CHAPTER-1

ALKALOIDS OF BERBERIS MACROSEPALLA
1.1 INTRODUCTION

*Berberis* (Berberidaceae), a genus of about 190 species with spiny deciduous, evergreen shrubs, with yellow wood and flowers is distributed mainly in the temperate countries of the world, excepting Australia and S. Africa. About 13 species of *Berberis* occur in India, mostly in the lower Himalayan region and in Assam, four species occur in central and southern India (Nilgiris) and one variety of *B. asiatica* Roxb. (var. Clarkeana C.K. Schneider), in the Parasnath hills of Chota Nagpur. Most of the *Berberis* species in India are known by the same vernacular name and possess similar properties. They are reported to be bitter tonic, alterative, astringent, stomachic and diaphoretic and as curative of piles. A thick extract is made from root bark, roots and lower stem wood by boiling in water, which is called 'Rasaut', 'Rasavanti' or 'Rasanjan'. It is used in acute conjunctivitis and chronic ophthalmia in India. The yield of Rasaut from *B. lycium* was about 15.4% of the weight of wood taken, and it contained moisture 25% and berberine 9.4%. In crude preparations much of berberine is decomposed due to over heating.

Rasaut mixed with bitter and alum or with opium and lime juice is painted over the eyelids in acute conjunctivitis and in chronic ophthalmia. The practice of washing unhealthy ulcers with Rasaut is very old. *Berberine sulphate* is highly toxic to *Leishmania tropica*. The tincture and decoction have long been used and reputed as effective antipyretics and antiperiodics but is not antimalarial.

Chopra *et al.* have studied the pharmacological properties of berberine. It is found to be moderately toxic to larger animals. The minimum lethal dose per kilogram body weight for rabbits is about 0.1 mg. It is stated to be mostly destroyed in rabbits, but in man considerable amounts appear in urine, a few hours after oral administration. The yellow barberry dye obtained from bark has been largely used in tanning and colouring leather. The roots of *B. asiatica* are still used locally by villagers. Some *Berberis* spp. are cultivated as ornamental plants for their foliage and flowers.

*B. macrosepalla*, a small shrub with spreading branches, attaining 2-4 ft. height is found in the interiors of the Sikkim Himalaya (12,000 - 13,000 ft). Leaves are obovate-oblong coarsely spinulose-toothed, margins thickened, peduncle slender. Leaves fascicled, 0.5-1.0 in. usually glaucous beneath, flowers rather large, peduncle curved, glabrous, berries large ovoid 0.5-0.75 in., red in colour, 6-10 seeded and stigma sessile.
A 90% ethanolic extracts of the leaves and stems of the plant when subjected to a wide range of biological activity at Central Drug Research Institute, Lucknow, India, exhibited significant antimicrobial, hypotensive, diuretic and antiamoebic activities. The LD₅₀ of the extract was found to be 316 mg/kg. The extract did not show CVS, CNS and spasmylotic activities. In the follow-up studies the activities were concentrated in the alkaloidal fractions of the ethanolic extract of the plant.

1.2 Previous Investigation

Literature survey revealed that various *Berberis* species have been extensively investigated for their alkaloidal constituents and resulted in the isolation of various 1-benzyltetrahydroisoquinoline derived alkaloids e.g. protoberberine, bisbenzylisoquinolines etc. The protoberberine alkaloid berberine (1) is a major constituent of the stem bark and roots of *B. edgeworthiana*, *B. insignis*, *B. lycium* and *B. orthobotrys*. Further the base has also been isolated from the roots of *B. aristata*, *B. asiatica* and *B. bhutanensis*. Berberine along with other protoberberines columbamine (2), jatrohhizine (3) and palmatine (4) had been isolated from *B. glauca*. Five alkaloids had been isolated from 'rasaut' of *B. aristata*, aromoline (5), oxyberberine (6), berberine chloride (1), oxyacanthine (7), and berbamine (8). The twigs of *B. darwinii* yielded berberine, jatrorrhizine, thalifindine (9), protopine (10) and quaternary aporphine base magnoflorine (11).

The root and bark of *B. vulgaris* furnished berberine, bisbenzylisoquinoline alkaloids oxyacanthine and berbamine. *B. asiatica* has yielded berberine and a phthalideisoquinoline alkaloid hydrastine (12). An isoquinoline derived alkaloid aconcaguine (13) a new proaporphine-benzylisoquinolines (+)-epiberbivaldine (14) and (+)-rupancamine (15) had been isolated from *B. actinacantha*. The aporphine alkaloids glaucine (16), isoboldine (17) and isocorydine (18) have also been reported from some *Berberis* spp.

1.3 Objective of Present Investigation

A perusal of literature revealed that *B. macrosepalla* have escaped the attention of chemists. The objectives of the present investigations was therefore to isolate and characterise the alkaloidal constituents present in the active fraction of alcoholic extract of the leaves and stems of *B. macrosepalla*.

1.4 Present Investigation

The leaves and stems of *B. macrosepalla* were collected from Chhangu lake, East Sikkim. The air-dried powdered plant material was extracted exhaustively with 95% ethanol at ambient temperature. The basic material from the ethanolic extractive was extracted with 10%
(1) $R_1 = O \cdot CH_2 \cdot O; R_2 = R_3 = OMe$
(2) $R = R_2 = R_3 = OMe; R_1 = OH$
(3) $R = OH; R_1 = OMe; R_2 = R_3 = H$
(4) $R = R_1 = R_2 = R_3 = OMe$
(9) $R \cdot R_1 = O \cdot CH_2 \cdot O; R_2 = OMe; R_3 = OH$

(5)

(6)

(7)

(8)

(10)
R = R_3 = OMe; R_1 = R_4 = OH; R_2 = H; R_5 = Me.

R = R_1 = R_2 = R_3 = Me; R_4 = R_5 = H

R = R_3 = OMe; R_1 = R_2 = OH; R_4 = R_5 = H

R = R_1 = R_3 = OMe; R_2 = R_5 = H; R_4 = OH.
HCl and was successively fractionated into hexane soluble, chloroform soluble A and n-butanol soluble B alkaloidal fractions. The alkaloidal mixtures A and B were purified by careful column chromatography over neutral Al₂O₃ (TLC control) and preparative thin layer chromatography (PTLC) over SiO₂ gel. The alkaloids isolated and characterised are recorded in Table-1.

### Table-1

**Compound isolated from the plant Berberis macrosepalla**

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>Alkaloids</th>
<th>Molecular formula</th>
<th>m.p. (°C)</th>
<th>[α]₁₀⁻²⁵</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I.</strong></td>
<td>Tetrahydroprotoberine alkaloids</td>
<td></td>
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<tr>
<td>(1.)</td>
<td>Alkaloid A</td>
<td>C₂₀H₂₁NO₄</td>
<td>133-134</td>
<td>-298°</td>
<td>Canadine (19)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(c, 0.85, CHCl₃)</td>
<td></td>
</tr>
<tr>
<td>(2.)</td>
<td>Alkaloid B</td>
<td>C₂₁H₂₅NO₄</td>
<td>142</td>
<td>-290.5°</td>
<td>Tetrahydro-palmatine (20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(c, 1.52, CHCl₃)</td>
<td></td>
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<tr>
<td><strong>II.</strong></td>
<td>Bisbenzylisoquinoline alkaloids</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3)</td>
<td>Alkaloid C</td>
<td>C₂₆H₄₂N₂O₆</td>
<td>182-183</td>
<td>+149°</td>
<td>Isotetrandrine (21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(c, 1.0, CHCl₃)</td>
<td></td>
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<tr>
<td>(4)</td>
<td>Alkaloid D</td>
<td>C₃₀H₄₀N₂O₆</td>
<td>212-213</td>
<td>-</td>
<td>Oxyacanthine (7)</td>
</tr>
<tr>
<td><strong>III.</strong></td>
<td>Quaternaryprotoberine alkaloids</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5)</td>
<td>Alkaloid E</td>
<td>C₂₁H₂₂NO₄</td>
<td>239-241</td>
<td>-</td>
<td>Palmatine (4)</td>
</tr>
<tr>
<td>(6)</td>
<td>Alkaloid F</td>
<td>C₂₀H₃₈NO₄</td>
<td>144-145</td>
<td>-</td>
<td>Berberine (1)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7)</td>
<td>Alkaloid G</td>
<td>-</td>
<td>215</td>
<td>-</td>
<td>Unidentified</td>
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</table>

#### 1.4.1 The Chemistry of Alkaloid A (19)

Alkaloid A (C₂₀H₂₁NO₄) (M⁺ 339) had in its IR spectrum, absorption bands at 2900, 2800, 1605 (Ar), 1240 (OMe), 940 (O-CH₂-O) cm⁻¹. The UV spectrum (λ_max 230 and 288 nm) of the base remained unchanged in the presence of alkali indicating the absence of phenolic hydroxyl group. The UV spectrum in conjunction with the IR spectrum of the base suggested the presence of a tetra- hydroprotoberberine nucleus. The base did not react with HCHO-HCO₂H and with CH₂N₂.
The integrated NMR spectrum of the alkaloid A showed the presence of 21 protons. Two aromatic methoxyl functions resonated at δ3.85, a doublet centered at δ4.30 (J = 15Hz) was assigned to the C₈-He (equatorial) proton. A singlet at δ5.90 appeared for methylenedioxy group. The aromatic region of the spectrum integrated for four protons. The ortho coupled C₁₁ and C₁₂ protons appeared as a doublet centered at δ6.56 (d,2H, J = 7.5 Hz) The two aromatic protons appeared as singlets at δ6.72 and 6.80 (s each, 1H each, C₁ -H and C₄-H).

The mass fragmentation pattern of alkaloid A was characteristic of tetrahydroprotoberberine undergoing facile fission at the two benzylic bonds as shown in Scheme-1. The molecular ion peak of the base was at m/z 339 (M⁺), and other intense peaks were at m/z 176 and 174. The minor peaks were at m/z 164 and 149. The data thus suggested that the methylenedioxy group was present at C-2,3 positions while two methoxyl groups were at C-9,10 positions of aromatic rings¹⁴,¹⁵.

The physical constants and spectral data described above suggested that the alkaloid A has a canadine (19) structure. The identity of alkaloid A with canadine was finally established by direct comparison (cotlc, mmp, uv, ir, nmr, [α]D and mass spectra) with an authentic sample¹⁶,¹⁷.

1.4.2 The chemistry of alkaloid B (20)

Alkaloid B (C₁₂₂₅NO₄) (M + 355) in its IR spectrum¹⁸, had absorption bands at 2900, 2800, 1605 (Ar) and 1240 (OMe) cm⁻¹. In the UV spectrum, the absorption maxima were at 230 and 284 nm which remained unchanged in the presence of alkali indicating the absence of phenolic hydroxyl group. The IR and UV spectra of the base suggested the presence of a tetrahydroprotoberberine¹²,¹³ nucleus. The base did not react with HCHO- HCO₂H and with CH₂N₂.

In integrated NMR spectrum of the alkaloid B there were 25 protons. Four aromatic methoxyl functions resonated at δ3.82 (s,3H), 3.83 (s,6H) and 3.87 (s,3H). Aromatic solvent induced shift, caused by the addition of a few drops of C₆D₆ to CDCl₃ solution, further separated the methoxyl signals which appeared as singlets at δ3.60,3.63, 3.68 and 3.80 respectively. A doublet (J = 15 Hz) centered at δ4.30 was assigned to the C₈-He (equatorial) proton. The aromatic region of the spectrum integrated for four protons. The ortho coupled C₁₁ and C₁₂ protons appeared as a doublet centered at δ6.67 (d,2H, J = 7.5Hz), the remaining two aromatic protons appeared as singlets at δ6.78 and 6.80 (s each,1H each, C₁-H and C₄-H).
Scheme - 1

Mass fragmentation pattern of Tetrahydroprotoberberines

\[ \text{19} \quad R \cdot R_1 = \text{CH}_2, \ m/z \ 339 (M^+) \]
\[ \text{20} \quad R = R_1 = \text{CH}_3, \ m/z \ 355 (M^+) \]

\[ \text{19a} \quad R \cdot R_1 = \text{CH}_2, \ m/z \ 174 \]
\[ \text{20a} \quad R = R_1 = \text{CH}_3, \ m/z \ 190 \]

\[ \text{19b} \quad R \cdot R_1 = \text{CH}_2, \ m/z \ 176 \]
\[ \text{20b} \quad R = R_1 = \text{CH}_3, \ m/z \ 192 \]

\[ m/z \ 149 \]
The $^{13}$C NMR spectrum of alkaloid B revealed the presence of 21 carbons. The four methoxyl carbons resonated at $\delta$55.76 (q, 2 × OMe), 56.05 (q) and 59.22 (q). The most downfield signal at $\delta$150.18 was due to C$_9$ aromatic carbon. The angular carbon(C13a) appeared at $\delta$59.94(d). The upfield signal in the spectrum at $\delta$29.08 was due to C$_5$.

The mass spectrum of the alkaloid B was characteristic of tetrahydropyroberberine$^{14,15}$ (Scheme-1). The molecular ion peak in the spectrum was at m/z 355 (M$^+$.). Other significant peaks in the spectrum were at m/z 324 (M$^+$.-31), 192, 190, 164 and 149.

The physical constants and spectral data described above suggested that the alkaloid B had a tetrahydropalmatine (20) structure. The identity of alkaloid B with tetrahydropalmatine was finally established by direct comparison (cotle, mmp, uv, ir, nmr, $[\alpha]$D and ms spectra) with an authentic sample$^{19}$.

1.4.3 The Chemistry of Alkaloid C (21)

Alkaloid C (C$_{38}$H$_{42}$N$_2$O$_6$) (M$^+$ 622) had absorption bands in UV spectrum at 229 and 282 nm. No change in UV spectrum was observed on addition of alkali suggesting the absence of phenolic hydroxyl group. Its IR spectrum had absorption bands at 3400, 3100, 2933, 1585 (Ar), 1235 (OMe), 840 (olefinic) cm$^{-1}$.

The integrated $^1$H NMR spectrum of alkaloid C confirmed the presence of 42 protons in the molecule. The well separated signals for two N-methyl groups at $\delta$2.28 and 2.58 indicate it to be a member of berbamine series because the alkaloids of the oxycanthine series have both these signals near $\delta$2.55$^{20,21}$. The most shielded methoxyl group at C-7 appeared at $\delta$3.14. The methoxyl group at C-6$'$ appeared at $\delta$3.60, that of C-6 at $\delta$3.74 and C-12 methoxyl group showed signal at $\delta$3.92. Ten aromatic protons were present between $\delta$6.00-7.53.

The mass spectrum of the alkaloid C had a weak but observable molecular ion at m/z 622. The mass fragmentation pattern (Scheme-2) was typical of a bisbenzylisoquinoline alkaloid$^{22-24}$. Double benzylic cleavage as shown in 21 gave the ion (21a) m/z 396. Loss of H and Me from ion (21a) yielded ions (21c) m/z 395 and (21d) m/z 381 respectively. Doubly charged ion (21b) m/z 198 formed from the molecular ion (M$^{2+}$) by loss of Me and OMe furnishes ion (21e) m/z 175.
Scheme - 2

Mass fragmentation pattern of Isotetrandrine

(21) m/z 622

(21a) m/z 396

(21b) m/z 198

(21c) m/z 381

(21d) m/z 381

(21e) m/z 175

(23c) m/z 395

m/z 364

m/z 349
The physical constants and spectral data described above, suggested that the alkaloid C had structure 21 corresponding to isotetrandrine (6,7,8,11+, 12-6, 7',12+). The identity was finally established by direct comparison (cotlc, mmp, uv, ir, nmr, [α]D and ms spectra) with an authentic sample25,26.

1.4.4 The Chemistry of Alkaloid D (7)

Alkaloid D(C37H40N2O6) (M+ 608) had, in its UV spectrum, absorption maxima at 229 and 282 nm. A bathochromic shift to 233 and 286 nm occurred on addition of alkali indicating, thus, the presence of a phenolic hydroxyl group. The IR spectrum of the base had absorption bands at 3440 (OH), 3020, 2940, 1238 (OMe), 1200 and 840 (olefinic) cm⁻¹.

The integrated ¹HNMR spectrum of alkaloid D confirmed the presence of 40 protons in the molecule. The signals for two N-methyl groups at δ2.55 and 2.60 suggested the alkaloid to be a member of oxyacanthine series21.

A methoxyl group at C-7 was shielded and appeared at δ3.18. A C-6' methoxyl was present at δ3.63 while C-6 methoxyl at δ3.78. The aromatic region integrated for 10 protons between δ6.3-7.4.

The mass fragmentation pattern (Scheme-2) of compound D was typical of a bisbenzylisoquinoline alkaloid27 with molecular ion at m/z 608. The other prominent peaks were at m/z 607,501 (M⁺-107), 417(M⁺-191), 416, 396, 395, 381, 364, 335, 198 (base peak), 192, 175. The ion peaks at m/z (M⁺-107) and (M⁺-191) are characteristic peaks of oxyacanthine type bases20 (Fig. I) and were different from berbamine type bases which have weak but characteristic peaks at m/z (M⁺-191) and(M⁺-137) (Fig.II).

The (M⁺-137) peak is usually less intense than the (M⁺-191) peak in the berbamine series, while in the oxyacanthine series it is the (M⁺-107) peak which is more intense than the (M⁺-191) peak20.

The physical constants and spectral data described above, suggested that the alkaloid D had structure 7 corresponding to oxyacanthine (6,7',11+, 12-6,7,8', 12+)25. The identity was finally confirmed by direct comparison (cotlc, mmp, uv, ir, nmr,[α]D and ms spectra) with an authentic sample28,29.
1.4.5 The Chemistry of Alkaloid E (4)

Alkaloid E (C\textsubscript{21}H\textsubscript{22}NO\textsubscript{4}) (M\textsuperscript{+} 351) exhibited absorption bands in UV spectrum at 236, 272 and 347 nm which remain unchanged in presence of alkali. The IR spectrum of the base had absorption maxima at 3350, 2900, 1600 (Ar), 1560, 1440, 1345, 1240 (OMe), 1140, 1100, 1010, 870 and 780 cm\textsuperscript{-1}.

The \textsuperscript{1}H NMR spectrum of the alkaloid E had signals for 4 aromatic methoxyl groups at \(\delta\) 3.80, 3.86, 4.00 and 4.04 respectively. Of the 4 aromatic protons, singlets for \(\text{C}_1\text{-H}\) and \(\text{C}_4\text{-H}\) were at \(\delta\) 6.97 and 7.58 respectively. The signals for \(\text{C}_{1\text{I}}\text{-H}\) and \(\text{C}_{1\text{2}}\text{-H}\) ortho coupled protons were at \(\delta\) 7.88 (J = 9Hz) and 8.07 (J = 9Hz) respectively. The low field signals at \(\delta\) 8.89 (s, 1H) and 9.73 (s, 1H) were assigned to the \(\text{C}_{1\text{3}}\text{-H}\) and \(\text{C}_{8}\text{-H}\) respectively.

The mass spectrum of the base had a molecular ion at m/z 352 (M\textsuperscript{+}). The other significant peaks in the spectrum were at m/z 351 (M\textsuperscript{+}-1), 350, 337 (M\textsuperscript{+}-15), 336 and 321 (M\textsuperscript{+}-31).

Alkaloid E on treatment with Sn/HCl or NaBH\textsubscript{4} furnished a tetrahydro derivative identical in all respects (cotlc, mmp, uv, ir, nmr and ms spectra) with tetrahydropalmatine (20). The physical and chemical constants of the base were almost identical with palmatine (4)\textsuperscript{30,31}. A direct comparison (cotlc, mmp, uv, ir, nmr and ms spectra) with an authentic specimen of palmatine finally established the identity.

1.4.6 The Chemistry of Alkaloid F (1)

Alkaloid F (C\textsubscript{20}H\textsubscript{18}NO\textsubscript{4}) (M\textsuperscript{+} 336) had absorption bands in UV spectrum at 228, 266 and 354 nm. No change in UV spectrum was observed on addition of alkali. Its IR spectrum had absorption bands at 3350,2900,1600 (Ar), 1500, 1380, 1330, 1300, 1245 (OMe), 1220, 960 (O.CH\textsubscript{2}.O) and 840 cm\textsuperscript{-1}.

The \textsuperscript{1}H NMR spectrum of the alkaloid F had signals for two aromatic methoxyl groups at \(\delta\) 4.1 and 4.18 respectively. A singlet was observed at \(\delta\) 5.59 for methylenedioxy group. Of the 4 aromatic protons, singlets for \(\text{C}_1\text{-H}\) and \(\text{C}_4\text{-H}\) were at \(\delta\) 6.9 and 7.7 respectively. A doublet for ortho coupled protons (\(\text{C}_{1\text{I}}\text{-H}\) and \(\text{C}_{1\text{2}}\text{-H}\)) appeared at \(\delta\) 8.01 (d, J = 9Hz). The signals for \(\text{C}_{1\text{3}}\text{-H}\) and \(\text{C}_{8}\text{-H}\) were at \(\delta\) 8.9 (s, 1H) and 9.9 (s, 1H) respectively.

The mass spectrum of the alkaloid F had a molecular ion at m/z 336 (M\textsuperscript{+}) and the other significant peaks were at m/z 335 (M\textsuperscript{+}-1), 321 (M\textsuperscript{+}-15) and 305 (M\textsuperscript{+}-31).
Alkaloid F on treatment with Sn/HCl or NaBH₄ furnished a tetrahydro derivative identical in all respects (cotle, mmp, [α]D, uv, ir, nmr and ms spectra) with canadine (19). The physico-chemical constants of alkaloid F were identical with berberine (1). A direct comparison of alkaloid F with authentic sample of berberine confirmed the identity (cotle,mmp,uv,ir,nmr and ms spectra)16,31.

1.4.7 The Chemistry of Alkaloid G

Alkaloid G in its IR spectrum had absorption bands at 3400, 2920, 1638, 1540, 1450, 1390, 1280, 1220, 960, 850 cm⁻¹. In the UV spectrum the absorption maxima were at 228, 277 and 322 nm. A bathochromic shift to 226,279 and 300 nm occurred on addition of alkali indicating, thus, the presence of a phenolic hydroxyl group.

The ¹H NMR spectrum of alkaloid G showed a singlet for one N-methyl group at δ2.8. Three methoxyl groups appeared as singlets at δ3.25 (s, 3H) and at δ3.4 (s, 6H). A doublet (J = 12 Hz) centered at δ4.25 was observed. A doublet (J = 6 Hz) appeared at δ6.3 for one proton. Singlet at δ6.45 and a doublet at δ6.55 (J = 6Hz) appeared for one proton each.

The mass spectra of the alkaloid G had molecular ion peak at m/z 433 (M⁺) and other significant peaks were at m/z 402, 393, 392, 377, 356, 347, 346, 209, 195, 122 and 105.

The structure elucidation of alkaloid G is in progress.

1.5 Biological activities

1.5.1. Antifungal activity

The following five strains of pathogenic fungi, of which 3 yeast-like viz., Candida albicans (CA), Cryptococcus neoformans (CN) and Sporotrichum schenckii (SS), two mycelial: one dermatophyte- Trichophyton mentagrophytes (TM) and an opportunistic pathogen- Aspergillus fumigatus (AF), have been employed for antifungal testing. All these strains were maintained on Sabouraud's agar slants32.

Palmatine, (-)-tetrahydropalmatine were devoid of any antifungal activity against C. albicans, C. neoformans, T. mentagrophytes and A. fumigatus. (dl)-Tetrahydropalmatine showed antifungal activity against two pathogens namely C. neoformans and A. fumigatus at a dose of 500 ug/ml while berberine showed antifungal activity against three pathogens viz., C. albicans, C. neoformans and A. fumigatus at a dose of 500 ug/ml.
1.5. 2. Antibacterial Activity

Five bacteria viz., *Streptococcus faecalis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* penicillin resistant (2500 units) were used for antibacterial screening. Nutrient broth was employed as test medium for bacteria.

(±)-Canadine and palmatine were found to be inactive against *S. faecalis*, *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *S. aureus* penicillin resistant (2500 units). (dl)- Tetrahydropalmatine showed very good antibacterial activity against four pathogens viz., *S. faecalis* and *S. aureus* (125 ug/ml), *K. pneumoniae* and *E. coli* (250 ug/ml) while berberine showed antibacterial activity against *S. faecalis* and *S. aureus* at a dose of 250 ug/ml.

1.5.3. The other pharmacological activities of isolated alkaloids:

Canadine is reported to exhibit analgesic and papaverine like spasmolytic activity and is a potentiator of antimitotic effect of colchicine. (-) Canadine methochloride also has shown some hypotensive activity in anesthetized cats and dogs.

Berberine showed a wide variety of pharmacological effects including respiratory stimulation, transient hypotension, convulsion cholinesterase, tyrosine decarboxylase and tryptophanase inhibitor. It acted as anti-anaemic agent and showed some cytotoxic and antineoplastic activity. Berberine is used in the treatment of gastro-intestinal disorders. The base has been used for cholera and infantile diarrhoea. Berberine chloride showed anthelmintic activity and is able to eliminate *Syphacia obvelata* from mice.

Tetrahydropalmatine inhibited the respiratory chain by interfering with the action of NADH oxidase. It showed analgesic, sedative and hypnotic activity.

Palmatine showed uterine contractant properties and bactericidal activity, produces antiarrhythmic, inotropic, adrenocorticotropic, anticholinesterase and analgesic effects in experimental animals.

Isotetrandrine showed *in vitro* antitumour activity against He La cells and Ehrlich ascites. Oxyacanthine showed antibacterial, antitubercular and *in vitro* antitumour activity against He La-S3 cells. It is a sympathicolytic agent, adrenaline antagonist, vasodilator. Oxyacanthine chloride at 1:10,000 dilution killed *Bacillus subtilis* and *Colpidium colpoda*. 
1.6 EXPERIMENTAL

Melting points were taken in Buchi 530 and are uncorrected. The optical rotations were measured on a Perkin-Elmer 241 polarimeter. The UV spectra were taken on Lambda-15 (Perkin-Elmer) recording photometer and the IR spectra were taken on a Perkin-Elmer infracord 157 or Beckman Ac-1 instrument. The $^1$H NMR spectra were taken on Perkin-Elmer R-32, CFT-20 (at 90, 60 MHz), and Bruker WM-400 (200 and 400 MHz) spectrometers in the solvents stated, using tetramethylsilane (TMS) as an internal standard and chemical shifts recorded in $\delta$ (ppm) units. $^{13}$C NMR were taken on Bruker WM-400 spectrometer (at 100.13 MHz). The mass spectra were recorded on JEOL-D 300 mass spectrometer.

Neutral Al$_2$O$_3$ and silica gel (Merck and Sisco) were used for column chromatography. Silica gel GF$_{254}$ was used for thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC). PTLC was carried out over glass plates (20x20 cm) containing silica gel (10-20g). Anhydrous sodium sulphate was routinely used for drying the organic solvents and all solvents were evaporated under reduced pressure below 50$^\circ$.

The alkaloids were visualised by spraying with dragendorff reagent$^{38,39}$ (Solution A - 0.85 g basic bismuth nitrate in 10 ml acetic acid and 40 ml H$_2$O, Solution B- 8g potassium iodide in 20 ml H$_2$O. Stock solution-equal volumes of A and B were mixed. Spray reagent- 1 ml stock solution mixed with 2 ml acetic acid and 10 ml water), by exposure to iodine vapours and by fluorescence in UV lamp.

PLANT MATERIAL

The leaves and stems of *Berberis macrosepalla* were collected from Chhangu lake, East Sikkim in October and was identified by Dr. M. P. Sharma of Central Drug Research Institute, Lucknow, India. A herbarium specimen is on deposit in the herbarium of Botany department of Central Drug Research Institute, Lucknow.

EXTRACTION AND FRACTIONATION

Air-dried, finely powdered plant material (1.2 kg) of *B. macrosepalla* was exhaustively extracted with 95% alcohol (5x4 lt.) at room temperature. The combined percolate was concentrated under reduced pressure below 50$^\circ$ to afford a dark green viscous mass (55g) which was extracted with 10% HCl (6 x 100 ml). The acidic extract was defatted with n-hexane (5 x 100 ml) and then basified with sodium carbonate (pH 8-9). The liberated bases were
extracted with chloroform (5 x 100 ml), washed with H₂O (4 x 50 ml), dried and solvent removed in vacuo to give the alkaloidal mixture A (6.5 g). The chloroform insoluble layer was extracted with n-butanol (3 x 100 ml). The combined n-BuOH layer was washed with H₂O (2 x 50 ml), dried and solvent removed under reduced pressure to give the alkaloidal mixture B (15.0 g).

**CHROMATOGRAPHY OF ALKALOIDAL MIXTURE A**

The alkaloidal mixture A (3 g) was dissolved in chloroform, adsorbed on neutral Al₂O₃ (8 g) and was put on a column of neutral Al₂O₃ (100 g) in hexane. The column was successively eluted by hexane with increasing proportions of ethylacetate and methanol in ethyl acetate. Total fractions (168) of 50 ml each were collected. The fractions were monitored by TLC and the results are presented in Table-2.

**Alkaloid A (19)**

The fractions 53-59, showing single spot on SiO₂ plates (solvent : CHCl₃ : MeOH ; 99 : 1) were mixed and the solvent removed. The crude base, thus obtained, was crystallized from CHCl₃ to give *alkaloid A (19)* (0.12 g), m.p. 133-134°; [α]D -298° (c, 0.85, CHCl₃).

<table>
<thead>
<tr>
<th>Frac. No.</th>
<th>Eluant</th>
<th>Weight (g)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-11</td>
<td>Hexane</td>
<td>0.02</td>
<td>Oily residue</td>
</tr>
<tr>
<td>12-34</td>
<td>Hexane:Ethylacetate (3:1)</td>
<td>0.16</td>
<td>-do-</td>
</tr>
<tr>
<td>35-52</td>
<td>Hexane:Ethylacetate (1:1)</td>
<td>0.08</td>
<td>-do-</td>
</tr>
<tr>
<td>53-59</td>
<td>Hexane:Ethylacetate (1:3)</td>
<td>0.15</td>
<td>Alkaloid A</td>
</tr>
<tr>
<td>60-65</td>
<td>Ethylacetate</td>
<td>0.25</td>
<td>Alkaloid A&amp;B</td>
</tr>
<tr>
<td>66-80</td>
<td>Ethylacetate:Methanol (98:2)</td>
<td>0.3</td>
<td>Alkaloid B</td>
</tr>
<tr>
<td>81-89</td>
<td>Ethylacetate:Methanol (94:6)</td>
<td>0.32</td>
<td>Complex mixture with traces of Alkaloid B</td>
</tr>
<tr>
<td>90-96</td>
<td>Ethylacetate:Methanol (90:10)</td>
<td>0.2</td>
<td>Mixture; Alkaloid C as major component</td>
</tr>
<tr>
<td>97-149</td>
<td>Ethylacetate:Methanol (85:15)</td>
<td>0.3</td>
<td>Alkaloid C&amp;D</td>
</tr>
<tr>
<td>150-158</td>
<td>Ethylacetate:Methanol (1:1)</td>
<td>0.4</td>
<td>Alkaloid E</td>
</tr>
<tr>
<td>159-168</td>
<td>Methanol</td>
<td>0.44</td>
<td>Alkaloid E &amp; F</td>
</tr>
</tbody>
</table>

Table-2

*Chromatography of Alkaloidal mixture A (3.0 g)*
UV $\lambda_{\text{EtOH}}^{\text{max}}$ : 230 and 288 nm
IR $\nu_{\text{KBr}}^{\text{max}}$ : 2900, 2800, 1605 (Ar), 1490, 1425, 1340, 1270, 1240, (OMe), 1075, 1035, 940 (O.CH$_2$.O) and 840(olefinic) cm$^{-1}$
$^1$HNMR(CDCl$_3$) : $\delta$3.85(6H,s,2xOCH$_3$),4.30(d,lH,C$_8$.H$^-$, $J$ = 15 Hz),5.90 (2H,s,O.CH$_2$.O),6.56 (d, 2H,C$_{11}$.H and C$_{12}$.H,$J$ = 7.5 Hz), 6.72 (1H,s,C$_1$.H) and 6.80(1H,s,C$_4$.H)
MS : m/z 339 (M$^+$), 338 (M$^+$.1), 324 (M$^+$.15), 176, 174, 164 and 149.

Alkaloid B (20)

The fractions 66-80, single spot on SiO$_2$ plates (solvent:CHCl$_3$:MeOH; 98:2) were mixed and the solvent removed, the residue thus obtained was crystallized from MeOH to yield alkaloid B (20) (0.24g) m.p.141-142°; [$\alpha$]$_D$ -290.5° (c, 1.52, CHCl$_3$).

UV $\lambda_{\text{MeOH}}^{\text{max}}$ : 230 and 284 nm.
IR $\nu_{\text{KBr}}^{\text{max}}$ : 2900,2800, 1605 (Ar), 1490, 1440, 1325, 1240(OMe), 140, 1080, 1020, 990, 850 and 780 cm$^{-1}$.
$^1$H NMR(CDCl$_3$) : $\delta$2.5-4.0(m,8H),3.82(s,3H,OCH$_3$),3.83 (s,6H, 2 x OCH$_3$), 3.87(s,3H,OCH$_3$),4.30(d,1H,C$_8$.He,$J$ = 15 Hz),6.67 (d,2H, C$_{11}$.H and C$_{12}$.H,$J$ = 7.5 Hz), 6.78 and 6.80 (s,each $^1$H each,C$_1$.H andC$_4$.H).
$^1$H NMR (CDCl$_3$-C$_6$D$_6$) : $\delta$2.6-4.1(m,8H),3.60(s,3H,OCH$_3$-C$_{10}$), 3.63 and 3.68(s each,3H each,OCH$_3$-C$_2$ and C$_3$),3.80(s,3H,OCH$_3$-C$_8$),4.30 (d, 1H,C$_8$.He, $J$ = 15Hz) and 6.45-6.89 (4H, Ar-H).
$^{13}$C NMR(CDCl$_3$) : $\delta$109.05 (d,C-1), 126.84 (s, C-1a), 147.58 (s, C-2), 145.12 (s, C-3), 111.61 (d, C-4), 129.87 (s,C-4a), 29.08 (t, C-5), 51.46 (t, C-6), 53.97 (t, C-8), 150.18 (s, C-9), 127.81 (s, C-9a), 147.58 (s, C-10), 111.12 (d, C-11), 123.76 (d, C-12), 128.60 (s, C-12a), 36.28 (t, C-13), 59.94 (d, C-13a), 55.76 (q,2 x OCH$_3$), 56.05 (q, OCH$_3$) and 59.22 (q,OCH$_3$).
MS : m/z 355 (M$^+$), 354 (M$^+$.1), 324 (M$^+$. 31), 192,190,164 and 149.

17
Alkaloid C (21)

The fractions 90-96, eluted with ethylacetate: methanol (90:10) were mixed and the solvent removed. The crude mixture so obtained was purified by preparative TLC (plates: silica gel; solvent: CHCl₃;MeOH; 95:5). The major band on the plates was cut off and extracted with CHCl₃:MeOH (3:1). Solvent was removed and the residue thus obtained was crystallized from MeOH to yield alkaloid C (21) (0.08g) as colourless prisms, m.p. 181-182°; [α]D + 149° (c, 1.0,CHCl₃).

\[ \text{UV } \lambda_{\text{max}}^{\text{MeOH}} : 229 \text{ and } 282 \text{ nm.} \]

\[ \text{IR } \nu_{\text{max}}^{\text{KBr}} : 3400, 3100, 2933, 1585 \text{(Ar), } 1235 \text{(OMe)} , 1072 \text{ and } 840 \text{ cm}^{-1}. \]

\[ 1^H \text{NMR(CDC}13) : \delta 0.28(3H,s,N-CH₃), 2.58(3H,s,N-CH₃), 3.14(3H,s, OCH₃-C₇), 3.60(3H,s, OCH₃-C₆'), 3.74(3H,s, OCH₃-C₆), 3.82(s,1H,C₁-H) \text{ and } 2.84(s,1H,C₁-H), 3.92(3H,s, OCH₃-C₁₂), 5.98(s,1H,C₈-H), 6.28(s,1H,C₅-H), 6.42(2H, s, C₁₀ and C₁₀'-H), 6.54(s,1H,C₃'-H), 6.65(s,1H,C₃'-H), 6.78(s,1H,C₁₄-H), 6.80(s,1H,C₁₃-H), 7.10(s,1H,C₁₃'-H), 7.27(s,1H,C₁₄'-H) \]

\[ \text{MS } : \text{m/z } 622 \text{ (M}^+), 607, 485 \text{ (M}^+-137), 431 \text{ (M}^+-191), 396, 395, 381, 198 \text{ (base peak), } 175 \text{ and } 174. \]

Alkaloid D (7)

The fractions 97-149, eluted with ethylacetate: methanol (85:15), were combined and solvent removed to afford a crude mixture which was further purified by preparative TLC (plates:silica gel; solvent:CHCl₃;MeOH; 90:10). The major band was cut off and eluted with CHCl₃:MeOH (90:10), solvent removed under reduced pressure. The residue was crystallized from MeOH to yield alkaloid D (7) (0.15g), m.p. 212-213°.

\[ \text{UV } \lambda_{\text{max}}^{\text{MeOH}} : 229 \text{ and } 282 \text{ nm.} \]

\[ \text{UV } \lambda_{\text{max}}^{\text{MeOH} + \text{NaOH}} : 233 \text{ and } 286 \text{ nm.} \]

\[ \text{IR } \nu_{\text{max}}^{\text{KBr}} : 3440(OH), 3020,2940,1280,1238(OMe), 1200 \text{ and } 840 \text{ cm}^{-1}. \]

\[ 1^H \text{NMR(CDC}13) : \delta 2.55(3H,s,N-CH₃), 2.60(3H,s,N-CH₃), 3.18(3H,s,OCH₃-C₇), 3.63(3H,s,OCH₃-C₆'), 3.68(1H,s,C₁-H), 3.78(3H,s, OCH₃-C₆'), 4.19(1H,s,C₁-H), 5.43(1H,s,C₁₀-H), 6.31(1H,s, C₅'-H), 6.34(1H,s,C₁₁'-H), 6.35(1H,s,C₅'-H), 6.63 \]
(1H,s,C₈-H), 6.76 (1H,s,C₁₄-H), 6.78(1H,s,C₁₃-H), 6.94(1H,s,C₁₃-H), 6.98(1H,s,C₁₀-H), 7.44(1H,s,C₁₀-H)

MS : m/z. 608 (M+), 607,504 (M+ -107), 417 (M+ -191),416, 396, 395, 381, 364, 335, 198 (base peak), 192, and 175.

Alkaloid E (4)

The fractions 150-158, single spot on SiO₂ plates (solvent: CHCl₃: MeOH; 85:15) were mixed and the solvent removed to afford the residue which was crystallised from MeOH to furnish alkaloid E (4) (0.36g) as yellow needles, m.p. 239-241°.

UV λ<sub>max</sub><sub>MeOH</sub> : 236, 272 and 347 nm
IR ν<sub>max</sub><sub>KBr</sub> : 3350, 2900, 1600(Ar), 1560, 1440, 1345, 1240 (OMe), 1140, 1100, 1010, 870 and 780 cm⁻¹

¹H NMR : δ3.16(m,2H,C₅-H₂), 3.80(s,3H,OCH₃), 3.86 (s, 3H, OCH₃), 4.00 (s,3H,OCH₃), 4.04(s, 3H,OCH₃), 4.88(m, 2H,C₆-H), 6.97 and 7.58 (each s,1H each, C₄-H and C₅-H), 7.88 and 8.07 (each d, 1H each, C₁₁-H and C₁₂-H, J =9Hz), 8.89 (s, 1H, C₁₃-H) and 9.73(s,1H,C₈-H).

MS : m/z 352 (M+), 351 (M+ -1), 350, 337 (M+ -15), 336 and 321 (M+ -31).

Reduction of Alkaloid E

To a solution of alkaloid E (4) (15 mg) in EtOH (7 ml) and conc. HCl (1 ml) was added tin metal (12 mg) and the mixture refluxed for 4 hr. The solvent from the resulting mixture was removed and H₂O added. The acidic solution was basified with Na₂CO₃ (pH8-9) and the liberated bases were extracted with CHCl₃ (15 x 3 ml). The CHCl₃ layer was washed with H₂O, dried and concentrated in vacuo give a tetrahydroderivative (10 mg), m.p. 139° (MeOH) which was found identical (mmp, uv, ir, nmr and ms) with an authentic sample of tetrahydropalmatine (20).

Alkaloid F (1)

The fractions 159-168, eluted with methanol were combined and the solvent removed. The crude residue, thus obtained was purified by PTLC (plates: silica gel, solvent: CHCl₃:MeOH;85:15). The major band was cut off from the plates and the residue obtained after removing the solvent was crystallised from MeOH to afford alkaloid F (1) (0.21g), m.p. 144-145°.
UV $\lambda_{\text{MeOH}}^{\text{max}}$ : 228, 266 and 354 nm.
IR $\nu_{\text{KBr}}^{\text{max}}$ : 3350, 2900, 1600 (Ar), 1500, 1380, 1330, 1300, 1245 (OMe), 1220, 960 (OCH2O) and 840 cm$^{-1}$.
$^1$H NMR : $\delta$ 4.1 (3H, s, OCH$_3$), 4.18 (3H, s, OCH$_3$), 5.59 (2H, s, OCH$_2$O), 6.9 and 7.7 (s, each 1H each, C$_1$-H and C$_4$-H), 8.01 (d, 2H, C$_{11}$-H and C$_{12}$-H, J = 9Hz), 8.9 (s, 1H, C$_{13}$-H) and 9.9 (s, 1H, C$_8$-H).

MS : m/z 336 (M$^+$), 335 (M$^+$-1), 321 (M$^+$-15) and 305 (M$^+$-31).

Reduction of alkaloid F

To a solution of alkaloid F (1) (15 mg) in EtOH (7 ml) and conc. HCl (1 ml) was added tin metal (12 mg) and the mixture refluxed for 4 hr. The solvent from the resulting mixture was removed and H$_2$O added, basified with Na$_2$CO$_3$ (pH 8-9) and the liberated bases were extracted with CHCl$_3$ (15x3 ml). The combined CHCl$_3$ layer was washed with H$_2$O, dried and concentrated under reduced pressure to give tetrahydroderivative (11mg), m.p. 168-169°C (MeOH) which was found identical (mmp, uv, ir, nmr and ms) with an authentic sample of canadine (19).

CHROMATOGRAPHY OF ALKALOIDAL MIXTURE B

The alkaloidal mixture B (10 g) was dissolved in n-butanol, adsorbed on neutral alumina (30 g) and was loaded on a column of neutral Al$_2$O$_3$ (300 g) in ethylacetate. The column was eluted with a mixture of ethylacetate and increasing proportions of methanol. A total of 152 fractions each of 100 ml were collected. Elution of the column was monitored by TLC. The results are presented in Table-3.

Alkaloid E (7)

The fractions 79-86 showing single spot on the tlc plate (solvent:CHCl$_3$:MeOH;85:15) were mixed and the solvent removed to give a residue (1.0g). The residue was crystallized from MeOH to yield Alkaloid E (4) (0.96g) as yellow needles., m.p. 239-241°C. The base was found identical with palmatine (mmp, uv, ir, nmr and ms spectra).

Alkaloid F (1)

Fractions 96-101 were mixed, the solvent removed and residue crystallized from MeOH to afford alkaloid F (1) as yellow needles (0.78g), m.p. 144-145°C. It was found identical with berberine (mmp uv, ir, nmr and ms spectra).
Table-3

Chromatography of Alkaloidal Mixture B (10.0 g)

<table>
<thead>
<tr>
<th>Frac. No.</th>
<th>Eluant</th>
<th>Weight (g)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-64</td>
<td>Ethylacetate</td>
<td>0.2</td>
<td>Oily residue</td>
</tr>
<tr>
<td>65-71</td>
<td>Ethylacetate: Methanol (95:5)</td>
<td>0.09</td>
<td>-do-</td>
</tr>
<tr>
<td>72-78</td>
<td>Ethylacetate: Methanol (90:10)</td>
<td>0.5</td>
<td>Mixture</td>
</tr>
<tr>
<td>79-86</td>
<td>Ethylacetate: Methanol (85:15)</td>
<td>1.0</td>
<td>Alkaloid E</td>
</tr>
<tr>
<td>87-95</td>
<td>Ethylacetate: Methanol (1:1)</td>
<td>1.8</td>
<td>Alkaloid E&amp;F</td>
</tr>
<tr>
<td>96-101</td>
<td>Ethylacetate: Methanol (1:3)</td>
<td>0.8</td>
<td>Alkaloid F</td>
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<tr>
<td>102-111</td>
<td>Methanol</td>
<td>2.1</td>
<td>Alkaloid F &amp; Complex mixture</td>
</tr>
<tr>
<td>112-140</td>
<td>Methanol : H₂O (98:2)</td>
<td>0.6</td>
<td>Mixture; Alkaloid G as major component</td>
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<tr>
<td>141-152</td>
<td>Methanol : H₂O (95:5)</td>
<td>2.2</td>
<td>Complex mixture</td>
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</table>

Alkaloid G

Fractions 112-140 eluted with methanol:H₂O (98:2) were mixed and the solvent removed. The crude base so obtained was purified by PTLC (plates: silica gel) solvent: CHCl₃:MeOH;1:3). The major band on the plates was cut off and eluted with MeOH and solvent removed to afford the residue which was crystallized from MeOH to give alkaloid G, (0.4 g) m.p. 215°.

UV \( \lambda_{\text{MeOH}} \max \) : 228, 277, 322 nm
UV \( \lambda_{\text{MeOH} + \text{NaOH}} \max \) : 205, 226, 279, 300 nm
IR \( \nu_{\text{KBr}} \max \) : 3400, 2920, 1638, 1540, 1450, 1390, 1310, 1280, 1250, 1220, 1070, 1050, 1000, 960, 850 cm⁻¹

\(^1\text{H NMR(DMSO-}d_6)\) : δ2.8 (3H,s,N-CH₃), 3.25 (s,3H, OCH₃), 3.4 (s,6H,3 × OCH₃), 4.25 (d, J = 12Hz, 2H), 6.3 (d, J = 6Hz, 1H), 6.45 (s, 1H), 6.55 (d, J = 6Hz, 1H).

REFERENCES