAIN THRESHOLD (mm/Hg)					
Wt(g) TIME (MINS)	0	15	30	45	60	75
20	6.2	5.8	8.3	9.3	5.7	4.7
17	5.7	7.8	10.5	10.8	7.8	8.0
26	4.0	5.2	7.5	8.0	6.5	5.2
27	3.5	6.0	6.8	8.5	6.2	6.3
24	4.8	6.2	8.2	9.2	6.4	6.0
22	5.2	6.2	8.5	9.4	7.0	6.4
ΣF_1	29.4	37.8	49.8	55.2	39.6	36.6
F_1	4.9	6.3	8.3	9.2	6.6	6.1
$\Sigma(F_1 - F_1)$	0.	0	0	0	0	0
$\Sigma(F_1 - F_1)^2$	5.2	4.06	7.78	4.54	2.62	6.52
S^2	1.04	0.812	1.556	0.901	0.524	1.304
S	1.0	0.9	1.3	0.9	0.7	1.1

F_2 FRUIT WATER EXTRACT 100MG/KG

	PAIN THRESHOLD (mm/Hg)					
Wt(g) TIME (MINS)	0	15	30	45	60	75
29	5.3	7.9	9.7	7.9	8.1	7.7
22	4.5	6.5	8.3	8.1	9.8	6.0
26	4.7	7.7	9.4	8.1	8.5	5.9
20	6.0	8.2	10.5	8.0	8.8	7.5
27	4.4	7.3	8.8	8.5	8.0	6.8
30	4.5	6.8	7.7	8.0	8.4	6.3
ΣF_2	29.4	44.4	54.6	48.6	51.6	40.2
F_2	4.9	7.4	9.1	8.1	8.6	6.7
$\Sigma(F_2 - F_2)$	0	0	0	0	0	0
$\Sigma(F_2 - F_2)^2$	1.98	2.16	4.58	0.222	2.14	2.94
S^2	0.396	0.432	0.916	0.044	0.428	0.588
S	0.6	0.7	1.0	0.2	0.7	0.8

F_3 FRUIT WATER EXTRACT 150MG/KG

	PAIN THRESHOLD (mm/Hg)					
Wt(g) TIME (MINS)	0	15	30	45	60	75
28	3.8	6.2	9.3	7.9	5.8	4.5
24	5.2	8.3	11.2	9.0	7.9	6.1
25	4.9	7.7	9.6	8.4	6.2	5.6
31	5.0	8.0	11.5	8.7	7.5	5.8
22	3.6	5.0	10.4	7.5	6.7	5.2
20	6.3	8.0	9.8	7.1	6.7	4.6
F_3	68.8	43.2	61.8	48.6	40.8	31.8
F_3	4.8	7.2	10.3	8.1	6.8	5.3
$\Sigma(F_3 - F_3)$	0	0	0	0	0	0
$\Sigma(F_3 - F_3)^2$	4.9	8.58	4.00	2.66	3.08	2.12
S^3	0.98	1.716	0.080	0.532	0.616	0.424
S	1.0	1.3	0.9	0.7	0.8	0.7

G SEEDS WATER EXTRACT 50MG/KG

	PAIN THRESHOLD (mm/Hg)					
Wt(g) TIME (MINS)	0	15	30	45	60	75
20	4.7	4.3	8.5	8.3	7.5	6.3
23	4.3	5.0	7.2	8.3	6.8	6.7
19	4.0	5.2	6.7	8.5	7.5	7.8
27	4.2	6.3	7.8	8.5	9.3	7.0
21	5.0	5.4	7.4	8.2	7.5	5.4
25	5.4	5.6	8.0	8.6	8.2	5.8
ΣG	27.6	31.8	45.6	50.4	46.8	36
G	4.6	5.3	7.6	8.4	7.8	6.5
$\Sigma(G - G)$	0	0	0	0	0	0
$\Sigma(G - G)^2$	1.42	2.2	2.02	0.12	3.68	4.71
S^3	0.42	0.44	0.404	0.024	0.736	0.942
S	0.5	0.7	0.6	0.2	0.9	1.0

ANALGESIC ACTIVITY (CALCULATIONS)
A CONTROL (NORMAL SALINE)

Zero Mins.

Wt(g)	A		A - A⁻	(A - A)²
23	4.6		- 0.1	0.01
26	5.0		0.3	0.09
21	4.2		- 0.5	0.25
22	4.5		- 0.2	0.04
22	5.4		0.7	0.49
31	4.5		- 0.2	0.04
	28.2		0	0.92

$A = \frac{28.2}{6} = 4.7$

$S^2 = 1/5 \times 0.92 = 0.184$

$S = 0.429 \approx 0.4$

15 Mins.

Wt(g)	A		A - A⁻	(A - A)²
23	4.3		- 0.8	0.64
26	6.4		1.3	1.69
21	4.5		- 0.6	0.36
22	5.0		- 0.1	0.01
22	4.7		- 0.4	0.16
31	5.7		0.6	0.36
	30.6		0	3.22

$A = \frac{30.6}{6} = 5.1$

$S^2 = 0.644$

$S = 0.802 \approx 0.4$

30 Mins.

Wt(g)	A		A - A⁻	(A - A)²
23	3.7		- 1.3	1.69
26	5.5		0.5	0.25
21	4.7		- 0.3	0.09
22	5.1		0.1	0.01
22	4.5		- 0.5	0.25
31	5.7		1.5	2.25
	30.0		0	4.54

$A = \frac{30.0}{6} = 5$

$S^2 = 0.908$

$S = 0.95 \approx 1.0$

45 Mins.

Wt(g)	A	A - $\bar{A}$	$(A - A)^2$
23	4.8	0	0
26	5.2	0.4	0.16
21	3.8	- 1.0	1.0
22	4.5	- 0.3	0.09
22	4.5	- 0.3	0.09
31	6.0	1.2	1.44
	28.8	0	2.74

$S^2 = 0.548$
$S = 0.74 \approx 1.7$

$A = \frac{28.8}{6} = 4.8$

60 Mins.

Wt(g)	A	A - $\bar{A}$	$(A - A)^2$
23	5.6	0.7	0.49
26	5.7	0.4	0.16
21	4.2	- 0.7	0.49
22	3.5	- 1.4	1.96
22	4.7	- 0.2	0.04
31	5.7	0.8	0.64
	29.4	0	4.26

$S^2 = 1.065 \approx 1.07$
$S = 1.03 \approx 1.7$

$A = \frac{29.4}{6} = 4.9$

75 Mins.

Wt(g)	A	A - $\bar{A}$	$(A - A)^2$
23	5.0	0.7	0.49
26	4.4	0.1	0.01
21	4.2	- 0.1	0.01
22	3.5	- 0.8	0.64
22	4.0	- 0.3	0.09
31	4.7	0.4	0.16
	25.8	0	1.40

$S^2 = 0.28$
$S = 0.53 \approx 0.5$

$A = \frac{25.8}{6} = 4.3$

B_1 METAMIZOL 10MG/KG

Zero Mins.

Wt(g)	B_1	$B_1 - B_1$	$(B_1 - B_1)^2$
25	4.0	- 0.2	0.04
20	4.5	0.3	0.09
17	4.0	- 0.2	0.04
21	3.6	- 0.6	0.36
22	5.0	0.8	0.64
31	4.1	- 0.1	0.01
	25.2	0	1.18

$B_1 = \frac{25.2}{6} = 4.2$

$S^2 = 0.236$

$S = 0.486 \approx 0.5$

15 Mins.

Wt(g)	B_1	$B_1 - B_1$	$(B_1 - B_1)^2$
25	4.7	- 0.9	0.81
20	6.8	1.2	1.44
17	5.3	- 0.3	0.09
21	5.4	- 0.2	0.04
29	6.2	0.6	0.36
23	5.0	- 0.4	0.16
	33.6	0	3.34

$B_1 = \frac{33.6}{6} = 5.6$

$S^2 = 0.668$

$S = 0.817 \approx 1.0$

30 Mins.

Wt(g)	B_1	$B_1 - B_1$	$(B_1 - B_1)^2$
25	8.0	0.5	0.25
20	9.3	1.8	3.25
17	7.3	- 0.2	0.04
21	6.9	- 0.6	0.36
29	7.2	- 0.3	0.09
23	6.3	- 1.2	1.44
	45.0	0	5.43

$B_1 = \frac{45.0}{6} = 7.5$

$S^2 = 1.086$

$S = 1.042$

45 Mins.

Wt(g)	B_1	$B_1 - B_1$	$(B_1 - B_1)^2$			
25	7.8	0.7	0.49	S^2	=	1.912
20	8.5	1.4	1.96	S	=	1.383
17	8.2	1.1	1.21		≈	1.4
21	7.3	0.2	0.04			
29	5.6	- 1.5	2.25			
23	5.2	- 1.9	3.61			
	42.6	0	9.56			

$$B_1 = \frac{42.6}{6} = 7.1$$

60 Mins.

Wt(g)	B_1	$B_1 - B_1$	$(B_1 - B_1)^2$			
25	5.9	0.1	0.01	S^2	=	0.092
20	6.3	0.5	0.25	S	=	0.3
17	5.8	0	0			
21	5.8	0	0			
29	5.6	- 0.2	0.04			
23	5.4	- 0.4	0.16			
	34.8	0	0.46			

$$B_1 = \frac{34.8}{6} = 5.8$$

75 Mins.

Wt(g)	B_1	$B_1 - B_1$	$(B_1 - B_1)^2$			
25	4.7	- 0.5	0.25	S^2	=	0.092
	5.1	- 0.1	0.01	S	=	1.056
17	7.3	2.1	4.41	S	=	1.042
21	4.6	- 0.6	0.36		≈	1.1
29	4.5	- 0.7	0.47			
23	5.0	- 0.2	5.58			
	31.2	0	5.58			

$$B_1 = \frac{31.2}{6} = 5.2$$

B_2 METAMIZOL 20MG/KG

Zero Mins.

Wt(g)	B_2		B_2- B_2	$(B_2- B_2)^2$			
28	4.0		- 0.4	0.16	S^2	=	2.664
23	5.7		0.7	0.49	**S**	=	0.815
19	3.7		- 1.3	1.69		≈	0.8
22	5.5		0.5	0.25			
20	4.7		- 0.3	0.09			
25	5.8		0.8	0.64			
	30.0		0	3.32			

$B_2 = \frac{30}{6} = 5$

15 Mins.

Wt(g)	B_2		B_2- B_2	$(B_2- B_2)^2$			
28	6.8		- 0.5	0.25	S^2	=	2.016
23	8.5		1.2	1.44	S	=	1.42
19	5.6		- 1.7	2.89			
22	8.8		1.5	2.25			
20	5.8		- 1.5	2.25			
25	5.3		1.0	1.00			
	43.8		0	10.08			

$B_2 = \frac{43.8}{6} = 7.3$

30 Mins.

Wt(g)	B_2		B_2- B_2	$(B_2- B_2)^2$			
28	7.0		- 0.7	0.49	S^2	=	0.896
23	8.1		0.4	0.16	S	=	0.947
19	7.8		0.1	0.01		≈	1.0
22	8.8		1.1	1.21			
20	6.2		- 1.5	2.25			
25	8.3		0.6	0.36			
	46.2		0	4.48			

$B_2 = \frac{46.2}{6} = 7.7$

45 Mins.

Wt(g)	B_2		$B_2 - B_2$	$(B_2 - B_2)^2$
28	7.5		0	0
23	8.7		1.2	1.44
19	7.8		0.3	0.09
22	8.3		0.8	0.64
20	5.9		- 1.6	2.56
25	6.8		- 0.7	0.49
	45.0		0	5.22

$B_2 = \frac{45.0}{6} = 7.5$

$S^2 = 1.044$

$S = 1.021 \approx 1.0$

60 Mins.

Wt(g)	B_2		$B_2 - B_2$	$(B_2 - B_2)^2$
28	6.2		0.3	0.09
23	7.5		1.6	2.56
19	5.7		- 0.2	0.04
22	6.8		0.9	0.81
20	4.2		- 1.7	2.89
25	5.0		0.9	0.82
	35.4		0	4.64

$B_2 = \frac{35.4}{6} = 7.5$

$S^2 = 0.928$

$S = 0.96 \approx 1.0$

75 Mins.

Wt(g)	B_2		$B_2 - B_2$	$(B_2 - B_2)^2$
28	5.7		0.4	0.16
23	6.7		1.4	1.96
19	4.8		- 0.5	0.25
22	4.5		- 0.8	0.64
20	3.8		- 1.5	2.25
25	6.3		1.0	1.00
	31.8		0	6.26

$B_2 = \frac{31.8}{6} = 5.3$

$S^2 = 1.252$

$S = 1.119 \approx 1.1$

B_3 METAMIZOL 30MG/KG

Wt	Zero Mins.			
Wt(g)	B_3	$B_3 - B_3$	$(B_3 - B_3)^2$	
31	4.2	- 0.2	0.04	S^2 = 0.26
28	4.5	+ 0.1	0.01	S = 1.140
29	4.7	0.3	0.09	≈ 1.1
24	5.2	0.8	0.64	
25	3.8	- 0.6	0.36	
21	4.0	- 0.4	0.16	
	26.4	0	1.30	

$$B_3 = \frac{26.4}{6} = 4.4$$

15 Mins.

Wt(g)	B_3	$B_3 - B_3$	$(B_3 - B_3)^2$	
31	6.5	0.2	0.04	S^2 = 0.192
28	6.2	- 0.1	0.01	S = 0.438
29	6.4	0.1	0.01	≈ 0.4
24	5.8	- 0.5	0.25	
25	7.0	0.7	0.49	
21	5.9	0.4	0.16	
	37.8	0	0.96	

$$B_3 = \frac{37.8}{6} = 6.3$$

30 Mins.

Wt(g)	B_3	$B_3 - B_3$	$(B_3 - B_3)^2$	
31	7.2	0.2	0.04	S^2 = 0.052
28	6.8	- 0.2	0.04	S = 0.228
29	7.0	0.0	0.00	≈ 0.2
24	6.7	- 0.3	0.09	
25	7.0	0.0	0.00	
21	7.3	0.3	0.09	
	42.0	0	0.26	

$$B_3 = \frac{42.0}{6} = 7.0$$

45 Mins.

Wt(g)	B_3	$B_3 - B_3$	$(B_3 - B_3)^2$
31	9.7	0.8	0.64
28	9.0	0.1	0.00
29	8.9	0.0	0.00
24	8.2	- 0.7	0.49
25	8.0	- 0.9	0.81
21	9.6	0.7	0.49
	53.4	0	2.44

$B_3 = \frac{53.4}{6} = 8.9$

$S^2 = 0.488$

$S = 0.699$

≈ 0.7

60 Mins.

Wt(g)	B_3	$B_3 - B_3$	$(B_3 - B_3)^2$
31	7.2	0.2	0.04
28	7.8	0.8	0.64
29	7.0	0.0	0.00
24	6.3	- 0.7	0.49
25	7.0	0.0	0.00
21	6.7	0.3	0.09
	42.0	0	1.26

$B_3 = \frac{42.0}{6} = 7.0$

$S^2 = 0.252$

$S = 0.502$

75 Mins.

Wt(g)	B_3	$B_3 - B_3$	$(B_3 - B_3)^2$
31	6.8	0.3	0.09
28	6.5	0.0	0.00
29	6.8	0.3	0.09
24	6.0	- 0.5	0.25
25	6.6	0.1	0.01
21	6.3	- 0.2	0.04
	39.0	0	0.48

$B_3 = \frac{39.0}{6} = 6.5$

$S = 0.096$

$S = 0.3098$

≈ 0.3

C ROOT 50MG/KG

Zero Mins.

Wt(g)	C	C- C	$(C- C)^2$
23	4.3	0.1	0.01
24	4.7	0.5	0.25
23	4.0	- 0.2	0.04
20	4.8	0.6	0.36
29	3.6	- 0.6	0.36
24	3.8	- 0.4	0.16
	25.2	0	1.18

$S^2 = 1.18$

≈ 0.236

$S = 0.486$

≈ 0.5

$C = \frac{25.2}{6} = 4.2$

15 Mins.

Wt(g)	C	C- C	$(C- C)^2$
23	6.3	- 0.3	0.09
24	6.6	0	0
23	7.3	0.7	0.49
20	7.7	1.1	1.21
19	5.2	- 1.4	1.96
24	6.5	- 0.1	0.01
	39.6	0	3.76

$S^2 = 3.76$

$= 0.752$

$S = 0.173$

≈ 0.2

$C = \frac{39.6}{6} = 6.6$

30 Mins.

Wt(g)	C	C- C	$(C- C)^2$
23	6.2	- 0.6	0.36
24	5.0	- 1.8	3.24
23	8.2	1.4	1.96
20	9.3	2.5	6.25
19	6.3	- 0.5	0.25
24	5.8	- 1.0	1.00
	40.8	0	13.06

$S^2 = 26.2$

$S = 0.323$

≈ 0.3

$C = \frac{40.8}{6} = 6.8$

45 Mins.

Wt(g)	C	C- C	$(C- C)^2$
23	6.8	- 0.8	0.64
24	6.5	- 1.1	1.21
23	5.5	- 2.1	4.41
20	11.3	3.7	13.69
19	6.2	- 1.4	1.96
24	9.3	1.7	2.89
	45.6	0	24.80

$C = \frac{45.6}{6} = 7.6$

$S^2 = 4.96$

$S = 2.227$

≈ 2.2

60. Mins.

Wt(g)	C	C- C	$(C- C)^2$
23	6.5	- 0.2	0.04
24	6.9	0.2	0.04
23	5.8	- 0.9	0.81
20	7.8	1.1	1.21
19	5.5	- 1.2	1.44
24	7.7	1.0	1.00
	40.2	0	4.54

$C = \frac{40.2}{6} = 6.7$

$S^2 = 0.908$

$S = 0.953$

≈ 1.0

75 Mins.

Wt(g)	C	C- C	$(C- C)^2$
23	6.0	- 0.3	0.09
24	6.0	- 0.3	0.09
23	5.0	- 0.5	0.25
20	7.0	0.7	0.49
19	6.0	- 0.3	0.99
24	7.0	0.7	0.49
	37.8	0	1.42

$C = \frac{37.8}{6} = 6.3$

$S^2 = 1.42$

$S = 0.107$

≈ 0.1

D STEM 50MG/KG

Zero Mins.

Wt(g)	D	D- D	$(D- D)^2$
21	4.7	- 0.1	0.01
25	4.5	0.1	0.01
16	4.7	- 0.1	0.01
21	4.8	0	0
24	4.8	0	0
20	4.9	- 0.2	0.04
	28.8	0	0.07

$S^2 = 0.014$

$S = 0.024$

≈ 0.1

$D = \frac{28.8}{6} = 4.8$

15 Mins.

Wt(g)	D	D- D	$(D- D)^2$
21	5.3	- 0.3	0.09
25	6.3	0.7	0.49
16	6.2	0.6	0.36
21	4.8	- 0.8	0.64
24	5.1	- 0.5	0.25
20	5.9	0.3	0.09
	33.6	0	1.92

$S^2 = 0.384$

$S = 0.124$

≈ 0.1

$D = \frac{33.6}{6} = 5.6$

30 Mins.

Wt(g)	D	D- D	$(D- D)^2$
21	6.7	0.5	0.25
25	8.7	2.5	6.25
16	5.2	- 1.0	1.00
21	5.3	- 0.9	0.81
24	5.5	- 0.7	0.49
20	5.8	- 0.4	0.16
	37.2	0	8.95

$S^2 = 1.79$

$S = 1.337$

≈ 1.3

$D = \frac{37.2}{6} = 6.2$

45 Mins.

Wt(g)	D	D- D	$(D- D)^2$
21	7.0	0.5	0.25
25	8.7	7.2	4.84
16	6.3	- 0.2	0.04
21	4.0	- 2.5	6.25
24	7.3	0.8	0.64
20	5.7	- 0.8	0.64
	39.0	0	12.66

$D = \frac{39.0}{6} = 6.5$

$S^2 = 2.532$

$S = 1.591 \approx 1.6$

60 Mins.

Wt(g)	D	D- D	$(D- D)^2$
21	5.8	- 0.9	0.81
25	6.5	1.6	2.56
16	3.8	- 1.1	1.21
21	4.8	- 0.1	0.01
24	4.7	- 0.2	0.04
20	3.8	- 1.1	1.21
	29.4	0	5.84

$D = \frac{29.4}{6} = 4.9$

$S^2 = 1.168$

$S = 1.081 \approx 1.0$

75 Mins.

Wt(g)	D	D- D	$(D- D)^2$
21	5.5	0.4	0.16
25	6.3	1.2	1.44
16	4.8	- 0.3	0.09
21	3.5	0.4	0.16
24	4.7	0.4	0.16
20	3.8	- 1.3	1.69
	30.6	0	3.70

$D = \frac{30.6}{6} = 5.1$

$S^2 = 0.74$

$S = 0.86 \approx 0.9$

E LEAVES 50MG/KG

Zero Mins.

Wt(g)	E	E - E	$(E- E)^2$
19	8.5	3.1	9.61
23	6.7	1.3	1.69
17	2.8	- 2.6	6.76
27	3.5	- 1.9	3.61
20	7.6	2.2	4.84
22	3.3	- 2.5	6.25
	32.4	0	32.76

$E = \frac{32.4}{6} = 5.4$

$S^2 = 6.552$

$S = 2.6$

15 Mins.

Wt(g)	E	E - E	$(E- E)^2$
19	9.0	1.3	1.69
23	10.4	2.7	7.29
17	4.2	- 3.5	12.25
27	7.3	- 0.4	0.16
20	9.6	1.9	3.61
22	5.7	2.0	4.00
	46.2	0	29.00

$E = \frac{32.4}{6} = 5.4$

$S^2 = 5.8$

$S = 2.408 \approx 2.4$

30 Mins.

Wt(g)	E	E - E	$(E- E)^2$
19	10.3	2.2	4.84
23	1.0	1.9	3.61
17	5.2	- 2.9	8.41
27	6.8	- 1.3	1.69
20	1.0	1.9	3.61
22	6.3	- 1.8	3.24
	48.6	0	25.40

$E = \frac{48.6}{6} = 8.1$

$S^2 = 5.08$

$S = 2.254 \approx 2.3$

45 Mins.

Wt(g)	E	E - E	$(E- E)^2$
19	9.5	0.8	0.64
23	8.8	0.1	0.01
19	8.5	- 0.2	0.04
27	8.3	- 0.4	0.16
20	8.7	0	0
22	8.4	- 0.3	0.09
	52.2	0	0.94

$E = \frac{52.2}{6} = 8.7$

$S^2 = 0.188$

$S = 0.434 \approx 0.4$

60 Min.

Wt(g)	E	E - E	$(E- E)^2$
19	6.8	1.0	1.0
23	5.8	0	0
17	5.5	- 0.3	0.09
27	5.8	0	0
20	5.7	- 0.1	0.01
22	5.2	- 0.6	0.36
	34.8	0	1.46

$E = \frac{34.8}{6} = 5.8$

$S^2 = 0.292$

$S = 0.540 \approx 0.5$

75 Min.

Wt(g)	E	E - E	$(E- E)^2$
19	5.8	0.9	0.81
23	5.8	0.9	0.81
17	4.7	- 0.2	0.04
27	3.2	- 1.7	2.89
20	5.2	0.3	0.06
22	4.7	- 0.2	0.04
	29.4	0	4.65

$E = \frac{29.4}{6} = 4.9$

$S^2 = 0.93$

$S = 0.364 \approx 0.4$

F_1 FRUIT 50MG/KG

Zero Mins

Wt(g)	F_1	$F_1 - F_1$	$(F_1 - F_1)^2$
20	6.2	1.3	0.69
17	5.7	0.8	0.64
26	4.0	- 0.9	0.81
27	3.5	- 1.4	1.96
24	4.8	- 0.1	0.01
22	5.2	0.3	0.09
	29.4	0	5.2

$F_1 = \frac{29.4}{6} = 4.9$

$S^2 = 1.04$

$S = 1.02$

≈ 1.0

15 Mins.

Wt(g)	F_1	$F_1 - F_1$	$(F_1 - F_1)^2$
20	5.8	- 0.5	0.25
17	7.8	1.5	2.25
26	5.2	- 1.1	1.21
27	6.0	0.3	0.09
24	6.8	0.5	0.25
22	6.2	- 0.1	0.01
	37.8	0	4.06

$F_1 = \frac{37.9}{6} = 6.3$

$S^2 = 0.812$

$S = 0.901$

≈ 0.9

30 Mins.

Wt(g)	F_1	$F_1 - F_1$	$(F_1 - F_1)^2$
20	8.3	0	0
17	10.5	2.2	0.64
26	7.5	- 0.8	0.64
27	6.8	- 1.5	2.25
24	8.2	- 0.1	0.01
22	8.5	0.2	0.04
	49.8	0	7.78

$F_1 = \frac{49.8}{6} = 8.3$

$S^2 = 1.556$

$S = 1.247$

≈ 1.3

45 Mins.

Wt(g)	F_1	$F_1 - F_1$	$(F_1 - F_1)^2$
20	9.3	0.1	0.01
17	10.8	1.6	2.56
26	8.0	- 1.2	1.44
27	8.5	- 0.7	0.49
24	9.2	0	0
22	9.4	0.2	4.54
	55.2	0	4.54

$F_1 = \frac{55.2}{6} = 9.2$

$S^2 = 0.901$

$S = 0.953 \approx 1.0$

60 Mins.

Wt(g)	F_1	$F_1 - F_1$	$(F_1 - F_1)^2$
20	5.7	- 0.9	0.81
17	7.8	1.2	1.44
26	6.5	- 0.1	0.01
27	6.2	- 0.4	0.16
24	6.4	- 0.2	0.14
22	7.0	- 0.4	0.16
	39.6	0	2.62

$F_1 = \frac{39.6}{6} = 6.6$

$S^2 = 0.524$

$S = 0.724 \approx 0.7$

75 Mins.

Wt(g)	F_1	$F_1 - F_1$	$(F_1 - F_1)^2$
20	4.7	- 1.4	1.96
17	8.0	- 1.9	3.61
26	5.2	- 0.9	0.81
27	6.3	0.2	0.04
24	6.0	- 0.1	0.01
22	6.4	0.3	0.09
	36.6	0	6.52

$F_1 = \frac{39.6}{6} = 6.1$

$S^2 = 1.304$

$S = 1.142 \approx 1.1$

G SEEDS 50MG/KG

Zero Mins

Wt(g)	G	G - G	$(G - G)^2$
20	4.7	0.1	0.01
	4.3	- 0.3	0.09
19	4.0	- 0.6	0.36
27	4.2	- 0.4	0.16
21	5.0	0.4	0.16
25	5.4	0.8	0.64
	27.6	0.0	1.42

$G = \frac{27.6}{6} = 4.6$

$S^2 = 0.284\ 23$

$S = 0.533 \approx 0.5$

15 Mins.

Wt(g)	G	G - G	$(G - G)^2$
20	4.3	- 1.0	1.00
	5.0	- 0.3	0.09
19	5.2	- 0.1	0.09
27	6.3	1.0	1.00
21	5.4	+ 0.1	0.01
25	5.6	+ 0.3	0.04
	31.8	0.0	2.20

$G = \frac{31.8}{6} = 5.3$

$S^2 = 0.44\ 23$

$S = 0.663 \approx 0.7$

30 Mins.

Wt(g)	G	G - G	$(G - G)^2$
20	8.5	0.9	0.81
	7.2	- 0.4	0.17
19	6.7	- 0.9	0.81
27	7.8	0.2	0.04
21	7.4	- 0.2	0.04
25	8.0	0.4	0.16
	45.6	0.0	2.02

$G = \frac{45.6}{6} = 7.6$

$S^2 = 0.404\ 23$

$S = 0.636 \approx 0.6$

45 Mins.

Wt(g)	G	G - G	$(G - G)^2$
20	8.3	- 0.1	0.01
	8.3	- 0.1	0.01
19	8.5	0.1	0.01
27	8.5	0.1	0.01
21	8.2	- 0.2	0.04
25	8.6	- 0.2	0.12
	50.4	0.0	0.12

$G = \frac{50.4}{6} = 8.4$

$S^2 = 0.024\ 23$

$S = 0.155 \approx 0.2$

60 Mins.

Wt(g)	G	G - G	$(G - G)^2$
20	7.5	- 0.3	0.09
	6.8	- 1.0	1.00
19	7.5	- 0.3	0.09
27	9.3	1.5	2.25
21	7.5	- 0.3	0.09
25	8.2	0.4	0.16
	46.8	0.0	3.68

$G = \frac{46.8}{6} = 7.8$

$S^2 = 0.736\ 23$

$S = 0.858 \approx 0.9$

75 Mins.

Wt(g)	G	G - G	$(G - G)^2$
20	6.3	- 6.3	0.04
	6.7	0.2	0.04
19	7.8	1.3	1.69
27	7.0	0.5	0.25
21	5.4	- 1.1	2.20
25	5.8	- 0.7	0.49
	39.0	0.0	4.71

$G = \frac{39.0}{6} = 6.5$

$S^2 = 0.942\ 23$

$S = 0.971 \approx 1.0$

F2 FRUIT 100MG/KG

Zero Mins.

Wt(g)	F_2	$F_2 - F_2$	$(F_2 - F_2)^2$
29	5.3	0.4	0.16
	4.5	- 0.4	0.16
26	4.7	- 0.2	0.04
20	6.0	1.1	1.21
27	4.4	- 0.5	0.25
30	4.5	- 0.4	0.16
	29.4	0	1.98

$F_2 = \frac{29.4}{6} = 4.9$

$S^2 = 0.396\ 22$

$S = 0.629 \approx 0.6$

15 Mins.

Wt(g)	F_2	$F_2 - F_2$	$(F_2 - F_2)^2$
29	7.9	0.5	0.25
	6.5	- 0.9	0.81
26	7.7	0.3	0.09
20	8.2	0.8	0.64
27	7.3	- 0.1	0.01
30	6.8	0.1	0.36
	44.4	0	2.16

$F_2 = \frac{44.4}{6} = 7.4$

$S^2 = 0.432\ 22$

$S = 0.657 \approx 0.7$

30 Mins.

Wt(g)	F_2	$F_2 - F_2$	$(F_2 - F_2)^2$
29	9.7	0.6	0.36
	8.3	- 0.8	0.64
26	9.4	0.3	0.09
20	10.5	1.4	1.96
27	8.8	- 0.3	0.09
30	7.9	- 1.2	1.44
	54.6	0	4.58

$F_2 = \frac{54.6}{6} = 9.1$

$S^2 = 0.916\ 22$

$S = 0.957 \approx 1.0$

45 Mins.

Wt(g)	F_2	$F_2 - F_2$	$(F_2 - F_2)^2$
29	7.9	- 0.2	0.04
	8.1	0	0
26	8.1	0	0
20	8.0	- 0.1	0.01
27	8.5	0.4	0.16
30	8.0	0.1	0.01
	48.6	0	0.22

$$F_2 = \frac{48.6}{6} = 8.1$$

$S^2 = 0.044$ 22

$S = 0.21$

≈ 0.2

60 Mins.

Wt(g)	F_2	$F_2 - F_2$	$(F_2 - F_2)^2$
29	8.1	- 0.5	0.25
	9.8	1.2	1.44
26	8.5	- 0.1	0.01
20	8.8	0.2	0.04
27	8.0	- 0.6	0.36
30	8.4	- 0.2	0.04
	51.6	0	2.14

$$F_2 = \frac{51.6}{6} = 8.6$$

$S^2 = 0.428$ 22

$S = 0.654$

≈ 0.7

75 Mins

Wt(g)	F_2	$F_2 - F_2$	$(F_2 - F_2)^2$
29	7.7	1.0	1.00
	6.0	- 0.7	0.49
26	5.9	- 0.8	0.64
20	7.5	0.8	0.64
27	6.8	0.1	0.01
30	6.3	- 0.4	0.16
	40.2	0	2.94

$$F_2 = \frac{40.2}{6} = 6.7$$

$S^2 = 0.588$ 22

$S = 0.767$

≈ 0.8

F_3 FRUIT 150MG/KG

Zero Min.

Wt(g)	F_3	$F_3 - F_3$	$(F_3 - F_3)^2$
28	3.8	- 1.0	1.00
	5.2	1.0	0.16
25	4.9	0.1	0.01
31	5.0	0.2	0.04
22	3.6	- 1.2	1.44
20	6.3	1.5	2.25
	28.8	0	4.90

$F_3 = \frac{28.8}{6} = 4.8$

$S^2 = 0.98 \quad 24$

$S = 0.9899$

$= 1.0$

15 Mins

Wt(g)	F_3	$F_3 - F_3$	$(F_3 - F_3)^2$
28	6.2	- 1.0	1.00
24	8.3	1.1	1.21
25	7.7	0.5	0.25
31	8.0	- 0.8	0.64
22	5.0	2.2	4.84
20	8.0	0.8	0.64
	43.2	0	8.58

$F_3 = \frac{43.2}{6} = 7.2$

$S^2 = 0.98$

$S = 1.31$

$= 1.3$

30 Mins.

Wt(g)	F_3	$F_3 - F_3$	$(F_3 - F_3)^2$
28	9.3	- 1.0	1.0
24	11.2	0.9	0.81
25	9.6	- 0.7	0.49
31	11.5	1.2	1.44
22	10.4	0.1	0.01
20	9.8	0.5	0.25
	61.8	0	4.00

$F_3 = \frac{61.8}{6} = 10.3$

$S^2 = 0.8$

$S = 0.894$

$= 0.9$

45 Mins.

Wt(g)	F_3	$F_3 - F_3$	$(F_3 - F_3)^2$
28	7.9	- 0.2	0.04
24	9.0	0.9	0.81
25	8.4	0.3	0.09
31	8.7	0.6	0.36
22	7.5	- 0.6	0.36
20	7.1	- 1.0	1.00
	48.6	0.0	2.66

$F_3 = \frac{48.6}{6} = 8.1$

$S^2 = 0.532$

$S = 0.729 \approx 0.7$

60 Mins.

Wt(g)	F_3	$F_3 - F_3$	$(F_3 - F_3)^2$
28	5.8	- 1.00	1.00
24	7.9	1.1	1.21
25	6.2	- 0.6	0.36
31	7.5	0.7	0.49
22	6.4	- 0.1	0.01
20	6.7	- 0.1	0.01
	48.6	0	3.08

$F_3 = \frac{40.8}{6} = 6.8$

$S^2 = 0.616$

$S = 0.785 \approx 0.8$

75 Mins.

Wt(g)	F_3	$F_3 - F_3$	$(F_3 - F_3)^2$
28	4.5	- 0.8	0.64
24	6.1	0.8	0.64
25	5.6	0.3	0.09
31	5.8	0.5	0.25
22	5.2	- 0.1	0.01
20	4.6	- 0.7	0.49
	31.8	0	2.12

$F_3 = \frac{31.8}{6} = 5.3$

$S^2 = 0.424$

$S = 0.651 = 0.9$

APPENDIX IV

TABLE I TEST FOR ANALGESIC ACTIVITY

MEAN PAIND THRESHOLD (mm/Hg) AND STANDARD DEVIATION

	TIME (MINS.)	0	15	30	45
Samples	**Tail Clip Method**				
W	Control (Normal Saline)	4.9 ± 1.3	5.4 ± 1.6	4.6 ± 1.3	4.4 ± 0.9
X	Metamizol 10mg/kg	6.25 ± 1.8	7.2 ± 3.5	9.65 ± 0.9	8.55 ± 1.1
Y_1	Root Water Extract 10mg/kg	4.05 ± 1.8	7.5 ± 3.1	5.8 ± 2.1	5.1 ± 2.0
Y_2	Root Water Extract 50mg/kg	4.4 ± 0.6	7.95 ± 0.4	9.45 ± 1.8	8.4 ± 4.1
Z_1	Stem Water Extract 10mg/kg	5.0 ± 0.42	4.85 ± 1.2	5.1 ± 1.6	5.85 ± 0.2
Z_2	Stem Water Extract 50mg/kg	4.6 ± 0.14	6.8 ± 2.1	7.7 ± 1.4	7.85 ± 1.2

TABLE II

ALGESIC ACTIVITY

MEAN PAIN THRESHOLD (mm/Hg) AND STANDARD DEVIATIONS

	TIME (MINS)	0	15	30	45	60	75
Samples	Tail Clip Method						
A	Control (Normal Saline)	4.7 ± 0.4	5.1 ± 0.8	5.0 ± 1.0	4.8 ± 0.7	4.9 ± 1.0	4.3 ± 0.5
B_1	Metamizol 10mg/kg	4.2 ± 0.5	5.6 ± 0.9	7.5 ± 1.4	7.1 ± 1.4	5.8 ± 0.3	5.2 ± 1.1
B_2	Metamizol 20mg/kg	5.0 ± 0.8	7.3 ± 1.4	7.7 ± 1.0	7.5 ± 1.0	5.9 ± 1.0	5.3 ± 1.1
B_3	Metamizol 30mg/kg	4.4 ± 1.1	6.3 ± 0.4	7.0 ± 0.2	8.9 ± 0.7	7.0 ± 0.5	6.5 ± 0.5
C	Roots 50mg/kg	4.2 ± 0.5	6.6 ± 0.2	6.8 ± 0.2[a]	7.6 ± 2.2	6.7 ± 1.0	6.3 ± 0.1
D	Stem 50mg/kg	4.8 ± 0	5.6 ± 01	6.2 ± 1.3	6.5 ± 1.6	4.9 ± 1.0	5.1 ± 0.9
E	Leaves 50mg/kg	5.4 ± 2.6	7.7 ± 2.4	8.1 ± 2.3	8.7 ± 0.4	5.8 ± 0.5	4.9 ± 0.4
F_1	Fruits 50mg/kg	4.9 ± 1.0	6.3 ± 0.9	8.3 ± 1.3	9.2 ± 0.9	6.6 ± 0.7	6.1 ± 1.1
G	Seeds 50mg/kg	4.6 ± 0.5	5.3 ± 0.7	7.6 ± 0.6	8.4 ± 0.2[b]	7.8 ± 0.9	6.5 ± 1.0
F_2	Fruits 100mg/kg	4.9 ± 0.6	7.4 ± 0.7	9.1 ± 1.0	8.1 ± 0.2[b]	8.6 ± 0.7	6.7 ± 0.8
F_3	Fruits 150mm/kg	4.8 ± 1.0	7.2 ± 1.3	10.3 ± 0.9	8.1 ± 0.7	6.8 ± 0.8	5.3 ± 0.7

Significant Difference, $P<0.05^a$, $P<0.001^b$