CHAPTER-2

HISTORICAL RESUME
Since 1902, when Haberlandt conceived the idea of totipotency considerable success has been achieved in the field of the plant tissue culture, in other words micropropagation. Several books and reviews have been appeared on the impact of tissue culture techniques on plant improvement (Bornman 1985, Bajaj and Sopory 1986, Zheng et al 2001). Recently Mohapatra and Rath (2004) studied phytochemistry of Bacopa monnieri under vitro condition.


Bacopa monnieri experimental material of present study is an important plant of exceptionally high medicinal value. Most of the work has been done on their comparative biochemical investigation in and vivo clonal propagation. In the following pages a brief resume of the important work done on its biochemical and tissue culture are given.
Morel & Martin (1952) developed the technique of meristem culture for in vivo virus eradication of Dahlia.

Skoog & Miller (1957) gave general concept that organ differentiation in plants is regulated by interplay of auxins & cytokinins, is universally applicable & should work as a guide when developing a medium for a new plant type. According to this hypothesis the nature of organogenic differentiation is determined by the hormones.

Morel (1960) described an in vitro method for clonally multiplying orchids at a fantastic rate. Culture excised shoot tips of Cymbidium instead of developing into a leafy shoot formed a spherule-like body with rhizoids at the base.

Murashige and Skoog medium (1962) supplemented with different combinations of growth regulators has been used for growth of herbaceous species. Multiple shoots were obtained from shoot tips (1-2 cm) derived from field-grown plants of Bacopa monnieri in Murashige and Skoog medium supplemented with 0.5 mg/litre within 6 days of culture, whereas in the case of Paederia foetida and Centella asiatica multiple shoots were obtained from field-grown plants in MS medium supplemented with 1.0 mg BAP/litre within 7 days of culture. (Singh et al, 1999). Sehrawat et al, 2001, reported induction of multiple shoots in Rauwolfia serpentina from shoot apices and nodal segments on MS medium supplemented with benzyladenine and NAA.
Chatterjee et. al (1963) and Singh et. al (1988) found out medicinal properties of *Bacopa monnieri*.


Georges Morel (1965) was the pioneer in applying shoot tip culture for micro propagation of *Orchid cymbidium*.

Champagnat et. al., (1966) the protocorms originated from epidermal cells of leaf in shoot-tip culture. Some of these protocorms proliferated & formed a cluster of up to a dozen protocorms.

Hackett (1969) & Gupta et. al. (1978) describes the propagation by adventitious bulb lets formation directly from aseptically cultured bulb lets formation and directly from aseptically cultured bulb-scale segment. In the cultures of whole scales about 10 bulb lets were formed within 15 days.

Kartha et. al., 1974 b; Roest & Bokelmann, (1975); Behki & Lesley, 1976; Murray et.al., (1977) adventitious buds can be induced on the leaf & stem cutting of even those species which are normally not propagated vegetatively, under influence of an appropriate combination of growth regulators, in culture.

Barlass & Skene (1978, 1980 a,b;) described a propagation method for grapes in which encised shoot tips measuring 1 mm in length (with two to three leaf primordial), were cut into nearly 20 pieces. In cultures each shoot-tip fragment formed multiple shoot adventitious from the swollen leaf bases.

McComb (1978); Zimmerman & Broome, (1980) observed that at higher levels cytokinins tend to induce callusing and/or adventitious bud formation which can endanger the clonal nature of the micro propagated plants in some system.

Takayama & Misawa (1982) described an in vitro method for mass propagation of Begonia X Hiemalis which allowed the production of over 1014 plants in a year from a single 7X7 mm segment of a young leaf.

Recently, Mohapatra and Rath (2004) studied phytochemistry of Bacopa monnieri under in vitro conditions.

M.J. O'neill et at. (1986) has reported anti-malarial activity of some quassionoids (obtained from Simaroubaceous plants) against *Plasmodium falciparum*. They found the Simaroubaceous plants are sources of malarial drugs.

Simmonds & Wlerry (1987) describe a liquid shake culture method for rapid multiplication of this species by adventitious bud formation.

An efficient protocol for plant regeneration from protoplast of pepperming was developed by jullien et al. (1998) by stepwise optimization of first cell division, microcallus formation M2, 4-D in and shoot differentiatio.n. Best results were obtained with M. Although liquid medium and benzy lademine (4 combination with NAA (2.5 was more efficient to support first protoplast divisions, solid medium was clearly more suitable to any system subsequent cell divisions leading to the formation of microcalluses (Jullien et al, 1988)

Arinima and Sinha (1989) examined free amino acids and proteins from 16 cultivars of *Vicia faba*. Studies reveal that differences in free amino acids
but argunul, aspartic acid, isoleucine, leucine, methinine serine and valine common in all. Total protein varied from 19% to 33 % in cultivars of V. faba.

Hassain M. Ahmed G et. al (1990) work done on rapid clonal propagation in Sesbania cannabina through in vitro shoot proliferation from shoot tip explants.

Jain A.K et. al. (1990) contributes in vitro propagation through axillary bud multiplication in different mulberry genotypes.

Hassain et. al. (1991) observed the effect of nature of explants & pH on in vitro propagation of some mulberry genotypes.

Islam R, Zaman A et. al. (1993) had seen the effect of various type of cytokinins & auxins in vitro propagation as an aid for cloning of M. laevigata.

Organogenesis has been reported in number of medicinal plants through callus culture. Callus was induced from leaves of Rauwolfia serpentina (Rauvolfia serpentine), on WCR supplemented with coconut milk (CM), extra nitrogen souce solution, biotin (10 gm/litre), BAP (2 mg/litre), NAA (0.8 mg/litre) and GA₃ (0.0001 mg/litre). Callus, subcultured on WRC supplemented with CM, BAP (0.1 mg/litre) and NAA (0.1 mg/litre), grew well and differentiated into roots. This cycle of differentiation was observed repeatedly on further subculture (Mahmood Riffat 1994). Calli derived from
nodal explants of Bacopa cultured on MS medium containing 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), when subcultured on MS medium containing 0.1 or 0.5 mg/l BA or 0.2 mg/l 2,4-D+0.1 or 0.5 mg/l kinetin, developed somatic embryos.

Jain and Kulshreshtha (1994) and Schulte et al (1972) studied Phytochemical analysis of this plant.

Sanago et al., (1997) has reported antimicrobial activity against the bacterial strains Haemophilus influenzae (6 strains), Staphylococcus aureus (5 strains), Streptococcus pneumonia (3 strains), Streptococcus pyogens (8 strains) and Moraxeela catarrhalalis (5 Strains) Through 13 plant species of their studied area (Mali). They concluded that the methanolic extract of these traditional plants is a potential carrier for antimicrobial substances.

Fature (1998) carried out in vitro shoot organogenesis from leaf disc in 2 weeks of culture on MS medium M indole 3- butyric 6-BA and 2.0.mannitol supplemented with 300 MM NAA 9.0 acid and transferred into medium with out mannitol containing 0.5 M thidiazuron. BA.

The somatic embryos germinated either on the same media or on MS basal medium, and the resulting plantlets were successfully transplanted to soil. (Tiwari et al, 1998).

Mathur and Kumar (1998) traced out possibilities increasing medicinal value with creating short sexual life cycle of tissue culture.
In Centella, shoots regenerated from the stem and leaf callus were rooted within 11 days in 1/2 strength MS basal salts supplemented with 0.5 mg/l indole-3-acetic acid and 2% (w/v) sucrose. About 85% of rooted plantlets were acclimatized and transferred to the greenhouse. (Patra et al, 1998). Tiwari et al, 2000, tried rooting on different media in Bacopa, i.e. MS media with or without hormones and found that rooting was highest (90%) on full-strength MS medium containing 2.46 uM IBA.

Daroker et al. (1999) has reported antibacterial activity of rose petals. They found that rose petals of several species of rose plants possess antibacterial activity.

The first two approaches lead to plantlets formation via organogenesis through production of unipolar shoots, which must then be further multiplied, followed by rooting in a multistage process. In contrast, somatic embryogenesis leads to the formation of a bipolar embryo through steps that are often similar to zygotic embryogenesis. Shoot multiplication is widely used for the clonal propagation using the above approaches; it has been possible to produce plantlets of over 70 angiosperms and 30 gymnosperms. Tissue culture techniques have been applied for the propagation of approximately 20% of 7000 known Ayurvedic plant species (Rajendra and D'Souza, 1999).

Surface sterilization is most important step before inoculation of explant. Different steps have been employed for treatment of explant. Shrivastava and Rajani, 1999, have described sterilization treatment of Bacopa, which includes use of 0.1% Mercuric chloride (w/v) for 2 minutes followed by
rinsing thoroughly with sterile distilled water. Different sterilization treatment was followed by Mathur and Kumar, 1998, in which leaves and stem explants were shaken for 10 minutes in Tween 20 (Ranbaxy) and Savlon (Johnson & Johnson in water for 10 minutes, rinsed in running water for 30 minutes, treated with 0.1% Mercuric for 3-4 minutes and washed several times with sterile water.

Multiple shoots were obtained on MS medium supplemented with auxins or/and cytokinins with or without coconut milk. Maximum number of plants was obtained on medium containing Kin/2-ip0 (0.1 mg/l) and Kin (1 mg/l) in shoot tip and nodal cultures respectively. (Tejavathi et al, 2001). Banerjee et al, 1999 reported in Centella that initial sprouting required the presence of BAP (2gm/l) and IBA (0.1 gm/l), however for induction of multiple shoots higher concentration of BAP (3 gm/l) and lower concentration of NAA (0.05 mg/l) is required.

In medicinal plants rooting of microshoots have been obtained in MS medium with IAA, IBA, NAA used singly or in combinations or when transferred to hormone free medium. Root induction in B. monnieri was observed in Murashige and Skoog medium supplemented with 0.5 mg BAP (benzyladenine)/litre within 6 days of culture. In the case of P. foetida and C. asiatica root induction was observed within 12 and 21 days of culture when single shoots of both the plant species were cultured on MS media supplemented with 0.25 mg BAP+0.5 mg IBA and 0.5 mg BAP+1.5 mg NAA/litre, respectively (Singh et al, 1999).

Reseva (1999) cultured mint on LS media supplemented with various growth regulators and natural mint plant extract (NMPE). Addition of 0.5
mg/q BAP and NMPE to the medium gave high multiplication rates at 4 week period. On LS medium in the presence of IAA (1 mg/l) and NMPE, single shoots with a root system were formed in the 4 week period. The plantlets obtained were suitable for planting out.

Rajani, M et al. (2000) has focused on the chemical properties and pharmacological activities of major chemical constituents of B. monnieri. A brief overview of its taxonomy and morphology, biotechnological approaches for the micropropagation and in vitro production of secondary metabolites of this plant are also discussed.


David Wheatley et al (2000) A number of medicinal plants are traditionally endowed with anxiolytic or sedative properties and, in the context of this review, both indications are considered since the former may induce a mood conducive to the latter. For any sleep-inducing drug to be effective, a tranquil ambience needs to be established a priori. Thus, physical ailments (i.e. pain), factors interfering with sleep (i.e. noise), psychological conditions causing stress, psychiatric illnesses (i.e. depression) and other drugs that interfere with sleep (i.e. caffeine) need to be controlled, if possible. Kava-kava is a well-established hypnotic drug, with a rapid onset of effect, adequate duration of action and minimal morning after-effects. However, reports of serious hepatotoxicity with this preparation have led to it being banned in most countries worldwide. On the other hand, side-effects with
valerian would appear to be bland indeed. However, it's slow onset of effect (2-3 weeks) renders it unsuitable for short-term use (i.e. 'jet-lag'), but it does have profound beneficial effects on sleep architecture (augments deep sleep) that may make it particularly suitable for long-term use and for the elderly. In a personal trial (not double-blind) in stress-induced insomnia, both kava and valerian improved sleep and the ill-effects of stress, and the combination of the two was even more effective for the control of insomnia. Aromatherapy (lavender, chamomile, Ylang-Ylang) would appear to improve sleep, but how practical a form of treatment this may be remains to be determined. The only other plant drug that may have some effect on sleep is melissa, but reports are too scanty to form any opinion about this. Based on animal experiments, passion flower (Passiflora) may have a sedative action, but the sedative action of hops has not been investigated in any detail. In conclusion, there is a need for longer-term controlled studies with some of these compounds (particularly valerian). Aromatherapy constitutes a tantalising possibility. In the interpretation of this review, it should be borne in mind that the evidence on which it is based is often incomplete or missing, but that is all that is available. Consequently some conjecture on the part of the author is inevitable and should be appreciated as such.

Tiwari et al, 2000, suggested that for micropropagation of Centella asiatica, plants were washed thoroughly for 30 mins under running tap water followed by removal of leaves, which was followed by soaking in the mixture of 1% cetrimide solution containing 150 mg/l Bavistin and 50 gm/l Trimethoprim for 25-30 mins. The explants were finally treated with 0.1% mercuric chloride for 3-4 mins followed by rinsing in sterile distilled water for 4-5 times.
Tiwari et al, 2000, proposed an efficient and rapid method using liquid shake cultures for in vitro propagation of *Bacopa*, a medicinally important herb. This was achieved by culturing nodal explants on liquid MS medium with or without 6-benzyladenine. Compared to single axillary shoot proliferation on a growth regulator free agarified medium, the respective liquid medium induced 4 or 5 shoots per nodal explant 4 weeks after culture. Addition of 6-benzyladenine (0.01-0.01 mg/l) resulted in the increase in morphogenetic response (number of shoots, mean shoot length and number of roots per node explant) in both the type of culture media.

Al-douri N.A.(2000) has reported an ethnobotanical survey which was carried out in different regions of Iraq following interviews with various traditional healers, who are called Attars, and various other people. Ninety-seven medicinal plants belonging to forty-three families are listed in alphabetical order giving the parts used and method of preparation according to their therapeutic use.

Tiwari et al, 2001, reported use of range of cytokinins for multiple shoot induction for *Bacopa*, with node, internode and leaf explants. Of the four cytokinins (6-benzyladenine, thidiazuron, kinetin and 2-isopentenyladenine) tested thidiazuron (6.8 um) and 6-benzyladenine (8.9 uM) proved superior to other treatments. Optimum adventitious shoot buds induction occurred at 6.8 rM thidiazuron where an average of 93 shoot buds were produced in leaf explants after 7 weeks of incubation.

Mahasneh (2001) has reported antimicrobial activity against the 4 bacteria and 2 fungal species through aqueous, ethanolic and butanolic extract of
aerial part of 10 plant species of their studied area. He also compared hot water extract, ethanol extract and butanolic extract for their antimicrobial activity. He conducted that butanolic extract is more potential carrier for antimicrobial substances.

Stough C. et al (2001) has reported extracts of *Bacopa monniera* have been reported to exert cognitive enhancing effects in animals. However, the effects on human cognition are inconclusive.

**OBJECTIVE:** The current study examined the chronic effects of an extract of *B. monniera* (Keenmind) on cognitive function in healthy human subjects.

**METHODS:** The study was a double-blind placebo-controlled independent-group design in which subjects were randomly allocated to one of two treatment conditions, *B. monniera* (300 mg) or placebo. Neuropsychological testing was conducted pre-(baseline) and at 5 and 12 weeks post drug administration.

**RESULTS:** *B. monniera* significantly improved speed of visual information processing measured by the IT task, learning rate and memory consolidation measured by the AVLT (P<0.05), and state anxiety (P<0.001) compared to placebo, with maximal effects evident after 12 weeks.

**CONCLUSIONS:** These findings suggest that *B. monniera* may improve higher order cognitive processes that are critically dependent on the input of information from our environment such as learning and memory.
Kaushik et al. (2002) has studied the medicinal values of traditional plant Neem, they analyzed the anti-inflammatory, immunostimulant, antiulceral, anti-fungal, anti bacterial, anti-carcinogenic, Hepatoprotective, anti-oxidant activities and effect on CNS.

Janovska et al. (2003) has reported anti-viral activities against Herpesviruses (HSV-1 & HSV-2) and Adenoviruses (ADV-3, ADV, & ADV-11) through flavonoids and aqueous extract of Caeselpinia pulcherrima plant. They found Caselpina pulcherrima, which possess anti-viral activities, may be derived from the flavonoids of quercetin.

Wali and Siddiqui (2003) developed a rapid and efficient protocol for the induction of multiple shoots from leaf and stem explants that were cultured in MS medium containing different concentrations of BAP or Kinetin, either alone or in combination with NAA or IAA.

Russo and F. Borrelli (2003) Bacopa monniera (BM), a traditional Ayurvedic medicine, used for centuries as a memory enhancing, anti-inflammatory, analgesic, antipyretic, sedative and antiepileptic agent. The plant, plant extract and isolated bacosides (the major active principles) have been extensively investigated in several laboratories for their neuropharmacological effects and a number of reports are available confirming their nootropic action. In addition, researchers have evaluated the anti-inflammatory, cardiotonic and other pharmacological effects of BM preparations/extracts. Therefore, in view of the important activities performed by this plant, investigation must be continued in the recently observed actions described in this paper. Moreover, other clinical studies
have to be encouraged, also to evidence any side effects and possible interactions between this herbal medicine and synthetic drugs.

Rani et al, 2003 reported that callus induction in Withania somnifera (L). Dunal was observed from hypocotyls, root, and cotyledonary leaf segments, grown on Murashige and Skoog (MS) medium supplemented with various concentrations and combinations of 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin (Kn). Maximum callusing (100%) was obtained from root and cotyledonary leaf segments grown on MS medium supplemented with a combination of 2 mg/l (9.1 uM) 2,4-D and 0.2 mg/l (0.9 uM) Kn. When hypocotyls segments were used as explants, callus induction was noticed in 91% of cultures, which showed regeneration on MS medium supplemented with 2 mg/l, 2,4-D and 0.2 mg/l Kn.

Phulan Rani et al. (2004) has reported antimicrobial activity against multidrug resistant Salmonella typhi through aqueous and methanolic extract of 54 Ayurvedic plants species of their studied area.

Root induction has also been reported in Phyllanthus amarus (Ghanti et al, 2004) using MS medium supplemented with a concentration of 0.5 gm/l of IBA. Rooted shoots were hardened on basal liquid medium and subsequently in sterile soil+vermiculite (1:1). In case of Withania somnifera (L) Dunal, shoots were rooted best (87%) on MS medium containing 2 mg l⁻¹ (9.9 uM) indole-3-butyric acid (IBA). The plantlets were transferred to the field after acclimatization and showed 60% survival. (Rani et al, 2003).
An efficient protocol has been established for rapid multiplication of *M. arvensis* axillary bud using Murashige and Skoog (MS) medium. Media prepared with tap water and commercial sugar and those prepared with double distilled water and tissue culture grade sucrose did not show significant difference in vitro induction of shoot/node and root/shoot. MS medium fortified with 4.44 M N6 – benzyladene (BA) and 2.32 Kinetin was optimum for proliferation of shoots (Sunada Kumari et al 2004)

M. Deepak et al (2004) has reported Bacoside A, the putative bioactive component of the Indian medicinal plant Bacopa monnieri, was found to be a mixture of saponins with bacoside A3 (1), bacopaside II (2), jujubogenin isomer of bacopasaponin C (3) and bacopasaponin C (4) as major constituents. An HPLC method together with an optimised extraction procedure was developed for the estimation of 1-4 in B. monnieri to enable standardisation of the latter. Concentration ranges of the analytes in samples of B. monnieri collected from different regions of India were 0.14-0.85% (w/w) (1), 0.12-0.69% (2), 0.05-0.72% (3) and 0.05-0.44% (4). The importance of using bacoside A, with known concentrations of 1-4, as a reference standard for the routine analysis of B. monnieri is highlighted. Two common flavonoids, luteolin and apigenin, were present in all samples of B. monnieri. Copyright © 2005 John Wiley & Sons, Ltd.

Kishore K. et al (2005) has reported to investigate the effect of bacosides (alcoholic extract of brahmi) on scopolamine (3 mg kg(-1), ip), sodium nitrite (75 mg kg(-1), ip) and BN52021 (15 mg kg(-1), ip) induced experimental amnesia in mice, using Morris water maze test, all the agents were administered 30 min before the acquisition trials on each day and repeated for 4 consecutive days, and on 5th day during the retrieval trials.
Bacosides on anterograde administration (before training) in mice, significantly decreased the escape latency time (ELT) during the acquisition trials for 4 consecutive days and increased the time spent (TS) in target quadrant during the retrieval trials on 5th day, and on retrograde administration (after training) bacosides were found not to affect TS significantly. Bacosides also significantly decreased the ELT and increased the TS in mice treated anterogradely with scopolamine and sodium nitrite. Bacosides did not exhibit any significant effect on TS of mice treated retrogradely with sodium nitrite. On the other hand, bacosides significantly increased the TS of mice treated retrogradely with BN52021. On the basis of the present results it can be concluded that bacosides facilitate anterograde memory and attenuate anterograde experimental amnesia induced by scopolamine and sodium nitrite possibly by improving acetylcholine level and hypoxic conditions, respectively. Beside this bacosides also reversed BN52021 induced retrograde amnesia, probably due to increase in platelet activating factor (PAF) synthesis by enhancing cerebral glutamate level.

Russo A. et al (2005) has reported Bacopa monniera (BM), a traditional Ayurvedic medicine, used for centuries as a memory enhancing, anti-inflammatory, analgesic, antipyretic, sedative and antiepileptic agent. The plant, plant extract and isolated bacosides (the major active principles) have been extensively investigated in several laboratories for their neuropharmacological effects and a number of reports are available confirming their nootropic action. In addition, researchers have evaluated the anti-inflammatory, cardiotonic and other pharmacological effects of BM preparations/extracts. Therefore, in view of the important activities performed by this plant, investigation must be continued in the recently
observed actions described in this paper. Moreover, other clinical studies have to be encouraged, also to evidence any side effects and possible interactions between this herbal medicine and synthetic drugs.

S.William decruse et al. (2005) gave an efficient protocol on single nodes from young top shoot of Decalepsis arayalpathra were cultured on Murashige and Skoog’s (MS) agar medium supplemented with 0.1 to 5.0 mg/Lt. BAP. All the concentrations of BAP induced single axillary shoot of carrying length. However, MS medium supplemented with 1.0mg/Lt. BAP support rapid growth and produced the longest shoots (6.8cm.) in 60 days. For further multiplication, the nodes and shoot tips from in vitro derived shoots were recultured on MS medium fortified with 0.5 mg/Lt. BAP, which produced 8 cm long shoots having 5-7 nodes in 30 days period. Further, the top microshoot cuttings (3-5cm long) with 2-3 nodes, subcultured on average of the MS medium supplemented with 1.5mg/Lt. IAA, produced on average of 6.3 roots in 30 days period. The rooted plants after hardening were reintroduced into their natural habit at kallar reserve forest, Thiruvananthapuram, After two year 89% survival of reintroduced plants was recorded.

Amanda Kumar (2005) has done a meristem specific promoter of the S-phase specific gene (cyco7) from sorghum bicolor by polymerase chain reaction (PCR) using a proof reading DNA polymerase.

Sangeeta Nath et al (2005) were overcome of exudation during shoot tip (1 cm) culture of Adhatoda vasica by modifying the pH of the media and by lowering the concentrations of ammonium ion and potassium ions.
Though shoot formation occurred in media supplemented with BA (2 mg/lt.) 96.67% of the shoots developed callus at the cut basal end and the explants turned brown and necrotic due to the phenolic exudates released into the medium. Development of callus tissue and browning was eliminated by culturing the shoot tip explants in primary MS medium supplemented with thiadiazuron (0.30mg/lt.) and coconut milk (15%) which also promoted development of shoots but with stunted curried leaves. Shoots (5.2) with healthy leaves were obtained when these shoots were transferred to a secondary MS medium supplemented with 0.5 mg/lt. BA. Incorporation of coconut milk (15%) in the secondary MS medium had a growth promotory effect. High frequency rooting (9.33) was recorded in MS basal medium. The rooted plantlets were hardened and established at 85% rate in pots.

Farrukh Aquil et.al. (2005), has reported antimicrobial activity against Antimethicillin resistant Staphyloccus aureus (MRSA) through 4 different plant species of their studied area.

Nair et.al., (2005) have reported antimicrobial activity against the 5 microorganism's species through 9 different endemic plant species of their studied area. They also reported comparison of hot water extract and methanolic extract for their antimicrobial activity. They conclulded that methanolic extract is more potential carreer for antimicrobial substances.

Rios et.al., (2005) has reported the past, present and future of medicinal plants both as potentional antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents. Sonia peria leite et.al. (2006) has reported the antimicrobial activity against 5 different...
species of human pathogenic bacteria and 17 fungal strains through the various organic and aqueous extracts of leaves of Indigofera suffruticosa. They suggested that aqueous extract of leaves of Indigofera suffruticosa obtained by infusion and can be used in the treatment of skin diseases caused by dermatophytes.

J Alzheimers (2006) has reported PSAPP mice expressing the "Swedish" amyloid precursor protein and M146L presenilin-1 mutations are a well-characterized model for spontaneous amyloid plaque formation. Bacopa monniera has a long history of use in India as an anti-aging and memory-enhancing ethnobotanical therapy. To evaluate the effect of Bacopa monniera extract (BME) on amyloid (Abeta) pathology in PSAPP mice, two doses of BME (40 or 160 mg/kg/day) were administered starting at 2 months of age for either 2 or 8 months. Our present data suggests that BME lowers Abeta 1-40 and 1-42 levels in cortex by as much as 60%, and reverses Y-maze performance and open field hyperlocomotion behavioral changes present in PSAPP mice. The areas encompassed by Congo Red-positive fibrillar amyloid deposits, however, were not altered by BME treatment. The data suggest that BME has potential application in Alzheimer's disease therapeutics.

Jyoti A. et al (2006) has reported Bacopa monniera is a nerve tonic used extensively in traditional Indian medicinal system "Ayurveda". Reports regarding its various antioxidative, adaptogenic and memory enhancing roles have already appeared in the last few decades. In the present study, aluminium chloride (AlCl$_3$) was used to generate neurotoxicity. We have investigated the neuroprotective effect of Bacopa extract against aluminium-induced changes in peroxidative products, such as thio-
barbituric acid-reactive substance (TBA-RS) and protein carbonyl contents and superoxide dismutase (SOD) activity. Effect on lipofuscin (age pigments) accumulation and ultrastructural changes were also studied. Bacopa effects were compared with those of l-deprenyl. Co-administration of Bacopa extract during aluminium treatment significantly prevented the aluminium-induced decrease in SOD activity as well as the increased oxidative damage to lipids and proteins. Protective effect was also observed at microscopic level. Fluorescence and electron microscopic studies revealed considerable inhibition of intraneuronal lipofuscin accumulation and necrotic alteration in the CA1 region of the hippocampus. Observations showed that Bacopa's neuroprotective effects were comparable to those of l-deprenyl at both biochemical and microscopic levels.

Channa S. et al (2006) has reported the ethanol extract of Bacopa monniera (Scrophulariaceae) exhibited marked anti-inflammatory activity against carrageenan-induced paw edema in mice and rats, an acute inflammatory model. To assess the possible mechanism of anti-inflammatory action against carrageenan, the ethanol extract was treated with chemical mediators (histamine, serotonin, bradykinin, prostaglandin E(2) and arachidonic acid)-induced edema in rats. The extract selectively inhibited prostaglandin E(2)-induced inflammation. Thus, it may be inferred that B. monniera possesses significant anti-inflammatory activity that may well be relevant for its effectiveness in the healing of various inflammatory conditions in traditional medicine.

Vikas Kumar (2006) has reported- although very few drugs are currently approved by regulatory authorities for treating multi-factorial ailments and
disorders of cognition such as Alzheimer's disease, certain plant-derived agents, including, for example, galantamine and rivastigmine (a semi-synthetic derivative of physostigmine) are finding an application in modern medicine. However, in Ayurveda, the Indian traditional system of medicine which is more than 5000 years old, selected plants have long been classified as ‘medhya rasayanas’, from the Sanskrit words ‘medhya’, meaning intellect or cognition, and ‘rasayana’, meaning ‘rejuvenation’. These plants are used both in herbal and conventional medicine and offer benefits that pharmaceutical drugs lack. In the present article, an attempt has been made to review the most important medicinal plants, including Ginkgo biloba, St John's wort, Kava-kava, Valerian, Bacopa monniera and Convolvulus pluricaulis, which are widely used for their reputed effectiveness in CNS disorders.

K. kalimuthu et al. (2006) have developed a high frequency, simple & rapid regeneration protocol from shoot tip and nodal explants of Vanilla plantifolia on Murashige and Skoogs medium supplemented with 6-benzylamino purine & coconut water.

Reddy et al (2006) gave an efficient protocol for micro propagation of Azadirachta indica A Juss. A medicinally important plant, has been standardized. Cotyledonary nodes (1 cm long) excised from 15-20 days old in vitro germinated seedling were used as explants. The seeds were germinated on half strength MS medium devoid of phyto hormones. Cotyledonary nodes were cultured on MS medium supplemented with different concentration of cytokinins (BA/TDZ/2-ip) and auxins (2, 4-d and NAA). Maximum shoot proliferation from single explants was obtained on MS media incorporated with TDZ (0.0015mm) 2,4-D(0.0005mm) adenine.
sulphate (40mg/l.), glutamine(100mg/l.) and thiamine HCL (10mg / lt.). In vitro produced shoots were induced roots on half strength MS medium supplemented with a range of IBA concentration(0.0005-0.005m). However, the highest frequency of root proliferation was observed on ½ MS medium supplemented with 0.002m IBA. The regenerates were transferred to field condition after acclimatization with a success rate of 80%.

Saleh Al-Qura'n, et al (2007) There are 87 species belonging to 59 genera and 33 plant families that were identified and presented in the area of study. The three largest families are: Labiatae (nine aquatic species), Compositae (seven species), and Salicaceae (seven species). The largest genera are Mentha (six species), Polygonum (five species), and Salix (five species). There are sixty-three folk medicinal aquatic species (73.3%) that are reputed to have similar therapeutic uses in neighbouring countries, while the 24 remaining species (26.7%) do not. Emerged species (living in close contact with water body) were the most frequently recorded, while amphibious, submerged or floating species were the least. The folk medicinal important value of aquatic species recorded was determined according to Friedman method. ROP values were higher than 50 in 21 species (24%), and therefore have the highest popularity in folk medicine. Less than three informants reported the 26 species (29.9%) that have therapeutic effects, and therefore, were excluded from further consideration. ROP values were less than 50 in 40 species (46.1%), and were considered nonpopular medicinal plants.

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Michael Adams et al (2007) has reported in traditional herbal medicine, numerous plants have been used to treat age-related cognitive disorders. In this review, we compiled available literature from four Swiss university libraries, scientific journals, and online database query's on plants and remedies used in traditional medicinal systems for such diseases. Over 150 plant species in various preparations and mixtures were found. European herbals from the 16th and 17th century alongside traditional Chinese and Indian medicinal works were the most prolific sources. The information is organised into geographic regions and when available the findings are discussed in the light of more recent scientific findings concerning their secondary metabolites and in vitro and in vivo activities relevant to dementia and Alzheimer's disease.

Pravina K. (2007) et al has reported BacoMind trade mark is an enriched phytochemical composition of Bacopa monniera (B. monniera), a common medicinal plant used in the traditional systems of medicine as a memory-enhancing agent. BacoMind trade mark was standardized with reference to bioactive compounds and was evaluated for short-term safety and tolerability in healthy adult volunteers. The study plan employed randomized, open label, dose escalation design. Each of 23 participants were orally given one single capsule of BacoMind trade mark daily for 30 days, i.e., 300mg for first 15 days and 450mg for next 15 days. Detailed examination of clinical, hematological, biochemical, and electrocardiographic parameters done in pre and post-treatment periods did not indicate any untoward effects in any of the treated volunteers. Mild adverse events related to gastrointestinal system were observed in the trial, which subsided spontaneously. BacoMind trade mark was found to
meet the safety criteria at the dose administered for the given duration of trial period in healthy adult volunteers.

Jyoti A. et al (2007) has reported Bacopa monniera is a perennial herb, and is used as a nerve tonic in ayurveda, a traditional medicinal system in India. Aluminium-induced neurotoxicity is well known and different salts of aluminium have been reported to accelerate oxidative damage to biomolecules like lipids, proteins and nucleic acids. The objective of the present study was to investigate whether Bacopa monniera could potentially inhibit aluminium toxicity in the cerebral cortex. Male Wister rats (8 months old) were administered with AlCl(3) orally at a dose of 50mg/kg/day in drinking water for 1 month. Experimental rats were given AlCl(3) along with Bacopa monniera extract at a dose of 40mg/kg/day. One group of rats was treated with l-deprenyl at a dose of 1mg/kg/day along with AlCl(3) treatment. We have observed that Bacopa monniera prevented accumulation of lipid and protein damage significantly, which resulted from aluminium intake. Decline in the activity of endogenous antioxidant enzymes associated with aluminium administration was also inhibited by Bacopa monniera extract. The potential of Bacopa monniera to inhibit Al-induced oxidative stress was observed to be similar to that of l-deprenyl, which was taken as standard. The potential of Bacopa monniera extract to prevent aluminium neurotoxicity was reflected at the microscopic level as well, indicative of its neuroprotective effects. These findings strongly implicate that Bacopa monniera has potential to protect brain from oxidative damage resulting from aluminium toxicity.

Shahin Sharif A. et al (2007) Currently there has been an increased interest globally to identify antioxidant compounds that are
pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity. Traditional herbal medicines form an important part of the healthcare system of India. Ayurveda, supposed to be the oldest medical system in the world, provides potential leads to find active and therapeutically useful compounds from plants. Considering the growing interest in assessing the antioxidant capacity of herbal medicine in this review we discuss about rarely reviewed 24 plants reported to have antioxidant properties. Some of the plants reviewed are part of multi-herbal preparations while others are used singly. Certain herbs like Amaranthus paniculatus, Aerva lanata, Coccinia indica and Coriandrum sativum are used as vegetables indicating that these plants could be source of dietary antioxidant supplies, which is another emerging area of research.

Birader et.al. (2007) has reported the antimicrobial activity against the different species of microorganism through the aqueous and alcoholic extraction of medicinal plant Triphala & Triphala mashi. They compared the phytochemical screening. In conclusion, it appears that Triphala mashi has non-specific antimicrobial activity.

Chi P. Ndi et.al. (2007) has reported antimicrobial activity against some Gram-positive, Gram-negative bacteria and yeasts through methanolic extract of 72 Eremophilla species of their studied area Australia.

Ushimaru et.al., (2007) reported the evaluation of the in-vitro antimicrobial activity of methanolic extracts of Allium sativum, Carophyllus aromaticus,
Zingiber officinale, Psidium gajava, Cymbopogon citrates and Mikania glomerata against E. Coli, Salmonella typhemurium, staphylococcus aureus. He found the highest anti Staphylococcus activity in Caryophyllus aromaticus plant extract and it was also effective against all bacterial strains tested.

Vukovic et.al. (2007) has reported antimicrobial activity against 13 bacterial and 3 fungal species through the essential oil and methonolic extract of Teucrium montanum plant. They concluded that the essential oil has antibacterial as well as antifungal effect.

Yujie Fu et al., (2007) has reported antimicrobial activity against 3 Gram-positive bacteria, 3 Gram-Negative bacteria and 2 fungi through essential oils of Clove and Rosemary plants. They also presented comparative study on the antimicrobial activity of essential oils of alone and in combination. They concluded that the combination of the essential oils is more effective against the microbes.

Mothana et.al., (2008) has reported antimicrobial activity against 3 Gram-positive bacteria, 2 Gram negative bacteria, one yeast spp. and 3 multi resistant staphylocous strains through 16 different endemic plant species of their studied area (Yemini). They also reported that the methanolic extract is more potential career for antimicrobial substances compared than hot water extract. transformation and analyzed for tissue specific expression. Incubation of kanamycin resistant tobacco shoots with GUS substrate resulted in intense blue coloration in the shoot tips root tips and root proving that the promoter sequences were enough to induce meristem specific activity of the reporter gene for sequential deletions from the 5 end
were carried out. The deletion analysis indicated that a 13 bp repeat first sequence was essential for regulation of meristem specific expression of the reporter gene.

Ya-Ling Hsu et al (2009) This study is the first to investigate the anticancer effect of dehydrocostuslactone ((3aS,6aR,9aR,9bS)-Decahydro-3,6,9-tris(methylene) azuleno[4,5-b]furan-2(3H)-one; DHE), a medicinal plant-derived sesquiterpene lactone, on hepatocellular carcinoma. Our results showed that DHE inhibits the proliferation of Hep G2 and PLC/PRF/5 cells by inducing apoptosis. DHE induces upregulation of Bax and Bak, downregulation of Bcl-2 and Bcl-XL, and nuclear relocation of the mitochondrial factors apoptosis-inducing factor (AIF) and endonuclease G (Endo G). DHE triggered endoplasmic reticulum (ER) stress, as indicated by changes in cytosol-calcium levels, PKR-like ER kinase (PERK) phosphorylation, inositol requiring protein 1 (IRE1) and CHOP/GADD153 upregulation, X-box transcription factor-1 (XBP-1) mRNA splicing, and caspase-4 activation. Enhancement of ER stress by DHE is through p38 and extracellular signal-regulated kinase1/2 (ERK1/2)-dependent manners and subsequently causes c-Jun N-terminal kinase activation, resulting in AIF and Endo G nuclear relocation. Both of IRE1 siRNA transfection and BAPTA-AM (1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis (acetoxy)methyl ester)) pretreatment inhibit DHE-mediated apoptosis, supporting the hypothesis that DHE induces cell death through ER stress. Importantly, animal studies have revealed a dramatic 50% reduction in tumor volume after 45 days of treatment. This study demonstrates that DHE may be a novel anticancer agent for the treatment of liver cancer.