INVESTIGATIONS OF SOME FAST GROWING PLANTS FOR PULP MANUFACTURE AND THEIR PHYTOCHEMICAL ANALYSIS

Thesis

Submitted In partial fulfilment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

By

R. MANAVALAN *M. Pharm.*

PHARMACY DISCIPLINE

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI *(Rajasthan)*

1981

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI, RAJASTHAN.

CERTIFICATE

This is to certify that the thesis entitled "INVESTIGATIONS OF SOME FAST GROWING PLANTS FOR PULP MANUFACTURE AND THEIR PHYTOCHEMICAL ANALYSIS" submitted by Mr. R.MANAVALAN, ID.No. 76S81501, for the award of the Ph.D. degree of the Institute, embodies original work done by him under my supervision.

Dated 24.6.1981.

(B.M. MITHAL)
Professor of Pharmacy.

ACKNOWLEDGEMENTS

I am indebted to Dr. B.M. Mithal, Professor of Pharmacy, B.I.T.S., Pilani, for guiding the research work and for active encouragement in planning and execution of work at all stages.

I am extremely thankful to Dr. C.K. Atal, Director, Regional Research Laboratory (RRL), Jammu-Tawi for providing me all the facilities and help to complete a part of research work at the RRL. I am also thankful to Dr. J.S. Chawla, Assistant Director, Cellulose and Pulp, and Dr. S.M. Anand, Scientist, RRL for their numerous suggestions and help. My sincere thanks are due to Dr. O.P. Suri, Scientist, Dr. K.L. Dhar, Assistant Director, Dr. K.A. Suri, Scientist, Dr. K.L. Bedi, Scientist, Dr. K.L. Thappa, Scientist and Dr. S.K. Agarwal, Scientist, RRL for their timely suggestions and encouragements. I acknowledge my sincere thanks to Dr. Dinesh, Assistant Director, RRL for providing me the spectral data of compounds.

I am also thankful to Dr. C.R. Mitra, Director, B.I.T.S. for permission to carry out a part of work at RRL, Jammu.

I am extremely thankful to Dr. S.S. Mathur, Group Leader, Pharmacy Discipline, B.I.T.S. for his timely encouragements in my work.

My sincere gratitude to other colleagues and well-wishers who have constantly encouraged me.

Dated 24.6.1981.

R. Manavalan

(R. MANAVALAN)

C O N T E N T S

CHAPTER I : INTRODUCTION.

INTRODUCTION

The plant kingdom continues to be a indefatigable source of raw materials which are at the very base of human economy. Man still depends to a very large extent on plants for foods, clothes, drugs, papers and many other industrially significant materials. It is true that human ingenuity has discovered some alternatives but the fundamental fact is that man, inspite of his technology and science, will always have to heavily lean on the world of plants.

Amongst the various natural polymers that man uses for different purposes perhaps the most outstanding material is cellulose. If one were to estimate the total quantity of cellulose used each year the figures would be astronomical. A largest quantity of cellulose is used for making paper and the next largest for rayon. With the advent of rayon and varied uses for cellulose derivatives, the demand for high grade alpha cellulose or rayon grade cellulose jumps up each year. The conventional raw material bamboo (Dendrocalamus species) for rayon grade cellulose cannot meet the demand. Hence search has been on for suitable alternative fast growing plants. Since 1957 fast growing angiosperms has been investigated for the manufacture of rayon grade pulp.

Literature survey revealed that a number of plants have been investigated for this purpose by earlier workers, prominent amongst which are:

Acacia decurrence[1]

Boswellia serata[2,3] (S.-Shallaki; H.-Luban, Salai)

Bagasse[4]

Ochroma lagopus[5]

Ochlandra travancorie[6]

Eucalyptus grandis[7]

Arundo donax[8] (H.-Baranal; B.-Gahanal; P.-Bansi)

Broussonetia papyrifera[9] (Paper mulberry)

Jute sticks[10]

Kydia calycina[11] (H.-Pola, Pula; T.-Vendai)

Mixture of hardwoods[12]

Acacia mollisina[13]

Poplars[14]

Abies pindraw[15]

Dillenia pentagyma[16]

Sterospermium suavedens[17] (S.-Abhipriya, Kala; H.-Padal, Pandri)

Eucalyptus citriodora[18] (Tel.-Talanoppi)

Pterocarpus marsupium[19] (H.-Banda, Bija; S.-Asana, Bijaka)

Melia azedarach[20] (S.-Dreka, Gairika; H.-Bakain)

Cymbopogon citratus[21] (S.-Bhustrina; H.-Gandhatrina)

and Anthocephalus cadamba[22] (H.-Kadamba; T.-Vellai-cadamba).

A survey of the rural area around Pilani (Rajasthan) showed that a number of fast growing plants are amongst the natural flora. Ten fast growing plants mentioned below were chosen to screen them as possible sources of high alpha cellulose or rayon grade pulp. Simultaneously wherever it was felt that the plants had not been investigated phytochemically the same was also carried out.

1. Crotalaria juncea (S.-Sana; H.-Sunn) - 1½, 3 and 7 months old collected from Regional Research Laboratory, Jammu, experimental farm.

2. Crotalaria retusa (H.-Ghunghunian; Tel.-Potugalli) - 6½ months old collected from RRL, Jammu, experimental farm.

3. Sesbania sesban (H.-Jayanthi; Tel.-Jalugu) - 1 year old collected from RRL, Jammu, experimental farm.

4. Sesbania roxburghii - 1 year old collected from RRL, Jammu, experimental farm.

5. Leptadenia pyrotechnica - wild plant collected from Rajasthan desert.

6. Crotalaria burhia (P.-Meini; Marathi-Ghagari) - wild plant collected from Rajasthan desert.

7. Populus casale I-488 - 5 years old collected from RRL, Jammu, experimental farm.

8. Lantana camara (Tel.-Pulikampa; Mal.-Arippu) - wild plant collected from Jammu area.

9. Broussonetia papyrifera (Paper mulberry) - 1½ years old collected from RRL, Jammu, experimental farm.

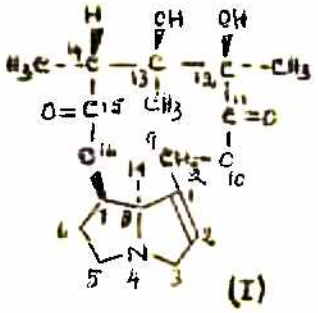
10. Hibiscus cannabinus (S.-Jali; H.-Patsan; Tel.-Gongura) - 6 months old collected from RRL, Jammu, experimental farm.

To qualify as a source of high grade alpha cellulose, a plant should yield atleast 88% of alpha cellulose. At the same time it should have less than 5% pentosan, less than 0.15% ash, less than 0.15% lignin, brightness of 85% G.E., viscosity (1% solution) 5-25 centipoise and copper number 1-1.25 as per TAPPI specification[23].

Crotalaria juncea[24] (Sunhemp), belonging to the family Leguminosae, is an erect, shrubby annual, 4 to 10 feet high with simple, narrow subsessile leaves and fairly large bright yellow flowers. The sunhemp, also known as Indian or Bombay hemp, is one of the commonly cultivated crops in India next in importance to jute as a source of bast fibre. The plant is cultivated all over India in rotation with grain or cash crops either for fibre or as a green manure crop. The sunhemp cultivated for fibres thrives best on well drained highland soils. The crop takes 4 to 4½ months to mature fully, when it can be harvested for seed. For fibre extraction, it has to be harvested earlier although opinions vary about the maturity for fibre. The fibre content of the plant is about 5% of the weight of the dry stem. Raw sunhemp has about 80% of cellulose. The average annual production of the fibre is about 100,000 lbs out of which 20-30% is exported. The sunhemp[25,26] had been reported to be a potential raw material for cellulose fibres. The plant contains a small quantity of pyrrolizidine alkaloids and its seeds[27] contain 34,6% crude protein, 4.3% fat, 41.1% starch, 8.1% fibres, and give an ash value of 3.3%. No investigations have so far been done to find whether this plant can be a source of high alpha cellulose. Hence

this task was undertaken and at the same time it was decided to carry out its phytochemical investigation of seeds.

Crotalaria retusa[28], belonging to the family Leguminosae, is an erect, robust, undershrub attaining a height of 15-20 feet. It is common throughout India, Ceylon and Malaysia. It is occasionally cultivated for its fibre which is used in canvas making in admixture with sunhemp. The fibre is tough and is amenable to improvement if attention is paid to the conditions of cultivation. The plant is popularly called Glory of Mahabaleshwara in Bombay and is cultivated in Florida and tropical regions of America for its ornamental flowers. It has been used in the treatment of scabies and impetigo. Evaluation of this plant for fibres[25,26] indicated it to be one of the good sources of cellulose. The seeds[29] have been reported to contain a toxic alkaloid of pyrrolizidine type, monocrotaline (I), to the extent of 8-10%. The biosynthetic studies[30] indicated that isoleucine and threonine were precursors for monocrotaline



(I)

12,13-dihydroxy - 12, 13, 14-trimethyl-crotal-1-enine.

Since no attempt has been made so far to find out whether it can be a source of high alpha cellulose, it was chosen for screening. Analysis of the fixed oil of its seeds was also undertaken since the same have not been analysed so far. Monocrotaline has been converted into pyrrolizidieneamides[31] which has been shown to be a hypotensive agent, pyrrolizidine esters[32,33] which are antimitotic and potent local anaesthetics, mono and diquaternary compounds[34,35] which have been reported to posses ganglion blocking activity and diplacine[36] which was reported to have curarae like activity.

Sesbania sesban[37], belonging to the family Leguminosae, is a soft wooded quick growing short lived shrub, 1.8 to 6 metres high, and cultivated throughout the plains of India upto an altitude of 1200 metres. The plant is extensively cultivated as a shade tree for turmeric, tea, sweet orange and cotton plants. Sesbania can grow under widely different conditions including waterlogged conditions and acid soils. Fibre from the bark is used for making ropes and contains cellulose 65.9%, lignin and pectin 24.3%, extractives 2.5%, and ash 2.2%. Leaves[38] of the plant contain protein 26.6%, ether extractives 5.8% and fibre 8.4%. Pigments[39] isolated from the flowers include a complex of cyanidin and

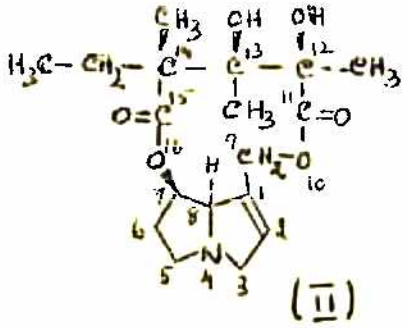
delphindin glycoside acylated with gallic acid and an unidentified acid. Seeds contain protein 33%, cellulose 28.3%, vitamin C 89.4 mg/100 gms and give total extractives 18.2% and ash 4.2%. A sample of seeds from Aligarh, on extraction with petroleum ether, yielded 5.3% of a greenish yellow oil with the following characteristics specific gravity 0.9241, refractive index 1.4805 at 25°C, saponification value 193.3, acid value 3.0, acetyl value 23.0 and iodine value 112.4. The oil[40] contained palmitic 9.0%, stearic 17.5%, lignoceric 1.9%, oleic 24.4%, linoleic 36.3% and linolenic acids 10.9%. The laboratory pulping studies of this plant indicated its potentiality for good grade pulp and so it was decided to screen this plant for high grade alpha cellulose.

Sesbania roxburghii[41], belonging to the family Leguminosae, is an erect, annual 1.8 - 3.6 metres high and is found in South East Asia and Malasyia. It occurs in lakes in the Central and Eastern Bengal and in marshes on the west coast. The plant is grown as a garden fence in Bengal. No previous report is available on its high alpha cellulose and phytochemical investigation of seeds. Hence the plant was chosen for screening.

Leptadenia pyrotechnica[42], belonging to the family Asclepidaceae, is a much branched, often leafless, shrub upto 1.8 metres high found chiefly in dry and sandy places in Punjab, Western Uttar Pradesh, Rajasthan and Northern parts of Bombay along the sea coast. The plant yields fibre used for rope making. It is reported to be suitable for paper manufacturing also. No work has been reported on this plant regarding its high alpha cellulose content and so it was chosen as one of the plants for investigations. The plant has also not been phytochemically investigated. Literature survey indicated that L. reticulata known as Jivanthi (Sanskrit), found in Punjab and Western peninsula of India, contains chemical compounds[43,44] such as hentriacontanol, alpha amyrin, beta amyrin, stigmasterol, gamma sitosterol (mixture of beta sterol and campsterol) and two flavonoids diosmetin and luteolin. The aqueous and alcoholic extracts[45] of this plant have been tested for antibacterial and antifungal activity and the aqueous extract[46] has been found to produce prolonged hypotensive action in dogs. Another plant L. spartum[47] on chemical investigation yielded 5-trioconten-17-one, beta sitosterol, stigmasterol, oleanolic acid, 18-pentatriacontanol and sugars such as glucose, fructose, maltose and sucrose. Since the

genera Leptadenia is promising for sterols and terpenoids, the serial parts of the plant L. pyrotechnica were chosen for phytochemical investigations.

Crotalaria burhia[48], belonging to the family Leguminosae, is a low, slender branched undershrub common in the dried parts of Sind, Baluchistan, Punjab, Rajasthan, Gujarat and Cambay. It yields a fibre similar to sunhemp but ropes made from it, though cheaper are weak. Till now, no scientific study has been made to obtain high alpha cellulose from this plant and so the plant was chosen for investigations. Literature[49] survey indicated that monocrotaline and croburhine (II) were isolated from the stems of this plant. Since the pyrrolizidine type of alkaloids are known to appear in the seeds of plants belonging to the family Leguminosae, the seeds of this plant C. burhia was selected for phytochemical investigation.



(II)

12,13-dihydroxy-14-ethyl-12,13,14-trimethyl-crotal-1-enine.

Populus casale I-488[50], belonging to the family Salicaceae, is a hybrid due to natural cross fertilization among Poplar species. Poplars are trees chiefly distributed in the North temperate zone. Some species occur in the sub-tropical regions. About ten species grow wild in India. Poplars are very fast growing plants with an anhual girth increment of 1.8 - 2.5 cm. Since this plant can be a source of timber, its cultivation has been tried in various places in India. Introduction of exotic Populus[51] into India has been attempted by the Forest Research Institute, Dehra Dunn since 1958. So far over 160 species and hybrids have been planted out of which 68 continue to grow. The trials have indicated that Poplars required a sub-tropical or temperate climate and soil with a high water content. They are strong light demanding plants and thrive much better under irrigation. The trial cultivation of P. casale I-488 at Jammu was successful. Several species of Poplar are known to provide pulp for making papers and high grade alpha cellulose. Hence this hybrid was chosen for the investigation as a potential source of high grade alpha cellulose.

Lantana camara[52,53], belonging to the family Verbanaceae, is a hairy, unarmed or slightly pricky -

shrub, 0.3 - 1.8 metres or more in height. It is a native of America and is cultivated as an ornamental or hedge plant. Numerous varieties and types of L. camara are met with and some of them are polyploids. The varieties and types are so intermixed that it is difficult to differentiate between them morphologically. Most of them have recurved prickles on the stem but under cultivation they become less prickly. Of the many known varieties of L. camara, three have been reported from India namely var. aculeata, var. mista and var. nivee amongst which var. aculeata is the most common. It thrives in areas where rain-fall exceeds 200 inches and also in comparatively dry localities with 30 inches rain-fall per annum. The plant has vigorous growth and regenerates quickly after cutting, transplanting or burning and forms a dense impenetrable thicket. It blossoms and bears fruits almost throughout the year. The plant is considered to be a nuisance due to its fast growth. Till now no attempt has been made to use this very fast growing plant as a source of cellulose and hence it was chosen for screening of high alpha cellulose content. The leaves[54] yield 0.2% of a volatile oil on steam distillation which has been reported to contain a

sesquiterpene (80%) probably caryophyllene, 1-alpha-phellandrene and small amounts of aldehyde and alcohols. The leaves[54] contain a toxic principle lantadene A (acryloyloxyoleanonic acid $C_{35}H_{52}O_5$, m.pt. 282-285°C) which causes acute photosensitization and severe itches in sheep. It also contains triterpenoid lantadene B[55] (dimethyl acryloyloxyoleanoic acid, $C_{35}H_{52}O_5$, m.pt. 293-294°C) which is inactive and a sterol lanamarene which is cardioactive. The barks of stems and roots[56] contain a quinine like alkaloid lantanine with strong antipyretic and antispasmodic properties. The volatile oils from the leaves and flowers of L. camara collected from different geographical sources showed differences in composition[57-62]. Keeping this in view, the volatile oil from the flowers of L. camara growing in Jammu was studied to know whether it contains any new constituents not reported earlier.

Broussonetia papyrifera[63], belonging to the family Moraceae, is a medium sized tree common in China and Japan where it is cultivated on the edges of the fields. It was introduced into India near Dehra Dunn and has spread to the surrounding parts. The tree prefers a cool climate and thrives best on moist soils. The best fibres of this plant are soft, lustrous and very strong. Viscose -

rayon pulp had been successfully prepared from this plant but the literature does not reveal the age of the plant used for the investigation and so 5 years old plant introduced at Jammu was chosen for the investigation of high alpha cellulose content.

Hibiscus cannabinus[64], belonging to the family Malvaceae is an erect herb with straight, slender, glabrous or prickly stem 8-12 feet high. It is indigenous to India and is cultivated mainly as a fibre crop. The plant is adoptable to different soils and climates and has vigorous growth and maturation rate. It has cellulose 88.2%, alpha cellulose 61.6% and hemicellulose 14.2%[65]. The pulping test[66] had shown that the fibre can be cooked with 15% caustic soda solution. Paper pulp[67] had been produced sucessfully from this plant. The above data had given impetus to work out this plant for high grade alpha cellulose. Whole seeds after milling and hydraulic pressing yielded 13% fatty oil. The fatty acid analysis[68] indicated palmitic 17.5%, stearic 45%, linoleic 25.5% and epoxyoleic acid 7% and the oil from some other geographical source[69] indicated decanoic 5.84%, palmitic 29.9%, stearic 3.08%, oleic 25.77% and linoleic acid 31.7%. Thus literature revealed that the composition of the oil varies with the geographical source and so the seeds

collected from H. cannabinus grown in Jammu region were extracted for oil which was subjected to analysis.

The plants taken for the high alpha cellulose investigation were not debarked based on the literature[70] evidence that increase in fibre yield is possible with the use of unbarked wood chips and also strength, brightness and contaminat levels remained comparable with debarked chips.

CHAPTER II : PREPARATION OF HIGH ALPHA CELLULOSE OR RAYON GRADE PULP.

PREPARATION OF HIGH ALPHA CELLULOSE OR RAYON GRADE PULP

The following fast growing plants were chosen for this study:

1. Crotalaria juncea
2. Crotalaria retusa
3. Crotalaria burhia
4. Sesbania sesban
5. Sesbania roxburghii
6. Leptadenia pyrotechnica
7. Populus casale I-488
8. Lantana camara
9. Broussonetia papyrifera
10. Hibiscus cannabinus

The plant materials 1, 2, 4, 5, 7, 9 and 10 were collected from the experimental farm of Regional Research Laboratory, Jammu-Tawi whereas numbers 3 and 6 were collected from areas around Pilani in Rajasthan. Number 8 material was collected from the regions around Jammu where it is abundant.

A. Analysis of Wood:

All the plant materials were air dried and 40-60 mesh powders were used for the determination of ether

extractives[71], alcohol-benzene extractives[72], hot water extractives[73] and holocellulose[74], lignin[75], pentosans[76] and ash contents as per TAPPI standards. For ether extractives, soxhlation of the powdered materials with diethyl ether were done. These values are measure of waxes, fats, resins, phytosterol and non-volatile hydrocarbons. For alcohol-benzene extractives, the extractions with alcohol-benzene mixture (1:2) were done by soxhlation. These values are measure of waxes, fats, resins and certain other ether insoluble components such as gums and other water soluble components. The hot water extractives are measure of organic salts, sugars, gums, protein like materials, galactans, tannins and pigments in the wood. The holocellulose was estimated[79] by heating the powdered materials with sodium chlorite and acetic acid on water bath for one hour, washing the materials followed by drying and weighing. For lignin estimation, air dried samples were kept with 15 ml of 72% sulphuric acid for two hours at 18 to 20°C. The contents were diluted with 500 ml of water, refluxed for 4 hours, filtered through Gooch crucibles and the residues dried at 105°C ± 3°C to constant weights and weighed. For pentosan estimation, the powdered materials were distilled with strong hydrochloric acid to distill off the furfural formed from pentosan. The furfural were precipitated with

phloroglucinol. The weights of the furfural phloroglucide were found out as usual using Gooch crucibles. The amounts of pentosan were estimated using the following formulae:

Pentosan = (a + 0.0052) f where a = wt. of the precipitate in grams and f = 0.985 if a is less than 0.03 gram, = 0.887 if a is less than 0.03 and 0.3, = 0.882 if a is more than 0.3 gram. For ash estimation, the powdered materials were ignited in tarred crucibles in a muffle furnace at 575°C for 3 hours, cooled and weighed. The results are given in Table 1.

Table 1 : Analysis of the Stems of Plant Materials.

Analysis	1.a	1.b	1.c	2	3	4	5	6	7	8	9	10
Ether extractive (%)	1.36	1.19	1.02	0.75	0.65	2.30	2.20	0.42	1.20	0.63	0.22	0.53
Alcohol-benzene extractive (%)	4.60	4.90	4.86	2.86	2.32	6.80	7.30	1.87	3.00	2.21	2.00	0.63
Hot water extractive (%)	6.80	7.30	7.45	5.90	6.80	8.00	8.60	10.80	3.20	7.02	5.20	5.60
Pentosan content (%)	21.00	20.90	20.50	20.60	16.70	22.00	25.40	21.50	23.00	22.00	20.10	1 .00
Lignin content (%)	23.90	24.80	24.00	26.00	20.10	25.70	25.50	18.00	1 .30	21.30	18.50	1 .30
Holocellulose content (%)	76.00	76.00	76.80	74.00	75.60	72.50	74.20	78.00	77.00	7 .60	79.00	7 .00
Ash content (%)	0.98	1.00	1.30	1.30	0.62	1.58	0.90	0.30	1.20	1.15	0.47	0.36

1.a Crotalaria juncea (1½ months old) 1.b C. juncea (3 months old) 1.c C. juncea (7 months old)

2. C. retusa (6½ months old) 3. C. burhia (wild plant) 4. Sesbania sesban (1 year old) 5. Sesbania roxburghii (1 year old) 6. Leptadenia pyrotechnica (wild) 7. Populus casale 1-488 (5 years old)

8. Lantana camara (wild) 9. Broussonetia papyrifera (1½ years old) 10. Hibiscus cannabinus (6 months old).

. Prehydrolysis of plant materials to determine conditions for maximum removal of pentosans:

The stems of the plant materials were chopped to small pieces and hundred gram samples were immersed in water keeping the material-water ratio 1:10 and autoclaved at 165°C for ½, 1, 1½, 2 and 2½ hours respectively. The time needed for the temperature to rise to 165°C was noted in each case. The yield of the materials and the pentosan contents[76] after prehydrolysis and the pH of the mother liquor were also ascertained and are given in Table 2. The main objective of the prehydrolysis was to reduce the pentosan to maximum possible extent without causing degradation of alpha cellulose.

Table 2 : Analysis of Plant Materials after Prehydrolysis.

Temperature = 165°C Time to rise 165°C in the autoclave = 1 hr. approximately.

Plant	Time(hrs)	Yield of Prehydrolysed materials(%)	Pentosan(%)	pH*
1.a				
a	0.5	91.0	15.5	5.6
b	1.0	87.3	14.0	5.6
c	1.5	84.6	16.3	5.7
d	2.0	76.3	8.8	5.7
e	2.5	75.0	8.5	5.8
1.b				
a	0.5	95.0	15.2	5.3
b	1.0	92.0	13.3	5.3
c	1.5	89.0	9.5	5.3
d	2.0	80.0	8.6	5.4
e	2.5	79.0	8.5	5.3
1.c				
a	0.5	96.3	13.3	4.8
b	1.0	93.5	12.4	4.7
c	1.5	89.0	9.3	4.8
d	2.0	76.3	8.5	4.6
e	2.5	76.0	8.4	4.3

Table 2 contd.

Plant	Time(hrs)	Yield of Prehydrolysed materials(%)	Pentosan(%)	pH*
2				
a	0.5	94.8	15.6	3.9
b	1.0	90.8	12.8	3.8
c	1.5	88.0	10.6	3.9
d	2.0 ✓	77.0	8.3	3.6
e	2.5	76.8	8.2	3.5
3				
a	0.5	91.1	13.3	3.9
b	1.0	88.8	12.4	3.9
c	1.5	87.7	12.0	3.9
d	2.0 ✓	77.6	9.3	3.0
e	2.5	73.3	9.5	3.1
4				
a	0.5	97.4	21.0	5.3
b	1.0	95.0	19.2	5.8
c	1.5	87.0	19.3	5.3
d	2.0 ✓	85.0	9.3	5.3
e	2.5	75.0	9.3	5.3

contd..

Table 2 contd.

Plant	Time (hrs)	Yield of prehydrolysed materials (%)	Pentosan (%)	pH*
5				
a	0.5	96.3	22.2	5.9
b	1.0	89.6	20.6	5.8
c	1.5	82.0	18.0	5.3
d	2.0 ✓	75.0	9.3	5.3
e	2.5	67.0	9.0	5.2
6				
a	0.5	91.1	13.3	5.8
b	1.0	88.8	12.4	5.7
c	1.5	87.7	12.0	5.6
d	2.0 ✓	77.6	9.3	5.6
e	2.5	73.3	9.5	5.5
7				
a	2.0	91.0	15.2	5.0
b	3.0 ✓	90.0	13.9	4.2
c	3.5	89.0	13.2	4.2

contd..

Table 2 contd.

Plant	Time(hrs)	Yield of Prehydrolysed materials(%)	Pentosan(%)	pH*
8				
a	0.5	96.0	15.0	4.7
b	1.0	87.0	9.5	4.7
c	1.5	80.0	8.4	4.6
d	2.0 ✓	79.0	8.4	4.5
e	2.5	76.0	8.2	4.2
9				
a	0.5	93.0	14.8	3.8
b	1.0	85.0	13.7	3.8
c	1.5	79.0	10.8	3.7
d	2.0 ✓	75.6	9.0	3.6
e	2.5	73.4	8.8	3.6
10				
a	0.5	95.0	16.2	4.9
b	1.0	83.0	10.8	5.0
c	1.5	80.0	10.5	4.5
d	2.0 ✓	79.0	8.7	4.5
e	2.5	76.0	8.2	4.5

* is for the liquid obtained after hydrolysis in the autoclave.

It was found that the optimal conditions needed for prehydrolysis was autoclaving at 165°C for 2 hours in the case of Crotalaria juncea, C. retusa, C. burhia Sesbania sesban, Sesbania roxburghii, Leptadenia pyrotechnica, Lantana camara, Broussonetia papyrifera and Hibiscus cannabinus and for 3 hours in the case of Populus casale I-488. and are tick marked (✓) in Table 2.

C. Kraft pulping of the prehydrolysed material to determine conditions for maximum removal of lignin:

The prehydrolysed samples were kraft (sulphate) pulped in an autoclave at 165°C for 4 hours using different percentages of kraft chemicals NaOH:Na_2S in the ratio of 3:1 based on oven dry weight of the materials. The material:liquor ratio was kept at 1:10. The pentosan[76], lignin[77], holocellulose[74] contents, permanganate number[78] (number of millilitres of tenth normal potassium permanganate solution consumed by one gram of moisture free pulp) and yields of the sulphate pulps were determined and given in Table 3. From the permanganate number, bleach requirements for hundred grams of prehydrolysed kraft pulps were calculated using the conversion table given in TAPPI standard. The optimal requirement of kraft chemicals needed for the prehydrolysed materials with less pentosan contents were tick marked (✓) in Table 3.

Table 3 : Analysis of material after Kraft pulping.

Temperature in autoclave = 165°C Duration = 4 hours.

Time to rise 165°C in the autoclave = 1 hr approximately.

Plant	Kraft chemicals* (%)	Pentosan (%)	Lignin (%)	Holo cellulose (%)	$KMnO_4$ No.	Yield of kraft pulp* (%)
1.a						
a	20	13.0	8.0	88.0	17	30.0
b	20	10.2	8.5	90.0	16	30.0
c	20	8.8	7.8	92.2	17	29.6
d	20 ✓	6.3	7.5	93.3	16	27.2
e	20	6.0	7.3	94.6	16	26.2
1.b						
a	20	13.3	8.0	90.0	17	32.0
b	20	9.4	8.5	91.5	18	30.0
c	20	8.4	7.8	93.0	17	29.5
d	20 ✓	6.4	7.5	94.0	17	27.2
e	20	6.3	7.3	94.6	17	26.2
1.c						
d	20	6.4	8.3	94.0	14	28.0
d	22 ✓	6.5	6.5	95.6	12	26.8

contd..

Table 3 contd.

Plant	Kraft chemicals* (%)	Pentosan (%)	Lignin (%)	Holocellulose (%)	$KMnO_4$ No.	Yield of kraft pulp** (%)
2						
d	21	5.3	7.6	91.6	12	25.0
d	22	4.8	6.9	91.8	11	24.8
d	23	4.6	6.8	92.0	12	23.0
d	24 ✓	4.0	4.8	92.5	11	22.3
d	25	4.2	4.7	92.9	10	22.8
3						
d	20	6.4	7.2	85.0	11	24.8
d	22 ✓	5.8	6.9	87.0	10	24.0
d	24	5.6	6.8	88.2	10	23.7
4						
a	20	12.0	10.0	86.0	16	28.0
b	20	13.0	9.0	90.0	17	27.0
c	20	12.3	9.0	92.0	16	26.8
d	20 ✓	9.3	7.8	90.0	15	22.5
e	20	8.4	6.1	93.0	16	20.6

contd..

Table 3 contd.

Plant	Kraft chemicals* (%)	Pentosan (%)	Lignin (%)	Holocellulose (%)	$KMnO_4$ No.	Yield of kraft pulp** (%)
5						
a	20	18.3	10.0	87.0	15	30.0
b	20	15.6	8.0	88.0	15	28.0
c	20	13.0	7.0	90.0	13	26.0
d	20 ✓	8.3	6.5	92.5	13	26.5
e	20	8.3	6.5	94.0	12	26.7
6						
a	20	8.4	4.5	82.0	18	25.0
b	20	6.4	3.5	83.5	16	24.5
c	20	6.3	3.0	90.5	14	24.0
d	20 ✓	6.2	2.9	91.0	13	23.0
e	20	6.2	3.0	91.0	13	18.0
7						
b	20	13.8	7.5	87.0	25	49.2
b	22	13.6	6.9	88.0	20	46.0
b	24	13.0	4.5	88.9	16	43.5
b	26 ✓	13.8	4.3	90.0	14	41.1
b	28	14.7	3.9	90.0	12	40.0

contd..

Table 3 contd.

Plant	Kraft chemicals* (%)	Pentosan (%)	Lignin (%)	Holocellulose (%)	$KMnO_4$ No.	Yield of kraft pulp** (%)
8						
d	25	8.5	4.5	84.0	11	32.0
d	26 ✓	6.0	4.0	87.0	10	30.0
d	27	6.0	4.0	90.0	9	28.0
9						
d	26	7.5	4.5	80.0	9	45.0
d	28 ✓	6.0	3.0	86.0	8	40.0
d	30	5.9	2.8	88.0	8	38.0
10						
d	24	8.2	4.5	80.0	12	40.0
d	26 ✓	8.0	4.0	86.0	11	38.0
d	28	7.5	3.5	86.0	10	36.0

* % of chemicals as sodium hydroxide and sodium sulphide in the ratio of 3:1 based on oven dry weight of prehydrolysed materials.

** expressed as percentage of oven dry raw materials.

4. Bleaching of the prehydrolysed kraft pulp for high alpha cellulose:

All the prehydrolysed kraft pulped samples were bleached by "CEHED" sequence and washed with sulpurous acid. The amounts of chlorine, hypochlorite and chlorine dioxide used for bleaching were 70%, 20% and 10% of the bleach requirement calculated on the basis of permanganate numbers.

In the first stage of bleaching the pulps were treated with chlorine water to get 3% pulp concentration, maintained at 30°C for one hour and washed thoroughly with water. The pulps suspended in water (3% concentration) were treated with 1.5% sodium hydroxide (based on oven dry weight of the pulp) kept at 70°C for one hour and washed throughly with water in the second stage. In the third stage the pulps were treated with sodium hypochlorite, diluted with water to get 3% pulp concentration maintained at 30°C for one hour and washed with water. The fourth stage was extraction stage similar to the second stage. In the fifth stage the pulps were treated with sodium chlorite and acetic acid, water was added to get 3% pulp concentration and the admixture maintained at 80°C for one hour and washed thoroughly with water followed by sulphurous acid treatment to stabilize the brightness.

2. Analysis of the high alpha cellulose pulps as per TAPPI specifications:

The high alpha cellulose pulps were analysed for alpha cellulose[79], lignin[77], ash, pentosan[76], copper number[80], brightness[81], viscosity[82] and the yield. The results are given in Table 4.

Alpha cellulose estimation:

Cellulose pulp consists of two arbitrarily defined carbohydrate fractions : the alpha fraction of high molecular weight which remains when a mixture of pulp and 8.3% sodium hydroxide solution is filtered after swelling the fibres in 17.5% sodium hydroxide solution and the hemicellulose fraction which contains short chain molecules and which remains in solution after the above treatment.

Three grams of pulp was treated with 75 ml of 17.5% sodium hydroxide solution for 45 minutes. The concentration of sodium hydroxide was then reduced to 8.3% and the mixture treated for another 30 minutes. The alpha cellulose was separated by filtration, washed, dried and weighed. The amount of alpha cellulose was calculated as a percentage based on moisture free pulp. The results were corrected by deducting ash and lignin contents of the pulp.

Copper number estimation:

The copper number is defined as the number of grams of the mettalic copper in the Cu_2O resulting from the reduction of copper sulphate by 100 gms of the pulp fibre. It is a measure of degradation of celluloses in pulp. For the estimation, Hagglund method of estimation was followed.

Brightness evaluation(%):

The brightnesses of the pulp samples were measured in a standard "reflection meter" in which magnesium oxide was used as the standard for brightness. The preparation of the test specimen and the measurement of brightness of the samples were done as per the TAPPI standards.

Viscosity determination:

A sample containing the calculated amount of dry pulp or cellulose was weighed and transferred to a glass or polyethylene container and fitted with a stopper or screw cap. Distilled water (25 ml) was added and the container was closed and shaken to wet out and disperse the sample. The air was swept from the container with a stream of nitrogen and without stopping the flow of nitrogen, 25 ml of cupriethylenediamine solution having 1M copper and 2M ethylenediamine was added. The container was closed tightly and shaken vigorously until the cellulose was completely dissolved. The viscosity of the

solution was measured in a falling ball viscometer. The viscosity 'y' was calculated using the formula : $y = t(\rho_1 - \rho_2)k$ where t = time expressed in seconds, ρ_1 = density of the ball g/cm^3, ρ_2 = density of the liquid g/cm^3 and k = instrument constant. By using the conversion table by Browning[7], the intrinsic viscosity $[\eta]$ of the samples were determined. The degree of polymerisation (DP) of the samples could be found approximately by the formula $DP = [\eta] \times 190$ and the average molecular weight by the formula : $M.Wt = DP \times 162$.

Table 4 : Analysis of the bleached pulps.

Plant	Alpha cellulose (%)	Lignin (%)	Ash (%)	Pentosan (%)	Copper No.	Brightness (%)GE	Yield* of pulp (%)	Viscosity**
1.a								
a	84.0	2.6	0.8	6.8	0.96	65.0	20.4	-
b	85.6	2.7	0.7	6.2	0.76	70.0	19.6	-
c	90.0	2.0	0.7	5.8	0.76	73.0	20.4	-
d	92.3	1.0	0.7	4.6	0.89	75.0	22.2	-
e	94.7	1.0	0.4	4.3	1.08	80.0	22.5	-
1.b								
a	85.0	2.5	0.8	6.2	0.90	63.0	23.4	-
b	87.3	2.8	0.6	6.9	1.00	63.0	23.4	-
c	91.6	2.0	0.5	5.9	1.00	76.0	23.8	-
d	94.7	2.8	0.5	9.1	1.14	80.0	22.5	-
e	85.2	1.0	0.5	3.7	1.25	82.0	22.6	-
1.c								
d	94.6	1.0	0.4	6.4	1.08	82.0	23.4	-
d	96.2	1.0	0.4	6.0	1.08	82.0	19.8	-

contd...

Table 4 contd.

Plant	Alpha cellulose (%)	Lignin (%)	Ash (%)	Pentosan (%)	Copper No.	Brightness (%)GE	Yield* of pulp (%)	Viscosity**
2								
d	93.0	2.2	0.7	3.3	0.76	80.0	20.0	-
d	93.2	2.0	0.6	3.5	0.76	78.0	20.4	-
d	94.6	1.0	0.5	2.8	0.89	79.0	19.3	-
d	95.2	1.0	0.5	2.5	1.08	91.0	19.4	-
d	95.7	1.0	0.5	2.5	1.08	82.0	19.2	-
3								
d	93.0	1.0	0.5	6.2	0.76	73.0	20.0	4.0
d	94.2	1.0	0.4	5.9	0.89	72.0	21.0	4.6
d	95.6	1.0	0.4	5.2	1.14	80.0	21.0	4.2
4								
a	78.0	4.0	1.1	3.2	0.50	73.0	23.6	4.8
b	84.0	1.5	1.8	3.5	0.80	73.0	22.9	4.6
c	92.0	2.6	1.2	3.0	0.90	72.0	23.0	4.8
d	94.0	2.8	1.3	3.2	1.14	78.0	19.8	4.8
e	94.4	1.6	0.3	2.7	1.21	80.0	18.7	4.6

contd...

Table 4 contd.

Plant	Alpha cellulose (%)	Lignin (%)	Ash (%)	Pentosan (%)	Copper No.	Brightness (%)GE	Yield* of pulp (%)	Viscosity**
5								
a	78.0	1.0	0.9	10.3	0.60	74.0	20.6	4.8
b	82.0	0.9	0.9	9.0	0.60	78.0	21.6	5.0
c	90.0	0.3	0.9	8.7	0.80	81.0	21.7	5.0
d	93.0	0.2	0.3	4.0	1.08	75.0	19.4	4.9
e	93.8	0.2	0.3	3.8	1.04	77.0	18.5	4.8
6								
a	76.0	3.8	0.2	6.3	0.64	73.0	16.7	4.3
b	82.0	2.0	0.3	6.4	0.76	72.0	15.5	4.0
c	94.0	0.2	0.2	3.7	0.89	75.0	19.4	3.8
d	94.3	0.2	0.2	2.7	1.14	75.0	19.8	3.1
7								
b	89.0	0.1	1.1	7.9	0.50	81.0	44.6	5.8
b	82.5	0.1	0.7	6.9	0.50	82.0	42.3	5.3
b	94.5	0.1	0.6	6.9	0.64	82.0	37.4	5.5
b	94.5	0.1	0.5	6.4	0.76	85.0	34.7	5.6
b	93.0	0.1	0.5	6.9	0.89	85.0	33.6	5.8

contd..

Table 4 contd.

Plant	Alpha cellulose (%)	Lignin (%)	Ash (%)	Pentosan (%)	Copper No.	Brightness (%)GE	Yield* of pulp (%)	Viscosity**
8								
d	94.0	1.0	0.5	3.5	0.63	83.4	30.6	5.4
d	96.0	0.5	0.4	2.5	1.02	84.4	28.0	4.9
d	94.0	0.5	0.4	4.0	1.02	86.0	25.0	5.9
9								
d	95.0	0.4	0.1	3.5	0.63	90.0	41.0	7.2
d	96.0	0.1	0.1	2.3	1.02	89.0	38.0	7.1
d	90.0	0.1	0.1	4.0	1.02	89.0	35.0	6.7
10								
d	95.0	0.4	0.2	3.5	0.63	90.0	41.0	7.1
d	96.0	0.2	0.2	2.8	1.02	89.0	38.0	7.1
d	96.0	0.2	0.2	4.0	1.02	89.0	35.0	6.7

* yield based on oven dry weight of the raw materials.

** viscosity of 1% solution by falling ball viscometer in centipoises.

If the bleached prehydrolysed kraft pulps pass all the specifications for rayon grade pulp as per TAPPI*, it is suitable for rayon grade pulp provided it should pass filterability test. Those pulps qualify only for alpha cellulose as against TAPPI specifications, it is recommended for high alpha cellulose pulp of chemical grade.

The pulps obtained from C. juncea (1.5 months, 3 months, and 7 months old), C. retusa, C. burhia, S. sesban, S. roxburghii, Leptadenia pyrotechnica, L. camara, H. cannabinus and P. casale I-488 were found to be suitable raw materials for high alpha cellulose whereas the pulp obtained from B. papyrifera was found suitable for rayon grade pulp.

---

* Alpha cellulose atleast 88%, less than 5% pentosan, ash 0.15%, lignin 0.15%, brightness 85% GE, viscosity of 1% solution 5 - 25 centipoise and copper No. 1 - 1.25.

CHAPTER III : PREPARATION OF MICROCRYSTALLINE CELLULOSE (MCC) FROM FAST GROWING PLANTS.

PREPARATION OF MICROCRYSTALLINE CELLULOSE (MCC) FROM FAST GROWING PLANTS.

Microcrystalline cellulose (MCC) is a very pure form of cellulose and is almost free from organic and inorganic impurities. It is a partially hydrolysed "level off DP cellulose" which is subsequently disintegrated mechanically and dried to form a colloidal flour. The pioneer worker Battista[83] had made through investigations and recommended for a number of applications. Besides that German and USA workers investigated its application in the field of nutrition, pharmacy and beverages. When tablets are made out of it, they retain their properties in humid atmosphere but instantly release their active components in contact with water. Products which are improved by the unique properties of MCC include tablets, stable dispersions, emulsions and gels. The most important use of MCC in pharmaceutical industry is as a tableting aid because of its inherent excellent binding, disintegrant and lubricating properties. MCC has been used as adsorbent in chromatography. In short MCC is a gift to pharmaceutical, food and cosmetic industries because of its inertness, freedom from organic and inorganic impurities and non-toxicity. Literature survey indicated that MCC can be made from soft wood

and hard wood high alpha cellulose pulps, prepared from waste materials such as saw dust and paper wastes. The MCC has been prepared from bamboo[84] (Dendrocalamus hamiltonii), Ekra read[84] (E. ravennea), staple cotton fibre[84] and news print waste[85]. The MCC from cotton was evaluated as a tablet excipient in comparison with modified starch and polyvinyl pyrrolidine[86]. Keeping in view the vast industrial utility of MCC, the high grade alpha cellulose pulp prepared from ten fast growing plants mentioned were converted into MCC.

Materials:

The high alpha cellulose prepared from the plants as per Chapter II were used for preparation of micro-crystalline cellulose.

Methods:

The high alpha cellulose was refluxed with 2.5N hydrochloric acid keeping the solid liquor ratio 1:40 to get hydrocellulose or "levelling off DP cellulose"[83]. The mechanical disintegration in water followed by drying was skipped. Instead the hydrocellulose was washed and mechanically disintegrated in alcohol-acetone (50:50) medium in a Hobert mixer at 3000 r.p.m. for half an hour. This product was filterable through

filter press and the filtered solvent could be reused for the subsequent batches. The MCC thus prepared was dried at room temperature and yielded a milky white product.

The MCC obtained was analysed for molecular weight[82], moisture content, organic solvent extractives, ash content[87], contents of calcium[88], chlorides[87], iron[87], copper[88], arsenic[68], lead[90] and sulphate[87]. Average diameter of the microcrystals were found by electron microscopy with the scanning electron microscope (SEM) set with low beam accelerating voltage 7.5 kv and given in Fig. 2, 3, 4 & 5. The micrographs of MCC were obtained from Tropical Products Institute, London. The bulk density[89], yield and other general properties are also reported. All the analytical data are given in Table 5 & 6.

Experimental Data:

a) Table 5 : Viscosity, Average Molecular Weight and Yield of the MCC Obtained:

S.No.	Plant	Intrinsic viscosity (η)	DP = (η)x90	Average M. wt. DP x162	Yield (%)
1	S. sesban	1.00	190	30780	70
2	S. roxburghii	1.00	190	30780	70
3	C. juncea	1.25	238	38556	75
4	C. retusa	1.25	238	38556	74
5	P. casale I-488	1.50	285	46670	75
6	C. burhia	1.00	190	30780	72
7	L. pyrotechnica	1.00	190	30780	72
8	L. camara	1.25	238	38556	73
9	H. cannabinus	1.25	238	38556	74
10	B. papyrifera	1.50	285	46670	75

b) Table 6 : Ash, Anion, Cation Contents and Bulk Density of MCC Obtained:

S. No.	Ash	Sulphated ash	Ca ppm	Cu ppm	Fe ppm	As ppm	Pb ppm	Cl^- (%)	SO_4^{--}	Bulk density g/litre
1	0.04	0.07	40	<4	<10	Nil	Nil	<0.035	<0.06	320
2	0.04	0.08	30	<4	<10	Nil	Nil	<0.035	<0.06	310
3	0.03	0.06	40	<4	<10	Nil	Nil	<0.035	<0.06	320
4	0.05	0.06	35	<4	<10	Nil	Nil	<0.035	<0.06	320
5	0.03	0.06	40	<4	<10	Nil	Nil	<0.035	<0.06	320
6	0.03	0.07	20	<4	<10	Nil	Nil	<0;035	<0.06	300
7	0.03	0.07	20	<4	<10	Nil	Nil	<0.035	<0.06	300
8	0.05	0.09	30	<4	<10	Nil	Nil	<0.035	<0.06	350
9	0.04	0.07	40	<4	<10	Nil	Nil	<0.035	<0.06	300
10	0.03	0.06	30	<4	<10	Nil	Nil	<0.035	<0.06	330

c) Average diameter of the microcrystals of MCC obtained by electron micrographs: For evidence, only the micrographs of MCC obtained from C. burhia, P. casale I-488, E. papyrifera and H. cannabinus are given in Fig. 2, 3, 4 & 5 respectively. The micrograph of a sample of high alpha cellulose is also given in Fig. 1 for comparison.

Fig. 1 Alpha cellulose.

Fig. 2 MCC.
Average diameter 4 µ.

Fig. 3 MCC.
Average diameter 6 µ.

Fig. 4 MCC.
Average diameter 3 µ.

Fig. 5 MCC.
Average diameter 3.5 µ.

a) All the samples were milky white, odourless, insoluble and dispersible in water. They were partially soluble and showed swelling in dilute alkalies, were insoluble and resistant to dilute acid, inert organic solvents and oils. Almost 98% of the samples passed through 100 mesh sieve and 65% through 300 mesh sieve when sieved on a mechanical stirrer for one hour. The alcohol soluble extractives in all were less than 0.05%.

From a review of the properties* of MCC samples it was evident that the same compared with the marketed varieties of MCC. Hence it was concluded that the high alpha celluloses prepared from S. sesban, S. roxburghii, C. juncea, C. retusa, P. casale, I-488, C. burhia, L. pyrotechnica, L. camara, H. cannabinus, B. papyrifera could be suitable materials for commercial production of MCC.

---

* Battista et al[83] prescribed the commercial MCC has molecular weight 30000 to 50000, moisture less than 5%, organic solvent extractibles less than 0.05%, ash less than 0.05%, calcium less than 40 ppm, chlorides less than 50 ppm, iron less than 10 ppm, and copper less than 4 ppm. The average diameter of the micro crystals ranged from few thousand Angstrom to ten microns. The British standard (BPC) specifies appearence should be clear white. It should have sulphated ash less than 0.1%, arsenic less than 0.35% and sulphate less than 0.06%.

[illegible] IV : [illegible] OIL [illegible] CF Crotalaria juncea, Crotalaria retusa, Sesbania roxburghii AND Hibiscus cannabinus.

[illegible] SEEDS OF Crotalaria juncea, Crotalaria retusa, Sesbania roxburghii and Hibiscus cannabinus

Oils and fats, besides their indispensable use in cooking, find a number of applications in soap making, candle making and in pharmacy as solvents for intramuscular injections, as ointment bases and to prepare oil derivatives as pharmaceutical additives. Search for seed oils is an active field of investigation for nutritive purpose and industrial applications. The seeds of the plants C. juncea, C. retusa, S. roxburghii, H. cannabinus were chosen for screening.

Methods:

a) Cleansed seeds were ground in a mixi and extracted with hexane for about 18 hours in a Soxhlet extractor. The resultant extract were filtered and dried over anhydrous sodium sulphate. The bulk of the solvent was distilled off under reduced pressure and last traces of solvent removed by passing nitrogen gas. The yields are reported as percentages of the weight of the seeds. (Table 7).

b) Physical characteristic such as color, refractive index (Abbe's refractometer), apparant specific gravity (using pyknometer), and optical rotation (Nilgar polarimeter) were found out and are reported in Table 7.

c) Acid value[87](A.V): To about 2 gms of accurately weighed oils 50 ml of 1:1 v/v mixture of neutral ethylalcohol and n-hexane was added. The admixture was boiled for 2 minutes and titrated against 0.1N alcoholic potassium hydroxide using phenolphthalein as the indicator. The acid values were calculated by the following formula:

$$A.V = \frac{56.1 \times a \times N}{p}$$

where a = ml of alcoholic KOH, N = normality of alcoholic KOH and p = wt. of the sample in gms. Results are given in Table 7.

d) Hydroxyl Value[87](H.V): Two grams of accurately weighed oil samples were heated at 100 ± 5°C in reflux set ups for 1 hours with 50 ml of acetylating reagent (25 gms of acetic anhydride made up to 100 ml with dry pyridine). Then 2 mls of distilled water were added along the inner sides of the condenser to wash down the aceticanhydride which was inturn washed down with alcohol. The contents of the flask were titrated against standard alcoholic KOH using phenolphthalein as the indicator and at the same time a blank was run.

The hydroxyl values were calculated using the formula:

$$H.V. = \frac{56.1 \times N \times t}{w}$$

where

t = Blank titre value - Experimental titre value, N = Normality of alkali, w = Wt. of the oil in gms. Results are given in Table 7.

e) Iodine value (I.V.)[87]: This value was found out by Wijj's method. The oil samples dissolved in dry chloroform or carbontetrachloride were treated with excess known quantities of iodine monochloride (Wijj's reagent) in flasks with air tight stoppers. The flasks were kept in dark for 60 minutes. Then the unreacted iodine monochloride was estimated by iodimetry. Blank determinations were done simultaneously under identical conditions. The iodine values were calculated using the formula:

$$I.V. = \frac{(Vb - Vs) \times N \times 100 \times f}{w}$$

where

Vb = Volume of $Na_2S_2O_4$ solution used in blank, Vs = Volume of the $Na_2S_2O_4$ unused by the oil samples, N = Normality of the $Na_2S_2O_4$ solution, f = Number of gms of iodine equivalent to 1 ml of $Na_2S_2O_4$ solution, w = Weight of the sample in gms.

Results are given in Table 7.

f) HBr titration of the oil: HBr titration is to find whether groups like epoxy, cyclopropane and cyclopropene are present in the oils.

Durbetakis reagent was prepared by diluting 30 ml of "Analar" grade acetic acid hydrobromide (50%) to one litre with "Analar" acetic acid and standardising against anhydrous sodium carbonate. Crystal violet dissolved in glacial acetic acid (0.1% solution) was used as the indicator.

About 0.3 to 0.5 gm of accurately weighed sample of oil in dry benzene was titrated against Durbitakis reagent at -4°C. The percentage of oxirane oxygen was calculated using the following formula:

$$\% \text{ oxirane oxygen} = 1.6 \times \frac{NV}{W}$$

The titration was continued in the same flask at 55°C with stirring for cyclopropene fatty acid. The values were calculated as sterculic acid/malvalic acid using the formulae:

$$\% \text{ sterculic acid} = 29.45 \times \frac{NV}{W}$$

$$\% \text{ malvalic acid} = 28.04 \times \frac{NV}{W}$$

where v is the number of ml of HBr solution of normality N, required for w gms of the sample. Results are given in Table 7.

g) halphen test: This test detects the presence of cyclopropene group in the fatty acids. It was done only on Hibiscus cannabinus oil after getting the positive result on HBr titration.

Eight drops of the oil were mixed with 8 drops of ... Halphen's reagent (1% solution of sulphur in CS_2 diluted with equal volume of alcohol) in a test tube. The contents were heated over a water bath for 45 minutes to remove CS_2. The color change from orange to red indicated the presence of cyclopropene acids. The color change with cottonseed oil was taken as standard under identical conditions.

h) Saponification equivalent (S.E.)[87]: Two to three grams of accurately weighed samples of oils were refluxed with 50 ml of alcoholic KOH solutions on a water bath for one hour and titrated against standard HCl using phenolphthalein as indicator. Blank was run simultaneously. The values were calculated using the formula:

$$S.E. = \frac{1000 \times w}{(Vb - Vc) \times N}$$

where w is the weight of the sample, Vb is the volume of HCl used in blank experiment, Vc is the volume of HCl used in experiment and N = normality of HCl acid. Results are given in Table 7.

i) Estimation of unsaponifiable matter: Two grams of the accurately weighed oil samples were saponified with 100 ml of 0.5N alcoholic solution for 1 - 2 hours. The alcohol was distilled off with occasional additions of water. The contents was then diluted to three times their volumes with distilled water and extracted with peroxide free solvent ether 3 to 5 times successively till the extracts gave no residue on evoporation. The combined ethereal extracts were washed with 5% NaOH solution and subsequently with water till the washings were neutral. The washed extracts were dried over anhydrous sodium sulphate, filtered and distilled to remove the solvent. The weights of the residues after drying at 80°C for one hour were determined and the percentage of unsaponifiable matters calculated and given in Table 7.

j) Thin layer chromatography (T.L.C.): This was done on plates coated with silica gel G. to distinguish between oxygenated and non-oxygenated fatty acids. The non-oxygenated fatty acids move faster than any of the oxygenated fatty acids and give a single spot irrespective

of chain length and unsaturation. The identification of the spots were done with reference to

a) Ground nut oil - a normal oil without any special functional groups.

b) Cotton seed oil - with cyclopropene group.

c) Triveraclin - with epoxy group

d) Castor oil - with hydroxyl group.

Results are given in Table 7.

k) Ultra-violet absorption spectroscopy[92]: The UV spectra were taken in "PYE UNICAM SP8-100" UV spectrometer. About 0.5 to 0.6 gram of accurately weighed oil samples were dissolved in cyclohaxane. Half ml of this solution has diluted with hexane to 25 ml to give a final concentration of 100-120 ug/ml. Ground nut oil peaks were used as the reference. The objective here was to detect conjugated dienes, alpha-beta unsaturated ketone, conjugated dienes or ketonic group. None of the oils showed any special groups as mentioned.

l) Infra-red spectroscopy (IR)[90,91]: For the spectra, "Perkin Elmer 377 Grating IR Spectrometer" was used. Ground nut oil was used as the reference.

The IR spectra with interpretation are given in figures 6,7,8 & 9 for C. juncea, C. retusa, S. roxburghii and H. cannabinus respectively.

Only in the case of H. cannabinus seed oil an additional peak at 1010 cm^{-1} was observed indicating the cyclopropene group.

m) NMR Spectroscopy[92]: The spectra of the oils were taken in Varian T-60A NMR spectrometer. The NMR spectra with interpretations are given in figures 10,11, 12 & 13 for C. juncea, C. retusa, S. roxburghii and H. cannabinus respectively.

Only the seed oil of H. cannabinus gave additional signal at 0.62 τ indicating the presence of cyclopropene group in fatty acid.

n) Gas Liquid Chromatography (GLC): This technique was used for the study of fatty acid composition of the seed fats studied.

The seed fats from C. juncea, C. retusa and S. roxourghii which were normal oils was esterified using CH_3OH/H_2SO_4 to prepare methyl esters of fatty acid for GLC. From the H. cannabinus oil which is having acid sensitive cyclopropene group, the methyl esters of mixed

acids were prepared by transesterification. For the transesterification, one gram of the oil in 50 ml of absolute methanol containing 1% sodium methoxide was refluxed for about 20 minutes and the methylesters were extracted with ether. The methylesters of the oils were treated with 60 ml of absolute methanol saturated with silver nitrate and the reaction was allowed to proceed at room temperature with stirring for 24 hours. Then the contents were diluted with water and extracted with ether. The extracts were dried over anhydrous sodium sulphate and the solvent evaporated in a stream of nitrogen gas.

The methylester samples were analysed using Perkin Elmer 881 gas chromatograph equipped with hydrogen flame ionisation detector. The conditions used were:

Neoplex - 400, 15% supported on chromosorb W as adsorbent

Column temperature : 190°C

Detector temperature : 250°C

Injection temperature: 270°C

Carrier gas : Nitrogen at the flow rate of 25 ml/minute.

Column specifications: Stainless steel, 2 meters long and $\frac{1}{8}$" diameter.

The individual fatty acids were identified with respect to methylester of lin seed oils (Fig. 14) The

chromatograms for other oils are given in figures 15, 16, 17 & 18. The percentage of individual fatty acids were evaluated by the triangular method of peaks from chromatograms given in Table 8.

The yield of seed oil of H. cannabinus was 20%. This indicated that this is one of the potential sources of seed oils for soap and candle manufacture. The GLC data showed that oils of C. juncea, C. retusa and S. roxburghii are rich in linoleate whereas H. cannabinus oil was rich in palmitate. The H. cannabinus oil also has cyclopropene fatty acid.

Experimental Data:

Table 7 : Physicochemical Characteristics of the Seed Oils.

Analysis	C.juncea	C.retusa	S.roxburghii	H.cannabinus
a) Color & yield	Dark yellow 3.73%	dark green 3.8%	yellowish brown 3.78%	light yellow 20%
b) $n_D^{25^\circ}$	1.4551	1.4551	1.4540	1.4563
c) Specific gravity	0.8884	0.9086	0.9218	0.9182
d) Optical rotation	Zero	Zero	Zero	Zero
e) Acid value	1.33	4.93	3.29	1.51
f) Hydroxyl value	Nil	Nil	Nil	Nil
g) Iodine value (I.V.)	100.2	116.8	189.1	117.5
h) HBr Titration at 4°C	Zero	Zero	Zero	Zero
at 55°C	Zero	Zero	Zero	0.3 ml*
i) Halphen test	Negative	Negative	Negative	Positive
j) Saponification equivalent	282.6	264.5	296.0	300.0
k) Unsaponifiable matter (%)	2.93	2.68	12.63	2.18
" I.V.	2.07	1.89	0.55	1.56
l) TLC**	← No oxygenated fatty acid →			
	Normal	Normal	Normal	Normal
m) UV Spectra	Normal	Normal	Normal	Normal + peak at 1010 cm^{-1}.
n) IR Spectra (Fig.6,7,8 &9)	Normal	Normal	Normal	Normal + signal at 0.62τ
o) NMR Spectra (Fig.10,11,12 & 13)				

* This is equivalent to 2.61% of cyclopropene fatty acid expressed as sterculic acid.

** Hexane:Diethyl ether:Glacial acetic acid = 70:30:1 as solvent system. The spray is chromic acid.

Table 8 : Component Fatty Acids of the Seed Oils by GLC (Fig. 15, 16, 17 & 18).

Methyl esters of component fatty acids.	S.juncea	C.retusa	S.roxburghii	H.*cannabinus
1) 11 : 0 Undeconate	Traces	Traces	Nil	Nil
2) 12 : 0 Laurate	Traces	Traces	Traces	Nil
3) 13 : 0 Tridecanate	1.16	2.03	Traces	10.90
4) 14 : 0 Myristiate	0.45	0.93	Traces	Nil
5) 15 : 0 Pentadecanate	0.63	1.12	Traces	6.36
6) 16 : 0 Palmitate	22.77	36.67	15.30	38.97
7) 16 : 1 Isomerate	Nil	Nil	Traces	Nil
8) 17 : 0 Margarate	Nil	0.89	Traces	Nil
9) 18 : 0 Stearate	4.15	1.30	2.35	Traces
10) 18 : 1 Oleate	15.07	13.68	15.06	37.70
11) 18 : 2 Linoleate	52.51	40.64	63.15	
12) 18 : 3 Linolenate	3.20	2.69	4.13	Nil
13) 20 : 0 Arachidate	Traces	Traces	Nil	Nil

* H. cannabinus seed oil has cyclopropene fatty acid around 4.68% expressed as sterculic acid in addition to the above mentioned fatty acids.

Chemistry : [illegible]

Of Leptadenia pyrotechnica.

...Isolation of LP-I from AERIAL PARTS OF Leptadenia pyrotechnica:

The aerial parts of L. pyrotechnica were coarsely powdered and extracted with ethanol in a soxhlet for 60 hours. The extract was distilled under reduced pressure to remove the solvent and the left behind residue was taken in dilute sulphuric acid (5%) and filtered. The acid insoluble residue was chromatographed over silica gel and petroleum ether (40°C-60°C), benzene, chloroform, ethyl acetate and alcohol as eluants. Each fraction was rechromatographed further to isolate phytoprinciples. After rechromatography only the benzene fraction yielded three crystalline principles which were identified as taraxerol (LP-I), beta sitosterol (LP-II) and fernenol (LP-III). Since LP-I and LP-III were isolated for the first time in the genus Leptadenia, detailed structural elucidation had been done by physical and chemical methods.

A) Identification of LP-I:

1. Solubility: The compound was soluble in petroleum ether, benzene, chloroform and insoluble in alcohol, hot water, 5% solution of sodium hydroxide, 5% sodium bicarbonate solution and in concentrated sulphuric acid.

2. Optical rotation: $[\alpha]_D$ 0° in chloroform

3. Elements[93]: Nitrogen, halogens and sulphur were absent.

4. Elemental Analysis: Analysis of the compound for elements gave the following values:

C = 84.46%; H = 11.79% and O = 3.75%.

5. Detection of Functional Groups:

a) Free Carboxylic Group[93]: The compound was insoluble in sodium hydroxide solution. This indicated the free carboxylic group was absent.

b) Phenolic Hydroxy Group[93]: The compound dissolved in alcohol did not give violet colour with neutral ferric chloride solution. This indicated the phenolic hydroxy group was absent.

c) Xanthate Test for Free Alcoholic Group[94]: 10 mgs. of the compound mixed with 50 mgs of KOH was heated in a test tube, cooled and few drops of ether added followed by dropwise addition of carboondisulphide. The development of yellow turbidity indicated the alcoholic group.

d) Free Carboonyl Group[93]: The compound did not (i) reduce Fehling's solution and (ii) give precipitate with 2,4-dinitrophenylhydrazine solution and so free carbonyl group was absent.

e) Ether Linkage:

Gibb's Test[95]: An alcoholic solution of the compound was treated with 10% H_2SO_4 (.2 ml), concentrated H_2SO_4 (2.0 ml) and gallic acid 1% solution in alcohol (.1 ml) and warmed on a water bath for 30 minutes. Absence of green colour indicated that no methylenedioxy group is present.

Vitali Test[96]: A little of the compound was taken in a test tube and treated with 2 drops of concentrated H_2SO_4/ 2 drops of benzoylperoxide in benzene. The inside of the stopper was charged with a drop of chromotropic acid - H_2SO_4 mixture and inserted in the tube containing the compound. The tube was heated in a paraffin bath at 170°C for 10 minutes. The suspended drop was wiped on a spot plate. The absence of violet colour indicated that no methylenedioxy group is present.

f) Ester Linkage:

Hydroxmate Test[94]: About 5 mg of the compound was added to 1% hydroxylamine hydrochloride (0.2 ml) in methanol in a test tube. To this 2N KOH in methanol was added till the contents were alkaline. The tube was heated to boiling, cooled and then 2N HCl was added to make it acidic. Absence of bluish purple colour on addition

of a drop of 10% ferric chloride solution indicated that ester linkage is absent.

g) Epoxide linkage[7]: To a drop of the solution of compound in dioxane one drop of potassium periodate solution containing silver nitrate was added in a black spot plate. Absence of turbidity indicated that epoxide linkage is absent.

h) Unsaturation: About 2 mg of the compound dissolved in neutral alcohol was treated with 2% solution of $KMnO_4$ and warmed on a water bath. The disappearence of permanganate colour indicated unsaturation in the compound.

The compound (2 mg) in neutral alcohol was treated with 1% solution of tetranitromethane (2 drops) in neutral alcohol. The appearence of yellow colour indicated the presence of double bond.

i) Test for sterol:

Liebermann-Burchard Test[98]: The compound (2 mg) in $CHCl_3$ was treated with few drops of acetic anhydride followed by addition of concentrated H_2SO_4. Development of play of colours indicated sterol type of compounds.

6. I.R. Spectral Data:

The I.R. (in $CHCl_3$) absorption bands and the assignments of various groups are given in Table 9.

Table 9 : I.R. Spectral Data of LP-I.

cm^{-1}	Assigned Groups
3650	OH stretching
1100, 1260, 1330	secondary OH bending and C-O stretching.
3030	C-H stretching alkene trisubstituted.
820	C-H bending alkene trisubstituted.
1660	C-C stretching alkene trisubstituted.

7. N.M.R Spectral Data:

The N.M.R spectrum of LP-I is given in Fig. 19 and had given the signals as given in Table 10.

Table 10 : N.M.R Signals of LP-I.

δ	No. of protons	Multiplicity	Protons assignment.
0.75, 0.95, 1.05, 1.4 - 1.6.	48	-	CH_3-CH_2 protons.
3.2 - 3.4	1	m	>CH-OH.
5.25 - 5.60	1	m	vinyl proton.

In the PMR spectrum of the compound after D_2O exchange (given in Fig. 20), the signal 3.2 - 3.4 δ disappeared and hence confirmed the presence of OH group.

. Mass Spectral Data[103,104]: The mass spectrum of LP-I is given in Fig. 21. It showed a prominent M^+ at 426 and the other characteristics fragments appeared at m/e 302, m/e 287, m/e 284, m/e 204 and m/e 60.

Some of the important fragments obtained are given in Fig. 22 which were in agreement with the structure of taraxerol[104].

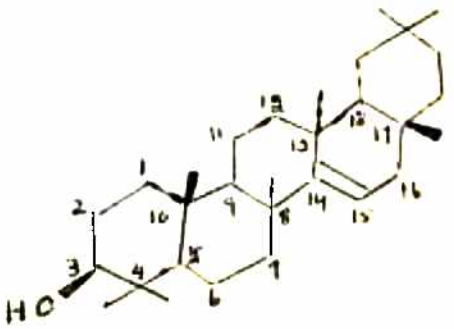
9. Melting Point: The compound LP-I was crystallized from benzene as needls. It gave a melting point of 282-283° which is in agreement with the melting point of tarexerol given in literature.

10. Preparation of LP-I Acetate and Identification: LP-I was refluxed with an excess of acetic anhydride and sodium acetate for sufficient period of time to acetylate it. The acetylated compound was precipitated in ice cold water, filtered, dried and crystallized in $CHCl_3$-CH_3CH mixture. The acetylated compound on TLC in benzene gave a Rf. of 0.75 compared to Rf. of 0.6 of the original compound. In the IR spectrum of the acetate, the 3650 cm^{-1} peak due to OH stretching of LP-I vanished and peaks 1725-1750 cm^{-1} (C=O stretching) 1455, 1375 cm^{-1} (methyl bending of acetate), 1020-1290 cm^{-1} (symmetric stretching of ester C-O-C) appeared. In NMR of acetate (given in Fig. 23) signals at 3.2 to 3.48 (due to alcoholic OH LP-I) disappeared and a new sharp signal appeared at 1.98 for 3 protons indicating monoacetylated product formation. The melting point of the compound was 304-305°. The MS of the LP-I acetate had been explained in Fig. 22.

11) Confirmation of LP-I Ketone as Tarexerone: Selective oxidation of secondary alcoholic group of LP-I was carried out by Zonn's reagent[101] (solution of chromic acid and

sulphuric acid in water) in acetone at 15-20°C without affecting the double bond. This ketone had absorption band at 1700 cm^{-1} in IR (KBr). In N.M.R ($CDCl_3$) given in Fig. 24, the signal at 3.2 to 3.4δ due to hydroxyl group of ... vanished. The results are in accordance with taraxerone isolated from Alder bark[102,103].

Based on the above evidence LP-I was confirmed as taraxerol[104-109] which is having structure as follows:



TARAXEROL

Isolean - 14 - en - 3β - ol.

2. Identification of LP-II:

LP-II was identified as beta-sitosterol[110-112] by its m.p. 140°C, $[\alpha]_D^{25}$ -37° (in $CHCl_3$), precipitation with digitonin, acetate m.pt. 127-128°C, $[\alpha]_D^{25}$ -41° (in $CHCl_3$). It was further confirmed by co-TLC, mix-TLC with authentic sample of beta-sitosterol.

3. Identification of LP-III:

1. Solubility: Soluble in petroleum ether, benzene, chloroform, ether, and insoluble in alcohol, hot water, 5% sodium hydroxide solution, 5% $NaHCO_3$ solution and in concentrated H_2SO_4.

2. Elements: Nitrogen, halogens and sulphur were absent.

3. Specific rotation: $[\alpha]_D^{25}$ -24° in $CHCl_3$.

4. Elemental analysis: Analysis of the compound for elements gave the following values:

C = 84.46%; H = 11.79% and O = 3.75%.

5. Functional Groups:

a) Free carboxylic group : Absent.

b) Phenolic hydroxy group : Absent.

c) Free alcoholic group : Present.

d) Free carbonyl group : Absent.

e) Ether linkage : Absent.

/ Ester linkage : Absent.

-. -- linkage : Absent.

. / Unsaturation : Present.

i/ Test for sterol : Positive.

6. IR Spectral Data:

The IR (in $CHCl_3$) absorption bands and the assignments are recorded in Table 11.

Table 11 : IR Spectral Data of the Compound LP-III.

cm^{-1}	Assigned Groups
3650	OH stretching
1100,1260,1330	secondary OH bending and C-O stretching.
3030	C-H stretching alkene trisubstituted.
820	C-H bending alkene trisubstituted.
1660	C-C stretching alkene trisubstituted.

7. NMR Spectral Data:

The NMR spectrum of the compound LP-III is given in Fig. 25 and gave the signals recorded in Table 12.

7. [illegible]:

The NMR spectrum of the compound LP-III in [illegible] gave the signals recorded in Table 12.

Table 12 : NMR Spectral Data of the Compound LP-III (in $CDCl_3$).

δ	No.of protons	Multiplicity	Protons assignment.
0.90, 0.95, 1.10, 1.20, 1.30, 1.70.	48	-	CH_3-CH_2 protons.
3.4 - 3.6	1	m	>CH-OH.
5.5 - 5.8	1	m	vinyl proton.

In the D_2O exchange NMR spectrum, the disappearence of 3.4 - 3.6 δ signal confimed the presence of O-H function.

8. Mass Spectral Data[113]: In the Mass spectrum (Fig. 26), the compound LP-III showed a prominent M^+ at 426 m/e and the other characteristic fragments appeared at m/e 411, m/e 408, m/e 341, m/e 273, m/e 259, m/e 247, m/e 323, m/e 253, m/e 248 and m/e 229.

Some of the important mass fragments obtained are explained in Fig. 27 which are in agreement with the structure of fernenol.

9. Melting point:

The compound LP-III was crystallized in acetone flakes and gave a melting point of 194°C.

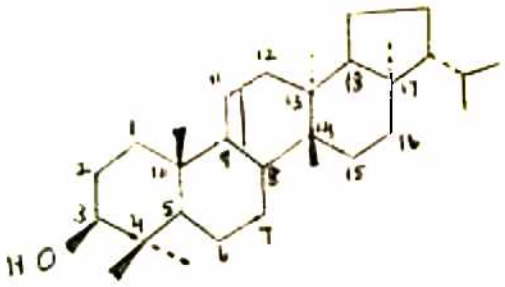
10. [illegible] Acetate and Identification:

[illegible] acetate prepared as described for LP-I was identified as fernenol acetate by its melting point 215-216°C, its $[\alpha]_D$ -10° in $CHCl_3$, its IR, NMR and mass spectrum. The mass spectrum of LP-III acetate showed the parent peak at m/e 468. The other prominent peaks are m/e 411, m/e 408, m/e 453, m/e 383, m/e 315, m/e 301, m/e 283, m/e 255, m/e 248 and m/e 229. The important fragmentation pattern of the peaks are described in Fig. 27.

11. Confirmation of LP-III Ketone as Fernenone:

The LP-III having secondary alcoholic -OH and a double bond was selectively oxidised with Zone's reagnet without affecting the double bond. The identity of the ketone was confirmed by IR and NMR. The melting point of the fernenone was found to be 184-185°C.

Based on the above evidences, the LF-III was confirmed as fernenol which is having structure as follows:



FERNENOL

Fern - 9 - (11) - en - 3 β - ol.

CHAPTER VI : VOLATILE OIL FROM LEAVES OF *Lantana camara*. COLLECTED FROM JAMMU.

[illegible] FROM FLOWERS OF Lantana camara COLLECTED [illegible].

Fresh flowers of L. camara, collected in the months of [illegible]ugust through November were subjected to steam distillation in a Clavenger's apparatus and the average yield of the volatile oil determined. Refractive index, specific gravity and optical rotations were determined and are reported in Table 13. Other values[87] were also determined as below and given in Taole 13.

Ester Value: (E.V.)

About 100 mg of the essential oil was accurately weighed in a 100 cc alkali resistant saponification flask. Five mls of neutral 95% alcohol were added and the free acids were neutralized with standarized 0.1N aqueous KOH with phenolphthalein as the indicator. Ten mls of 0.5N alcoholic KOH were then added an air condenser attached and the contents reflexed for 2 hours on a water bath. Thereafter, the flask was cooled and excess alkali titrated with standarized 0.5N hydrochloric acid. Blank was also run. The ester value was calculated using the following formula:

$$E.V. = \frac{m \times 28.05}{w}$$ where m = volume, in ml, of 0.5N alcoholic KOH to saponify the esters and w = weight of the substance in gms.

Ester value after acetylation:

10 gms of the oil and 20 mls of acetic anhydride were refluxed for 2 hours in a 200 ml flask using air condenser. This was then mixed with 600 mls of water contained in a large beaker and was boiled for 30 minutes and cooled. Then it was transferred to a separator and the lower layer was rejected. The acetylated product was washed successively three times with 50 mls of water, twice with 50 mls of solution of sodiumbicarbonate and then with 50 mls portions of warm solution of sodium-chloride (50-70°C) until the washings were no longer acid to litmus paper. The acetylated product was dried over anhydrous sodium sulphate and its ester value determined.

Acetyl Value:

This value is the difference between ester value after acetylation and ester value of the oil.

Carbonyl Value:(C.V.)

Hydroxylamine hydrochloride method was used to determine this value. About 100 mgms of the oil was accurately weighed in a 100 cc saponification flask and 20 mls of 0.5N hydroxylamine hydrochloride solution added to it. The flask was kept for 24 hours and the liberated hydrochloric acid titrated with 0.5N alcoholic KOH solution

until the original greenish shade of the hydroxylamine solution was obtained. Blank titration was also done simultaneously.

$$\text{S.V.} = \frac{156.25 \times \text{Normality of alkali} \times \text{Amount of alkali}}{\text{Wt. of the oil in gms} \times 10}$$

Thin Layer Chromatography:

The TLC of the oil alongwith authentic samples were done to identify compounds present in the oil. Observations are given in Table 13.

Gas Liquid Chromatography:

It was carried out on Perkin Elmer F.E.-811 gas chromatographic equipment using a silicone column, nitrogen as carrier gas and hydrogen flame ionisation detector. The temperature of the column was raised from 90°C to 195°C and the rate was 5°/minute. Results are given in Table 14.

x erimenta[illegible]:

Table 13 : Physico-chemical characteristics of Volatile oil from flowers of L. camara obtained from Jammu.

Properties	Jammu sample
a) Colour	Light yellow
b) Yield	0.1025%
c) Refractive Index (29°C)	1.4880
d) Specific gravity	0.8861
e) Optical rotation	Zero
f) Acid value	4.2550
g) Ester value	4.6000
h) Ester value after acetylation	22.8000
i) Acetyl value	17.8000
j) Carbonyl value	18.32%
k) Thin layer chromatography over silica gel (Benzene as solvent system; vanillin-sulphuric acid as spray reagent).	Menthen-1-ol, caryophyllene oxide, citronellal, elemol and limonene identified with authentic samples.

: GLC analysis of volatile oil from flowers of

C. camara obtained from Jammu.

Consistuents	Jammu sample
1-Alpha-phellandrene	+
p-cymene	+
	-
	-
	-*
	-*
	-*
	-*
	+
	+
	-*
	+
Geranicl	+
Caryophyllene	+
	+
	+
	+
	+
	+
	+
Elemol	+
Caryophyllene oxide	
Limonene	

+ = present

-* = absent but are present in oil samples of other geographical places. (57-62).

The GLC graph of volatile oil from the flowers of L. camara showed thirty six compounds of which only fourteen could be identified with authentic samples by comparison with their retention time in the graph. Compared with Allahabad, Delhi, Kerala and Japan oil samples a few more constituents namely citronellal, menthen-1-ol, elemol, caryophyllene oxide and limonene in the Jammu oil have been identified. This indicated the variation in the constituent of volatile oils due to the change in geographical source.

CHAPTER VII : ALKALOIDS FROM SEEDS OF *Crotalaria burhia*.

ALKALOIDS FROM SEEDS OF *Crotalaria burhia*.

Two alkaloids namely monocrotaline and croburhine have been reported from the stem of *C. burhia*[49]. Since many plant species of the genus *Crotalaria* are well known for existence of alkaloids in the seeds, the seeds of *C. burhia* were analysed for alkaloids.

The seeds collected from Pilani (Rajasthan) were soxhlated with petroleum ether (40°-60°C) to remove fixed oils and fats and subsequently extracted with alcohol. The alcoholic extract was distilled under reduced pressure to remove the alcohol. The residue was taken in 1N sulphuric acid and filtered. The filtrate was extracted with chloroform to remove impurities and coloring matters. Subsequently the filtrate was made alkaline with ammonia and extracted with successive portions of chloroform. The combined chloroform extracts were distilled under reduced pressure. The TLC of the residue ($CHCl_3$: CH_3OH : NH_3 = 85 : 14 : 1 as solvent system) gave two spots with Dragendroff's reagent with Rf values 0.5 and 0.6.

Since the TLC gave positive results, the residue was chromatographed over silica gel using solvents of increasing polarities. The ethanol-chloroform (20 : 80)

fraction yielded one crystalline principle (CB-I) and ethanol-chloroform (35 : 65) fraction yielded another crystalline principle (CB-II).

Identification of CB-I:

This CB-I was crystallized from chloroform and gave a m.pt. of 167-168°C which was same as that of croburhine. The compound was identified as croburhine[49] by mix m.pt., Co-TLC and mix-TLC with an authentic sample of croburhine. (refer the structure II in the Introduction)

For further confirmation, IR and NMR spectra of the compound CB-I were taken. The IR spectrum (in KBr) of the CB-I showed peaks at 1485 cm^{-1} (CH_2 bending), 2900 cm^{-1} (CH_2 stretching), 1680 cm^{-1} (C = C stretching), 1737 and 1720 cm^{-1} (ester carbonyl saturated) and 3350 cm^{-1} (OH stretching). The NMR spectrum of the CB-I taken in $CDCl_3$ showed signals at 0.75δ (t, $\underline{H_3}C - CH_2 - {}^{14}\overset{|}{C} -$), 1.17$\delta$ (s, $H_3C - {}^{14}\overset{|}{C} -$), 1.45$\delta$ ($\underline{H_3}C - \overset{|}{C} - OH$), 2.13$\delta$ (m, $-{}^{6}CH_2-$), 2.20δ (m, $-O\underline{H}$), 3.25δ (m, $- {}^{5}CH_2-$), 3.85δ (m, $- {}^{8}\overset{|}{C}H -$), 4.83$\delta$ (q, $- {}^{9}CH_2-$), 5.25δ (m, $- {}^{7}\overset{|}{C}H -$) and 6.12$\delta$ (m, $- \overset{|}{C} = {}^{2}\underline{C}H-$)

Identification of CB-II:

This CB-II was crystallized from acetone. It gave a m.pt. of 201°C which was same as that of monocrotaline. The compound was identified finally to be monocrotaline[29,49] by

mix m.pt., Co-TLC, and mix-TLC with an authentic sample of monocrotaline. (refer the structure I in the Introduction).

The compound CB-II, identified as monocrotaline, showed the following IR peaks (in KBr) to support the identity: 1485 cm^{-1} (CH_2 bending), 2900 cm^{-1} (CH_2 stretching), 1680 cm^{-1} (C = C stretching), 1737 and 1728 cm^{-1} (ester carbonyl saturated), and 3350 cm^{-1} (OH stretching). The NMR spectrum of the compound CB-II in $CDCl_3$ had given signals at 0.95δ (d, $\underline{H_3}C$ - ^{14}CH -), 1.10δ (s, $\underline{H_3}C$ - ^{4}C - OH), 2.13δ (m, - $^{6}CH_2$ -), 2.20δ (m, - O$\underline{H}$), 3.25δ (m, - $^{5}CH_2$ -), 3.85δ (m, - ^{8}CH -), 4.71δ and 4.87δ (q, - $^{9}CH_2$ -), 5.08δ (m, - ^{1}CH -) and 6.03δ (m, - ^{1}C = $^{2}\underline{C}H$ -).

CHAPTER VIII : SUMMARY AND CONCLUSIONS.

SUMMARY AND CONCLUSIONS

Chapter I gives a brief survey of the fast growing plants chosen for the study. Chapter II summarises the methods used for production of high alpha cellulose or rayon grade pulp from the ten fast growing plants namely Crotalaria juncea, Crotalaria retusa, Crotalaria burhia, Sesbania sesban, Sesbania roxburghii, Leptadenia pyrotechnica, Populus casale I-488, Lantana camara, Broussonetia papyrifera and Hibiscus cannabinus. Chapter III describes the production of microcrystalline cellulose from the high alpha cellulose pulp obtained from the above ten plants. Chapter IV incorporates the study of fixed oils obtained from seeds of C. juncea, C. retusa, S. roxburghii and H. cannabinus for physical characteristics and fatty acid components by GLC. Chapter V describes the isolation and identification of terpenes and steroids from the aerial parts of L. pyrotechnica. Chapter VI gives the analysis of volatile oil obtained from flowers of L. camara for physical characteristics and constituent compounds by GLC. Chapter VII describes the isolation and identification of alkaloids from the seeds of C. burhia.

Amongst the fast growing plants mentioned above only B. papyrifera came up to the TAPPI specifications for

rayon grade pulp. The rest of the plants were found to be suitable for high alpha cellulose pulps of chemical grade. The optimal conditions of prehydrolysis and kraft pulping and the yields of the pulps have been worked out in each case.

Microcrystalline cellulose (MCC) was prepared from the high alpha celluloses of the plants by a modified method. After hydrolysis of the alpha celluloses with 2.5N hydrochloric acid for 15 minutes, the obtained hydrocelluloses were mechanically disintegrated in alcohol-acetone (50:50) mixture for half an hour and filtered. The residues remaining on the filters were dried and found to be good variety of MCC. The current method of mechanically disintegrating the hydrocellulose in water and subsequent drying it by spray drier, freeze drier or drum drier etc. adds to the cost of the material. In the method discussed in the thesis the solvent is recoverable and can be used for successive batches. As such this method could be cheaper and less cumbersome. The MCC prepared by this method compared well with the marketed varieties of MCC in molecular weights, viscosities, average diameter of crystals etc.

Fixed oils from the seeds of C. juncea, C. retusa, S. roxburghii and H. cannabinus gave yields of 3.73%,

3.8%, 3.78% and 20% respectively. The H. cannabinus oil though present in higher amounts showed the presence of cyclopropene fatty acid and as such cannot be recommended for edible purposes. However it may be possible to use it for soap and candle making. The oils in other plants are in too low amounts to be economical. The H.cannabinus oil was found to be rich in palmitate (38.97%) whereas C. juncea, C. retusa and S. roxburghii seed oils are rich in linoleate (52.51%, 40.64% and 63.15% respectively).

Aerial parts of L. pyrotechnica showed the presence of taraxerol (LP-I), beta-sitosterol (LP-II) and fernenol (LP-III). The beta-sitosterol was identified by comparison with an authentic sample. The taraxerol and fernenol were identified by physical and chemical methods, spectral methods such as IR, NMR and MS and preparation of derivatives such as acetates and ketones. The compounds have been isolated for the first time from this plant.

Volatile oil from flowers of L. camara collected from Jammu was found to have citronellal, menthen-1-ol, elemol, caryophyllene oxide and limonene. These constituents have not been reported in oils of L. camara growing in Allahabad, Delhi or Kerala. This indicated the variation in the constituents of volatile oils due to change in geographical source.

The seeds of C. burhia were found to contain alkaloids namely monocrotaline and croburhine. These were identified using authentic samples. These alkaloids were reported to be present in the stems of the same plant.

In conclusion it may be said that the ten fast growing plants analysed have good potentialities as probable sources of rayon grade/chemical grade alpha cellulose. Some of them which have been analysed have been found to have interesting chemical constituents and warrant further analysis.

REFERENCES

1. Bhat, R.V., Singh, [illegible], Jaspal, N.S. and Gopalkrishnan, [illegible], Cellulose research symposium II, C.S.I.R., 1956.

2. Guha, S.[illegible], Singh, [illegible] and Saxena, V.B., Indian Pulp and Paper, 1960, 64(12), p. 565.

3. Pande, G.C., Koutu, [illegible], Kothari, B.L., Jayasankar, A.K., Singh, I. and Jadav, [illegible], Indian Pulp and Paper, 1972, 2(5 & 6), p. 9-16.

4. Guha, [illegible], Singh, [illegible] and Saxena, V.B., Indian Pulp and Paper, 1960, 15(6), p. 379.

5. Ibid., Indian Pulp and Paper, 1961, 16(2), p. 141.

6. Bhat, R.V. and Viramani, A.C., Indian Pulp and Paper, 1961, 16(3), p. 159.

7. Guha, S.R.D. and Singh, [illegible], Indian Pulp and Paper, 1963, 18(2), p. 187.

8. Ibid., Indian Pulp and Paper, 1963, 18(6), p. 12.

9. Guha, S.R.D. and Madan, R.M., Indian Pulp and Paper, 1964, 18(12), p. 675.

10. Sen, [illegible], Das Gupta, [illegible] and Sankar, P.B., Research and Industry, 1964, 9(5), p. 126-127.

11. Gupta, ..., Indian Pulp and Paper, 1966, 21(6), p.403.

12. Singh, ... and Bhatia, ..., Indian Pulp and Paper, 1967, 21(10), p. 617-618.

13. Singh, ..., Indian Forester, 1967, 93(7), p. 486-488.

14. Chawla, J.S., Sharma, A. and Krishnaswami, S.P., Indian Pulp and Paper, 1967, 22(4), p. 263-265.

15. Guha, S.K.D., Singh, ... and Gupta, R.K., Indian Pulp and Paper, 1968, 22(7), p. 397-400.

16. Pande, G.C., Indian Pulp and Paper, 1969, 23(11), p.617-620.

17. Gupta, M.K., Indian Pulp and Paper, 1970, 24(8), p. 351-355.

18. Chawla, J.S., Sharma, A.N. and Krishnaswami, S.P., Indian Pulp and Paper, 1970, 24(7), p. 7-9.

19. Gupta, M.K., Indian Pulp and Paper, 1971, 25(7), p. 453-455.

20. Pande, G.C., Routu, S.R., Singh, I, Jadav, H.P. and Singh, ..., Indian Pulp and Paper, 1972, 26(10), p. 143-148.

21. ...ins, ... and Chawla, J.S., Indian Pulp and Paper, 1978, 33(1), p. 3-5.

22. Anonc, ... and Chawla, J.S., Indian Pulp and Paper. 1979, 33(5), p. 29-32.

23. Standards and Suggested Methods by Technical Association of Paper and Pulp Industry (TAPPI), 1948.

24. The Wealth of India, Raw Materials, Council of Scientific and Industrial Research, India, 1950, 2, p. 373-381.

25. Nelson, G.H., Clark, T.F., Wolff, I.A. and Quentin Jones, TAPPI, 1966, 1, p. 49.

26. Raymond, L., Clark Thomas, F., Kwolek William, F., Wolff, Ivan, A. and Quentin Jones, TAPPI, 1970, 53, p. 1697.

27. Bulletin. Imp. Inst., London, 1921, 19, p. 454.

28. The Wealth of India, Raw Materials, Council of Scientific and Industrial Research, India, 1950, 2, p. 382.

29. Subhash Kumari, Kapur, K.K. and Atal, C.K., Current Sciences, 1966, 35(21), p. 66.

30. Robin David, J., Bale Nicholas, M. and Crout David, N.G., J. Chem. Soc. Perkin Trans, 1974, 18, p. 2082-2086.

31. Rao, P.G., Sawhney, ...S. and Atal, C.K., Indian J. Pharm., 1975, 37(5), p. 127.

32. Puri, S.C. Sawhney, R.S. and Atal, C.K., Indian J. Pharm., 1972, 34(5), p. 36.

33. Suri, O.P., Sawhney, R.S., Gupta, O.P. and Atal, C.K., Indian J. Pharm., 1975, 32(2), p. 36.

34. Suri, A.A., Sawhney, R.S. and Atal, C.K., Indian J. Pharm., 1976, 38(1), p. 23.

35. Suri, A.A., Sawhney, R.S. and Atal, C.K., Indian J. Pharm., 1974, 36(4), p. 90.

36. Grashahonka, V.M. and Lisityn, Y., Achievement of Soviet, Medicine, Foreign Language Publishing House, Moscow, 1960.

37. The Wealth of India, Raw Materials, Council of Scientific and Industrial Research, India, 1972, 9, p. 298.

38. Farooq, et al., J. Sci. Fd. Agric., 1954, 5, p. 498.

39. Brij Singh, et al., Indian J. Ver. Sci., 1959, 29(2 & 3), p. 32.

40. Farooq, et al., J. Sci. Fd. Agric., 1954, 5, p. 499.

41. The Wealth of India, Raw Materials, Council of Scientific and Industrial Research, India, 1972, 9, p. 299.

42. Ibid., 1952, 2, p. 73.

43. Krishna, A.V.G., Venkata Rao, E. and Venkata Rao, D., Indian J. Pharm., 1973, 35(6), p. 209.

44. Krishna, A.V.G., Venkata Rao, E. and Venkata Rao, D., Planta Medica, 1975, 27, p. 395.

45. Patil, A.P. and Dantwala, A.S., Indian J. Pharm., 1958, 20, p. 241-244.

46. Agarwal, S.L., Deshmankar, B.S., Verma, S.C.L. and Saxena, S.P., Indian J. Med. Res., 1960, 48(4), p. 457-464.

47. Chemical Abstracts published by American Chemical Society, 1975, 83(7), 65374h.

48. The Wealth of India, Raw Materials, Council of Scientific and Industrial Research, India, 1980, 2, p. 373.

49. [illegible], A.K., Sawhney, R.S. and Atal, C.K., Indian J. Pharm., 1975, 13, p. 835-836.

50. The Wealth of India, Raw Materials, Council of Scientific and Industrial Research, India, 1962, 6, p. 33-34.

51. Paper No. I.F. 3.3, 11th All India Sivicultural Conference from the Central Sivilculturist, Forest Research Institutes, Dehra Dunn.

52. The Wealth of India, Raw Materials, Council of Scientific and Industrial Research, India, 1962, 5, p. 31-32.

53. Iyengar, Current Sciences, 1932, 1, p. 206.

54. Chemical Abstracts published by American Chemical Society, 1944, 38, p. 6297 and 1948, 42, p. 9086.

55. Barton, et al., J. Chem. Soc., 1954, p. 3989 and 1956, p. 4160.

56. Joshi and Nagan, J. Sci. Indust. Res., 1952, 2, p.261.

57. Moudgil, K.B. and Vridhachalam, P.N., Perfumery and Essential Oil Record, 1922, 13, p. 173.

58. Kufuku, K., Ikeda, T. and Fugita, Y., J. Chem. Soc., (Japan), 1934, 55, p. 305.

59. [illegible] and Le, G., Bull. Imp. Inst., 1934, 32, p. 253.

60. [illegible], Ind. Perfumer, 1960, 4, p. 17.

61. [illegible], J. Indian. Inst. Sci., 1914-1918, 1, p. 55-95.

62. [illegible], Al. Moghazyshoail, [illegible], Wasset,,G.M. [illegible] Al. [illegible], Planta Medica, 1972, 21(3), p. 283-288.

63. [illegible], Council of Scientific & Industrial Research, India, 1948, 2, p. 230.

64. Ibid., 1950, 5, p. 77-84.

65. Chemical Abstracts published by American Chemical Society, 1930, 24, p. 6023.

66. Lathrop Nelson, Indian Pulp and Paper, 1954-55, 9. p.27.

67. Chemical Abstracts published by American Chemical Society, 1968, 68, 14231s.

68. Ibid., 1975, 83, 128743a.

69. Ibid., 1976, 64, 151662k.

70. Auchter, R.J., Approved Technical Articles by Forest Products Laboratory (Madison, Wes 53705), Forest Service, U.S. Department of Agriculture.

71. Standards and Suggested Methods by Technical Association of Paper and Pulp Industry (TAPPI), T5m-39.

72. Ibid, T5m-39.

73. Ibid, T5s-52.

74. [illegible], Methods of Wood Chemistry, Interscience Publishers, New York, 1967.

87. British Pharmacopoeia, Her Majesty's Stationery Office, London, 1963.

88. Rodier, J., Analysis of Water, John Wiley and Sons, New York, 1975, p. 181-286.

89. Parfitt, G.D. and Singh, K.S.W., Characterization of Powder Surfaces, Academic Press, London, 1976, p.132-133.

90. Bellamy, L.J., The Infrared Spectra of Complex Molecules, John Wiley and Sons, London, 1960.

91. Nakanishi, K., Infrared Absorption Spectroscopy, Holden-Day Inc., San Francisco, 1962.

92. Scott, A.I., Interpretation of UV Spectra of Natural Products, Pergamon Press, London, 1964; Dyer, J.R. Application of Absorption Spectroscopy of Organic Compounds, Prentice Hall of India Private Limited, New Delhi, 1974.

93. [illegible], [illegible], Text book of Practical Organic Chemistry, Ballentyne and [illegible], London, 1962,

94. Cheronis, D.N. and Entrikin, B.J., Identification of [illegible] Compounds, [illegible] Science Publishers, New York [illegible], 1.33.

95. [illegible] ch, E. [illegible], Modern Methods of Plant Analysis, Springer Verlag, Berlin, 1955, 4, p. 387.

96. Feigel, [illegible], Spot Tests in Organic Analysis, Elsevier Publishing Company, 19[illegible].

97. Ross, J.A., Stanley, D.T., William, E.E. and Alexander, E.C., J. Amer. Chem. Soc., 1956, 7[illegible], p. 4075.

98. Gilman, H., Organic Chemistry, London, 1950, 2, p.139.

99. Budzikiewicy, H., Wilson, J.M. and Djerassi, J. Amer. Chem. Soc., 1963, 85, p. 3688.

100. Silverstein, [illegible], Bassler, G.G. and Morrie, T.C., Spectrometric Identification of Organic Compounds, John Wiley Sons Inc., New York, 1968.

101. Braude, E.A. and Faucett, J.[illegible], Organic Synthesis Collective Volume, 1965, 4, p. 698.

102. Beaten, et al., Tetrahedron, 1958, 2, p. 246.

103. Keller, et al., Helv. Chem. Acta., 1950, 33, p.1050.

104. [illegible]ute[illegible], Arch. Pharm, 1900, 238, p. 555.

105. Zellner, Weiss. Sitzber, Akad. Wieswein, 1923, 132, p. 25[illegible].

106. [illegible]rows and [illegible]s, J. Chem. Soc., 1938, p. 2042.

107. Du[illegible]stu[illegible], et [illegible]l., Aust. J. Chem., 1953, 6, p. 321.

108. Bude, et al., J. Org. Chem., 1961, 26, p. 274.

109. Beaten, et al., J. Chem. Soc., 1955, p. 2131.

110. Bernstein and Walter, J. Org. Chem., 1937, 2, p. 231.

111. Shoppu, J. Chem. Soc., 1968, p. 1032.

112. Elsevier's Encyclopedia of Organic Chemistry, 14s, 1808s, 1954.

113. Nishimoto, K., Ito[illegible] and Natori, S., Tetrahedron, 1968, 24, p. 735-752.

114. Kundu, S.K. and Mrs. Chatterjee, A., Tetrahedron Letters, 1966, p. 1043-1047.

115. Nishimoto, K., Natori, S. and Ohnolo, T., Chem. Pharm. Bull., 1966, 14, p. 97.

*****