CHAPTER 2

LITERATURE REVIEW

- GENERAL
- CENTELLA ASIATICA LINN.
- BACOPA MONIERRA LINN.
- EVOLVULUS ALSINOIDES LINN.
- CONVOLOVULUS PLURICAULIS CHOIS.
- REFERENCES
Since ancient times, a number of herbal medicines have been used in the treatment of CNS disorder and many studies have been carried out in the search of suitable plant drugs and their multiherbal formulations that would be effective in improving the performance of CNS. *Evolvulus alsinoides*, *Convolvulus pluricaulis*, *Centella asiatica* and *Bacopa monniera* are the important traditional drug in Ayurvedic and Unani system of medicine. Because of their utility in our traditional system of medicine it has been extensively investigated by the research workers.

2.1 CENTELLA ASIATICA LINN.

2.1.1 Description of the Plant

It is distributed in tropical and subtropical regions of India particularly in damp, shady places and can commonly be seen along marshy banks of rivers, streams, ponds, irrigated fields etc. In Himalayas it grows wild in natural habitat round the year. The aerial parts of the plant are used as a drug, either as such or after processing to its derivatives - extracts and pharmaceutical preparations. It is a prostrate herb perennial with rooted nodes and long internodes; leaves simple with elongated petioles and sheathing leaf bases, broadly cordate, reniform, creanate or sinuate, toothed, flower pink, almost sessile, 3-5 in fascicled umbels; fruits laterally compressed with two mericarps having 7-9 subsimilar ridges.

2.1.2 History

*Centella asiatica* Linn is an important constituent of the Ayurvedic materia medica and frequently mentioned in Charaka Samhita (1000 A.D.).
Bhavapракашa (16th Century A.D.) has given its therapeutic uses. Brahmī has been mentioned in three Ayurvedic works: Charaka (1941) andSusruta (1972). In classical Indian Ayurvedic Literature it is recommended as one of the RASAYANA (rejuvenator).

2.1.3 Synonyms


2.1.4 Common Names in Different Languages

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>Indian pennywort</td>
</tr>
<tr>
<td>Hindi</td>
<td>Brahmamanduki, Bemsag</td>
</tr>
<tr>
<td>Kannada</td>
<td>Urage, Vondelaga</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Muttil, Kutannal, Mutakan</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Mandukparni, Munduki</td>
</tr>
<tr>
<td>Tamil</td>
<td>Vallarai</td>
</tr>
<tr>
<td>Telugu</td>
<td>Brahmī, Sarawatakū</td>
</tr>
</tbody>
</table>

2.1.5 Geographical Distribution

The plant is indigenous to the warmer regions of both hemispheres, including Africa, Australia, Cambodia, Central America, China, Indonesia, the Lao People's Democratic Republic, Madagascar, the Pacific Islands, South America, Thailand, Southern United states of America, and Viet Nam. It is especially abundant in the swampy areas of India, the Islamic Republic of Iran, Pakistan, and Srilanka up to an altitude of approximately 700m (The Indian Pharmaceutical Codex, 1953).
2.1.6 Purity Tests (Vietnam Materia Medica, 1972)

- Foreign organic matter : Not more than 2%
- Total ash : Not more than 19%
- Acid-insoluble extractive : Not less than 6%
- Water-soluble extractive : Not less than 6%
- Alcohol-soluble extractive : Not less than 9.5%

Pesticide residues

Pesticide residue is established in accordance with national requirements. Normally, the maximum residue limit of aldrin and Herba Centellae is not more than 0.05mg/kg (European Pharmacopoeia, 1997).

Heavy metals

Recommended lead and cadmium levels are not more than 10 and 0.3 mg/kg, respectively, in the final dosage form of the plant material (WHO, 1998; Abdul et al., 2002).

2.1.7 Medicinal Uses

Uses supported by clinical data

Treatment of wound, burns and ulcerous skin ailments and prevention of keloid and hypertrophic scars (Reynolds, 1993). Extracts of the plant have been employed to treat second and third degree burns. Extracts have been used topically to accelerate healing, particularly in cases of chronic postsurgical and post-trauma wounds (Gravel, 1965; Bhat et al., 1977). Extracts have been administered orally to treat stress induced stomach and duodenal ulcers (Karting, 1988; Bhakuni et al., 1969).

Uses described in pharmacopoeas and in traditional systems of medicine

In the 19th century the plant and its extracts were incorporated into the Indian Pharmacopoea and recommended not only for wound healing (Wealth
of India, 1950) but especially for the treatment of skin lesions and disease such as leprosy, eczema and psoriasis (Bhattacharya, 1956b).

The plant enjoys considerable reputation in Indian system of medicine as diuretic, alterative and tonic. The plant is acrid, bitter, sweetish, laxative, cooling, antipyretic, improve appetite, voice, memory, cures leucoderma, anemia, urinary discharges, diseases of blood, bronchitis, inflammation, fever, biliousness, enlargement of spleen, thirst, asthma, smallpox and is used in insanity. In some parts of India, the people are in the habit of taking the powdered dried leaves with milk for improving memory and its alterative tonic. The leaves are said to be useful in syphilitic skin diseases both externally and internally (Kirtikar and Basu, 1976; Bhattacharya, 1956c).

The drug is of anti-spasmodic effect and is a powerful stimulant of circulatory chiefly affecting the vessels of skin and mucous membrane; it is useful in the treatment of some heart diseases (Brequet, 1987). In large doses, it is a stupefying narcotic and in some case produces cephalagia or vertigo with a tendency to coma (Khan et al., 1979; Bhattacharya et al., 1978).

Miscellaneous

In Ceylon, *Cantella asiatica* is used as cover crop in tea and rubber plantations (Wealth of India, 1962).

**TABLE 2.1: USES OF CENTELLA ASIATICA IN FOLK REMEDIES**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Reported uses</th>
<th>Locality</th>
<th>Mode of administration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>In dysentery</td>
<td>Bombay &amp; Meghalaya (India)</td>
<td>3-4 leaves with cumin &amp; sugar ponded &amp; applied to naval</td>
<td>Kirtikar &amp; Basu, 1935</td>
</tr>
<tr>
<td>2.</td>
<td>Applied to tumors &amp; boils</td>
<td>Meghalaya (India)</td>
<td>Plant paste is given</td>
<td>Kirtikar &amp; Basu, 1935</td>
</tr>
<tr>
<td>S.No.</td>
<td>Reported uses</td>
<td>Locality</td>
<td>Mode of administration</td>
<td>References</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------</td>
<td>-------------------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>3.</td>
<td>For skin eruption</td>
<td>Konkan area</td>
<td>A poultice made from juice of plant mixed with Cadamba bark, bitter &amp; black cumin is applied</td>
<td>Kirtikar &amp; Basu, 1935; Bioteau et al., 1957</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(India)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>In cough</td>
<td>Meghalaya</td>
<td>Syrup prepared by boiling leaves along with Ginger &amp; Black pepper</td>
<td>Kirtikar &amp; Basu, 1935</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(India)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Tonic for women after delivery</td>
<td>Nilgiri (India)</td>
<td>Leaf juice with palm jaggery</td>
<td>Abrahaam, 1981</td>
</tr>
<tr>
<td>6.</td>
<td>In dysentery</td>
<td>Orissa (India)</td>
<td>2-3 spoonful of leaf paste administered in an empty stomach for 2-3 days</td>
<td>Saxena et al. 1981</td>
</tr>
<tr>
<td>7.</td>
<td>To improve memory</td>
<td>Rajasthan (India)</td>
<td>Leaves of Centella asiatica</td>
<td>Singh &amp; Pandey, 1980</td>
</tr>
<tr>
<td>8.</td>
<td>In Hook-worm infestations in childrens</td>
<td>Indonesia</td>
<td>Plant juice</td>
<td>Singh &amp; Pandey, 1980</td>
</tr>
<tr>
<td>9.</td>
<td>In the treatment of colic</td>
<td>Indonesia</td>
<td>Juices of plant along with juice of Achillea viscosum, Phyllanthus nituri &amp; Phyllanthus urinaria</td>
<td>Perry &amp; Metzger, 1980; Stierle et al., 1995</td>
</tr>
<tr>
<td>10.</td>
<td>Stimulant</td>
<td>Malay Peninsula</td>
<td>Plant along with Cyprus rotundus</td>
<td>Perry &amp; Metzger, 1980; Suchitra et al., 1999</td>
</tr>
<tr>
<td>11.</td>
<td>In whooping cough and bronchial congestion</td>
<td>Indonesia</td>
<td>Infusion along with Picria felterrae</td>
<td>Perry &amp; Metzger, 1980; Sumaryono et al., 1991</td>
</tr>
</tbody>
</table>
2.1.8 Phytochemical Work Reported on *Centella asiatica*

**TABLE 2.2: GROUP OF COMPOUNDS PRESENT IN CENTELLA ASIATICA**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Saponins</td>
<td>Bhattacharya &amp; Lythgoe, 1949; Bhattacharya, 1956; Rao &amp; Seshadri, 1969; Rastogi <em>et al.</em>, 1960</td>
</tr>
<tr>
<td>3.</td>
<td>Essential oils</td>
<td>Bhattacharya, 1956a</td>
</tr>
<tr>
<td>5.</td>
<td>Anthocyanins</td>
<td>Martinelli, 1980</td>
</tr>
<tr>
<td>6.</td>
<td>Nitrogen &amp; Sulphur containing pectine</td>
<td>Bhattacharya, 1956a; Berlin <em>et al.</em>, 1988a</td>
</tr>
<tr>
<td>7.</td>
<td>Free amino acids</td>
<td>George &amp; Gananthuin, 1975</td>
</tr>
<tr>
<td>10.</td>
<td>Different salts and sugars</td>
<td>Bhattacharya, 1956a; Berlin <em>et al.</em>, 1983</td>
</tr>
</tbody>
</table>

Saponin present in *Centella asiatica*

The plants growing in different parts of India show variation in their total saponin content. The similar type of variation is prevalent in the plants of the other geographical regions as well.

**TABLE 2.3: SAPONIN CONTENT OF THE VARIOUS INDIAN SAMPLES OF CENTELLA ASIATICA**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Source</th>
<th>Saponin content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hardwar</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>Dehradun</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>Jammu</td>
<td>8.0</td>
</tr>
<tr>
<td>4</td>
<td>Trivandrum</td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>Madras</td>
<td>2.3</td>
</tr>
<tr>
<td>6</td>
<td>Hyderabad</td>
<td>1.1</td>
</tr>
<tr>
<td>7</td>
<td>Lucknow</td>
<td>2.2</td>
</tr>
</tbody>
</table>
The most common saponin, asiaticoside $\text{C}_{35}\text{H}_{88}\text{O}_{23}$, has been isolated from the fresh leaves, as colorless needles, m.p. 232°C. The yield of fresh leaves is 0.7-1.2g/Kg (Boiteau et al., 1949; Shakoor et al., 1994). It is a rhamno-glucosyl derivative of the triterpenic acid, asiatic acid. The water soluble derivative of asiaticoside is oxyasiaticoside. Some other saponins isolated from this plant are listed in Table 2.4.

**TABLE 2.4: SAPONINS ISOLATED FROM CENTELLA ASIATICA**

<table>
<thead>
<tr>
<th>Saponin</th>
<th>Constituents</th>
<th>M.P.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asiaticoside</td>
<td>Asiatic acid, glucose, rhamnose</td>
<td>230 – 233°C</td>
<td>Mohatu et al., 1987; Polonsky, 1968; Si-Qu &amp; Huci-Fang, 1980</td>
</tr>
<tr>
<td>Madecassoside</td>
<td>Madecassic acid, glucose, rhamnose</td>
<td>ND</td>
<td>Henry et al., 1967; Meknight et al., 1991; Mersinger et al., 1988</td>
</tr>
<tr>
<td>Centelloside</td>
<td>Centellic acid, glucose, fructose</td>
<td>ND</td>
<td>Bhattacharya, 1956; Meyer et al., 1987; Mizusaki et al., 1971</td>
</tr>
<tr>
<td>Brahmoside</td>
<td>Brahmic acid, glucose, rhamnose, arabinose</td>
<td>242°C</td>
<td>Rastogi &amp; Dhar, 1963; Morris, 1986; Nair et al., 1986; Nessler, 1994; Sethi, 1997</td>
</tr>
<tr>
<td>Brahminoside</td>
<td>Brahmic acid, glucose, rhamnose, arabinose</td>
<td>222°C</td>
<td>Rastogi &amp; Dhar, 1963; Nicolaou et al., 1994; Nitsch et al., 1969; Okabe et al., 1967</td>
</tr>
<tr>
<td>Thankuniside</td>
<td>Thankunik acid, glucose, rhamnose</td>
<td>239°C</td>
<td>Dutta &amp; Basu, 1962; Pal et al., 1996; Peterson et al., 1993</td>
</tr>
<tr>
<td>Isothankuniside</td>
<td>Isothankunik acid, glucose, rhamnose</td>
<td>250°C</td>
<td>Dutta &amp; Basu, 1968; Sato et al., 1987; Samiulla et al., 2001</td>
</tr>
<tr>
<td>Asiaticoside-A</td>
<td>Terminolic acid, glucose, rhamnose</td>
<td>ND</td>
<td>Sahu et al., 1989; Sato et al., 1984; Schubel et al., 1989; Sen et al., 1993</td>
</tr>
</tbody>
</table>

ND: Not determined

**Triterpenic acid isolated from Centella asiatica**

Several pentacyclic triterpenic acids have been isolated and characterized from this plant. They occur either in free state or as aglycones of the naturally occurring saponins.
TABLE 2.5: TRITERPENIC ACID ISOLATED FROM CENTELLA ASIATICA

<table>
<thead>
<tr>
<th>Acids</th>
<th>M.P.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asiatic acid</td>
<td>241°C</td>
<td>Si-Quo &amp; Hsieh-Fang, 1980</td>
</tr>
<tr>
<td>Madecassic acid</td>
<td>ND</td>
<td>Par, 1969; Kinnerseley et al., 1980</td>
</tr>
<tr>
<td>Brahmic acid</td>
<td>293°C</td>
<td>Singh &amp; Rastogi, 1968; Kupchan et al., 1972; Kurz et al., 1980</td>
</tr>
<tr>
<td>Isobrahmic acid</td>
<td>263°C</td>
<td>Rastogi &amp; Dhar, 1963; Kutchan et al., 1994</td>
</tr>
<tr>
<td>Thankunic acid</td>
<td>314°C</td>
<td>Dutta &amp; Basu, 1962; Leistner, 1975</td>
</tr>
<tr>
<td>Isothankunic acid</td>
<td>288°C</td>
<td>Dutta &amp; Basu, 1968; Mangatal et al., 1989</td>
</tr>
<tr>
<td>Betulic acid</td>
<td>308°C</td>
<td>Rastogi &amp; Dhar, 1963; Matsubara et al., 1989</td>
</tr>
<tr>
<td>Centoic acid</td>
<td>256-261°C</td>
<td>Bhattacharya &amp; Lythgoe, 1949</td>
</tr>
<tr>
<td>Centelic acid</td>
<td>ND</td>
<td>Bhattacharya &amp; Lythgoe, 1949</td>
</tr>
<tr>
<td>6-hydroxyasiatic acid</td>
<td>285-288°C</td>
<td>Sahu et al., 1989; Kutney et al., 1983</td>
</tr>
<tr>
<td>Terminolic acid</td>
<td>300°C</td>
<td>Sahu et al., 1989; Mastuda et al., 2001</td>
</tr>
</tbody>
</table>

ND: Not determined

Polyacetylenes

The following polyacetylenes (I to V) have been isolated from this plant and their structures established (Schulte et al., 1973). These are C15 compounds with alcoholic OH and acetyl functions.

\[
\text{Me-(CH}_2\text{)}_4\text{-CH=CH-CH}_2\text{-(C=C)}_2\text{-CH=CH-CH}_2\text{OAc}
\]

I

\[
\text{Me-(CH}_2\text{)}_4\text{-CH=CH-CH-(C=C)}_2\text{-CH=CH-CH}_2\text{OAc}
\]

II

\[
\text{Me-(CH}_2\text{)}_4\text{-CH-CH-CH-(C=C)}_2\text{-CH-CH=CH}_2\text{OH}
\]

III
Sterols and lipid compounds

The plant is reported to possess stigmasterol, stigmasterone and stigmasterol-β-D-glucopyranoside (Srivastava & Shukla, 1996; Srivastava et al., 1997). An unsaturated acid dotriacent-8-en-l-oic acid and a cyclohexane derivative 11-oxohenisanyl-cyclohexane have been isolated and characterized from this plant (Srivatsava & Shukla, 1996). The herb yielded an essential oil of green color possessing the strong odour of the original herb, sitosterol, a resinous substance and fatty oil (Chavadej et al., 1994; Chen, 1969). The fatty oil consisted of glycerides of oleic, linoleic, linolenic, palmitic, stearic and lignoceric acid (Walima & Katti, 1937; Brinkhaus et al., 2000).

The bitter principle, vallarine and pectic acid were reported from the leaves and roots (Corey et al., 1980; Cragg et al., 1993). The plant has been reported to contain ascorbic acid (Joachin & Panditatesckere, 1940).

Nitrogen containing constituents

The plant has yielded glycine, aspartic acid, glutamate, serine and alanine occurred in large amount compared to other amino acids in the leaves, petiole and stolons (George & Ganarethianom, 1975; Kingsbury et al., 1991). In the roots, relatively large quantities of glutamic acid, threonine, alanine, glysine, histidine and aminoibutyric acid could be detected (Camron et al., 1993; De Carolis et al., 1993). The presence of lysine, glutamic acid, phenyl alanine, alanine, aspartic acid and serine has been demonstrated by two dimensional TLC and gas chromatography (Centallani & Vacchi, 1981).
Flavonoids

The plant has been reported to contain hyperin (Nakoki & Morita, 1960). The leaves were found to contain 3-glucosylquercetin, 3-glucosylkaempferol and 7-glucosylkaempferol.

Miscellaneous

A yellowish-green colored essential oil has been found in alcoholic extract. It possesses the strong odour of herb from the alcoholic extract fatty oil (Igbavboa et al., 1985), sitosterol (Ikuta et al., 1969), tannin and resinous substances (Inamdar et al., 1996) have been detected. The fatty oil consists of glycerides of oleic (Inoue et al., 1984), linolic (Inoue et al., 1963), linolenic, lignoceric, palmitic and stearic acid (Walima & Katti, 1937; Kao et al., 1975). From the dry plant an alkaloid hydrocotyline (C_{22}H_{33}O_{8}N, m.p. 210-212°C) has been isolated. The yield was found to be 0.0016%. The herb contains a bitter principle vallarine and pectic acid in leaves and roots (Deus et al., 1984; Dutta et al., 1967; Ellis, 1983; Holton et al., 1994; Hou et al., 2002). Plants also possess an unidentified thermostable factor, composed of many components. Its nature is still not known. An enzyme, thiaminase isolated from the plant, catalyzes the splitting of the thiamine molecule into thiazole and pyrimidine moieties (Ellis et al., 1996; Fukui et al., 1983). The plant is also rich in vitamin-C and carotene. The total ascorbic acid content in the plant was found to be 13.8% (Basu et al., 1947; Rattanapanone et al., 1974; De-Eknamkul et al., 1984).

Six constituents, brazhmoside, brhaminoside, brahmic acid, isobrahmic acid, betulic acid and stigmosterol were isolated from Centella asiatica in pure state (Rastogi et al., 1960; Dell et al., 1989; Denis et al., 1988).

Khan et al. (1989) examined the status of trace and minor elements in some Bangladeshi foodstuff including Centella asiatica using proton induce X-ray emission (PIXE) and radioisotopes induce florescence techniques. In this
connection in *Centella asiatica*, Mn, Fe, Ni, Cu, Zn, Se, As, Br, Rb, Sr, Mo, and Pb were determined.

Diallo *et al.* (1991) developed a method of separation of asiaticoside and madecassoside from *Centella asiatica* by direct coupling of high-speed counter current chromatography to thin layer chromatography.

Variation in the chemical composition of Indian samples of *C. asiatica* was studied by Suryaprakash & Tiruvenkata (Suryaprakash *et al.*, 1969).

Anathbandhu & Ranjit (1991) determined the asiaticoside from the leaves of ten ecotype of *C. asiatica* in India. They studied genotype effect and the correlation between the genomic diversity of this compound, which has tremendous medicinal value against leprosy. An alkaloid hydrocotylin, C_{22}H_{38}O_{8}N, m.p. 110-112°C has been isolated from this plant in variation (Govindachari *et al.*, 1972; Han *et al.*, 1994). The possibility of utilizing the selected genotypes of sub-template Himalayas for commercial exploitation were also examined.

The structure of two new triterpenoid trisaccharides asiaticoside-A and asiaticoside-B from *Centella asiatica* have been elucidated as the [O-α-Lrhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl (1→6)]-O-β-pyranose ester of 2α, 3α, 6α, 23α-tetrahydroxy-urs-12-ene-28-oic acid and the [O-α-Lrhamnopyranosyl-(1→4) glucopyranosyl (1→6)] O-β-D-glucopyranose ester of 2α, 3α, 6α, 23α-tetrahydroxylean-12-ene-28-oic acid by spectroscopic anlysis (Funk *et al.*, 1990; Furuya *et al.*, 1972; Gerardy *et al.*, 1993; Hashimoto *et al.*, 1993; Heide *et al.*, 1989; Henry *et al.*, 1987; Hirata *et al.*, 1989).

Gunther & Wagner (1996) developed a new method of quantitative determination of triterpenes in extracts and phytopreparations of *Centella asiatica* (L.) urban employing HPLC. The method is suitable for finger print analysis and standardization of all *Centella asiatica* preparations.
Antioxidative activity of various extracts from different parts of *Centella asiatica* including leaves petioles (stolon) and roots, using three types of solvents (ethanol, water, light petroleum), were evaluated using a linoleic acid model system and the thiobarbituric acid test. Result showed that the ethanol extract of all parts of *Centella asiatica* exhibited significantly (P<0.05) higher antioxidative activity than the water extract, while the light petroleum ether showed negligible activity. Increasing the concentration of the extract (1000-3000 ppm) resulted in increase in antioxidative activity of both the ethanol and water extract. From 3000 ppm upward, antioxidative activity of the ethanol extract was not significantly different (P<0.05) from that of alphatocopherol. Roots showed the highest activity of the parts tested. The antioxidative activities of the ethanol extract were found to be stable upto 50°C and exhibited optimum activity at neutral pH (Abdul Hamid *et al.*, 2002; Hara *et al.*, 1989).

A comparative chemical analysis of ten Indian ecotypes of *Centella asiatica* was carried out to estimate asiaticoside content in leaves. The highest amounts of asiaticosides were obtained in ecotypes II and X of subtemperate Himalaya (0.114% and 0.105% respectively). Subsequently, two newly reported B-chromosomes were noted in these races besides their normal karyotype. The influence of genomic constituents on asiaticoside contents was analyzed. The asiaticoside contents differed significantly among the ecotypes examined, reflecting their genome variation. The possibility of utilizing the selected genotypes of sub temperate Himalaya for commercial exploitation was considered (Anathbandhu *et al.*, 1991).

The essential oil isolated by steam-distillation from the aerial parts of *Centella asiatica* was analyzed by capillary GC and GC-MS. Forty one compounds were identified, sesquiterpenoids accounting for 79.7% of the oil.
The main constituents of the oil were betacaryophyllene (26.8%), alphahumulene (33.7%) and germacrene (10.0%) (Wong & Tan, 1994).

The technique of electrospray characterization for Centella asiatica extract has been developed. Extracts of plant was examined by electrospray mass spectrometry (ESI-MS). This technique allowed identification of the examined plant. More specifically, triterpenes were detected rapidly at concentrations in the range 0.1-1μg/ml, using 0.2-1 mg/ml of plant extract (Mauri et al., 2000).

Centella asiatica plants were collected in Malaysia. The edible parts were analyzed for moisture, protein, fat, ash and minerals. Centella asiatica leaves contained 82.6% water, 1.8% crude protein, 1.7% fat and 2.4% ash on a fresh weight basis. The stems and roots of Centella asiatica contained 90.3 and 81.2% water and 0.8 and 1.7% crude protein. K, Ca and Mg were the most abundant minerals in Centella asiatica leaves in terms of dry matter. The leaves had higher concentrations of minerals than the roots and stems. In comparison with other fruits and vegetables, Centella asiatica has high levels of Fe (848 ppm), Zn (292 ppm) and Ca (9248 ppm). It is suggested that consumption of Centella asiatica as vegetable may supplement daily human mineral requirements.

From the aerial parts of Centella asiatica bioactive triterpenes viz. asiaticoside, asiatic acid, madecassic acid and madecassoside were isolated by employing high performance liquid chromatography (Dutta et al., 2001).

A new triterpenoid glycoside 3-O-[α-L-arabinopyranosyl]-2α, 3β, 6β, 23α tetrahydroxy urs-12-ene-28 oic acid (I) accompanied by 6β-hydroxy asiatic acid and asiatic acid were isolated from Centella asiatica. The structure of I was established by physiochemical data. Compound (I) exhibited dose-dependent growth inhibitory activity against larvae of Spilarctia oblique but was not as active as azadirachtin (Shukla et al., 2000).
Some of the chemical constituents present in *Centella asiatica*

- **Asiaticoside**
- **Madecassic acid**
- **Brahmic acid**
- ** Asiatic acid**
- **Terminolic acid**
- **Isothankunic acid**
- **11-Oxoheneicosanyl cyclohexane**
- **Dotriacont-8-en-1-oic-acid**

### 2.1.9 Pharmacological Work Reported on *Centella asiatica*

The pharmacological activity of *Centella asiatica* is thought to be due to several saponin constituents, including asiaticoside, asiatic acid, and madecassic acid (Karting, 1988). Asiaticoside stimulates the epidermis by
activating the cells of the malpighian layer in porcine skin, and by keratinization in vitro (May, 1968).

Extract of Centella asiatica effectively treated stress induced stomach and duodenal ulcers in humans (Karting, 1988), Oral adminisration of Centella asiatica extract to rats produced a dose dependant reduction in stress induced gastric ulceration and the antiulcer activity was similar to that of famotidine. The mechanism of action appears to be associated with a central nervous system depressant activity of Centella asiatica, owing to an increase in the concentration of GABA (γ-aminobutyric acid) in the brain (Chatterjee et al., 1992).

A 70% ethanol extract of the drug administered intrperitoneally to mice produced anticonvulsant activity (Adesina, 1982).

Infusion of the plant is used in India and Madagascar in the treatment of leprosy and is known to ameliorate the symptoms of disease and to improve the general health of the patient. The usual dose for oral administration is 5-10 grains of the plant powder thrice daily. In large doses, the drug is a stupefying narcotic, producing giddiness and sometimes coma (Wealth of India, 1950).

The ethanolic extract was reported to have depressant effect in rat in toxic doses. The glycosidal fraction displayed sedative effect in rat. It decreased the tone and diminished the amplitude of contraction of the isolated ileum of rabbit and albino rat at a concentration of 1:2500 to 1:500.

In a concentration of 1:5000 to 1:500, it produced 50-90% inhibition of acetylcholine-induced spasm in isolated rats ileum. In anaesthetized dog, it produced slight respiratory stimulant, hypotension and bradycardia (Malhotra et al., 1961). The ethanolic extract of the whole plant showed anti-protozoal property against Entamoeba histolytica (Dhar et al., 1968).
Dandiya and Sakina (1990) gave the psychopharmacological profile of *Centella asiatica* extract and reported that the plant extensively used in Indian traditional medicine for mental ailments, has been found to possess potent CNS depressant properties as a result of a battery of pharmacological test. In addition it was found to possess an antidepressant ion which appeared to be mediated through the D<sub>2</sub> receptor and a cholinomimetic action which is blocked by atropine and chlorpromazine (Dandiya & Sakina, 1990).

The anti-stress activity of Indian and Mauritian sample was determined in albino rats by assessing the adrenocortical response following one hour of immobilization stress (Upadhyay *et al*., 1991). The adrenocortical response to immobilization stress with both samples was significant. The activity was further verified recently (Sharma *et al*., 1996).

Khosla *et al.* (1996) screened *Centella asiatica* for its putative antistress activity in a battery of experiments. Ethanol extract of both drugs at 100 mg/kg exhibited significant anti-stress activity in all the parameters studied, compared with diazepam at 2.5mg/kg.

The alcoholic extract was used as suspension in 10% gum acacia in water. The intraperitoneal LD<sub>50</sub> in albino rats was found to be 1.93 (1.64-2.27) g/kg. Death occurred 12-24 hours after the drug administration. The extract had depressant effect in rats in toxic doses only but had no analgesic activity. The extract, in concentration of 1:1000 to 1:500, had relaxant action on isolated rats ileum (Malhotra, 1961).

A double blind clinical trial was conducted on 30 mentally retarded children who were free from epilepsy and other neurological conditions to study the effect of the drug in general mental ability. The result indicated a significant improvement in both general ability and behavioral pattern when the drug was administered for a short period of 12 weeks (Apparao *et al*., 1973).
Brahmoside was found to possess sedative action in rats equivalents to that minor tranquillizer. The action appeared to be mainly on the cholinergic mechanism (Ramaswamy et al., 1970).

The hydroalcoholic extract of C. asiatica showed anxiolytic /sedative effect in elevated plus maize, potentiation of the hypnotic effect of pentobarbitone and anticonvulsant activity against phenylentrazol-induced convulsion. The oral LD₅₀ of the extract in rats was found to be higher than 675 mg/kg indicating a high therapeutic index. In addiction, chronic oral administration also exhibited a low toxicity for C. asiatica (Sertie, 1977).

The effect of aqueous extract of C. asiatica fresh leaves on learning and memory was studied in albino rats using two-compartment passive avoidance task. The effect of this extract on this extract on the content of nor-epinepherine (NE), dopamine (DA), 5-HT in the brain and on the levels of their metabolites both in the brain and urine were also assessed. Significant improvement was observed in the 24-hour retention in the drug treated group compared to the saline administered controls. The concentration of NE, DA and 5HT and their metabolite in the brain were decreased significantly in drug treated group. The urine metabolite levels were also significantly decreased except total 3-methoxy - 4 - hydroxy - phenylglycol (MHPG). These results indicate that C. asiatica causes an overall decrease in the turnover of central monoamines, implicating the involvement of NE, DA and 5HT systems in learning and memory process (Nalini et al., 1992).

The drug has been tried in a group consisting of 25 patients anxiety neurosis. A six week treatment with this drug provided not only significant relief in symptoms but also a quantitative reduction in the anxiety level, leading to improved mental function studied in terms of fatigue rate and immediate memory span. Thus the drug appears to be a antianxiety agent (Singh et al., 1981).
The aqueous extract of the *C. asiatica* has been analysed for its anti-anxiety activity against reference standard (Diazepam) in mice. The extract (25 mg/kg) i.p. decreased spontaneous motor activity and delayed pentyline terazole induced convulsions in mice. This activity was comparable to diazepam. (Diwan et al., 1991).

Experimental studies on psychotropic effect in rats showed significant barbiturate hypnosis potentiation effect and anti-convulsant activity, besides producing significant alteration in the neurochemistry of the brain (Singh et al., 1981).

The effect of the whole plant powder on growth pattern and some biochemical constituents of blood and issue was studied on albino rats fed on low protein diet (5%) level. The drug prevented the mortality rat due to gross protein deficiency. It increases the blood protein nitrogen and prevented the fatty infiltration of liver (Shrivastava et al., 1997).

Poultice of fresh leaves was applied on cuts and wounds, which accelerated wound healing (Borthakur et al., 1996). The ethanolic extract showed anti-filarial activity in dogs injected with *Ditrofilaria immitis*. Activity may result from the biotransformation of plant constituents by the digestive enzymes of the host (Chakarborty et al., 1996).

The plant commonly used in Ayurvedic system of medicine for the treatment of various diseases including cancers. The methanolic extract has shown significant cytotoxicity (Babu et al., 1995). To Ehrlichacites tumor cells and Daltons lymphoma ascites tumor cells, *in-vitro* cytotoxicity assay showed significant cytotoxicity with both crude and acetone fraction towards various transformed cell lines. Similarly the activity was significantly enhanced in long term exposure to fibroblast cells. The radioisotopic studies using (3H)-thymidine, uridine and leucine revealed that both acetone fraction and
fluorescent compound have direct effect on DNA synthesis as seen by the decreased incoperation of thymidine. More interestingly, both fractions exhibited a slight increase in transcription and translation in the tumor cell during cell death.

Enhancement of attachment of microcarriers Tpa production of fibroblast cells in a serum - free medium by addition of extract of C. asiatica was studied by Kim et al., (1993).

Hexane and etylacetate fractions of the methanoextractives displayed significant anti-bacterial activity against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and P. cichorii. Two of its isolated metabolites stigmasterol and dotriacont-8-en-loic acid inhibited V. subtilis, E. coli and P. aeruginosa (Shrivastava et al., 1997).

Ravokatra et al. (1974) tested the activity of asiaticoside in the healing of perforating duodenal ulcer induced by chemical treatment in young male wistar rats. The oral application of asiaticoside at three hours after rats were given mercaptoethylamine (65mg/100gm) to induce ulcers, reduced the illness of the rats by 50%. Apparently asiaticoside hastened the scar formation in the duodenal tissue through production of new connective tissue that had suffered a deregulation after the poisoning with mercaptoethylamine.

Asiaticoside has yielded encouraging results in the treatment of leprosy (Bailey, 1945). It probably acts by dissolving the waxy covering of Bacillus leprae, the bacteria thus become fragile and may easily be destroyed by the tissues or by some other drug. The results of the injection of the solution are reported to be remarkable. The leprosy nodules are broken down, diffuse infiltration disappear, perforating ulcer and lesions on the finger heal and most remarkable of all, the eye lesions are rapidly cured if the treatment is given before the posterior chamber of the eye is involved.
Brahmic acid, a biologically active triterpenoid present in the plant has therapeutic values in ulceration, extensive wounds and eczema (Asakawa et al., 1982) and inhibitory effect on the biosynthetic activity of fibroblast cells (Vecchiae et al., 1984).

Preliminary experiments carried out by Chin et al. have shown that the plant possesses insecticidal property (Chin et al., 1994). The n-hexane, ethyl acetate and n-butanol fractions of the methanol extractive exhibited 77, 59 and 85% feeding deterreny against Spilosoma obliqua at 2% concentration. Stigmasterol isolated from the hexane fraction showed 96, 89, 80, 68, 50 and 40% feeding deterreny against Spilosoma obliqua at 5000, 2500, 1750, 900, 450 and 250 ppm concentration. Similarly stigmasterol-β-D-glucopyranoside displayed 97, 90, 87, 56, 49 and 45% feeding deterreny against Spilosoma obliqua at 2500, 1250, 700, 300, 150 and 80 ppm concentration (Srivastava and Shukla, 1996).

The glycoside brahmoside and brahminoside possess antispasmodic properties besides being synergic with tremorine (Ramaswamy et al., 1970).

The expressed juice of Centella asiatica has shown antifertility activity in experimental animals (Dutta and Basu, 1968).

Asiaticoside has been used medicinally for leprosy and Lupes and experimentally for tuberculosis (Boiteau et al., 1949). Repeated injections of 0.5 ml of 4% aqueous asiaticoside reduces the number of tubercular lesion in the liver, lungs, nerve ganglia and spleen. The effect of asiaticoside on guinea pig skin has also been observed and toxicity to skin in vitro was investigated. It was found that asiaticoside was not much toxic but it failed to influence the mortality and healing of experimental burns in mice. (Lawrence, 1967a,b). Estimation of doses with anthrone and asiaticoside was determined and optimum condition for the reaction was established in 0-100μg samples (Rahandrahaha et al., 1963).
Another glycoside, madecassoside and its triterpine acid madecassic acid, was also found to have very good anti-inflammatory activity. An anti-fertility activity has also been reported (Dutta & Basu, 1968).

Two phases of healing were observed in rats with iterative wounds, an initial lengthing followed by a contraction of the wound. It was observed that the applying of C. asiatica titerpinoid extract (100mg/kg/day, orally) shortened the lengthening phase while having little effect on the contracting phase (Poizot & Dumex, 1978). The extract also promotes epitheliazation and a very early decrease in the wound surface area. The asiatic acid has hormonal (estrogenic and cortico-suprarenal), cicatrizing and bactericidal (acid resistant bacteria, particularly Mycobacterium tuberculosis and M. leprae as well as gram positive cocci) properties (Ratsinmanga and Boiteau, 1963).

The juice mixed with petroleum jelly makes a good liniment for rheumatism. Asiatic acid, madecassic acid and asiaticoside, terpinoids with an ursane skeleton were tested separately and in combination on skin human fibroblast collagen I synthesis in vitro. In the absence of ascorbic acid, the number as well as each individual component stimulated collagen I synthesis to a similar extent. In the presence of ascorbic acid, the level of collagen I secretion was higher for each individual component and for the mixture. A comparison for asiaticoside and asiatic acid shows that the sugar moiety of the molecule does not seem to be necessary for this biological activity.

Antibacterial activity of C. asiatica has been reported by Shukla et al. (1997). It has been reported that Hexane and ethyl acetate extracts displayed significant inhibitory activity against B. subtilis, E. coli and P. aeruginosa.

Flavanoid glycoside-3-glucosyl-quercetin (I) and 3-glucosyl-kaempferol (II) were isolated from C. asiatica and were found to possess wound healing and anti-inflammatory properties (Praum et al., 1993).
Crude extract of *C. asiatica* was found to inhibit angiotensin converting enzyme (ACE) by more than 50% and thus possessed anti-hypertensive activity (Hausen *et al.*, 1993).

In Egypt 102 patients with various type of bladder lesion caused by bilharzia (*Shistosoma haematobuim*) were injected intramuscularly each day for one to three months with a 2% titrated extract of the plant *C. asiatica*. In patients with superficial bladder ulcer, early bilharzial lesion the treatment encouraged healing in about 75% cases with little scar formation. The treatment also aided healing in patients whose bladder lesion had been treated surgically (Aziz, 1973).

Grimaldi *et al.*, (1990) performed the pharmacokinetics of total triterpenic fraction *C. asiatica*. After single and multiple administration to healthy volunteers. A new assay for ascorbic acid was given.

Analysis of a clinically important interaction between phenytoin and shankhapushpi, an ayurvedic preparation containing *C. asiatica* as one of its constituents was done by Danderkar and co-workers (1992).

A novel form for its intramuscularly fraction of titrated extract of *C. asiatica* in a mixed micellar system was developed by Kim and co-workers (2001). Titrated extract of *C. asiatica* (TECA), a drug use in treating systemic scleroderma, is poorly water soluble. A conventional dosage form for the intramuscular injection of TECA, propylene glycol (PG)- based TECA solution. To improve the solubility of TECA and reduce pain after injection, mixed micellar system composed of 10% surfactant mixture (Tween 20 and Tween 85) and 90% Phosphate buffer saline, ph 7.0 (PBS) were prepared. As the ratio of Tween 20 to Tween 85 increased from 0:10 to 4:6, followed by an abrupt decrease in size above the ratio of 6:4. Furthermore, the micellar system prepared with Tween 20 and Tween 85 at the ratio of 6:4, 8:2 or 10:0
could solubilize TECA more than 10mg/ml and the resultant dropeed size were less then 2μm. No significant changes were observed in the dropeed sizes and asiaticoside contents in these micellar formulations during storage, indicating these systems are stable for at least 60 days. Their osmotic pressures were remarkably lower than those of PG- based TECA solution and similar to that of saline solution, irrespective of dilution ratios. Most importantly, they markedly reduced the number of writhes compared with PG- based TECA solution after injection to mice. All of these results suggest that three TECA micellar formulations prepared with Tween 20 and Tween 85 improved the solubility of TECA and reduced pain following injection, possibly due to decrease in osmotic pressure. Thus, these micellar formulations composed of optimum ratios of Tween 20 and Tween 85 may have a potential as dosage form for the intramuscular injection of a poorly water soluble TECA.

Thirty -one species used in traditional medicines reported to be used as anti-hypertensives or diuretics, from different regions of the world (China, India, and South America), were investigated. The bioassays were based on inhibition of ACE (dipeptidyl carboxypeptidase), as measured from the enzymatic cleavage of the chromophore-fluorophore-labeled substrate dansyltriglycerine and diglycine. The crude extract of C. asiatica inhibited the enzyme more than 50% (Hansen et al., 1995).

A number of Thai medicinal plants, recommended as remedies for herpes virus infection and used in a primary health care were investigated for their intracellular activities against herpes simplex virus (HSV). C. asiatica showed both anti-HSV1 and 2 activities, as determined by the plaque inhibition assay. An inhibition of the production of the production of infectious HSV-2 virions from the infected cells could also be demonstrated. Combinations of each of these reconstituted extracts with 9-(2-hydroxymethyl)
guanosine (acyclovir, ACV) resulted either in subadditive, additive, or synergistic interactin, against HSV-2, depending on the dose of ACV used. Furthermore, the inhibitory effect of this plant extract was also substained by flow cytometric analysis of virus-specific antigens in the infected cells. The active constituent present in *C. asiatica* extract was determined to be asiaticoside (Yoosook, 1999).

The effect of *C. asiatica* (collected from India) on behavioral change in rats exposed to environmental stress (pen field maze and thirsty rats conflicts tests) was investigated. Individual fed on diet containing *C. asiatica* showed significant changes in behavioral changes induced by stress (Valsala, 1998).

The activity of asiaticoside, isolated from *C. asiatica*, was studied in normal as well as delayed type wound healing. The result indicates that asiaticoside exhibits significant wound healing activity in normal as well as delayed type healing models and is the main active constituent of *C. asiatica*.

Asiaticoside from *C. asiatica* exhibits good wound healing activity. The effect of asiaticoside on the concentration of antioxidants in wounds of rats was investigated to explore the possible involvement of antioxidants in asiaticoside-induced wound healing. Asiaticoside appeared to enhance increased antioxidant levels at an initial stage of healing which may be an important contributory factor to the healing properties of this substance (Shukla et al., 1999b).

Evaluation of tropical formulations of aqueous extract of *C. asiatica* on open wounds in rats was done by Sunil Kumar et al. (1998). Formulations (ointment, cream or gel) or an aqueous extract of *C. asiatica*, when applied topically, 3 times a day for 24 days to open wounds in rats, increased cellular proliferation and collagen synthesis at the wound side, as evidenced by increased in collagen content and tensile strength. Treated wound epithelialized
faster and the rate of wound contraction was higher compared with control wounds. The best formulation for promoting healing was the gel formulation. Aqueous, methanolic and chloroform extracts of *C. asiatica* were investigated for their effect on cognitive functions in rats. Male Wistar rats (200-250mg) were used to study the effect on learning and memory using shuttle box, step through, step down and elevated plus maze paradigms. Therefore, further experiments were conducted with aqueous extract using 100, 200, 300 mg/kg doses in different paradigms of learning and memory. All doses of aqueous extract increased the number of avoidances in shuttle box and prolonged the step through latency in step through apparatus in a dose dependent manner, while only two doses (200 and 300 mg/kg) of aqueous extract showed significant increase in the step down latency in step down apparatus and transfer latency in elevated plus maze (Kumar & Gupta, 2002).

The effect of single oral administration of different preparations of *Centella asiatica* were evaluated for their anticonvulsant profile in the maximal electroshock seizure in rats at 1, 3, 6 and 24 h after administration and pentyleneetetrazole (PTZ) test in mice and rats. The ED$_{50}$ dose of Phenytoin (30mg/kg) was used for comparison. The crude drug of *Centella asiatica* (500mg/kg) showed higher activity than the crude drug at 3 and 6 h but there was no anticonvulsant activity at 1 h (Sudha *et al.*, 2002).

The efficacy of *Centella asiatica* in alleviating cardiovascular, hepatic and renal damages in rats was studied. Twenty four male Sprague-Dawley rats were fed for 8 weeks with 4 different diets: normal commercial rat pellets (N), 1% *Centella asiatica* diet (C), 10% *Centella asiatica* diet (P) and 1% cholesterol plus 10% *Centella asiatica* diet (CP). Serum cholesterol and triglycerides (triglycerols) in C and CP rats were significantly higher (p<0.05) than in P and N rats. The creatine Kinase (CK) levels of C, CP and P rats were significantly high (p<0.05) in weeks 4 and 8, indicative of early cardiocytic
degeneration due to cholesterol deposition in the artery walls of hypercholesterolaemic rats (Suhaila et al., 2001).

A study was conducted to evaluate the possible anti-ulcerogenic activity of fresh juice of Centella asiatica against ethanol, aspirin, cold-restrain stress and pyloric ligation induced gastric ulcers in rats. The drug given orally in doses of 200 and 300 mg/kg twice daily for five days, showed significant protection against all the above experimental ulcer models and the results were comparable with those elicited by sucralfate (SF, 250mg/kg, per oral, BD X 5 days) (Sairam et al., 2001a).

Centella asiatica was found to be one of the most promising therapies of complementary therapies of chronic venous insufficiency (Pitter, 2001).

Protection against radiation-induced conditioned taste aversion by Centella asiatica was studied by Shobi and Goel (2001). To protect against the adverse effects of radiation, Centella asiatica (aqueous extract) was tested and compared with ondansetron (Emeset), a standard antiemetic drug. It is suggested that Centella asiatica could be useful in preventing radiation-induced behavioural changes during clinical radiotherapy.

Veerendra Kumar and Gupta (2003) have demonstrated that Centella asiatica has cognitive-enhancing and anti-oxidant properties in normal rats. Rats treated with Centella asiatica showed a dose dependant increase in cognitive behaviour in both paradigms. The present findings indicate that an aqueous extract of Centella asiatica is effective in preventing the cognitive deficits, as well as the oxidative stress, caused by STZ in rats.

Oral treatment with 50 mg/kg/day of crude methanol extract of Centella asiatica for 14 days significantly increased the anti-oxidant enzymes, like superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPx), and anti-oxidants like glutathione (GSH) and ascorbic acid decreased in lymphoma-bearing mice (Jayashree et al., 2003).
Total triterpenic fraction of *Centella asiatica* (TTFCA) is effective in improving venous wall alterations in chronic venous hypertension and in protecting the venous endothelium. TTFCA is active on connective tissue modulation, improves the synthesis of collagen and other tissue proteins by modulating the action of fibroblasts in the vein wall and stimulates collagen remodulating in and around the venous wall. This is due to the modulating action of TTFCA on fibroblasts as shown by experiments on the growth of human embryonal fibroblasts (Incandela *et al.*, 2001).

The double-blind, placebo-controlled study was undertaken to evaluate the anxiolytic activity of Gotu Kola (*Centella asiatica*) in healthy subjects. In this study, the authors evaluated the effects of Gotu Kola on the ASR in humans. The results revealed that compared with placebo, Gotu Kola significantly attenuated the peak ASR amplitude 30 and 60 minutes after treatment. Gotu Kola had no significant effect on self rated mood, heart rate, or blood pressure. These preliminary findings suggest that Gotu Kola has anxiolytic activity in humans as revealed by the ASR (Bradwejn *et al.*, 2000).

### 2.1.10 Clinical Pharmacology

The first clinical investigation on the medicinal application of this plant and its extracts were completed during the early 1940's. Boteau and Ratsimamanga (1956) had investigated the action of asiaticoside on cicarization of experimentally induced wounds. A comparison of treated and controlled wounds during the different phases of the cicatrization process indicated that asiaticoside substantially hastened the progress of healing. Asiaticosides works selectively by stimulating rapid and healthy growth of the reticuloendothelium.

A double blind clinical trial was conducted in 43 normal adults to study the rasayan effect of the drug. It increased the mean level of RBC, blood
sugar, serum cholesterol, vital capacity and total protein. The increase in the haemoglobin percentage was quite high and statistically significant. The drug also decreased the mean blood urea level and a moderate decrease in serum acid phosphate was observed (Apparao et al., 1967; Apparao et al., 1969).

Local application of an extract of the drug to second and third degree burns expected healing, prevented the shrinking and swelling caused by infection and further inhibited hypertrophic scar formation (Farnsworth, 1992).

Twenty-two patients with chronic infected skin ulcers were treated with a cream containing 1% extract of *Centella asiatica* (Boiteau and Ratsimamanga, 1956). After 3 weeks of treatment, 17 of the patients were completely healed and the ulcer size in the remaining 5 patients was decreased. Another trial using the same cream preparation demonstrated similar results (Boiteau and Ratsimamanga, 1957).

A standardized extract of *Centella asiatica* was reported to treat ulcer cruris (indolent leg ulcers) effectively in clinical trials (Huriez, 1972).

Oral administration of *Centella asiatica* or asiaticoside and potassium chloride capsules was reported to be a effective as dapsone therapy in patients with leprosy (Chakrabarty and Deshmukh, 1976). In a controlled study of 90 patients with perforated leg lesions owing to leprosy, application of a salve of the plant produced significantly better results than a placebo.

Clinical studies of herb *Centella asiatica* in the treatment of various venous disorders has demonstrated a positive therapeutic effect. In patients suffering from venous insufficiency who were treated with extract of drug, venous distension and oedema improved significantly, as compared with controls (Lythoge and Trippett, 1949).
2.2 BACOPA MONNIERA LINN.

2.2.1 Description of the Plant

It is a glabrous, succulent, small, prostrate or creeping annual herb, found throughout India in wet, damp and marshy areas. It has been in use as a potent nerve tonic for rejuvenating mental health and promoting intellect and memory since times immemorial.

2.2.2 History

Although, this plant has been mentioned in the religious, social and medical treatises of India since the time of Atharva-Ved (800 BC), the first, clear reference to its effect on intellect and memory is to be found in Charak Samhita written in the 1st century A.D., where brahmi is prescribed as a cure for mental retardation leading to psychosis (10:62). Implicit in the pharmacological properties ascribed to brahmi is the cognitive enhancing and anxiolytic effects of this plant. The other classical Ayurvedic treatise, i.e., Susruta Samhita precisely describes brahmi as efficacious in loss of intellect and memory.

2.2.3 Synonyms

*Bacopa monniera* (Linn.) Wettst. is also known as *Herpestis monniera* (Linn.) (Fam. Scrophulariaceae).

2.2.4 Common Names in Different Languages

<table>
<thead>
<tr>
<th>Language</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>Thyme leaved Gratiola</td>
</tr>
<tr>
<td>Hindi</td>
<td>Manduk Parni</td>
</tr>
<tr>
<td>Kannada</td>
<td>Nirubrahmi, Valabrahmi, Ondelaga, Mandukparni</td>
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<tr>
<td>Malayalam</td>
<td>Bbahmi</td>
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<tr>
<td>Sanskrit</td>
<td>Saraswati, Kapotavamka</td>
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</table>
2.2.5 Early Investigations

The plant has been investigated in several Indian laboratories for its neuropharmacological effects. Malhotra and Das (1959) reported a sedative effect of glycosides named by them as hersaponins. Aithal and Sirsi (1961) found that the alcoholic extract, and to a lesser extent the aqueous extract of the whole plant exhibited tranquilizing effects on albino rats and dogs. Prakash and Sirsi (1961), on the other hand, have found that the alcoholic extract of the plant and chlorpromazine improved the performance of rats in motor learning. Sinha (1971) has reported that a single dose of the glycoside hersaponin is better than pentobarbitone in facilitating acquisition and retention of brightness discrimination reaction. It is difficult, however, to interpret these results in the context of the available known traditional claims of improving learning and memory.

Therefore, the evaluation of the traditional claims of Brahmi was initiated by investigating the effect of the ethanolic extract on the acquisition, consolidation and retention of three newly acquired behavioural responses in albino rats (Singh and Dhawan, 1982). The memory enhancing effect of the ethanolic extract was traced to a mixture of triterpenoid saponins, designated as bacosides A and B. Both bacosides A and B showed single spots on TLC over silica gel, while bacoside A (Chatterjee et al., 1963, 1965) was obtained as colorless needles, bacoside B (Chatterjee et al., 1963) was a colorless powder.
In the investigations, taken up later (TLC and HPLC on reversed phase) showed that bacoside A was a mixture of at least three saponins which were designated as bacosides A1 (Kulshreshta and Rastogi, 1973), A2 (Kulshreshta and Rastogi, 1974) and A3 (Chandel et al., 1977). Methods have been developed for quantitative determination of bacoside A content in the extract by UV spectroscopy (Pal and Sarin, 1992) and HPLC (Pal et al. 1998).

To monitor the seasonal variations of bacosides, fresh plant material was collected every month, extracted with ethanol and fractionated. It was carried over a period of fourteen months. TLC of the n-butanol fractions of the ethanolic extracts of the plant was used to monitor bacosides. From this study it was concluded that bacoside A predominates in March and April whereas both bacosides A and B are available in May. In the rest of the months other compounds start appearing and disappearing (Rastogi et al., 1996).

Goel et al. (2003) reported the prophylactic and curative effects of standardized extract of Bacopa monniera in various gastric ulcer models. The effect was due to augmentation of the defensive mucosal factors like increase in mucin secretion, life span of mucosal cells and gastric antioxidant effect rather than on the offensive acid-pepsin secretion. The present study includes evaluation of standardized BME (bacoside A content--35.5 +/− 0.9) on other contributing factors towards ulcerogenesis. BME in the dose of 1000 µg/ml showed anti-Helicobacter pylori activity in vitro and in the dose of 10 µg /ml increased in vitro of prostanoids (PGE and PGI2) in human colonic mucosal incubates. It may be concluded that these factors may contribute to antiulcerogenic activity of BME.

Russo et al. (2003) reported the free radical scavenging capacity of a methanol extract of Bacopa monniera and the effect on DNA cleavage induced by H2O2 UV-photolysis was investigated. In addition, we examined whether this plant extract is capable of reducing the hydrogen peroxide-induced
cytotoxicity and DNA damage in human non-immortalized fibroblasts. It showed a dose-dependent free radical scavenging capacity and a protective effect on DNA cleavage. These results were confirmed by a significant protective effect on H2O2-induced cytotoxicity and DNA damage in human non-immortalized fibroblasts. The antioxidant capacity of BM may explain, at least in part, the reported antistress, immunomodulatory, cognition-facilitating, antiinflammatory and antiageing effects produced by it in experimental animals and in clinical situations and may justify further investigation of its other beneficial properties. Moreover, this experimental evidence suggests that because of its antioxidant activity, this Ayurvedic drug may be useful in the treatment of human pathologies in which free radical production plays a key role.

Rai et al. (2003) reported the investigations on the adaptogenic property of a standardized extract of *Bacopa monniera* against acute (AS) and chronic stress (CS) models in rats. Panax root powder (Panax quinquefolium) was taken as a standard. Male SD rats, weighing 180-200 g, exposed to immobilization stress for 150 min once only for AS and for seven consecutive days in CS, were fed with *B. monniera* or Panax root powder daily for 3 days in AS and for 7 days in CS, 45 min prior to each exposure of stress. Rats were sacrificed immediately after stress, the blood was collected, and the plasma was separated out for biochemical estimation. Adrenals, spleen, and thymus were dissected for organ weight and stomach for ulcer score. AS exposure significantly increased the ulcer index, adrenal gland weight, plasma glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase (CK) but significantly decreased the spleen weight. Pretreatment with *B. monniera* at 40 mg/kg per oral significantly reduced the AS-induced increase in the ulcer index, adrenal gland weight, plasma glucose, AST, and CK. A dose of 80 mg/kg po significantly reversed the AS-induced changes in adrenal gland weight, spleen weight, plasma glucose, ALT, and
AST. Panax root powder, 100 mg/kg po, significantly reversed the AS-induced changes in spleen weight, plasma ALT, AST, and CK. CS exposure resulted in a significant increase in the ulcer index, adrenal gland weight, plasma AST, and CK with a significant decrease in the thymus and spleen weight, plasma triglyceride, and cholesterol. Pretreatment with low dose of *B. monniera* extract at 40 mg/kg significantly reversed changes in ulcer index and plasma AST only, whereas the pretreatment with higher dose significantly reversed CS-induced changes in ulcer index, adrenal gland weight, CK, and AST. Panax root powder significantly reversed CS-induced increase in ulcer index, adrenal gland weight, CK, and AST. On the basis of our result, it is concluded that the standardized extract of *B. monniera* possesses a potent adaptogenic activity.

Channa *et al.* (2003) reported that various fractions and sub-fractions isolated from Bacopa monniera produced significant inhibition of carbachol-induced bronchoconstriction, hypotension and bradycardia in anaesthetized rats. All these showed more potency towards inhibition of tracheal pressure compared to either blood pressure or heart rate. The sub-sub fraction and compound 1 caused greater inhibition of tracheal pressure and heart rate compared to blood pressure. Thus, overall bioassay-directed fractionation of *B. monniera* improved the bronchodilatory activity in various fractions and compound 1 (2-219x) in anaesthetized rats. In vitro, the KCl-induced contraction was equally inhibited by crude extract, petroleum ether and methanol fractions on trachea suggesting bronchodilatory activity remained the same in fractions. On pulmonary artery petroleum ether, dichloromethane and methanol fractions produced 2-2.6 times more vasodilatation compared to crude extract of *B. monniera*. Subsequent sub-fractions failed to show the existence of broncho-vasodilatory activity, however, the CHCl₃/MeOH sub-fraction significantly reduced the acetylcholine-induced contraction on ileum. Both the methanol fraction and CHCl₃/MeOH sub-fraction caused marked
reduction of barium chloride, potassium chloride and calcium chloride induced contraction on guinea-pig ileum, indicating their interference with Ca\(^{++}\) ion movement. Thus, it may be concluded that various fractions derived from *B. monniera* possess broncho-vasodilatory activity, which is attributed mainly to inhibition of calcium ions.

Chakravarty *et al.* (2003) reported three new saponins, designated as bacopasides III, IV and V have been isolated from *Bacopa monniera* and their structures have been elucidated as 3-O-alpha-L-arabinofuranosyl (1→2)-beta-D-glucopyranosyl jujubogenin (1), 3-O-beta-D-glucopyranosyl (1→3)-alpha-L-arabinopyranosyl jujubogenin (2) and 3-O-beta-D-glucopyranosyl (1→3)-alpha-L-arabinopyranosyl pseudojujubogenin (3) mainly on the basis of two dimensional (2D) NMR and other spectral analyses.

Hou *et al.* (2002) reported two new saponins, 3-O-[6-O-sulfonyl-beta-d-glucopyranosyl-(1→3)]-alpha-l-arabinopyranosyl pseudojujubogenin (1) and 3-O-[alpha-l-arabinofuranosyl-(1→2)]-alpha-l-arabinopyranosyl jujubogenin (2), a new matsutaka alcohol derivative, (3R)-1-octan-3-yl-(6-O-sulfonyl)-beta-d-glucopyranoside (3), a new phenylethanoid glycoside, 3,4-dihydroxyphenylethyl alcohol (2-O-feruloyl)-beta-d-glucopyranoside (4), and a new glycoside, phenylethyl alcohol [5-O-p-hydroxybenzoyl-beta-d-apiofuranosyl-(1→2)]-beta-d-glucopyranoside (5), were isolated from *Bacopa monniera*. Their structures were established by NMR, MS, and chemical methods.

Chakravarty *et al.* (2002) reported three new phenylethanoid glycosides, viz. monniersides I-III (1-3) along with the known analogue plantainoside B were isolated from the glycosidic fraction of *Bacopa monniera*. Their structures were elucidated mainly on the basis of two dimensional (2D) NMR spectral analyses.

The antistress effect of bacosides of Brahmi (*Bacopa monnieri*), dissolved in distilled water, was studied in adult male Sprague Dawley rats by
administering oral doses of 20 and 40 mg/kg for 7 consecutive days. In half of the animals treated with 20 or 40 mg/kg of BBM, stress was given 2 h after the last dose. Stress was also administered to the animals treated with distilled water alone. BBM, at both doses, did not induce a significant change in the expression of Hsp70 in any brain region studied while stress alone produced a significant increase in the Hsp70 expression in all the brain regions. A significant decrease in the activity of superoxide dismutase (SOD) was evident in the hippocampus with the lower dose of BBM and in animals given stress alone, while an increase in the activity of SOD was observed in the brain regions with the higher dose of BBM. An increase in the activity of cytochrome P450 dependent 7-pentoxyresorufin-o-dealkylase (PROD) and 7-ethoxyresorufin-o-deethylase (EROD) was observed in all the brain regions after exposure to stress alone and with both doses of BBM although the magnitude of induction of P450 expression was less with a higher dose of BBM. Interestingly, stress when given to the animals pretreated with BBM for 7 days resulted in a decrease in Hsp70 expression in all the brain regions with a significant decrease occurring only in the hippocampus. Likewise the activity of SOD was found to be further reduced in all the brain regions in the animals treated with the lower dose of BBM followed by stress. However, when stress was given to the animals pretreated with the higher dose of BBM, a significant increase in the enzyme activity was observed in the cerebral cortex and in the rest of the brain while the activity of SOD was reduced to a much greater extent in the cerebellum and in the hippocampus. Likewise, the activity of P450 enzymes was found to be restored to almost control levels in the animals given stress and pretreated with the higher dose of BBM, while a lesser degree of induction, compared with animals treated with BBM or stress alone, was observed in the animals pretreated with the lower dose of BBM and given stress. The data indicate that BBM has potential to modulate the activities of Hsp70, P450 and SOD thereby possibly allowing the brain to be prepared to act under adverse conditions such as stress (Chowdhuri et al., 2002).
Nathan et al. (2001) examined the acute effects of an extract of *Bacopa monniera* on cognitive function in normal healthy human subjects. The study was a double-blind, placebo-controlled independent group design in which subjects were randomly allocated to one of two treatment conditions, *Bacopa monniera* (300 mg) (n = 18) or placebo (n = 20). Neuropsychological testing was conducted before and 2 h after drug administration. No significant changes were found on any of the tests. The findings suggest that *Bacopa monniera*, at least for the dose administered, has no acute effects on cognitive functioning in normal healthy subjects.

The standardized extract was reported earlier to have significant antioxidant effect, anxiolytic activity and improve memory retention in Alzheimer's disease. Presently, the standardized methanolic extract of *Bacopa monniera* (bacoside A - 38.0±0.9) was investigated for potential antidepressant activity in rodent models of depression. The effect was compared with the standard antidepressant drug imipramine (15 mg/kg, ip). The extract when given in the dose of 20 and 40 mg/kg, orally once daily for 5 days was found to have significant antidepressant activity in forced swim and learned helplessness models of depression and was comparable to that of imipramine (Sairam et al., 2002).

The methanolic extract of *Bacopa monniera* standardized to bacoside-A content (percentage-38.0±0.9), when given in the dose of 10-50 mg/kg, twice daily for 5 days, showed dose-dependent anti-ulcerogenic on various gastric ulcer models induced by ethanol, aspirin, 2 h cold restraint stress and 4 h pylorus ligation. BME in the dose of 20 mg/kg, given for 10 days, twice daily showed healing effects against 50% acetic acid-induced gastric ulcers. Further work was done to investigate the possible mechanisms of its action by studying its effect on various mucosal offensive acid-pepsin secretion and defensive factors like mucin secretion, mucosal cell shedding, cell
proliferation and antioxidant activity in rats. BME 20 mg/kg showed no effect on acid-pepsin secretion, increased mucin secretion, while it decreased cell shedding with no effect on cell proliferation. BME showed significant antioxidant effect per se and in stressed animals. Thus, the gastric prophylactic and curative effects of BME may be due to its predominant effect on mucosal defensive factors (Sairam et al., 2001b).

Successive petroleum ether, chloroform, methanol and water extracts of *Bacopa monnieri* were tested (*in vitro*) for mast cell stabilising effect. The methanolic fraction exhibited potent activity comparable to disodium cromoglycate, a known mast cell stabiliser (Samiulla et al., 2001).

*Bacopa monniera* has been evaluated alone and in combination with phenytoin for its effect on (a) passive-avoidance (PA) task; (b) maximal electroshock seizures; and (c) locomotor activity in mice. Phenytoin (PHT, 25 mg/kg po x 14 days) adversely affected cognitive function in the PA task. BM extract (40 mg/kg x 7 days), given along with phenytoin in the second week of the two-week regimen, significantly reversed PHT-induced impairment. Both acquisition and retention of memory showed improvement without affecting its anticonvulsant activity. The observed cognitive effects of PHT and BM were found to be independent of motor stimulation. The results provide evidence for potential corrective effect of BM in cognitive deficit associated with PHT therapy (Vohora et al., 2000).

The effect of a standardized extract of *Bacopa monniera* Linn. was assessed on rat brain frontal cortical, striatal and hippocampal superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) activities, following administration for 7, 14 or 21 days. The effects induced by this extract (bacoside A content 82% ± 0.5%), administered in doses of 5 and 10 mg/kg, orally, were compared with the effects induced by (-) deprenyl (2 mg/kg, p. o.) administered for the same time periods. Bacopa monniera (BM)
induced a dose-related increase in SOD, CAT and GPX activities, in all the brain regions investigated, after 14 and 21 days of drug administration. On the contrary, deprenyl induced an increase in SOD, CAT and GPX activities in the frontal cortex and striatum, but not in the hippocampus, after treatment for 14 or 21 days. The results suggest that BM, like deprenyl, exhibits a significant antioxidant effect after subchronic administration, which unlike the latter, extends to the hippocampus as well. The results suggest that the increase in oxidative free radical scavenging activity by BM may explain, at least in part, the cognition- facilitating action of BM, recorded in Ayurvedic texts, and demonstrated experimentally and clinically (Bhattacharya et al., 2000).

Two new dammarane-type jujubogenin bisdesmosides, bacopasaponins E and F of biological interest have been isolated from the reputed Indian medicinal plant *Bacopa monniera* and defined as 3-O-[beta-D-glucopyranosyl (1→3)[alpha-L-arabinofuranosyl(1→2)]alpha-L-arabinopyranosyl]-20-O-(alpha-L-rabinopyranosyl) jujubogenin and 3-O-[beta-D-glucopyranosyl (1→3)[alpha-L-arabinofuranosyl(1→2)]beta-D-glucopyranosyl]-20-O-alpha-L-rabinopyranosyl) jujubogenin respectively by spectroscopic methods and some chemical transformations (Mahato et al., 2000).

Dar et al. (1999) reported the calcium antagonistic activity in ethanol extract of *Bacopa monniera*. The plant extract inhibited the spontaneous movements of both guinea-pig ileum (IC50 = 24±4 μg/ml) and rabbit jejunum (IC50 = 136±9 μg/ml). A marked reduction in acetylcholine- and histamine-induced responses (0.0001-10 μM) in the ileum was evident in the presence of extract (260 μg/ml). The acetylcholine (1 μM)-induced contraction in the ileum was also inhibited by the extract (100-700 μg/ml) in a concentration dependent way (IC50 = 285±56 μg/ml). All these results indicate a direct action of the extract on smooth muscles. Calcium chloride-induced responses in the rabbit blood vessels and jejunum were attenuated in the presence of
plant extract (10-700 μg/ml) implying a direct interference of plant extract with influx of calcium ions in the cells. However, the lack of modification of either noradrenaline- or caffeine-induced contractions in the presence of extract suggests that extract has no detectable effect on mobilization of intracellular calcium. These results indicate that spasmolytic effect of the B. monniera extract in smooth muscles is predominantly due to inhibition of calcium influx via both voltage and receptor operated calcium channels of the cell membrane.

Reversed-phase high-performance liquid chromatographic separation coupled to (structurally informative) spectroscopic methods like NMR and MS and an efficient bioassay have been used to determine the active compounds from a crude fraction of Bacopa monniera. The fraction containing a mixture of saponins with closely related structures was found to show a significant anthelmintic activity against Caenorhabditis elegans (used as a model test organism for determining anthelmintic activity). The activity was correlated to two dammarane type triterpenoidal saponins containing at least three sugar units. The optimization of separation for 1 mg of the crude sample on column and the sensitivity of on-flow one- and two-dimensional NMR experiments to the high-molecular-mass compounds (890-930) has been demonstrated (Renukappa et al., 1999).

A new dammarane-type pseudojujubogenin glycoside, bacopasaponin D, has been isolated from Bacopa monniera and defined as 3-O-[alpha-L-arabinofuranosyl(I->2)beta-D-glucopyranosyl]pseudojujubogenin by spectroscopic methods and some chemical transformations. The 13C signals of the saponin were assigned by DEPT, 1H-1H COSY and HSQC techniques (Garai et al., 1996a).

Tripathi et al. (1996) reported the effect of alcohol and hexane fraction of Brahmi has been studied on FeSO₄ and cumene hydroperoxide induced
lipid peroxidation. Alcohol fraction showed greater protection with both inducers. Results were compared with known antioxidants tris, EDTA and a natural-antioxidant vitamin E. The effect of Brahmi was also examined on hepatic glutathione content. The mechanism of action could be through metal chelation at the initiation level and also as chain breaker. The results suggested that Brahmi is a potent antioxidant. The response of Brahmi was dose dependent. Tris, an hydroxyl trapper did not show any protection in comparison to Brahmi where as EDTA and vitamin E did protect against FeSO₄. In experimental conditions 100 micrograms Brahmi extract (alcoholic) was equivalent to 247 µg of EDTA (0.66 µM) and 58 micrograms of vitamin E. Interestingly Brahmi only slightly protected the autooxidation and FeSO₄ induced oxidation of reduced glutathione on lower doses 100 µg/ml and below, but on higher concentrations it enhanced the rate of oxidation.

Three new dammarane-type triterpenoid saponins, bacopasaponins A, B and C, of biological interest have been isolated from the reputed Indian medicinal plant Bacopa monniera and identified as 3-O-alpha-L-arabinopyranosyl-20-O-alpha-L-arabinopyranosyl-jujubogenin, 3-O-[alpha-L-arabinofuranosyl (1→2)alpha-L-arabinopyranosyl] pseudojujubogenin and 3-O-[beta-D-glucopyranosyl (1→3)[alpha-L-arabinofuranosyl (1→2)]alpha-L-arabinopyranosyl]pseudojujubogenin by spectroscopic methods and some chemical transformations. The hitherto undetermined configurations at C-20 and C-22 of pseudojujubogenin were elucidated by phase-sensitive ROESY, and 1H and 13C signals of the saponins were assigned by DEPT, 1H-1H COSY, HSQC and HMBC techniques (Garai et al., 1996b).

A new triterpenoid saponin, bacoside A3, a constituent of bacosides the saponin mixture of Bacopa monniera, was isolated and characterized. Its structure was established as 3-beta-[O-beta-D-glucopyranosyl(1→3)-O-[alpha-L-arabinofuranosyl(1→2)]O-beta-D-glucopyranosyl]oxy] jujubogenin
by chemical and spectral analyses. The cis-isomer of ebelin lactone was also obtained as one of the artefacts of the aglycone and its structure revised (Rastogi et al., 1994).

The effects \textit{Bacopa monniera} Linn on the learning performance of rats have been studied in different conditioning schedules by administering an aqueous suspension of an alcoholic extract (40 mg/kg, p.o.) for three or more days. The first schedule induced a labile behaviour using a shock-motivated brightness-discrimination reaction. The brahmi-treated group showed better acquisition, improved retention and delayed extinction (p<0.01-0.05). Similarly, in an active conditioned flight reaction, the drug-treated animals showed a shorter reaction time than the controls (p<0.01). Also in the continuous avoidance response the drug-treated group performed better than the controls (p < 0.01-0.05). Our findings are in conformity with the Ayurvedic claims and indicate that \textit{Bacopa monniera} can improve the performance of rats in various learning situations (Singh et al., 1982).

2.2.6 Memory Enhancing Effect

Both the extract as well as bacosides was evaluated for their nootropic activity. In adult male rats the extract (40mg/kg per oral) was given for three consecutive days for those cognitive tests eliciting labile form of behavior where the training was completed in one session and the same dose was administered initially for three consecutive days and then every third day for the cognitive tests eliciting a stable behavior. This test was completed in several sessions, comprising of one session per day. Thus a single test eliciting a labile behavior formation utilized a brightness discrimination reaction in a semi-automatic Y-maze. In this test significant effect was observed on all the three temporo-spatial parameters, i.e., acquisition, consolidation and retention. The initial test for a stable behavior was conditioned avoidance test using a sound cue as conditioned stimulus and foot shock as unconditioned
stimulus. In this test also the animals learnt to escape foot shock quickly (6 days versus 10 days in control) and the reaction time was lower from day 4 onwards. The final test was a continuous avoidance test where no exteroceptive stimulus was used and the animals were trained to postpone a foot shock by pressing a lever. A stable baseline behavior was achieved by day 20 in this test but this did not happen in the control animals (Singh and Dhawan, 1992).

Details studies were carried out with bacosides A and a mixture of bacosides A and B (Singh et al., 1988). The labile test used was the brightness discrimination reaction in a semi-automatic Y maze as was done with the extract. The stable base-line behavioral tests included the active conditioned avoidance test used earlier and a conditioned taste aversion test employing aversion to lithium chloride in water deprived rats as the cue. The bacosides produced a dose-related effect, similar to that of the extract in all the three tests. The effect of 10 mg/kg of bacosides was equipotent to 40 mg/kg of extract (Singh et al., 1988).

A study is reported on the effects of Bacopa monniera on human memory. Seventy-six adults aged between 40 and 65 years took part in a double-blind randomized, placebo control study in which various memory functions were tested and levels of anxiety measured. There were three testing sessions: one prior to the trial, one after three months on the trial, and one six weeks after the completion of the trial. The results show a significant effect of the Brahmi on a test for the retention of new information. Follow-up tests showed that the rate of learning was unaffected, suggesting that Brahmi decreases the rate of forgetting of newly acquired information. Tasks assessing attention, verbal and visual short-term memory and the retrieval of pre-experimental knowledge were unaffected. Questionnaire measures of everyday memory function and anxiety levels were also unaffected (Roodenrys et al., 2002).
2.2.7 Effect on Early Phases of Memory Consolidations

Based on experimental evidences, it has been postulated that memory exists in two forms, viz, short-term and long-term. Both these forms start simultaneously and deficit in retention curve is observed at a point where these two forms overlap. This hypothesis was tested in the learning model of foot shock motivated brightness discrimination reaction in the Y-maze and found that the reaction curve instead of being V-shaped was W-shaped, i.e., there were deficits occurring at two points, viz., 1.5h and 4.0h training relearning intervals. Therefore, it was presumed that, at least for this experimental models, there are perhaps three forms of memory, i.e. short-term memory-STM- (few seconds to minutes) and long-term memory-LTM- (few hours to days) and in between exists an intermediate form of memory-IM- (few minutes to hours). The two deficits occur at those two points, where, first, the STM overlaps with IM and second, where IM overlaps with LTM. The bacosides in a dose of 20mg/kg per oral when given 30 min prior to training abolished these deficits when the relearning was done at various time intervals after training. A support dose of 10 mg/kg per oral given 30 min prior to the 24h-relearning test produced a significant enhancement of retention (Singh and Dhawan, 1992). These results confirm that the facilitating effect of bacosides is due to their ability to consolidate the retention at the earliest form, i.e., STM. This facilitating effect persists when the other two forms, i.e. IM and LTM occur. These results suggested that the facilitating effect of bacosides is mainly due to their ability to consolidate the retention of a learnt behavior at the earliest form, i.e., STM. This facilitating effect persisted when the other two longer lasting forms of memory were getting consolidated.

2.2.8 Anti-Amnesic Effect

A disruption of memory during the consolidation phase can be attained by a variety of procedures or agents (amnesic agents). Most of the
procedures (e.g. electro-convulsions) or drugs (e.g. scopolamine) produce retrograde amnesia but some (hypoxia, diethylthiocarbamate) can cause transient amnesia when applied before training (De Zazzo and Tully, 1995). As the retrograde amnesia is clinically more relevant, bacosides were tested in 3 quintessential models. The bacosides were needed in a slightly higher dose (20mg/kg per oral for 3 days) to attenuate retrograde amnesia produced by electroconvulsive shock, immobilization or scopolamine in the brightness discrimination test (Singh and Dhawan, 1997). Another investigation was done by Manjerekar (1996), on the effect of bacosides on scopolamine induced amnesia in rats and mice employing elevated plus maze test, step down passive avoidance test and the Cook and Weidley test. It was found that a single dose of 10mg/kg bacosides to be either equipotent to or more effective than similarly administered piracetam (150mg/kg), cyclandelate (200 mg/kg) or Ginkgo biloba or Withanla somnifera extracts (100 mg/kg per oral for 4 days) in attenuating the amnesic effect of scopolamine. This was a consistent finding in the species used for all the three test models. Kapoor and co-workers (1999) have also found Bacopa monniera extract to be more potent than extract of Ginkgo biloba in counteracting the atropine induced transient amnesia on transfer latency in mice.

2.2.9 Effect on Alzheimer’s Model of Learning Impairment

Bhattacharya et al. (1999) have studied the effect of Bacopa monniera extract on rat models of Alzheimer’s disease. Lesions were produced either by intracerebroventricular injection of 15mg colchicine according to the method of Emerich and Walsh, (1990) or by the injection of 10mg ibotenic acid according to the method of Takahashi et al. (1992) in nucleus-basalis-magnocellularis. Oral administration of the brahmi extract in doses of 5-10 mg/kg during this period markedly reduced the magnitude of memory deficits caused by the 2 neurotoxins. Besides, the treatment also reversed the
colchicine-induced reduction in acetylcholine concentration, choline acetylase activity and muscarinic receptor binding in hippocampus and frontal cortex. Singh and co-workers (1979a) reported an increase in acetylcholine concentration in the frontal cortex of normal rats given 40 mg/kg of Bacopa extract orally once daily for 15 days. The acetylcholine level was reduced in other parts of the brain in their study.

2.2.10 Anxiolytic Effect

Singh et al. (1979b; 1980) were first to suggest an anti-anxiety effect of the brahmi extract based in its effect on gross behavior and prolongation of barbiturate hypnosis in rats. The anxiolytic activity of the ethanolic extract has been further investigated by Bhattacharya and Ghoshal (1998). The effect has been studied in the open field, elevated plus maze, social interaction and novelty suppressed feeding latency tests in rats. A dose related anxiolytic activity was observed in doses devoid of producing any motor deficit. It was, however, very much weaker than lorazepam. Shanker and Singh (2000) have also reported mild anxiolytic activity by observing effect on barbiturate sleeping time, amphetamine hyperactivity, clonidine induced fighting and biting, secondary conditioned response and survival under hypoxia. Shukla and co-workers (1987) have studied CNS effect of an Ayurvedic formulations based on leaves of Bacopa monniera (72%) with some added aromatics. The effect were studied in rats and mice and in both the species they observed a prolongation of the barbiturate sleeping time and anticonvulsant and anti nociceptive effects with high doses. Vohora et al., (1997) have localized the analgesia activity in a new diterpene, bacosine, isolated from this plant, but did not found any effect on the CNS with bacosine. The bacosides have a mild antidepressant activity as evident by reversal of reserpine syndrome and effect on immobility time in the swimming test in mice (Shanker and Singh, 2000). In EEG studies alterations were seen in the faster a, b and slower q
activities in several brain regions of conscious rats. The changes were opposite to those observed in pathological senility (Singh et al., 1990).

2.2.11 Other Pharmacological Effect

Bacosides exhibited significant anti-stress activity in doses 3-4 times higher than those required for nootropic effect. They improved duration and performance in the swimming endurance test, increased survival under hypoxic conditions and protected animals against morphine induced toxicity (Singh et al., 1996). Mild anti-inflammatory activity in doses devoid of gastric irritability has also been reported (Jain et al., 1994). Protection against both gastric and duodenal ulcers in several experimental models in rats has been reported with the whole plant powder (Kumar and Goel, 2000) as well as with fresh juice (Sairam et al., 2000). The ethanol extract has a relaxant effect on trachea, pulmonary artery and aorta of guinea pig and rabbit in-vitro (Dar and Channa, 1997). They later reported similar effects in guinea pig ileum and rabbit jejunum. The response of rabbit blood vessels to calcium was antagonized (Dar and Channa, 1999). However, the concentrations used in all these studies are rather high. The alcoholic extract also exhibited anti cancer activity against Walter carcinosarcoma 25% in rats (Bhakuni et al. 1969).

Tripathi and co-workers (1996) demonstrated a concentration related anti-oxidant effect of the ethanolic extract of the plant against ferrous sulphate and cumene hydroperoxide induced lipid peroxidation in rat liver homogenate. Bhattacharya et.al. (2000) have found an increase in superoxide dismutase, catalase and glutathione peroxidase activity in the frontal cortex, striatum and hippocampus following oral administration of the ethanolic extract of the plant in rat. The effect was also found to be dose dependent. The bacosides increase protein and serotonin level and decrease in norepinephrine concentration in hippocampus, hypothalamus and cerebral cortex. Bacosides,
however, have no effect on the binding of specific ligands to catecholamines, serotonin, acetylcholine or NMDA receptors in neuronal membrane preparations from the rat brain (Singh and Dhawan, 1997a). Immobilization leads to an increased expression of heat shock proteins (HSP) in cerebellum, cerebral cortex and hippocampus in decreasing order. Ethanolic extract of Bacopa monniera has similar, but much weaker effect per se. Prior administration of the extract, however, markedly reduced the stress-induced expression of HSP, especially in the cerebral cortex and hippocampus. Bacosides also effectively protect against stress induced increase in the level of lipid peroxides in several areas including the hypothalamus and cerebral cortex (Singh and Dhawan, 1997b).

2.2.12 ACUTE AND REGULATORY TOXICITY

The LD₅₀ of the ethanolic extract has been determined in rats and mice by both oral and i.p. routes. It was >2.5g/kg body weight by oral route in both the species, but by i.p. route it was much lower and was found to be 205 mg/kg [with the confidence limit (C.L.) ranging from 230-182] in rats and 224 mg/kg (C.L. 260-193) in mice. The oral LD₅₀ of bacosides in mice was 774 mg/kg and therefore it is evident that the extract has much lower toxicity than bacosides. Therefore, further toxicity studies were done with the standardized extract. Chronic toxicity studies were performed following oral administration with 2.5, 5.0 and 10 times the effective nootropic dose of the extract of a rodent (rat) and a non rodent (rhesus monkey) species for 90 days. A regular monitoring and record of the various haematological, biochemical and gross behavioral changes were regularly recorded. The terminal autopsy was followed by gross and microscopic examination of all viscera in accordance with the regulatory requirements. The extract was found to be safe (Singh and Dhawan, 1997b).
2.2.13 Clinical Trials

In spite of extensive use of the plant by the practitioners of Ayurveda there have been no well designed clinical trials so far to evaluate its efficacy as a cognitive enhancing agent. The safety and tolerability of bacosides in 51 healthy human volunteers were assessed (Asthana et al., 1996). They have found that single oral doses of 20-200 mg or 100 and 200 mg once daily for 4 weeks were safe and did not produce any reaction or side effect. Subsequently a placebo controlled double blind phase II clinical trials in 36 children of Attention Deficit Hyperactivity Disorder were conducted. The children received either a placebo or 50 mg of the extract twice daily for 12 weeks. The children receiving the extract showed significant improvement in scores in several test systems and there were no side effects. Clinical trials were initiated in elderly cases of Age Associated Memory Impairment (AAMI) at 3 medical centers, and 30 patients completed a 16-week study at BRD Medical College, Gorakhpur. A significant improvement in logical memory, digit forward and paired associated learning has been observed from fourth week onward in the group treated with the standardized extract without showing any side effect (CDRI Annual Report, 2000-2001).

2.3 EVOLVULUS ALSINOIDES LINN.

2.3.1 Description of the Plant

*Evolvulus alsinoides* (Fam. Convolvulaceae) is a perennial herb with a small woody branched root stock and occurs in tropical and sub tropical countries. *Evolvulus alsinoides* is available in the market as "Shankhpushpi". Various important medicinal properties have been attributed to this plant and it is frequently used in the indigenous medicine. The herbal physicians believe that this plant has the power to strengthen the brain and memory. The whole plant is used as infusion in the quantity of half a cupful twice daily.
The leaves are made into cigarettes and smoked in chronic bronchitis and asthma. The roots are used by the Santhals in intermittent fever of childrens. In medagasker, the root is considered antidiarrhotic.

### 2.3.2 Geographical Distribution

It is an attractive plant with perfusion of blue flowers, commonly growing amidst grass in waste places and met with throughout India and Ceylon.

### 2.3.3 Common Names in Different Languages

<table>
<thead>
<tr>
<th>Language</th>
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<tbody>
<tr>
<td>Hindi</td>
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<td>Gujarati</td>
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<td>Medagasc ter</td>
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</table>

### 2.3.4 Importance in Ayurveda

According to Ayurvedic literature the plant begins to flower in the beginning of summer and flowers abundantly throughout the summer. One of the "Tridosha", "Pitta" is most common during summer and the drug has been claimed to cure it. The following 'Shlokas' are highly of the medicinal importance of Shankhapushpi in Ayurveda.

#### भावप्रकाश-निषिद्दु

1. "शाङ्खपुष्पी तु शांखाहवा मांगल्य कुमारादपि च"
2. "शाङ्खपुष्पी सतः मेयन्य, कुश्या मानस रोग हृद रसायनी, कपामोण्या, स्मृति, कालित वलानिवा"
2.3.5 Therapeutic Value

The whole plant is reported to bitter, pungent, alexiatric and anthelmintic. It is used in bronchitis, biliousness, epilepsy, leucoderma, teething of infants, and loss of appetite. The plant is used as a febrifuge and as an alternative to oil to promote the growth of hair (Kirtikar et al., 1935).

2.3.6 Early Investigations

The in-vitro evaluation of the alcoholic extract of *Evolvulus alsinoides* revealed its marked antiulcer and anticyatatonic activity. There is a significant decrease in free acidity and volume of gastric content of the extracts treated rats when compared with control. It also caused a highly significant increase in the pH of gastric content. It is evident from the results that the extract produced reduction in the intensity of gastric ulceration as observed from reduced ulcer index in the alcoholic extract treated groups. Alcohol extract showed good anticyatatonic activity at 5 to 90 minutes and its activity was decreased after 90 minutes. Alcohol extract exhibited both antiulcer and anticyatatonic activity and appears to have the potential used to control ulcer formation which is a major disease that effect human gastrointestinal tract. This extract may perhaps useful to treat Parkinsons symptoms such as akinesia, rigidity and tremors (Purohit et al., 1996).

Karandikar et al. (1959) have reported in detail the macroscopic and microscopic aspects of *Evolvulus alsinoides*. Vardan et al. (1958) have reported the presence of alkaloides in *Evolvulus alsinoides*. They also isolated β-sitosterol.
2.4 CONVOLVULUS PLURICAULIS CHOIS.

2.4.1 Description of the Plant

*Convolvulus pluricaulis* Chois. (Fam. Convolvulaceae) is commonly known as "Shankhpushpi" in Hindi, is employed in the Ayurvedic system as a brain tonic in insanity and neuraesthesia.

2.4.2 Geographical Distribution

It grows on the waste land in the plains of Punjab, Bihar and Madhya Pradesh. The herb produces flowers during the months of September and October which are white to light pink in colour.

2.4.3 Synonyms

*Convolvulus pluricaulis* Chois is also known as *Convolvulus microphyllus* Sieb. (Fam. Convolvulaceae).

2.4.4 Common Names in Different Languages

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The whole plant is reported to bitter, pungent, alexiatric and anthelmintic. It is used in bronchitis, biliousness, epilepsy, leucoderma, teething of infants, and loss of appetite. The plant is used as a febrifuge and as an alternative to oil to promote the growth of hair (Kirtikar et al., 1935).

2.4.6 Early Investigations

Sairam and co-workers (2001a) reported the potential anti-ulcerogenic effect of juice of fresh whole plants of C. pluricaulis (CPJ) against various experimental gastric ulcer models induced by ethanol, aspirin, 2 hr cold restraint stress and 4 hr pyloric ligation in rats. The drug was given orally twice daily for five days in the doses of 375 and 750 mg/kg body weight. CPJ showed anti-ulcerogenic effect at both doses in all the experimental gastric ulcer models and was comparable to the reference drug sucralfate (250 mg/kg). Gastric juice secretion and mucosal studies were undertaken to find out the possible mechanism of action of antiulcer effect by studying its effects both on offensive and defensive mucosal factors. The antiulcerogenic effect of CPJ was found to be due to augmentation of mucosal defensive factors like mucin secretion, lifespan of mucosal cells and glycoprotiens rather than on the offensive factors like acid-pepsin.

An investigation was made to evaluate the role of Convolvulus pluricaulis root extract in the regulation of hyperthyroidism in female mice. Its possible site of action was also studied. L-Thyroxine treatment for 30 days increased serum concentrations of thyroxine (T4) and triiodothyronine (T3). The activity of hepatic 5'-monodeiodinase (5'-DI) and glucose-6-phosphatase (G-6-Pase) was also enhanced. On the other hand, administration of the plant extract either alone or with L-T4, decreased serum T3 concentration and the activity of hepatic 5'-DI and G-6-phase, without marked alteration in hepatic
lipid peroxidation, indicating the possible regulation of hyperthyroidism by the plant extract. It appears that the action of the plant extract on thyroid function is primarily mediated through the inhibition of 5'-DI enzyme activity (Panda et al., 2001).

The water soluble fraction of plant caused a marked and prolonged hypotension in dogs and inhibited the frog myocardium (Chaturvedi et al., 1966). Barar and Sharma (1965) confirmed the findings of early workers and reported that the extract caused transient depression of thyre amphibian and mammalian myocardium. It had spasmylytic activity on smooth muscles except in bronchial muscle. It potentiated the response of acetylcholine on bronchial and skeletal muscles. The extract did not exhibit any sedative or hypnotic property but potentiated significantly the effect of barbiturate and abolished conditioned avoidance response without significantly affecting the escape response, like other tranquillisers. The extract caused a reduction in the fighting behaviour in mice but was devoid of analgesic activity although it potentiated morphine analgesia (Sharma et al., 1965). These workers suggested that the extract might act by its action on subcortical areas of the brain.
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