Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 1, No. 3, January : 2024

STRUCTURAL STUDY OF BETULIN - EXTRACTED FROM ACTINOPTERIS - DICHOTOMA

Dr. Rashmi Shrivastava , senior lecturer , Dept of Applied sciences and humanity
IIMT College of Polytechnic, Greater NOIDA

Abstract
The rhizome and fronds of Actiniopteris dichotoma Bedd. has been reported to treat worm infestations, skin diseases, antifertility, diarrhoea and dysentery in Ayurveda. The present investigation involves the macroscopy, microscopy, physicochemical evaluation and preliminary phytochemical studies of Actiniopteris dichotoma Bedd. These observations will help in carrying out further research on this plant.
Betulin and its methyl ester may be considered potential anti-inflammatory agents. All of the compounds are more effective in the decrease of IL-6 secretion.
Keywords : Actinopteris dichotoma (plant material) , investigation , different techniques

Background
The emergence and spread of resistant microbes continue to be a major public health concern. Effective treatment alternatives, particularly from traditionally used medicinal plants, are needed.

Objective
The main objective of this research paper is to conduct structural study and analysis of Betulin using different techniques of spectroscopy to learn its importance.

Introduction
The natural sources of vegetative origin has been investigated by man from the immemorial time as a primitive source of food, shelter and medicine. His worry to be get control of diseases; led him to search for curative values of plants, as they are easily and abundantly available. The search for medicinal plants has continued through centuries. Our Ayurvedic system of medicine describes the medicinal importance of several hundred plant species.
From these primitive practices; extended and refined, there have developed modern industries based on the isolation of minor products of vegetal origin and their application to the economic needs of the civilized world. The late nineteenth century perhaps represented the period of highest utility of industrial phytochemistry. It has subsequently receded under competition with synthetic organic chemistry. The importance of natural products 1s still much with us, however, as both giant industries and minor efforts, the local commodities familiar through generations make their elects felt preferences for Among the most important secondary plant products of the past must surely have been those associated with disease. It would seem that, at one time or another, almost every plant of Europe, at least was thought to have had medicinal properties; even though few survived into modern medical practice and fewer still into our present pharmacopeias. Nevertheless, there are some, such as Digitalis, Papaver-somniferum and the loganiaceous sources of the bisquaternary alkaloids of curare; which in chemically purified form still remain vital members of our materia medica.

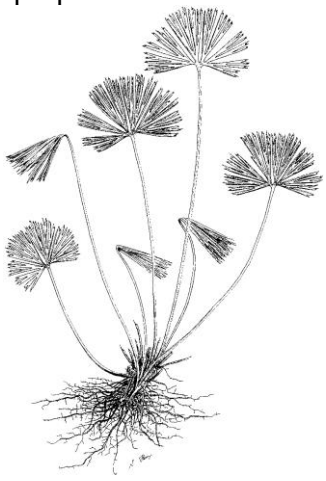
1.1 Introduction of the Plant
One of the most interesting and valuable part of Chemistry lies in the study of medicinal plants.
The economic importance of plants in human affairs is immense and has been so since the dawn of man. Historically, and prehistorically, the principal uses of plants have been as sources of food, of materials, for shelter and construction, of fiber for fabrics and paper, and of oxidizable material for fuel. Presumably, however the early searches for edible plants turned up those which were not only non edible but were also acutely toxic, as well as those even more interesting species which induced

peculiar but not wholly unpleasant sensations. Between these fell many that, although often poisonous possessed in smaller doses powers to relieve pain, reduce fatigue or fight symptoms of diseases.
Medicinally, the plants of Polypodiaceace family are very important.
Mostly the plants of polypodiaceace family are carminative, expectorant, insecticidal and anthelmintic in nature.
Actiniopteris-dichotoma is a very important medicinal plant of Polypodiaceace family. This plant is commonly known as "Morpankhi" in Hindi and in Sanskrit it is known as "Mayurshikha"
The plants of polypodiaceace family are a heterogenous assemblage of greatly specialized members of several distinct stock and are therefore a highly unnatural family. The family serve as an excellent example of a polyphylitic group; - a group which is tied together by the attainment of certain advanced characters which have been selected as a taxonomic basis
The fossil record bears out this conclusion which has been drawn from the evidence of comparative study for no members of the family are known before the mesozoic era.

1.2 Description of various species of Actiniopteris are as follows:

Actiniopteris - dichotoma:

This plant is commonly known as Morpankhi and the name of this plant in different languages are given below:
Eng. - Peacock's tail
Hin. - Mayursikha,
Mal. - Morpankhi
San. - Mayurasikha
Tam. - Mayilatumsikha , Nanmukappullu
DISTRIBUTION: Throughout India and very common in the lower hills of Attapaddy up to 600m in Nilgiris.
THE PLANT: Sori linear, elongated, submarginal indusium the same shape as the sorus, folded over it, placed one on each side of the narrow segments of the frond, opening towards the midrib, a herbacious single species like a miniature palm, stipes densely taifted 2-6 inches long, fronds like fans 1-1,1/2" inches deep, composed of numerous dichotomous segments which are rush like in texture, the veins few and sub-parallel with the indistinct midrib. The segments of the fertile midrib longer than those of the barren. One found throughout India specially in peninsula, in dry rocky places below 3000 feet elevation.
PARTS USED : Whole plant.
PROPERTIES AND USES: The plant is bitter, astringent, sweet cooling, acrid, constipating, anthelmintic haemostatic, antileprotic and febrifuge. It is useful in vitiated conditions of "kapha and pitta", 'diarrhoea', 'dysentery', 'helminthiasis', "leprosy', 'haemoptysis', haematemesis, skin diseases, diabetes and fever.
This plant is accredited with antibiotic properties and is used in some places as an antifertility drug.



Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 1, No. 3, January : 2024

Experimental Analysis

STRUCTURAL STUDY AND ANALYSIS OF COMPOUND (BETULIN - terpenoid - extracted from Actiniopteris-dichotoma)

The elemental analysis and molecular weight determination by mass spectrum, M 456) of the compound establish the molecular formula $C_3H_{48}O_3$.

It is optically active.[a]," +14° (pyridine).The compound is of triterpenoidalin nature; as it responds to characteristic colour reactions, e.g. a yellow colour changing to red. (Salkowski reaction), a red violet color.(Tschugajew reaction), a red-violet colour. Liebermann-Burchard reaction)

violet colour. (Brieskorn reaction), a violet - red colour. (Tschugajew reaction) ' , a reddish violet. (Noller's reaction)

and a light pink colour changing to violet.

The compound seems to be pentacyclic in nature as it gives a violet colour with 2:6-di-tert-butyl-p-cresol in ethanol'20

The molecular formula and IR spectrum 21,122 of the compound also indicate its pentacyclic nature.

When heated with acetic anhydride and pyridine, the compound forms an acetate, m.p.290-291°C. The acetate on analysis has been found to be C₃₀H47020COCH₃ indicating it to be a monoacetate of the compound. The formation of monoacetyl derivative indicates the presence of one hydroxyl group in the compound. The presence of a hydroxyl group in the compound is further confirmed by its IR, absorption band at 3448 cm*' and bands at 1739 and 1240 cm' (acetate)with no peak in the hydroxyl region in the IR spectrum of its monoacetate.

The compound forms an insoluble salt with potassium hydroxide and a methyl ester m.p.220-222°C with diazomethane indicating the presence of carboxyl group is further confirmed by the absorption band at 1700 cm' in its IR spectrum and a band at 1718cm* (ester carbonyl)in the IR spectrum of its methyl ester. The molecular weight (M+ at m/e 470)of the methyl ester suggests the presence of one -COOH group in the compound. This is further supported by a NMR signal at 83.62(a singlet for 3 protons of the ester group).

The compound decolourises bromine water in carbon tetrachloride; thereby indicating the presence of unsaturation in it. The compound neither gives a characteristic colour with tetranitromethane (Ruzicka reaction) nor shows any high terminal

UV absorption typical 24 of the 12:13 ~ double bond present in the triterpenes of the a ~ and ~ amyrin series. The compound therefore appears to be a member of the lupeol group. This conclusion is supported by the fact that theIR spectrum of the compound shows absorption bands at 1641 and 885 cm' which are in agreement with the characteristic frequencies of ethylenic double bond between position 20:29 in pentacyclic triterpenes of lupeol group 122.

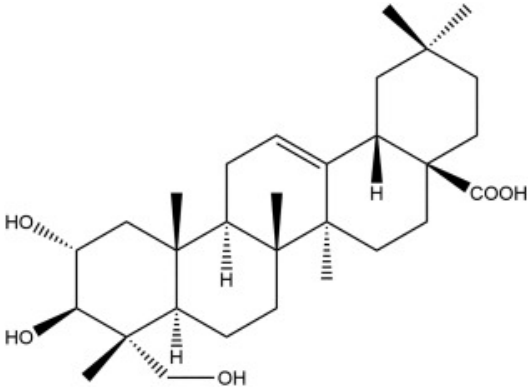
The presence of 20:29 ~ double bond in the side chain as CH_2-C-CH_2 is further confirmed by the two protons signal centered at & 4.70(2H) and a sharp singlet at

§ 1.75(3H) corresponding to methyl protons in NMR spectrum of its methyl ester 125,126

The absorption bands at 2933, 1381 and 1362 cm' in the IR spectrum of the compound indicates the presence of methyl group. The NMR spectrum of methyl ester exhibits signals at 8 0.75(3H), 0.77(3H), 0.90(3H), 1.0(3H) and 1.15(3H) for five tertiary methyl groups.

Thus the above facts conclusively indicate the compound to be a pentacyclic triterpenes of lupene series with one hydroxyl and one carboxyl group. The compound may therefore, be represented as

Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 1, No. 3, January : 2024



The problem is to ascertain the positions of -OH and -COOH Groups.

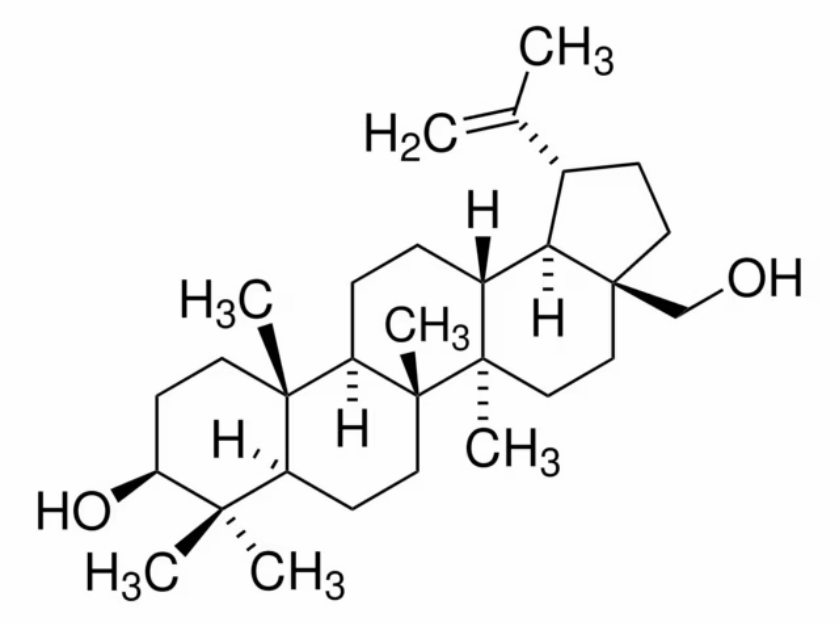
Position of -OH Group:

The methyl ester on oxidation with chromic acid forms an oxidative product, m.p. 165-169°C which responds to positive zimmermann Test 28 for 3-Keto group. This indicates the presence of a secondary -OH group at C3. This is also in agreement with the observation that most of the naturally occurring hydroxy triterpenic acids bear a secondary alcoholic group at C3.

2.2 Position of -COOH Group:

The methyl ester of the compound on saponification with 10% methanolic KOH solution for 8 hours at reflux temperature results in partial hydrolysis (85% of the ester is recovered unchanged). However, its saponification with diethylene glycolic KOH regenerates the original compound, m.p.316-318°C. The above facts clearly indicates that the -COOH group is highly hindered and is attached to C17. The presence of -COOH group at C17 in the compound is also supported by a signal at 83.62 in NMR spectrum of its methyl ester 25,126

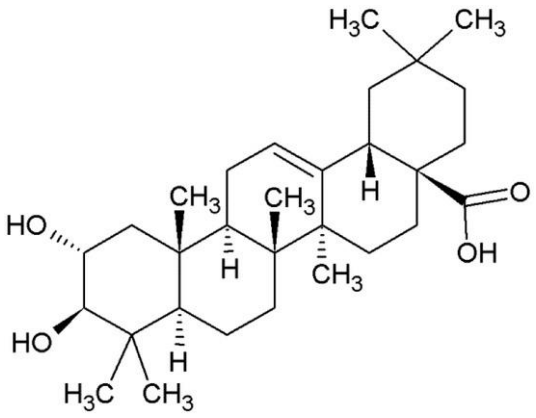
The methyl ester on reduction with LiAlH4 in a mixture of tetrahydrofuran : ether (1:4v/v) furnishes a diol, $C_{30}H_{50}O_2$, m.p.249-250°C. The diol is identified as Betulin by mixed melting point and superimposable IR spectra with that of authentic sample of Betulin. The structure of the diol (betulin) is well known and is represented as



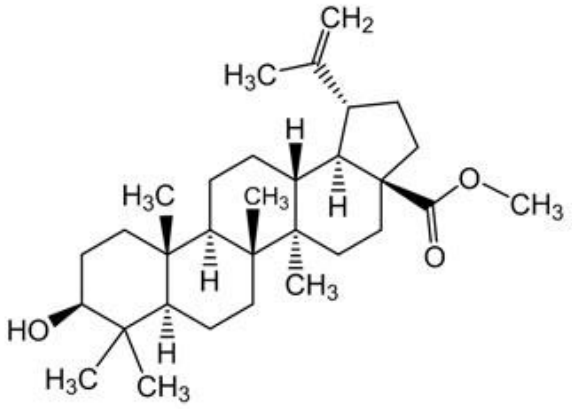
The -CH_2OH group at C17 in betulin results from the reduction of

Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 1, No. 3, January : 2024

-$COOCH_3$ group of the methyl ester. This confirms that -$COOCH_3$ group in methyl ester is linked to C17 and accordingly, the triterpenic acid contains
-COOH group at C17. The structure of the triterpenic acid and its methyl ester may therefore be represented as -



Triterpenic acid - Betulin



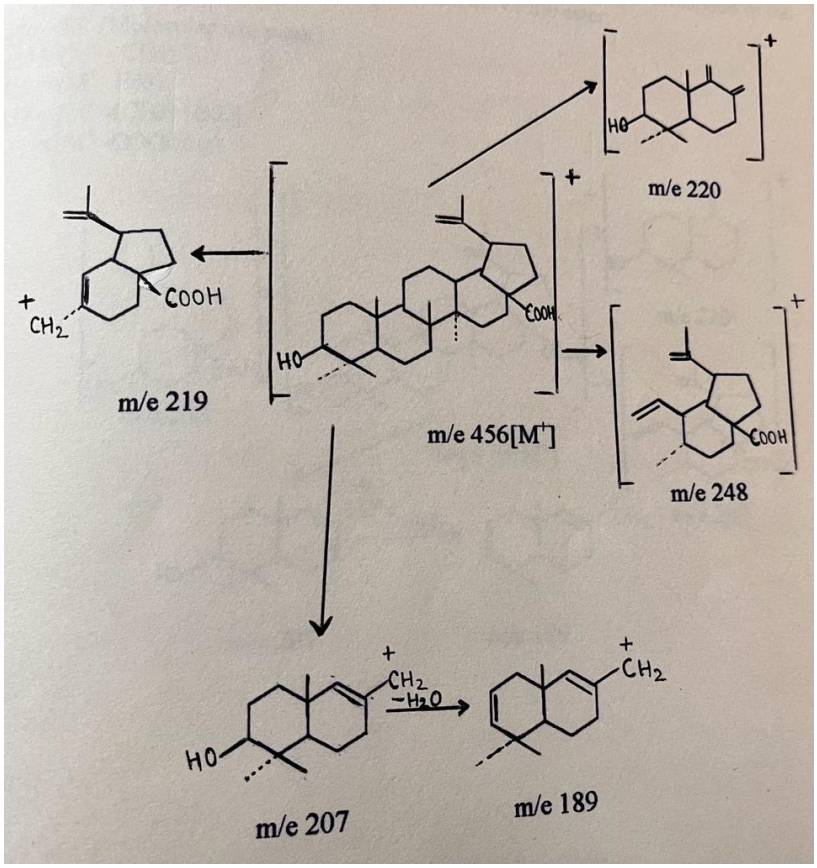
Methyl ester of Betulin

On reviewing the literature, it is found that the above structure of the triterpenic acid and its methyl ester represent betulinic acid 3ß-hydroxylup-20(29)-en-28-oic acid] and methyl betulinate [Me-3ß-hydroxylup-20(29) en-28 oate] respectively. The identity of the triterpenic acid with betulinic acid is finally confirmed by the following observations:
1. The UV and IR spectra of the triterpenic acid and its methyl ester are identical with those of betulinic acid and methyl betulinate respectively.
2. The melting points of the triterpenic acid and its methyl ester are not depressed when mixed with authentic samples of betulinic acid and methyl betulinate respectively.
3. The NMR spectrum and mass fragmentation pattern of the methyl ester of the triterpenic acid are in full accord with those of methyl betulinate.

2.3 Mass Spectrometric Studies of the compound

The formation of some major ions appearing at m/e 456, 441, 438, 423, 411, 248, 220, 219, 207 and 189(base peak) in the mass spectrum of the acid may be formulated as follows:
456 = M* (Molecular ion peak)
441 = (M*-CH_3)
438 = (M-H_2O)
423 = [M*(CH_3+H_2O)]
411 = (M*-COOH)

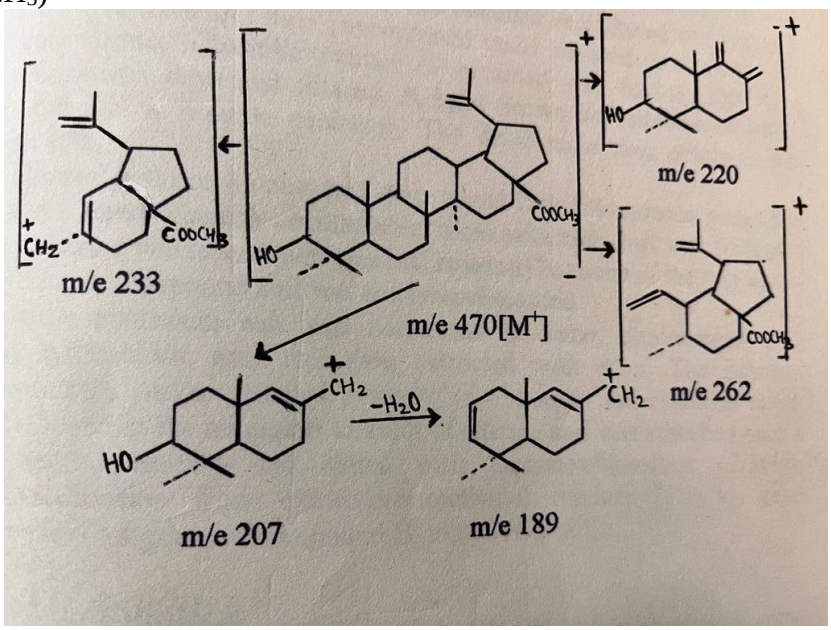
Industrial Engineering Journal
ISSN: 0970-2555
Volume : 53, Issue 1, No. 3, January : 2024



2.4 Mass Spectrometric Studies of the Methyl Ester of the compound

The prominent peaks at m/e 470, 455, 452, 437, 411, 262, 233, 220, 207 and 189(base peak) appearing in the mass spectrum of the ester correspond to the following ions which result due to fragmentation of the ester:

470 = M (Molecular ion peak)
455 = (M* -CH_3)
452 = (M* -H_2O)
437 = [M^* CH+H_2O)]
411 = (M*-$COOCH_3$)



Elemental Analysis of the compound

Found	Calculated for $C_{30}H_{48}O_3$
C = 78.86%	C = 78.94%
H = 10.51%	H = 10.52%

Molecular weight = 456 (by Mass Spectrum)

UV Spectrum Of The Compound
205 mu (ethanol)
Nmax
IR Spectrum Of The Compound

Position of absorption band	Assignment
3448 (s,b)	hydroxyl group
2933(s), 2850(w)	-CH3, -CH2
1700(s)	Carboxyl group
1460(s)	Cyclohexene -CH2
1392(m), 1381(m), 1362(m) 1320(m), 1295(w), 1274(m)	Triterpenic acid type
885(s)	Vinylidine type at Position 20:29
1641(sh)	>C= CH2

s-Strong, b-Broad, w-Weak, m-Medium, sh-Sharp

2.5 Elemental Analysis of Acetyl derivative

Found	Calculated for $C_{30}H_{47}O_2.OCOCH_3$
C = 77.24%	C = 77.10%
H = 10.21%	H = 10.09%

Acetyl group percentage

Found	Calculated for $C_{30}H_{47}O_2.OCOCH_3$
8.36%	8.62% (For one acetyl group)

IR Spectrum of acetyl derivative
The IR spectrum showed main absorption peaks at makr. 2940, 2855 (-CH_3, -CH_2-), 1739(acetate carbonyl), 1704 (carboxy carbonyl), 1642 (unsaturation), 1240(acetate streching) and 885(exocyclic methylene group >C=CH_2)cm' (IR)

Elemental analysis of methyl ester.

Found	Calculated for $C_{31}H_{50}O_3$
C = 78.92 %	C = 79.14 %
H = 10.56 %	H = 10.64 %

Molecular weight = 470 (by mass spectrum)

IR spectrum of methyl ester
The IR spectrum of methyl ester showed main absorption peaks at /max 3555 (hydroxyl), 1718 (esterCarbonyl), 1641 (unsaturation), 1460(-CH_2-), 1362 (-C-me) and 885(>C=CH_2) cm"

NMR Spectrum 125,126 of methyl ester
NMR spectrum of methyl ester was recorded in $CDCl_3$ on a
60Mc varian
spectrometer using TMS as internal standard.

Signal at 8 value	Assignment
0.75(s)	(3H) Tertiary methyl group
0.77 (s)	(3H) Tertiary methyl group
0.90(s)	(3H) Tertiary methyl group
1.00(s)	(3H) Tertiary methyl group
1.15(s)	(3H) Tertiary methyl group
1.75(s)	(3H) me occurring as me -C=CH_2
3.62(s)	(3H) -$COOCH_3$
4.70(m)	(2H) Vinylic proton occurring as me -C=CH_2

s- singlet m-multiplet

Elemental analysis

Found	Calculated for $C_{30}H_{50}O_2$
C=81.23 %	C=81.44 %
H=11.18 %	H=11.31 %

IR Spectrum of diol (Betulin)
Kr. 3509 (s,b), 2967(s), 1645(sh), 1462(m), 1383(m), 1110(sh), 1087(w), 1019(m), 990(w), 975(w) and 885(s) cm'.

Conclusion:
Betulin and its methyl ester may be considered potential anti-inflammatory agents. All of the compounds are more effective in the decrease of IL-6 secretion. However, the efficacy of COX-2 inhibition mainly was connected with Bet-Lys. Besides, after stimulation with basic betulin derivatives (Bet-Lys, Bet-Orn), the cells were less sensitive to the IFNγ stimulation, due to the polymerization of the interferon receptor. Moreover, the hydrophilic drugs were cytotoxic towards cells in much lower concentrations than the hydrophobic Betulin. In summary, Betulin and its methyl ester present an interesting alternative to dexamethasone in the attenuation of macrophages' inflammatory response.

However, further *in vivo* studies are required to validate the potential clinical use of the natural compounds.

References:

1. Stout. George H, Schultes Richard Evans 'Importance of plant chemical in Human affairs' p (381-82)
2. Finar. I. L 'Organic Chemistry', Longmann Green & Co. 582, (1962).
3. Tonkin & Coworkers Nature' London. 156. P 30). 1945.
4. Knowlton. et al. J. Am. Med. AssoC 14. 239 (1949)
5. Shamo. R.L. 'J.Am. Med Sci' 539, (1946)
6. Hesse Manfred. (Inst of org. chem; Univ. of Zurich) 'Alkaloid chemistry' p (89-90), 27; (1978)
7. Frank E. Humerslag "The technology and chemistry of alkaloid' 1; (1950).
8. Hesse Manfred. (Inst. Of org. chem.; Univ. of Zurich) 'Alkaloid chemistry' 89-90; 2-2 (1978)
9. Frank. E. Humerslag "The technology and chemistry of alkaloid' 5, (1950)
10. 'An Introduction to Economic Botany' p (85), 1985
11. Stout H, George & Schultes Richard Evans Importance of Plant chemical in Human affairs' P (381)
12. Gangulee H.C., Kar A.K 'College Botany' Vol -II, P (942), (1982)
13. Eames J. Morpholo gy of vascular plants' p (275) (1982)
14. 'Indian Medicinal Plants' A compendium of 500 species Vol 1
a. (1993) P (49-51)
15. 'Hand Book to the ferns of British India' By Beddoma R.H
a. kashyapa k., Ramesh Chandra * The useful plants of India'(1994)
16. Batta charjee S.K 'Handbook of Medicinal Plants'
a. Cole A.R.H. and Thornton D.W 'I Chem. Soc' 1332, (1957)
17. Ruzicka L, Liebige, Ann Chem. 25, 471 (1929)