Medicinal and Aromatic Plant Science and Biotechnology ©2008 Global Science Books

Coleus spp.: Micropropagation and *In Vitro* Production of Secondary Metabolites

Avinash Nagpal[1*] • Balwinder Singh[2] • Sanjeev Sharma[3] • Gita Rani[4] • Gurdeep Singh Virk[1]

[1] Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143 005, Punjab, India
[2] Department of Biotechnology, Khalsa College, Amritsar, India
[3] Department of Botany, Lyallpur Khalsa College, Jalandhar, India
[4] Department of Chemistry, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India

Corresponding author: * **avnagpal@rediffmail.com**

ABSTRACT

Coleus spp. have long been prized for their colorful foliage, which may combine shades of green, yellow, pink, red and maroon. They are plants for all locations. Besides their ornamental nature, *Coleus* spp. are also valued in folk medicine. *C. blumei* Benth., a natural hybrid of several *Coleus* spp. is an ornamental plant growing all over the world in an enormous number of different cultivars that vary in color and shape of the leaves, and is also known for its medicinal properties, in particular rosmarinic acid, one of its most important secondary compounds. Rosmarinic acid has been shown to exhibit antioxidative, anti-inflammatory and antiallergic activities in mammalian systems. *C. forskohlii* Briq. is a medicinally important plant valued for the production of forskolin, a diterpenoid, present in root tubers of the plant and known to lower blood pressure and intraocular pressure. *C. parviflorus* Benth. (country potato) has the potential of developing into a crop similar to potato. An ample amount of work has been reported on micropropagation of different *Coleus* spp. from different explants like shoot tips, leaf segments, nodal and internodal segments, etc. Furthermore, large-scale production of rosmarinic acid and forskolin from cell cultures of *C. blumei* and *C. forskohlii*, respectively, has also been reported. Genetic transformation using *Agrobacterium* has been reported to enhance rosmarinic acid and forskolin production in transformed cultures of *C. blumei* and *C. forskohlii*.

Keywords: *C. blumei*, *C. forskohlii*, forskolin, RA, rosmarinic acid, tissue culture

CONTENTS

INTRODUCTION

The word 'Coleus' brings to mind mental images of colorful foliage and is derived from the Greek word "Keleos" meaning sheath referring to a typical characteristic of coleus plants where the filaments fuse at the bottom to form a sheath around the style (de Loureiro 1790; Petersen 1994). The Genus *Coleus* belongs to the family, *Lamiaceace* (mint family) and is a close relative of peppermint, spearmint, salvia, basil, thyme, oregano and Swedish ivy. *Coleus* species are native to tropical and subtropical regions of Asia, Africa, Australia, East Indies, Malay-Archipelago and the Philippines (Lebowitz 1985; Petersen 1994; Vasishta 1994). Some species, especially those with colorful foliage (**Fig. 1**), are grown as ornamentals all over the world. Most *Coleus* species grow as herbaceous perennials while others may be subshrubs or lowshrubs. Plants of the genus *Coleus* vary in size from smaller types that will reach only 30 cm height to tall bushy types reaching 90 cm. The stems of all these plants are square in cross section. The leaves are opposite and 1-4 inches long, toothed, stalked or stalkless and variegated. The flowers are asymmetrical, bracteate, complete, zygomorphic, mostly blue or lilac-blue and are borne on terminal spiked stalks. Calyx – five, gamosepalous and corolla – five, gamopetalous, tubular and two-lipped. The upper lip is often two-lobed while the lower one is three-lobed and is longer. The lower lip also contains two pairs of deflexed stamens and a style while the upper lip forms a more or less attracting mechanism for insects. Stamens – four with filaments fused at their bottom to form a tube en-

Received: 5 December, 2007. Accepted: 26 April, 2008.

Invited Review

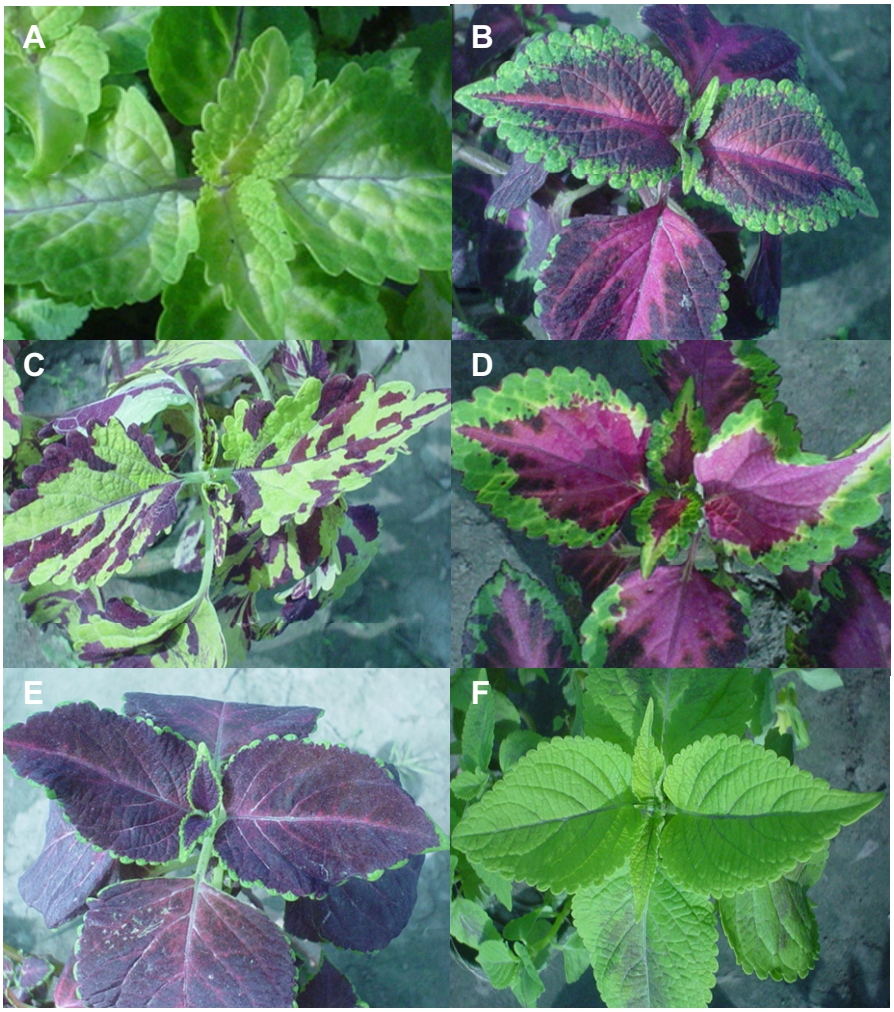

Fig. 1 *Coleus* species with colorful foliage. (**A-E**) Different cultivars of *C. blumei* – An erect branched undershrub with quadrangular stems, leaves variegated, flowering May–August. (**F**) *C. forskohlii* – An upright branched perennial herb with fleshy fibrous roots, quadrangular stem, leaves narrowed into petioles, flowers in terminal, spike-like racemes.

circling the style. The anthers – monothecous and joined. Gynoecium – bicarpellary syncarpous, the only style has two stigmas and the fruits are four seedlike nutlets (Everett 1981; Petersen 1994; Vasishta 1994).

Economic importance of *Coleus* species

Economically, the members of the genus *Coleus* are of great importance acting as source of medicines, providing food and as ornamentals (Schultze 1986; Engprasert *et al.* 2004). As recorded in ancient Sanskrit texts, different species of *Coleus* have been used for the treatment of a variety of diseases including heart diseases, abdominal colic, respiratory disorders, painful micturitions, insomnia, convulsions, skin problems, worms, etc. (Dube *et al.* 1981; Ammon and Muller 1985; de Souza and Shah 1988). **Tables 1** and **2** enlist medicinal properties and various bioactivities of different species of *Coleus* respectively. Some species e.g. *C tuberosus* and *C. forskohlii* produce tubers that are eaten in the same way as potatoes (http://en.wikipedia.org/wiki/Coleus). The leaves of *C. forskohlii* and *C. ambonicus* have been used as condiment for a long time (Schultze 1986). The use of *Coleus* as an ornamental herbaceous plant dates back to well before the Victorian era. *Coleus* species are prized, both for indoors and outdoors, for their colorful foliage which may combine shades of green, yellow, pink, red and maroon. The plants are more commonly grown as pot plants but are also a great favourite for the window base garden and a few dwarf types are suitable for carpet bedding. A few less known species are grown for their handsome flowers.

In this review, work on micropropagation of different *Coleus* species and *in vitro* culture of *C. forskohlii* for production of forskolin and *C. blumei* for production of rosmarinic acid is reviewed.

MICROPROPAGATION OF *COLEUS* SPECIES

Micropropagation is one of the key tools of plant biotechnology that explains the totipotent nature of plant cells. The concept of totipotency was proposed by Haberlandt (1902) and was unequivocally demonstrated for the first time by Steward (1958). Micropropagation generally involves four distinct stages, each with a specific set of requirements. These include (i) initiation of aseptic cultures, (ii) shoot multiplication, (iii) rooting of *in vitro* raised shoots, and (iv) hardening and field transfer of *in vitro* raised plants. Significant features of this technology are its enormous multiplication capacity in a relatively short time-span (useful for conservation strategies); production of healthy and disease-free plants and its ability to generate propagules all-year round (Martin 1985; Dhawan and Bhojwani 1986). Only a few publications that deal with microprogation of different *Coleus* species can be found in the literature (Sharma *et al.* 1991; Anbazhagan *et al.* 2005; Rajasekharan *et al.* 2005; Rani *et al.* 2006; Srinivasan *et al.* 2006). **Table 3** summarizes the literature on the microprogation of different *Coleus* species.

Hervey and Robbins (1978) reported the development of plants from leaf discs of variegated *C. blumei* and its relation to patterns of leaf chlorosis. Comer and Leonardo (1981) used a simple tissue culture protocol to examine phloem differentiation in pith explants of *C. blumei*. They observed the differentiation of sieve tube elements in pith parenchyma when pith blocks of *C. blumei* were used as explants and placed onto agar medium containing sucrose and indole-3-acetic acid (IAA). The concentration of growth regulator had little influence on the differentiation process, while increasing the availability of sucrose resulted in an enhancement of phloem differentiation. The authors observed that phloem and xylem differentiation began at the

Table 1 Medicinal properties of different *Coleus* species.

Name of species	Medicinal properties	Reference
Coleus aromaticus	Seasoning meat dishes, in food products	Uphof 1959
C. amboinicus	Skin problems and against worms	de Souza and Shah 1988
C. aromaticus	Chronic cough, asthma	CSIR 1992
C. amboinicus	Treatment of burns and as a poultice for centipedes and scorpion bites	Morton 1992
C. amboinicus	Skin allergies	Harsha *et al*. 2003
C. aromaticus	Antispasmodic, stimulant, stomachic, headache, fever, epilepsy, dyspepsia	Khory and Katrak 1999; Morton 1992
C. aromaticus	Reproductive problems	Lans 2007
C. barbatus	Decreases gastric secretion in rats	Fischman *et al*. 1991
C. esculentus	Anthelmintic	Kokwaro 1993; Burkill 1995; Allemann *et al*. 2004
C. forskohlii	Pneumonia	Bouquet 1969
C. forskohlii	Heart diseases, abdominal, colic and respiratory disorders, insomnia, convulsions	Sharma 1956; Chuneker 1960
C. forskohlii	Hypotensive agent with spasmolytic, cardiotonic and platelet aggregation inhibitory activity	Bhat *et al*. 1977
C. forskohlii	To treat malaria and break fevers	Holdsworth 1977; Kokwaro 1993; Rwangabo 1993; Rajendran *et al*. 1999
C. forskohlii	Alzheimer's disease	Baslas and Kumar 1981; Adachi and Hayashi 1996
C. forskohlii	Heart diseases, spasmodic pain and convulsions	Dubey *et al*. 1981
C. forskohlii	Treats thromboembolic platelet disorders	Agarwal and Parks 1982
C. forskohlii	Asthma (Bronchodilator)	Bruka 1983; Lichey *et al*. 1984
C. forskohlii	Therapeutic potential for glaucoma	Caprioli and Sears 1983; Burstein *et al*. 1984; Caprioli *et al*. 1984
C. forskohlii	Treatment of congestive heart failure	Bristow *et al*. 1984
C. forskohlii	Therapeutic potential for bronchial asthma, bronchospasmolytic activity	Lichey *et al*. 1984
C. forskohlii	Bronchitis	Boily and van Puyvelde 1986; Cos *et al*. 2002
C. forskohlii	Antiinflammatory	Dohadwalla 1986; Rupp *et al*. 1986
C. forskohlii	Cardiovascular diseases, hypertension	Kansal *et al*. 1978; Dubey *et al*. 1997
C. forskohlii	Inhibits thrombocyte aggregations, decreases intraocular pressure	Mersinger *et al*. 1988
C. forskohlii	Gastro-intestinal infections	Baerts and Lehmann 1989; Gupta *et al*. 1993
C. forskohlii	To relieve cough	Baerts and Lehmann 1989; Yoganarasimhan 2000; Chifundera 2001; Neuwinger 2000
C. forskohlii	Ear and eye infections	Baerts and Lehmann 1989
C. forskohlii	Phytotherapeutic, antiglaucoma and cardiotonic	de Souza 1993
C. forskohlii	Treatment of wounds and ringworms	Githinji and Kokwaro 1993; Chifundera 2001
C. forskohlii	Antiallergic activities through passive cutaneous anaphylaxis inhibition	Gupta *et al*. 1993
C. forskohlii	Treatment of bathing babies suffering from measles	Kokwaro 1993
C. forskohlii	Teeth and gum disorders	Hulme 1954; Kokwaro 1993
C. forskohlii	Genitourinary infections	Rwangabo 1993; Neuwinger 2000
C. forskohlii	General respiratory ailments	van Puyvelde *et al*. 1994
C. forskohlii	Anti-ageing effects in combination with tocotrienol and antioxidants	Adachi *et al*. 1996
C. forskohlii	Promotes lean body mass and treating mood disorders	Majeed *et al*. 1998
C. forskohlii	Asthma, glaucoma, eczema	Suryanayanan and Pai 1998
C. forskohlii	To relieve cold	Rajendran *et al*. 1999
C. forskohlii	Tonsillitis	Neuwinger 2000
C. forskohlii	Nausea	Hamill *et al*. 2003
C. forskohlii	Stomachaches, dyspepsia, nausea and vomiting	Yoganarasimhan 2000
C. forskohlii	Relaxative effects on isolated guinea pig tracheal spiral *in vitro*	Shan *et al*. 2008
C. xanthanthus	Rheumatic arthritis, colds, coughs, neurasthenia, scabies, snakebites and tuberculosis	Mei *et al*. 2002

same time in the system and that the concentration of sucrose determined the relative production of phloem cells.

Smith (1984) examined the developmental potential of excised primordia (1-4) and expanding leaves (5-8) of *C. blumei* Benth. cultured on medium containing IAA or kinetin. Mandler-Henger (1988) established callus cultures of *C. forskohlii* on B_5 medium (Gamborg *et al.* 1968) supplemented with 2,4-D (2,4-dichlorophenoxyacetic acid; 2.3 µM) and kinetin (6-furfurylaminopurine or KN; 0.93 µM). After different periods of subculturing, shoots were induced on B_5 medium supplemented with 5 µM 6-benzylaminopurine (BAP) for 3 weeks. For rooting, the shoots were transferred to medium containing 5 µM indole-3-butyric acid (IBA) and plantlets so obtained were successfully transferred to soil. Reuff *et al.* (1988) reported the cryopreservation of *C. blumei* suspension and callus cultures. Scaramuzzi *et al.* (1990) also reported *in vitro* propagation of two *C. blumei* varieties from crops and subcrops of vegetative fragments. Similarly Bejoy *et al.* (1990) reported *in vitro* shoot regeneration in *C. parviflorus.* Marcotrigiano *et al.* (1990) studied nucleus-controlled leaf variegation among *C. blumei* cultivars propagated by seed as well as shoot cultures on MS medium (Murashige and Skoog 1962) supplemented with 1-3 mg/l 6-benzyladenine (BA). The study revealed that micropropagation might induce epigenetic and/or heritable changes in leaf variegation. Cultivars/variants with nonpattern anthocyanin variegtion (NAV) were found to be less stable than cultivars with pattern anthocyanin variegation (PAV). Collins *et al.* (1990) reported that their method of "Direct selection" produced a cell line tolerant to 90 mM NaCl that continued to be resistant to NaCl even after 6 subcultures in the absence of NaCl.

Sen and Sharma (1991) reported shoot multiplication from shoot tip explants of aseptically germinated seedlings of *C. forskohlii* cultured on medium supplemented with 2 mg/l BA. Shoot multiplication was further enhanced with a gradual decrease in the level of BA and its final omission after 4 months. The addition of different auxins at 0.5 mg/l along with BA did not yield better results. The study demonstrated the potential of shoot culture *in vitro* and the use of micropropagated plants for the production of forskolin. Sharma *et al.* (1991) achieved *in vitro* clonal multiplication

Table 2 Bioactivities of different *Coleus* species.

Name of species	Type of bioactivity	Reference
Coleus aromaticus	Antioxidant, anticlastogenic and radioprotective effects	Rao *et al.* 2006
C. aromaticus	Antioxidant and free radical scavenging activity	Kumaran and Kurunakaran 2006
C. aromaticus	Antimicrobial	Deena *et al.* 2002
C. blumei	Antioxidative, anti-inflammatory, antimutagen, antibacterial and antiviral	Parnham and Kesselring 1985
C. forskohlii	Antispasmodic effect	Camara *et al.* 2003
C. forskohlii	Positive inotropic, lowers hypertension	Lindner *et al.* 1978; de Souza and Shah 1988
C. forskohlii	Lowers blood pressure and intra-ocular pressure and has anti-inflammatory properties	Shah *et al.* 1980
C. forskohlii	Adenylate cyclase activating properties	Metzger and Lindner 1981; Seamon *et al.* 1981; Seamon and Daly 1986
C. forskohlii	Antimetastatic	Agarwal and Parks 1983
C. forskohlii	Anticancer (tumour metastases)	Agarwal and Parks 1983; Asolkar *et al*. 1992
C. forskohlii	Antimicrobial activity	Bos *et al.* 1983; Alasbahi *et al.* 1999; Castillo and Gonzalez 1999
C. forskohlii	Anti-inflammatory, antiasthmatic, antiglaucoma	Rupp *et al.*1986; Dohadwalla 1986
C. forskohlii	Positive inotropic, positive chronotropic and hypotensive	Mersinger *et al*. 1988; Sasaki *et al*. 1998
C. forskohlii	Antibacterial, antiviral and antifungal activity	Boily and van Puyvelde 1986; van Puyvelde *et al*. 1994; Vlietinck *et al*. 1995
C. forskohlii	Antithrombotic agent, antiplatelet aggregatory effect	de Souza 1993
C. forskohlii	Anti-HIV inhibition activity	Kusumoto *et al*. 1995
C. forskohlii	Antiaging and antioxidant	Adachi *et al*. 1996
C. forskohlii	Antisenescence	Udagawa *et al*. 1996
C. kilimandcharis	Antidrepanocytant	Mpiana *et al*. 2007
C. xanthanthus	Antitumour activity	Kupchan *et al*. 1969 Zelnik *et al*. 1977
C. xanthanthus	Cytotoxic activity	Mei *et al*. 2002
C. zeylanicus	Antimicrobial	Deena *et al*. 2002

Table 3 Summary of literature on micropropagation of different *Coleus* species.

Name of species	Explant	Medium	Response	Reference
Coleus blumei	Nodal segments	MS + BA / MS + BA + IAA	Shoot multiplication	Zagrajski *et al*. 1997
C. blumei	Leaf	MS + BA (2 mgl^{-1}) + NAA (1 mgl^{-1})	Callus, plantlets	Ibrahim *et al*. 1992
C. blumei	Nodal segments	MS + BA (2 mgl^{-1}) + NAA (1 mgl^{-1})	Shoot formation	Rani *et al*. 2006
	Shoots	MS + IBA (2 mgl^{-1})	Rooting	
C. forskohlii	Leaf	B5 + 2,4-D (2.3 µM) + KN (0.93 µM)	Callus formation	Mandler-Hanger 1988
		B5 + BA (5 µM)	Shoot induction	
C. forskohlii	Shoot tips of seedlings	MS + BA (2 mgl^{-1})	Shoot multiplication	Sen and Sharma 1991
C. forskohlii	Nodal segments	MS + KN (2 mgl^{-1}) + IAA (1 mgl^{-1})	Shoot induction and	Sharma *et al*. 1991
	Shoots	MS + IAA (1 mgl^{-1})	multiplication	
			Rooting	
C. forskohlii	Shoot tips	MS + IAA (1 mgl^{-1}) + BA (1.5 mgl^{-1}) MS + IAA (1 mgl^{-1}) + KN (2 mgl^{-1})	Callus formation and adventitious shoots	Asamenew and Narayanaswamy 2000
C. forskohlii	Stem tips	MS + IAA (0.57 µM) + KN (0.46 µM)	Shoot multiplication	Bhattacharya and Bhattacharya 2001
C. forskohlii	Leaf	MS + KN (2.4 µM)	Callus formation	Reddy *et al*. 2001
	Callus	MS + KN (4.6 µM) + NAA (0.54 µM)	Shoot multiplication	
	Shoots	½ MS (No PGR)	Rooting	
C. forskohlii	Leaf	MS + BA (1 mgl^{-1}) + NAA (2 mgl^{-1})	Callus formation	Anbazhagan *et al*. 2005
		MS + BA (1 mgl^{-1})	Shoot induction	
C. forskohlii	Shoot tips	MS + NAA (0.54 µM) + BA (8.87 µM)	Shoot multiplication	Rajasekharan *et al.* 2005
	Nodal segments			
C. forskohlii	Leaf	MS + monuron + diuron	Callus formation	Srinivasan *et al*. 2006
		MS + monuron (2 mgl^{-1})		
Coleus × hybridus	Seed	MS + BA (1-3 mgl^{-1})	Shoot induction	Marcotrigiano *et al*. 1990

2,4-D = 2,4-dichlorophenoxyacetic acid; BA = 6-benzyladenine; IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; KN = kinetin; NAA = α-naphthaleneacetic acid; MS = Murashige and Skoog medium (1962); PGR = plant growth regulator.

of *C. forskolii* by culture of nodal segments on MS medium supplemented with 2.0 mg/l KN and 1.0 mg/l IAA. Shoots multiplied at a rate of 12 fold every six weeks. The shoots were rooted by transfer to MS medium containing 1.0 mg/l IAA. Ibrahim *et al.* (1990) compared the level of salt tolerance in *C. blumei* cv. 'Xenia Field' plants produced either from an in *vitro* or an *in vivo* selection method. A rooting test based on the ability of cuttings derived from *in vitro* as well as *in vivo* raised plants to form roots in NaCl concentrations up to 50 mM revealed that *in vitro* derived cuttings performed better than seedling (*in vivo*) derived cuttings. Bajrovic *et al.* (1993) reported the stimulation of growth of callus cultures of *C. blumei* with 1(6-purinyl)-2,5-dimethylpyrrole. Zagrajski *et al.* (1997) studied shoot induction from nodal, internodal and leaf explants of *C. blumei* with the use of BA alone or in combination with IAA. They reported that nodal explants were best for shoot induction and multiplication while internodal and leaf explants did not give consistent results. Asamenew and Narayanaswamy (2000) reported a rapid initiation and proliferation of callus from shoot tip explants of aseptically grown *C. forskohlii* cultured on MS medium containing IAA (1.0 mg/l) and BA (1.5 mg/l). Adventitious shoots (17.33) were obtained from compact greenish callus on passage to MS basal medium containing various concentrations and combinations of IAA and KN. But, the best response was observed in medium containing IAA (1.0 mg/l) and KN (2.0 mg/l). The shoots rooted by their transfer to hormone-free MS medium and developed into normal plantlets.

Bhattacharya and Bhattacharya (2001) developed a protocol that led to the formation of complete plantlets of *C. forskohlii* within 35-40 days by culturing shoot tip explants

on MS medium supplemented with 0.5 µM IAA and 0.46 µM KN through direct multiplication at a rate of 12.5 shoots/explant. The shoots were rooted and micropropagated plantlets were successfully established in soil after hardening with a high survival rate.

Reddy *et al.* (2001) established a protocol for shoot organogenesis and mass propagation of *C. forskohlii* from leaf-derived callus. Optimal callus was developed from mature leaves on MS medium supplemented with 2-4 µM KN alone. For regeneration of shoots, callus was transferred to MS medium containing 4.6 µM KN and 0.5 µM NAA. Bauer *et al.* (2002) developed a rapid and efficient protocol for the genetic transformation of *C. blumei* using co-cultivation of leaf explants with *Agrobacterium tumefaciens* and *A. rhizogenes*. Successful transformation depended on specific bacterial strain-plant genotype combi-nation and co-cultivation treatment. The wild type B6S3 bacterial strains employed gave the maximum efficiency. Strains C58C1 (pArA4abc), GV3101(pGV2215), 8196 and A281 were also effective. Among several co-cultivation conditions tested, the most efficient treatment was as fol-lows: excised leaf explants were incubated immediately in bacterial suspension for 5 min and were co-cultured with *Nicotiana tabacum* crown gall callus for two days. Plant hormone autotrophy, PCR analyses and hybridization analysis confirmed genetic transformation of the cultures. Sixteen different transgenic callus and cell cultures were maintained for more than four years in the absence of exogenous growth regulators and antibiotics without deterioration in the growth rate.

Asamenew and Narayanaswamy (2004) presented a method for rapid *in vitro* propagation of *C. forskohlii* by culture of shoot tips and nodal segments on MS medium supplemented with various concentrations of KN. Best response for shoot formation was obtained with 1.0 mg/l KN. Rooting was best with 1.0 mg/l IAA. Microtubers were also produced on *in vitro* rooting medium. Anbazhagan *et al.* (2005) developed a protocol for indirect regeneration of *C. forskohlii* from leaf explants. Maximum callus induction was obtained on MS medium supplemented with BA (1 mg/l) along with NAA (2 mg/l). Regeneration of shoots was observed after 7 weeks of initial culture. Maximum number of shoots was obtained on medium supplemented with 1.0 mg/l BA alone with the mean number of shoots as 23.80 ± 1.47. Rajasekharan *et al.* (2005) developed a protocol for *in vitro* propagation of *C. forskohlii* from shoot tips and single node cuttings using MS medium supplemented with NAA (0.54 µM) and BA (8.87 µM). Shoot multiplication was amplified with a gradual decrease of BA concentration, leading to its final omission after 4 months. Srinivasan *et al.* (2006) used various urea-derived herbicides and different cytokinin analogues to determine their effects on callusing response and shoot regeneration capacity of *C. forskohlii.* The herbicides, monuron and diuron evoked profuse callusing response from coleus leaf segments on MS medium. Shoot regeneration by monuron (2.0 mg/l) showed a maximum of 3 multiple shoots/explant with a frequency of 75% whereas diuron (0.5 mg/l) showed a higher frequency of shoot regeneration (90%) with a mean number of 6 shoots/explant. A rapid and highly effective method for micropropagation from nodal segments and shoot tip explants of *C. blumei* was established in our own laboratory (Rani *et al.* 2006) using MS medium containing BA (2 mg/l) and NAA (1 mg/l). Shoot tips proved to be better explants than nodal segments by having a high rate of shoot induction and more shoots. Shoot multiplication was achieved on the same medium as used for shoot initiation. Multiplied shoots rooted best on MS medium supplemented with indole-3-butyric acid (IBA) (2 mg/l). Micropropagated plantlets were successfully established in soil after hardening, with a 100% survival rate.

COLEUS FORSKOHLII AND FORSKOLIN

Coleus forskohlii

C. forskohlii (Willd.) Briq. [syn. *Plectranthus barbatus* Andr., *Plectranthus forskohlii* Briq., *Plectranthus forskalaei* Willd., *Plectranthus kilimandschari* (Gürke) H.L. Maass., *Plectranthus grandis* (Cramer) R.H. Willemse, *Coleus forskohlii* Briq., *Coleus kilimandschari* Gürke ex Engl., *Coleus coerulescens* Gürke and *Coleus barbatus* (Andr.) Benth. (Lukhoba *et al.* 2006)], commonly known as 'Gandeer', is an upright, herbaceous, branched aromatic, perennial herb reaching a height of 30-60 cm (de Souza and Shah 1988). The roots reach up to 20 cm in length and are 0.5-2.5 cm thick. They are tuberous, fasiculated, conical, fusiform, straight and strongly aromatic. Leaves, usually pubescent, narrow into petioles. Racemes are perfect, flowers, very showy bluish to pale lavender colored. Calyx, fine-toothed and deflexed in the fruit; corolla, pale blue, bilabiate, the lower lobes elongated and concave; stamens, monadelphous; ovary, four-parted; pollination, cross-pollination by means of wind and insects. The plant grows wild in the mountain slopes of Nepal, India and Thailand. According to Valdes *et al.* (1987), the plant originated from the Indian subcontinent and has been distributed in Egypt, Arabia, Ethiopia, East Africa and Brazil. In India, it is mostly cultivated in Tamil Nadu, Gujarat and Karnataka. *C. forskohlii* thrives best in red, sandy loam soils, which are porous and well drained with a pH ranging from 5.5 to 7. It does not require very fertile soils and can be economically grown on soils with marginal fertility. Humid climate with relative humidity ranging from 83-95% and a temperature range of 10-25°C is ideal for the crop in locations such as in Belgam (Karnataka) and Gujarat. The required annual rainfall is 100-160 cm, mainly between June and September. The crop also performs well in less humid and warmer regions of South India like Salem or Coimbatore, where it is grown as an irrigated crop (http://www.tnsmpb.tn.gov.in).

C. forskohlii has been used in indigenous systems of medicine as a remedy for heart diseases, abdominal, colic and respiratory disorders, painful micturition and certain CNS disorders such as insomnia and convulsions (Sharma 1956; Chunekar 1960; Bhat *et al.* 1977; Dubey *et al.* 1981, 1997). Its root extract has been found to possess antisenescence agent (Udagawa *et al.* 1996). Thus it finds great usage in antiaging cosmeties with the antioxidant tocotrienol (Adachi *et al.* 1996). Its further use in promoting lean body mass, treating mood disorders (Majeed *et al.* 1998) and its anticancer activities (Agarwal and Parks 1982; Asolkar *et al.* 1992) have drawn our attention towards this drug. The roots are a good source of various diterpenes i.e. coleonol, forskolin and barbatusin (Shah *et al.* 1980; Trivedi *et al.* 1982; Singh *et al.* 1984; Mukherjee *et al.* 1996; Yanagihara *et al.* 1996). These diterpenes are useful for curing different ailments including cardiovascular diseases, hypertension (Kansal *et al.* 1978, Dubey *et al.* 1997) asthma, glaucoma (Suryanayanan and Pai 1998) and Alzheimer's disease (Baslas and Kumar 1981; Adachi and Hayashi 1996).

Srivastava *et al.* (2002) carried out a detailed pharmacognostical study of roots of this plant procured from different geographical zones of India.The study included macro- and microscopical characters, fluorescence analysis of powder, physico-chemical values, preliminary phytochemical screening of different extracts and finally the establishment of a fingerprint profile of the crude drug through thin layer chromatography for the detection of chemical marker components. The authors found an almost four-fold increase in the percentage of sugar and starch in southern samples while the protein percentage was almost twice as high as in northern samples. Heavy metal (Co, Mn, Cd and Cu) accumulation was higher in samples from the southern part of the country, which may be due to high industrial activity or edaphic factors in Andhra Pradesh and Tamil Nadu. The percentage Pb was quite high in the Uttar Pradesh sample which may be due to some edaphic factor or

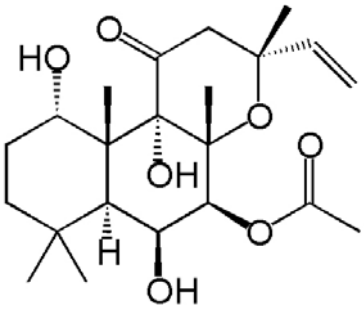

Fig. 2 Chemical structure of forskolin.

vehicular pollution in that particular region. Thus it was concluded that geographical variations play a crucial role in the quantitative alteration of parameters affecting the quality of the drug.

C. forskohlii is an ancient root drug recorded in Ayurvedic medicine under the Sanskrit name 'Makandi' and Mayini (Shah 1998). Upon closer investigation into the plant's pharmacological properties, it was revealed that *C. forskohlii* roots contain forskolin (**Fig. 2**), a compound that belongs to the chemical class of diterpenes (Bhat *et al.* 1977).

Most of the pharmacological properties of this plant are associated with the presence of forskolin. Forslean, an extract prepared from roots of *C. forskohlii* is a registered trademark and patented product of Sabinsa Corp., USA. It constitutes a minimum of 10% forskolin and is meant to promote lean body mass, fat loss and weight loss. Maintaining lean body mass is expected to have a positive impact on long term cardiovascular risk and life span, and hence becomes essential to maintain good health (Narayanan *et al.* 2002). Shen and Xu (2005) isolated two new diterpenoids, forskolin I and J from *Coleus forskohlii* (Willd.) Briq. collected from the Yunnan Province. Spectral analysis, including 1D and 2D NMR techniques showed them to be 1α,6β-diacetoxy-7β,9α-dihydroxy-8,13-epoxylabd-14-en-11-one and 1α,9α-dihydroxy-6β,7β-diacetoxy- 8,13-epoxylabd-14-en-11-one. Xu and Kong (2006) isolated labdane diterpenoid, 12 hydroxy-8, 13*E*-laddadien-15-oic acid from *C. forskohlii* using various chromatographic techniques and spectroscopic methods. Yang *et al.* (2006) reported three other diterpeniods from *C. forskohlii* viz. forskolin G (2), forskolin H (3), forskolin I (4). Their structures were elucidated as 1 α, 6 β-diacetoxy-8,13-epoxylabd-14-en-11-one, 1α-hydroxy-6β,7β-diacetoxy-8,13-epoxylabd-14-en-11-one and 1α, 9 α-dihydroxy-6 β, 7 α-diacetoxy-8,13-epoxylabd-14-en-11-one on the basis of spectral data.

Forskolin

Forskolin is a polyhydroxy labdane diterpeniod (Bhat *et al.* 1977) with a number of promising biological and pharmacological activities. In the last 20 years, forskolin has been successfully evaluated in numerous clinical studies, including bronchial asthma, cardiovascular health, glaucoma, sexual dysfunction in men and dermatological diseases. Chemically, forskolin is 7β-acetoxy-8, 13-epoxy 1α, 6β, 9α-trihydroxy-labd-14-en-11-one (Paulus 1980a, 1980b). Despite the fact that successful total synthesis of forskolin has been reported, it is not available by chemical synthesis because of its complex nature. Recently it has been recommended for reducing body fat while maintaing lean body mass (Ding and Staudinger 2005). The existence of forskolin was first reported by two independent groups of scientists during their screening programs for useful medicinal plants of India. During their study on tuberous roots of *C. forskohlii* they reported the presence of a bioactive component, a labdane diterpene, which was called colenol by one group (Tandon *et al.* 1977; Dubey *et al.* 1981) and forskolin by the other (Bhat *et al.* 1977; Paulus 1980a). Later, both of these compounds, colenol and forskolin, were shown to be the same substance and the name forskolin was retained (Saksena *et al.* 1985). Forskolin content has been shown to vary substantially (0.01-0.44%) among different genotypes with an average content of 0.1% of dry wt. of root tubers (Vishwakarma *et al.* 1988).

Forskolin is the only plant-derived compound known to have unique adenylate cyclase activating properties (Metzger and Lindler 1981, Seamon *et al.* 1981, Seamon and Daly 1986) resulting in an increase in levels of cyclic AMP (cAMP) which affects heart action, blood flow and intraocular pressure (Ammon and Muller 1985; Christenson *et al.* 1995). cAMP is also nicknamed as the 'second messenger', referring to its broad range of activity in the body's life-sustaining reactions. Due to its cAMP-dependent effects, forskolin has been considered for its development as an agent for the treatment of congestive cardiomyopathy (Bristow *et al.* 1984; Rupp *et al.* 1986) and glaucoma by lowering intraocular pressure (Caprioli and Sears 1983;

Table 4 Summary of literature on *in vivo/in vitro* production of forskolin from *C. forskohlii*.

Source	Medium	Forskolin content	References
Suspension culture	B_5 + 0.4 mgl^{-1} IBA	0.03%	Mersinger *et al*. 1988
	B_5 + 0.2 mgl^{-1} KN + 0.4 mgl^{-1} IBA	0.025%	
Roots	-	0.04-0.44%	Vishwakarma *et al*. 1988
Shoot tips	MS + BA	0.009%	Sen and Sharma 1991
Micropropagated plantlets		0.01%	
Tubers of micropropagated plants	-	0.1%	Sharma *et al*. 1991
Root cultures	B5	0.1%	Krombholz *et al*. 1992
Shoot, shoot-forming callus mass	MS + BA	0.01%	Sen *et al*. 1992
Micropropagated plants		0.013%	
Root callus	MS + IAA (0.5 mgl^{-1}) + IBA (0.5 mgl^{-1}) + glycine (5 mgl^{-1}) + CH (200 mgl^{-1})	0.075%	Tripathi *et al*. 1995
Tuberous roots	-	1.15-7.87 µg/mg/dw	Yanagihara *et al*. 1995
Tumorous callus	B_5O	0.02%	Mukherjee *et al*. 1996
Rhizogenic callus	B_5 + CH	0.011%	
Root culture	B_5 + IBA	0.041%	
Hairy roots	WPM	1.6 mg/flask	Sasaki *et al*. 1998
Rhizogenic tumourous line	B_5O + CH 2.0 gl^{-1}	2.3mg g^{-1}	Mukherjee *et al*. 2000
Rooty teratoma line	B_5O + CH 2.5 gl^{-1}	1.7 mg g^{-1}	
Roots of *in vitro* raised plants		0.12%	Reddy *et al*. 2001
Transformed rhizogenic line (GCO-RCH-10)	B5 + GA_3 (1mgl^{-1})	8.9- fold	Mukherjee *et al*. 2003
Cell suspension line GSO-5/7-K	B5 + BA (2 mgl^{-1})	6- fold	
Root line (RC-ST-2/16)	B5 + GA_3 (0.5 mgl^{-1})	4.3-fold	
Stem powder	-	0.103%	Saleem *et al.* 2005
Roots	-	0.097%	Saleem *et al*. 2006

BA = 6-benzyladenine; CH = casein hydrolysate; GA_3 = gibberellic acid; IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; KN = kinetin; WPM = woody plant medium

Burstein *et al.* 1984) and as a bronchodilator in asthmatics (Bruka 1983; Lichey *et al.* 1984; Kreutner *et al.* 1985). It has also been used to treat thromoboembolic platelet disorders (Agarwal and Parks 1982; Christensen *et al.* 1995), and tumour metastatis of certain cancers (Agarwal and Parks 1983). Other bioactivities associated with forskolin include antiallergic (Kreutner *et al.* 1985), anti-inflammatory (Dohadwalla 1986), antiobesity (Han *et al.* 2005), platelet aggregation inhibition (Agarwal and Parks 1982) and hypotensive (Dubey *et al.* 1981). **Table 4** summarizes literature on *in vivo/in vitro* production of forskolin.

Biosynthesis and estimation of forskolin

The only source of forskolin is still the roots of wild or cultivated *C. forskohlii* plants (Asamenew and Narayanaswamy 2000; Mukherjee *et al.* 2000; Kukreja and Garg 2007; Reddy *et al.* 2007). Even though the complete synthesis of forskolin has been reported (Ziegler *et al.* 1987; Corey *et al.* 1988; Delpech *et al.* 1996; Asada *et al.* 2000) it is not yet a commercially viable venture because of its complicated structure. Production of monoclonal antibodies against forskolin has been reported by Sakata *et al.* (1994) and Shoyama (1993). Yanagihara *et al*. (1995) determined the relationship between forskolin content and growth temperature of clonally propagated plants of *C. forskohlii.* Quantitative determination of forskolin content in the plants cultivated in different temperatures was investigated by ELISA using monoclonal antibodies against forskolin. Temperature clearly affected the production of forskolin demonstrating that the content of forskolin was higher at 20°C than at 15 or 30°C. The highest forskolin content (7.87 pg/mg dwt) was found in the base of tuberous root at 20°C. This content was approximately twice compared to that of 30°C, and nearly three times higher than that of 15°C. They also investigated the distribution of forskolin in the whole plant. The highest forskolin content was found at the base of the tuberous root which decreased towards the tip. Yanagihara *et al.* (1996) further presented the production of monoclonal antibodies (MAB) against forskolin and a sensitive assay system using ELISA. They further investigated the correlation between forskolin accumulation and growth rate using clonally propagated shoots. Forskolin content was determined using antibodies in an ELISA test which could detect forskolin in the range of 5 µg to 5 mg. Among different plant parts (flowers, racemes, leaves, stem, tuberous roots and roots) of the plants analyzed, tuberous roots and the stem base contained higher amounts of forskolin than other organs. The forskolin content in the stem decreased gradually towards top of the shoots. Engprasert *et al.* (2004) reported molecular cloning and functional expression of geranyl-geranylpyrophosphate (GGPP) synthase from *C. forskohlii.* GGPP synthase is thought to be involved in the biosynthesis of forskolin, which is primarily synthesized in the leaves and subsequently accumulated in the stems and roots. Schaneberg and Khan (2003) developed a rapid method using reversed-phase liquid chromatography for quantitative analysis of forskolin in *C. forskohlii*. The limit of detection was 1.5 mg/ml. The method was successful in the quantitative and qualitative evaluation of the marker compound in *C. forskohlii* plant material and in market products claiming to contain *C. forskohlii*. Sailo and Bagyaraj (2005) studied the effectiveness of 11 different arbuscular mycorrhizal (AM) fungi on the growth, nutrition and forskolin content of *C. forskohlii*. *Coleus* plants raised in the presence of most of these fungi showed an increase in growth, and phosphorous and forskolin content over those grown in the absence of AM fungi. The increase in the root biomass because of inoculation with *G. bagyarajii* and *S. calospora* was 51.25 and 33.75% respectively compared to uninoculated controls. The increase in shoot biomass was 71.36 and 47.38% due to inoculation with *G. bagyarajii* and *S. calospora* respectively. The P content of the shoot differed significantly among the treatments, maximum P occurring in the shoot of plants inoculated with *G. bagyarajii* and the least in uninoculated plants. The forskolin concentration in the root was highest in plants inoculated with *G. bagyarajii* (0.93%) and *S. calospora* (0.92%). Roots of uninoculated control plants had the least forskolin concentration (0.53%). Among different AM fungi tested, *Glomus bagyarajii* was found to be the best AM symbiont for inoculating *C. forskohlii* followed by *Scutellospora calospora*. Saleem *et al.* (2006) reported a simple and rapid process to produce a highly pure form of forskolin on a laboratory scale from *C. forskohlii* roots using common laboratory chemicals. This work made use of activated charcoal in the purification of forskolin in a reverted phase methodology.

In vitro production of forskolin

Since *C. forskohlii* is the only known plant source for forskolin, indiscriminate collections have led to severe depletion of this plant from wild habitats (Shah *et al.* 1980; Vishwakarma *et al.* 1988) and it is listed as one of the endangered plant species vulnerable to extinction (Sharma *et al.* 1991; Asamenew and Narayanaswamy 2000). Attempts have been made to standardize and use plant tissue culture techniques to meet ever increasing demands of pharmaceutical industry for production of forskolin. Forskolin production in untransformed suspension cultures of *C. forskohlii* has been reported by Mersinger *et al.* (1988). They observed that forskolin production required the induction of certain stages of differentiation in the direction of root formation. High producing plants of *C. forskohlii* were used for initiation of callus cultures on B_5 medium supplemented with 0.5 mg/l 2,4-D and 0.2 mg/l KN under continuous light. These cultures were transferred to B_5 medium supplemented with different concentrations and combinations of 2,4-D, KN, casein hydrolysate, IBA or BAP to establish suspension cultures. Production of forskolin was observed in the cultures maintained on hormone-free medium or those supplemented with a combination of IBA and KN or BAP. Maximum forskolin production (0.03%) was observed in medium containing 0.4 mg/l BAP alone followed by 0.025% in medium containing combination of IBA (0.4 mg/l) and KN (0.2 mg/l). One limiting factor of this finding was that the suspension cultures lost their capacity to produce forskolin after 3-4 years and new cultures had to be established. The two cell strains cultivated in continuous light or darkness (light and dark strain respectively) were shown to behave differently with respect to forskolin production. Forskolin production was higher in the light strain than in the dark strain. For the light strain maximum forskolin production (0.15%) was observed in the second cultivation period (14 days) whereas the dark strain attained maximum production (0.05%) only in the fourth induction period. The production of forskolin was coupled to morphological changes of the cultures. The 'light strain' formed large hollow cell aggregates with hairy structures inside, where as the 'dark strain' formed short roots. Mersinger *et al.* (1988) further tried to scale up the production of forskolin by liquid cultures of *C. forskohlii* in 20 L and 200 + airlift fermenters. The cultures were initiated in shake flasks for the first 14 days (induction period) and were then transferred to 20 L bioreactors containing the same medium. Forskolin production initiated at day 5 and reached a maximum value of 0.073% by the end of cultivation period. In a 200 L bioreactor, maximum forskolin (0.03%) content was observed at day 26. The lower forskolin content observed in a 200 L bioreactor as compared to a 20 L bioreactor might be the result of mechanical stress in the large bioreactor. In an attempt to obtain variation with respect to forskolin content in regenerated plants, Mandler-Henger (1988) treated the calli of *C. forskohlii* raised on B5 medium with different doses of mutagenic UV. Forskolin content was estimated in regenerated agar-grown plants and plants transferred to and grown in soil. Some regenerants showed a 1.5- to 2-fold increase in forskolin content compared to the untreated control. An interesting finding of this study was that more variation was visible with respect to leaf morphology and ar-

rangement.

Sharma *et al.* (1991) achieved *in vitro* clonal multiplication of *C. forskolii* by culture of nodal segments on MS medium and found that the forskolin content (0.1%) in tubers of micropropagated plants was same as that found in wild plants. Investigators have also used more or less differenttiated organ culture systems for production of forskolin (Krombholz *et al.* 1992; Sen *et al.* 1992). Krombholz *et al.* (1992) established root cultures of *C. forskohlii* by inducing roots in internode primary callus cultures maintained on medium B_5 without phytohormones and then transferred to liquid medium supplemented with 1 mg/l IBA and 60 mg/l casein hydrolysate. The growth of root cultures, which consisted of long, highly branched, glaucy looking roots, was rather slow, triplicating their dry weight after every 3 weeks. The amount of forskolin produced ranged from 127 to 1200 mg/kg dry mass depending on culture strain used. The addition of IBA to the medium resulted in increased production of forskolin e.g. IBA at 4.2 mg/l resulted in a yield of forskolin at 4-9 mg/kg dry mass and a stable root morphology. Krombholz *et al.* (1992) also established transformed root cultures using *Agrobacterium rhizogenes* strain 15834. They observed typical hairy root morphology and opine production in such cultures in the first 6 months. These cultures grew faster than untransformed root cultures; the biomass multiplied 20 times in 3 weeks on B5 medium. Though the forskolin yield was nearly the same as with untransformed cultures, yet due to better growth, overall production of forskolin was much higher (4.5 mg/l) for transformed root cultures. Cultivation of root cultures was also scaled up in 20 L glass jar bioreactors fitted with aeration, cutting and stirring devices. Growth of cultures (both untransformed and transformed) and forskolin production was generally better in large reaction vessels.

Sen *et al.* (1992) made a comparison of forskolin production in different *in vitro* culture systems of *C. forskohlii*. Shoot tips, callus and root tip cultures were established on MS and White's basal medium (White 1963) with and without plant growth regulators (PGRs). Shoot differentiating cultures established in the presence or absence of BAP revealed more or less similar amounts of forskolin as that of micropropagated plants. Root organ suspension cultures showed traces of forskolin production. Multiple shooting and formation of unorganized tissue was observed in shoot tip explants obtained from aseptically germinated seedlings. Addition of BAP (0.5-2.5 mg/l) was shown to promote shooting but did not affect the production of forskolin. Callus cultures were initiated on MS medium and then transferred to White's medium with 1 mg/l BAP and 1 mg/l NAA where they became friable, whitish and rhizogenic after 2 months. The rhizogenic callus did not produce forskolin indicating that root differentiation and forskolin production are not necessarily coupled. Excised root tips from *in vitro* micropropagated multiple shoots when cultured on ¼ MS basal medium supplemented with 0.5 mg/l IBA, formed an entangled mass of roots with primary and secondary laterals. Root cultures also did not produce forskolin (or only traces were found). This observation of forskolin production in shoots and shoot-forming callus but not or only traces in rhizogenic callus and root cultures is interesting, because naturally forskolin is mainly found in root tubers. Hence, forskolin production seemed to not depend exclusively on the correct differentiation of plant material. Other factors, too, seemed to influence forskolin production. The PGR composition of the medium has also been shown to play an important role in the expression of secondary metabolites. Nevertheless, cell and organ culture systems may be suitable to produce forskolin independently from plants and therefore may serve as a source for this compound in the future. 1,9-dideoxyforskolin (DDF) is another component in the root tubers of *C. forskohlii* but is pharmacologically inactive. This compound is also found in cell culture and can be transformed into active forskolin by introduction of the essential hydroxyl-groups 1α and 9α by the fungus *Scophlariopsis* and chemical acetylation (Ganguli 1986; Nadkarni *et al.* 1986; Inamdar *et al.* 1989). In another study, Tripathi *et al.* (1995) reported the production of coleonol (forskolin) by root callus cells of *C. forskohlii.*

In an attempt to determine the potential of tumorous cultures for production of forskolin in *C. forskohlii*, Mukherjee *et al.* (1996) reported initiation of transformed cultures of *C. forskohlii* using *A. tumefaciens.* The resulting tumour tissue and shooty teratomas were cultured *in vitro*. Forskolin was detected in tumorous callus (0.002%), rhizogenic callus (0.11%) and root cultures (0.14%) but not in shooty teratoma. This study provided another evidence of root tissue as the site of forskolin synthesis and accumulation. The authors opined that forskolin synthesis and accumulation in tumorous *C. forskohlii* cultures may permit the elucidation of diterpene metabolism in this species. Later, Mukherjee *et al.* (2003) reported an increase in the yield of forskolin in transformed root, rhizogenic calli and cell suspension cultures of *C. forskohlii* under the influence of various PGRs, viz. IAA, IBA, NAA, 2,4-D, KN, BAP, gibberellic acid (GA_3) and auxin conjugates IAA-ala, IAA-gly, IAA-phe and IAA-asp. Different cultivars responded differently to different plant growth regulators. GA_3 at 1.0 mg/l resulted in an 8.9-fold increase in forskolin content in transformed rhizogenic line GCO-RCH-10 while for root line RC-ST-2/16, GA_3 at 0.5 mg/l caused a 4.3-fold increase and for a cell suspension line GSO-5/7-K, BAP at 2 mg/l caused 6-fold increase in forskolin content. Growth and morphology were also found to be affected by the PGRs studied. A seven-fold increase in biomass was obtained in rhizogenic line GCO-RCH-10 with 0.5 mg/l GA_3. The shoot culture line ST-2/51/D showed prolific growth in the presence of all cytokinins but no forskolin was detected in the shoot cultures treated with any of these hormones. In another study, Li *et al.* (2005) reported *in vitro* production of forskolin by *C. forskohlii* hairy root cultures.

Kukreja and Garg (2007) studied the potential of hairy root cultures developed from leaf explants of *C. forskohlii* upon infection with *A. rhizogenes* strains A4 and LBA9402 for the production of forskolin. Among the two bacterial strains studied, strain A4 was more virulent and resulted in a transformation frequency of 97% within 7-8 days of inoculation whereas for strain LBA9402, hairy roots emerged after 10-12 days of inoculation with a transformation frequency of 75%. These roots were excised and grown on B_5 medium containing antibiotics cephalexin and ampicillin (1 mg/ml) in order to eliminate bacteria and for further growth. However this medium (B_5) was less responsive for root growth and biomass production. Hence all hairy root lines were transferred to hormone-free WP liquid medium which supported maximum hairy root biomass (6.33 g). Addition of IBA (1.0 mg/l) further increased the root biomass by 50% more reaching 9.33 g. Forskolin content was higher for root lines of A4 strain than for LBA 9402. It ranged from 0.011 to 0.11% for different lines of A4 strain and from 0.005% to 0.083% for LBA9402. Similarly the level of immediate precursor, 9-deoxyforskolin was also higher for lines of A4 strain.

COLEUS BLUMEI AND ROSMARINIC ACID

Coleus blumei

C. blumei is commonly grown in gardens throughout the world as an ornamental plant because of its colorful foliage. It occurs in an enormous number of different cultivars which vary in color and shape of the leaves. It is an erect branched undershrub, with quadrangular stems. Leaves, 5-12 x 4-8 cm, ovate-acuminate, crenate-serrate, rounded at the base, variegated; flowers, pale purple in close whorls on terminal solitary or paniculate racemes; calyx, 2-lipped with the upper lip large, commonly grown in gardens for its ornamental variegated foliage (Singh 1986). The flowers are borne in terminal spike-like racemes. The leaves are variegated and make them good ornamental plants (Vasishta 1994).

The nomenculature of *C. blumei* is very confusing as different names have been in use for the same species. *C. blumei* is probably a natural hybrid of several *Coleus* species with its origin in South East Asia. This plant was described for the first time by Linnaeus (1763) who assigned the name *Ocimum scutellanoides*. Then Brown (1810), named it as *Plectranthus scutellarioides*, the name later on used by Blume (1826) for another species. Bentham (1832-1836) then considered these two plants as different species and placed them in the genus *Coleus* with the species names as *C. blumei* and *C. scutellarioides.* Siebert and Vosa (1896) placed *C. blumei*, *C. scutellarioides*, *C. atropurpureus*, *C. bicolor*, *C. verschaffelti* and *C. hybridus* as subspecies under the species *C. scutellarioides*. Therefore, the legitimate name for the plant is *Coleus scutellarioides* (L.) Bentham (Lebowitz 1985). Nevertheless, several publications on this species have used the name *C. blumei* and the same name is used in this communication.

According to Gardener (1985), this plant was brought to Europe from Java in 1851 for its ornamental value and since then is being used as an ornamental plant in Europe. Availability of a number of varieties with striking colorful foliage and easy method of propagation through cuttings have made it a very popular plant for ornamental purposes. In India, Indonesia and Mexico, *C. blumei* is used as a medicinal plant (Schultze 1986). The medicinal value of this plant is attributed to rosmarinic acid, one of the most important secondary compounds synthesized by this plant. It is present in all plant parts and is supposed to act as a preformed, constitutively accumulated defence compound by virtue of its antimicrobial properties (Szabo *et al.* 1999).

Rosmarinic acid

Rosmarinic acid (R-*O*-caffeoyl-3,4-dihydroxyphenyllactic acid; RA), is an ester of caffeic acid and 3,4,-dihydroxyphenyllactate, which, besides chlorogenic acid, is one of the most common caffeic acid esters in the plant kingdom. RA and similar compounds have been known as 'Labiatengerbstoffe' (a type of tannin) even before the chemical structure of RA was elucidated. Two Italian chemists Scarpati and Oriente (1958) isolated RA for the first time as a pure compound and named it according to the plant from which it was isolated, *Rosmarinus officinalis*. These scientists also elucidated the molecular structure of RA, showing it to be an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Scarpati and Oriente 1958, 1960). They further showed that caffeic acid part of RA is derived from phenylalanine whereas 3,4-dihydroxyphenyllactic acid is derived from tyrosine. RA has been shown to exhibit antiallergic, antibacterial, antiviral, antioxidant and anti-inflammatory activities (Gracza *et al.* 1985; Parnham and Kesselring 1985; Engelberger *et al.* 1988; Peake *et al.* 1991; Kumaran and Karunakaran 2006). The anti-inflammatory properties of RA are thought to be based on the inhibition of lipoxygenases and cyclo-oxygenases and the interference of RA with the complement cascade (Parnham and Kesselring 1985). RA has been shown to be rapidly eliminated from the blood circulation after intravenous administration. It was also shown to possess very low toxicity in mice (Parnham and Kesselring 1985). It has been used in preparations against Herpes simplex infections (Wolbling and Leonhardt 1994), as a putative cancer-protective compound in food (Ho *et al.* 1994) or as a potential preservative in food and cosmetics (D'Amelio 1999). Inside the plants, RA is thought to be a part of the defense system against fungal and bacterial infections and predators. RA is also active against acute respiratory distress syndrome (ARDS) and other shock conditions (Bult *et al.* 1985; Parnham and Kesselring 1985). Further, the antioxidative activity and low toxicity make RA an interesting compound for food industry. It has applications as a food preservative and in medicine due to its functional properties as an antioxidant and antimicrobial (Deighton *et al.* 1993; Madsen and Bertelsen 1995; Frankel *et al.* 1996; Shetty *et al.* 1997). It was reported that in bulk corn oil rosemary extract containing carnosic acid and RA, showed significantly more antioxidant activity than carnosol (Frankel *et al.* 1996).

RA is a well known constituent of members of the families *Boraginaceae* and *Lamiaceae* (Petersen and Simmonds 2003) mainly the subfamilies *Saturejoideae* and *Nepetoideae* (Litvinenko *et al.* 1975; Molgaard and Ravn 1988; Zinsmeister *et al.* 1991) and also in other families like *Blechinaceae* (Hausler *et al.* 1992), *Zosteraceae* (Ravn *et al.* 1994), *Potamogetonaceae* and *Cannaceae* (Petersen and Simmonds 2003). According to Lamaison *et al.* (1990) species of the genera *Sanicula* (Apiaceace), *Lycopus*, *Melissa*, *Mentha*, *Origanum and Salvia* (Lamiaceace) contain RA in large amounts (more than 3%) based on dry weight. RA has also been shown to occur in lower plants such as hornworts (Takeda *et al.* 1990). This distribution of RA confirms that its occurrence can not be used as a chemotaxonomical marker to differentiate among families. One question still remains open as to whether the ability to synthesize and/or accumulate RA has evolved rather early in phylogeny of land plants or whether it has been developed independently in several taxa.

Biosynthesis and estimation of RA

Investigations on the biosynthesis of RA were started by Ellis and Towers (1970) who identified two aromatic amino acids, phenylalanine and tyrosine, as the precursors for RA in plants of *Mentha*. Their study showed that phenylalanine was incorporated into the caffeic acid part of RA whereas tyrosine gave rise to 3,4-dihydroxyphenyllactic acid. Since the precursors for the biosynthesis of RA are the amino acids phenylalanine and tyrosine (Ellis and Towers 1970), only one of the four hydroxyl groups is brought into the molecule by the precursors themselves; the other three hydroxyl groups are introduced during the biosynthesis of RA (Pabsch *et al.* 1991; Petersen *et al.* 1993). The enzymes involved in the biosynthesis of RA have been successively identified, isolated and characterized. Petersen *et al.* (1993) proposed a scheme for the biosynthetic pathway in cell cultures of *C. blumei* (**Fig. 3**).

Phenylalanine derived pathway: Phenylalanine is the precursor for the caffeic acid moiety of RA (Ellis and Towers 1970; Razzaque and Ellis 1977). It has been suggested that the general phenylpropanoid metabolism is involved in the biosynthesis of RA (Razzaque and Ellis 1977). The first enzyme involved in this pathway is phenylalanine ammonia-lyase (PAL), which catalyzes the oxidative deamination of phenylalanine to form *t*-cinnamic acid and ammonia. This is a soluble enzyme with high affinity towards its substrate phenylalanine. Razzaque and Ellis (1977) have shown that accumulation of RA in cell suspension cultures of *C. blumei* coincided with an increase in PAL activity. This study has demonstrated the rapid conversion of the phenyl propanoid amino acids into RA in the younger cultures. Histochemical examination of the *Coleus* cultures, however, has revealed that the bulk of the RA is accumulated in vesicles and extracellular deposits. The next step in this pathway is catalyzed by cinnamic acid 4-hydroxylase (CAH) which catalyzes hydroxylation of *t*-cinnamic acid in position 4 to form 4-courmaric acid. The enzyme activity essentially depended on molecular oxygen and NADPH. Cinnamic acid 4-hydroxylase, a cytochrome P450-dependent enzyme, is the first hydroxylase of the RA biosynthetic pathway introducing the 4-hydroxyl group into cinnamic acid. CAH is one of the three membrane-bound, cytochrome P450-dependent hydroxylases characterized in microsomal preparations from cell cultures of *C. blumei* (Petersen 1997). 4-courmaric acid is converted to the activated precursor 4-coumaroyl-CoA, the final intermediate of the phenylalanine derived pathway by the activity of hydroxy-cinnamoyl: CoA ligase (CHCL) (Karwatzki *et al.* 1989).

Tyrosine derived pathway: The first step of the tyrosine derived pathway is catalysed by tyrosine aminotransferase (TAT) and involves the transamination of tyrosine with the

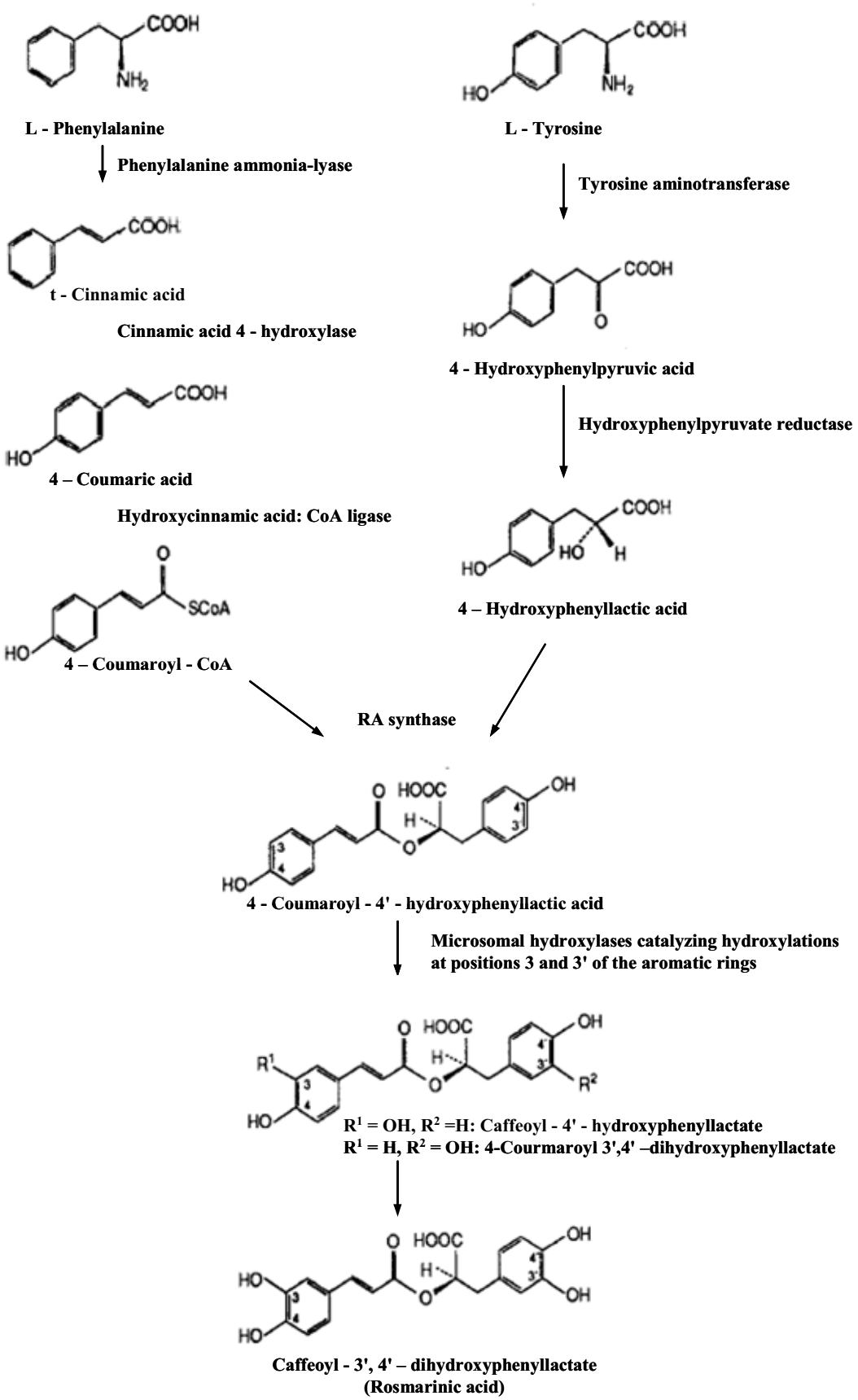

Fig. 3 Biosynthetic pathway for rosmarinic acid.

help of 2-oxoglutarate giving rise to 4-hydroxyphenylpyruvate (pHPP) and glutamate. Tyrosine aminotransferase (TAT) has been purified from *Anchusa officinalis* cell cultures (de Eknamkul and Ellis 1987). pHPP is further metabolized by hydroxyphenylpyruvate reductase (HPPR) to 4-hydroxyphenyllactate (pHPL) with help of NAD(P)H (Petersen and Alfermann 1988; Häusler *et al.* 1991; Meinhard 1991). HPPR has been characterized by Hausler *et al.* (1991) from suspension cultures of *C. blumei*. Kim *et al.* (2004) reported a more advanced purification method of this enzyme which eventually led to the isolation of a full length cDNA clone coding for a protein with HPPR activity from *C. blumei.* Kim and Petersen (2002) cloned a full-length cDNA of hydroxyphenylpyruvate dioxygenase (HPPD) from cell suspension cultures of *C. blumei.* HPPD transforms 4-hydroxyphenylpyruvate to homogenistate and therefore, com-

petes for the same substrate as HPPR, an enzyme of RA biosynthesis from *C. blumei*.

Ester formation: The enzyme catalyzing the formation of the ester from two precursors synthesized from phenylalanine and tyrosine is rosmarinic acid synthase (RAS) which transfers the hydroxycinnamoyl moiety from hydroxycinnamoyl-CoA to the aliphatic hydroxyl group of hydroxyphenyllactate. RAS was first isolated and described from cell cultures of *C. blumei* (Petersen and Alfermann 1988). The natural substrates of this enzyme presumably are 4-coumaroyl-CoA and 4-hydroxyphenyllactate resulting in the first ester, 4-coumaroyl-4'-hydroxyphenyllactate (pC-pHPL). Therefore, the systematic name of RAS (hydroxylcinnamoyl-CoA:hydroxyphenyl-acetate hydroxycinnamoyltransferase) would be 4-couma-royl-CoA:4-hydroxyphenylactate 4-coumaroyl-transferase (Petersen 1991). Berger *et al.* (2006) reported a modified method to purify RAS from suspension cells of *Coleus blumei* Benth. (Lamiaceae) and then is the first report of the cDNA-cloning of RAS which eventually led to the identification of peptide sequences, cloning of a corresponding cDNA by a PCR-based approach and expression of active RAS. 4-coumaroyl-4′-hydroxyphenylacetate is afterwards hydroxylated in positions 3 and 3′ of the aromatic rings by membrane bound cytochrome P450-dependent hydroxylases, i.e hydroxycinnamoyl hydroxyphenylactate 3- and 3′- hydroxylases.

RA is readily soluble in alcoholic solvents such as methanol and ethanol, but also to a limited extent in water. Small-scale isolation is usually performed by disrupting dried *Coleus* cells in mixture of methanol or ethanol and water by boiling or sonication (Razzaque and Ellis 1977; Ellis *et al.* 1979; Petersen and Alfermann 1988). According to Ulbrich *et al.* (1985), RA can easily be extracted with ether or ethyl acetate from acidic aqueous phases. For large-scale isolation of RA from bioreactor-grown cell cultures, hot water extraction (pH 3, 80°C) of the harvested cells is performed. The extract was then applied to an Amberlite XAD-2 column which was washed with water. RA was eluted from the column with 40% methanol in water, the eluate evaporated to dryness, and redissolved in water. RA crystals with a purity of about 95% could be harvested from this solution (Ulbrich *et al.* 1985).

Determination of RA can be performed by a variety of methods, e.g. TLC, HPLC, spectrophotometry, and gas chromatography. TLC is mainly used for the qualitative determination of RA, and several systems of stationary and mobile phases are described (Scarpati and Oriente 1958; Hiller 1965; Ellis and Towers 1970; Hanefeld and Herrmann 1976; Ellis *et al.* 1979; de Eknamkul and Ellis 1984; Fukui *et al.* 1984; Gracza and Ruff 1984; Winterhoff *et al.* 1988; Banthorpe *et al.* 1989; Sumaryono *et al.* 1991b). RA can be detected by its blue fluorescence under UV or by several other detection reagents. However, TLC is not commonly used for the quantitation of RA. A very easy method for the quantitative determination of RA is spectrophotometry (Hiller 1965; Razzaque and Ellis 1977; de Eknamkul and Ellis 1984; Ellis 1985, Lopez-Arnaldos *et al.* 1995), since RA in ethanolic solutions absorbs strongly at 333 nm. HPLC is a rapid, easy, and sensitive method for the quantitative determination of RA. Several systems of columns and isocratic or gradient elution have been described (Fukui *et al.* 1984; Gracza and Ruff, 1984; Whitaker *et al.* 1984; Gracza *et al.* 1985; Ulbrich *et al.* 1985; Verotta 1985; Adzet *et al.* 1988a, 1988b; Petersen and Alfermann 1988; Reuff *et al.* 1988; de Tommasi *et al.* 1991; Hippolyte *et al.* 1991; Sumaryono *et al.* 1991a, 1991b). Guedon and Pasquier (1994) developed a reversed phase HPLC method for qualitative and quantitative analysis of RA in leaves of *Mentha* × *pipertia*. Usually reversed-phase columns are used together with solvent systems containing acetonitrile or methanol and water. To elute RA in the fully protonated form, acids such as phosphoric acid or acetic acid are added in order to lower the pH of the solvents to about 3. Depending on the complexity of the mixtures in which RA has to be determined, isocratic or gradient elutions are used. GC determination (Reschke 1983) of RA needs derivatization and is therefore not commonly used.

Exarchou *et al.* (2001) described a combination of advanced nuclear magnetic resonance (NMR) methodologies for the analysis of complex phenolic mixtures with particular emphasis on caffeic acid and its ester derivative, RA. The combination of variable-temperature two dimensional proton-proton double quantum filter correlation spectroscopy (^{1}H-^{1}H DQF COSY) and proton-carbon heteronuclear multiple quantum coherence (^{1}H-^{13}C HMQC) gradient NMR spectroscopy allowed the identification and tentative quantification of caffeic acid and RA at 243 K in extracts from plants of the *Lamiaceae* family, without resorting to previous chromatographic separation of the components. Quantitative results obtained in this study were in reasonable agreement with reverse phase HPLC measurements.

In vitro production of RA

C. blumei as such is not used for isolation of RA, but cell cultures of this plant have been extensively used for commercial production of RA (Ulbrich *et al.* 1985). **Table 5** summarizes literature on *in vitro* production of RA from *C. blumei*. Suspension cultures from this species were the first ones used for an attempted biotechnological production of RA (Razzaque and Ellis 1977; Zenk *et al.* 1977). These authors used B_5 medium (Gamborg and Eveleigh 1968; Gamborg *et al.* 1968) for the establishment of callus and suspension cultures. The basal medium was supplemented with 1 mg/l 2,4-D and 0.1 mg/l KN (Razzaque and Ellis 1977). Zenk *et al.* (1977) studied the effect of addition of 35 differently substituted phenoxyacetic acids (10^{-5} M) on RA production and found that 2,4-D had the highest effect by promoting the production of RA by 40%. The amount of RA accumulated by suspension cultures of *C. blumei* is highly dependent on the amount of carbohydrates added to the culture media (Zenk *et al.* 1977; Ulbrich *et al.* 1985; Petersen and Alfermann 1988; Gertlowski and Petersen 1993). According to Zenk *et al.* (1977) highest RA concentrations were found in medium with 7% sucrose. This positive effect of high sucrose concentrations on the accumulation of RA was exploited by Ulbrich *et al.* (1985) who established a two-phase culture system for the production of RA by cell cultures of *C. blumei* in bioreactors. In the growth phase, the suspension cultures produced biomass but nearly no RA in a modified B5-medium with 2% sucrose. The cells when transferred to production medium consisting of a 5% sucrose solution, started to accumulate RA but grew only slowly. With this system, the authors were able to isolate about 100g of RA (purity 97%) from 32 L of production medium after 8 days in the growth medium followed by a production period of 11 days. The RA content of the *C. blumei* cells reached 21% of the dry weight. Petersen and Alfermann (1988) observed RA content of up to 19% of the cell dry weight were found in *C. blumei* suspension cultures grown in medium with 4% sucrose. Petersen and Alfermann (1988) also used similar system for the maintenance of *C. blumei* suspension cultures and RA synthesis. B5-medium with 2% sucrose was used for routine subculturing. According to Yokoyama and Yanagi (1991) cell suspension cultures of *C. blumei* range among the highest producing plant cell cultures with respect to secondary product formation since they reported an accumulation of RA up to 21% of the cell dry weight in cell cultures of *C. blumei*. In contrast to these studies de Eknamkul and Ellis (1988) have shown that RA synthesis by cultures of *Anchusa officinalis* and another strain of *C. blumei* do not depend on medium sugar content. In these cultures, highest accumulation of RA was reached with 3% sucrose in the medium. Glucose, fructose and equimolar mixtures of these two sugars had the same effect on RA accumulation in heterotrophic *A. officinalis* cultures as sucrose.

Petersen (1991) reported the accumulation of RA up to 19% of cell dry weight from cell cultures of *C. blumei* grown in medium with 4% sucrose. The effect of increased

Table 5 Summary of literature on *in vitro* production of rosmarinic acid from *C. blumei*.

Source	Medium	Rosmarinic acid content	Reference
Cell suspension culture	B_5 + 2,4-D 1 mgl^{-1} + 0.1 mgl^{-1} KN	8-11%	Razzaque and Ellis 1977
Suspension culture	B_5	3.6 gl^{-1}	Zenk *et al*. 1977
Suspension cells	B_5	100 mg	Ulbrich *et al*. 1985
Hairy root culture	B5 + MeJA (0.01 mM)	9.5 mg per flask	
Suspension culture	CB_2- medium with 2% sucrose	20 mg /l	Petersen and Alfermann 1988
Suspension culture	B_5 + 2% sucrose; B_5 + 5% sucrose	2 $mgml^{-1}$; 12%	Gertlowski and Petersen 1993
Suspension culture	B_5 medium with 0.1% DMSO and 40 g l^{-1} sucrose	1.0 gl^{-1}	Martinez and Park 1993
Suspension culture	CB_2- medium with 2% sucrose	20 mgl^{-1}	Petersen *et al*. 1995
Suspension culture	CB_2- medium with 2% sucrose	-	Petersen 1997
Suspension culture	CB_2- medium with 2% sucrose	2.1%	Szabo *et al*. 1999
Transformed callus culture	MS medium	11%	Bauer *et al*. 2004

2,4-D = 2,4-dichlorophenoxyacetic acid; DMSO = dimethyl sulfoxide; MeJA= methyl jasmonate; KN = kinetin

sucrose concentrations on RA accumulation was also elucidated by Gertlowski and Petersen (1993) in a suspension culture of *C. blumei.* They observed that sucrose had highest stimulating effect on growth and RA accumulation, followed by glucose and fructose. An increase in concentration of sucrose from 1 to 5% resulted in an increasing positive effect on growth and RA synthesis in the cell cultures with a maximum RA content (12% of the dry weight) in medium with 5% sucrose. However, RA accumulation obviously did not reach its highest level at 6% sucrose. The start of RA synthesis by the cell cultures seemed to be regulated by the growth limitation when a nutrient, e.g. phosphate was depleted from the medium. The rate of RA accumulation was related to the amount of carbon left in the medium when growth ceased. The depletion of phosphate from the medium on day 4 to 5 of the culture period coincided with the start of RA synthesis and accumulation. This is in contrast to the results obtained for reproducing cell cultures of *A. officinalis* (de Eknamkul and Ellis 1988), which showed that other nutrients may influence the onset of RA production as well. Gertolwski and Petersen (1993) further demonstrated that equimolar amounts of glucose and fructose, 1 or 2% of each, as well as 2 or 4% glucose or fructose separately did not have the same effect on the acumulation of RA as sucrose. The differences were more prominent in media with 4% total sugar concentration. Glucose alone sustained about the same RA accumulation in the cells as sucrose, but dry weight accumulation was lower. A mixture of glucose and fructose resulted in lower growth and RA content and the lowest yield was observed in medium with fructose alone. This is in contrast to results of Ulbrich *et al.* (1985) who also tested the effect of different sugars on growth and RA accumulation of suspension cultures of *C. blumei.* They reported higher RA yields in medium with fructose than with glucose. However, in this experiment also sucrose sustained the highest RA production. Petersen *et al.* (1994) also reported an accumulation of very high amounts of RA from suspension cultures of *C. blumei* cultured in medium with elevated sucrose concentrations.

C. blumei cultures preconditioned with 0.1% dimethyl sulfoxide (DMSO) can release secondary metabolites with viability maintenance when permeabilized by higher DMSO concentrations of 0.5-1.5% (Park and Martinez 1992). This finding opened a new possibility of continuous plant cell culture with simultaneous removal of secondary metabolites in an immobilized cell, trickle bed reactor, which had been used for alcohol production by microbial cells. Martinez and Park (1993) analyzed the effect of sucrose concentration on cell viability, cell growth, RA production, sugar consumption, product yield, and cell yield of preconditioned and non-preconditioned *C. blumei* in a suspension culture. They also suggested a possible strategy of sugar feeding in an immobilized cell, trickle bed reactor. The study revealed that batch suspension cultures of *C. blumei* cells preconditioned with 0.1% DMSO maintained viability at above 85% during a 14-day culturing period for initial sucrose concentrations of 30-70 g/l. At 60 g/l sucrose, cells grew rapidly with a doubling time of 10.7 h, and dry cell mass reached a maximum of 15.6 g/l. Broad optima of cell yield and RA were observed at 40-60 g/l sucrose concentration. Sucrose in the medium was hydrolyzed to glucose and fructose by extracellular and/or cell-bound invertase, and glucose was taken up by the cells at a higher rate than fructose. Even at higher concentrations of sucrose (40-70 g/l) used, sucrose consumption over 14 days did not exceed 33 g/l.

Several authors showed that RA accumulation can furthermore be enhanced by the addition of fungal elicitors (e.g. yeast extract (YE), preparations from *Pythium aphanidermatum*) or methyl jasmonate (MeJA). This suggests that RA could have a role in plants as a defence compound against pathogens and herbivores. Szabo *et al.* (1999) observed an approximately three-fold enhanced accumulation of RA in suspension cultures of *C. blumei* treated with either an elicitor preparation from the culture medium of the phytopathogenic oomycete *P. aphanidermatum* or with MeJA. The specific activities of phenylalanine ammonia lyase and RAS were also enhanced after addition of the fungal elicitor. The addition of MeJA transiently increased activities of phenylalanine ammonia lyase and hydroxyphenylpyruvate reductase, whereas the activity of RAS was not stimulated and the activity of tyrosine aminotransferase was slightly and constantly enhanced. MeJA stimulated RA accumulation not only when added directly to the culture medium, but also when it could reach the cells only via the gas phase.

The production and biosynthetic regulation of RA had also been studied in suspension cells of *A. officinalis* (Mizukami and Ellis 1991), *Lithospermum erythrorhizon* (Mizukami *et al.* 1993), hornwort *Anthoceros agnestis* (Vogelsang *et al.* 2006), root cultures of *Salvia officinalis* (Hippolyte *et al.* 1992), *Ocimum basilicum* (Tada *et al.* 1996) and *Heliotropium peruvianum* (Motoyama *et al.* 1996). Morimoto *et al.* (1994) studied RA production in callus tissue and regenerated plants of *Salvia microrrhiza* and reported higher concentration of RA in 15-week-old micropropagated plants. It has been reported that RA biosynthesis was affected by various elicitors in a number of plant species e.g. RA production was induced by MeJA in *C. blumei* (Szabo *et al.* 1999) and *L. erythrorhizon* (Mizukami *et al.* 1993) cell suspension cultures, by acetylated salicylic acid (SA) in *Origanum vulgare* tissue cultures (Andarwulan and Shetty 1999), by YE in *Agastache rugosa* cell suspension cultures (Kim *et al.* 2001), *S. miltiorrhiza* hairy root cultures (Chen *et al.* 2001), *L. erythrorhizon* (Mizukami *et al.* 1992), *Lavandula vera* (Georgiev *et al.* 2006) cell suspension cultures and *Orthosiphon aristatus* (Sumaryono *et al.* 1991a) cell suspension cultures and by vanadyl sulphate in *Lavandula vera* cell suspension cultures (Georgiev *et al.* 2006). In contrast to these studies RA content has been shown to be reduced by YE in *Salvia miltiorrhiza* cell suspension cultures (Chen and Chen 2000a, 2000b).

Yang and Shetty (1998) reported the stimulation of RA biosynthesis in oregano (*O. vulgare*) clonal line O-1 in response to proline, proline precursors (ornithine and arginine), and proline analogue (azetidine-2-carboxylate, A2C). Exogenous treatment with proline and proline precursors in the

presence or absence of proline analogue A2C, significantly enhanced RA content. Concurrently higher levels of endogenous proline were observed compared to control. Analogue (A2C) treatment alone stimulated highest levels of RA without any increase in endogenous proline. The stimulation of RA synthesis in response to proline or proline precursors with or without A2C suggested that deregulation and enhancement of proline synthesis or proline oxidation may be important for RA biosynthesis in oregano. This stimulation of RA biosynthesis provided strong clues that proline synthesis may be linked to stimulation of the pentose phosphate pathway, driving key precursor metabolites toward shikimate and phenylpropanoid pathways. RA-stimulating compounds also enhanced total phenolics and hardened stem tissues, indicating possible lignification due to polymerization of phenolic metabolites. In the medium used for maintenance of the cell cultures with only 2% sucrose, RA content remained at a basic level of about 2% of cell dry weight. The effect of YE as a fungal elicitor or of MeJA on the accumulation of RA has been described for cell cultures of *Orthosiphon aristatus* (Sumaryono *et al.* 1991a; Sumaryono and Proksch 1993) and *Lithospermum erythrorhizon* (Mizukami *et al.* 1992, 1993). YE enhanced RA accumulation up to 10-fold in cell cultures of *O. aristatus* independent of the growth stage. Highest RA levels were reached 72–96 h after addition of YE to the cell cultures. In cell cultures of *L. erythrorhizon*, RA accumulation was stimulated two- to three-fold by YE, with maximum levels 24 h after addition. The addition of 100 mM MeJA led to a ten-fold stimulation of RA ac-cumulation in *Lithospermum* cells after 48–72 h.

Kintzios *et al.* (2003) reported that leaf derived suspension cultures of sweet basil, *O. basilicum* L. accumulated RA up to 10 mg g^{-1} dry wt, a value up to 11 times higher than in callus cultures or in leaves of donor plants. Mohagheghzadeh *et al.* (2004) obtained RA from *Zataria multiflora* tops extract. Various *in vitro* cultures were established on MS or Modified Tobacco (MT) medium containing growth hormones. The results indicated that cultures of *Z. multiflora* biosynthesize RA (55-355 mg/100 g dry wt.) and the higest accumulation were reached on MT media containing NAA 2 mg/l. Rady and Nazif (2005) determined RA content of the *in vitro* produced plants of *Ocimum americanum*. They observed that MS medium with BA at a concentration of 1 mg/l and 0.25 mg/l IAA supported maximum RA production in plants produced from cultures grown on that medium. Li *et al.* (2005) examined the growth and RA production by *C. forskohlii* hairy root cultures in various liquid media. The hairy root cultures showed good growth in hormone-free MS medium containing 3% (w/v) sucrose (MS medium), and Gamborg B5 medium containing 2% (w/v) sucrose (B5 medium). RA yield reached 4.0 mg (MS medium) and 4.4 mg (B5 medium) after 5 weeks of culture in a 100 ml flask containing 20 ml of each medium. Hairy root growth and RA were also investigated after treatment with various concentrations of YE, SA and MeJA. RA production in a 100 ml flask containing 20 ml B5 medium reached 5.4 mg (1.9 times more than control) with treatment of 0.01 or 1% (w/v) YE, and 5.5 mg (2.0 times more than control) with treatment of 0.1 mM SA. The maximum RA content of 9.5 mg per flask (3.4 times more than control) was obtained in the hairy roots treated with 0.1 mM MeJA. These results suggested that MeJA is an effective elicitor for production of RA in *C. forskohlii* hairy root cultures.

FUTURE PERSPECTIVES

Coleus species have long been exploited for their aesthetic and medicinal values. The ever increasing demands of different *Coleus* species, especially *C. blumei* and *C. forskohlii*, for different purposes has necessitated our immediate concern for production of disease free plants, rapid multiplication of elite clones and faster introduction of novel cultivars with desirable traits. In this regard, *in vitro* propagation procedures should be suitable for conservation and large-scale commercial cultivation of *Coleus* species. At present, many reproducible protocols for *in vitro* propagation of *Coleus* species are available but the new challenges that should be addressed include cost efficiency, automation and control and optimization of microenvironment. It is, therefore, important to bring about further improvements in the existing tissue culture protocols. *Coleus* species are known to produce interesting secondary compounds e.g. *C. blumei* synthesizes rosmarinic acid (RA) and *C. forskohlii* produces forskolin. Attempts have been made to use plant tissue culture techniques for the production of forskolin and RA. Both these compounds are known for a number of promising biological and pharmacological activities. There is a need to undertake systematic studies on cell suspension cultures and transformed root cultures to enhance the production of therapeutically important forskolin and RA by employing a variety of elicitors, optimization of microenvironment and somaclonal variations coupled with *in vitro* induced mutagenesis. The study of secondary metabolism in plant cell cultures is a challenging field with many opportunities ahead. New tools of functional genomics combined with metabolomics and proteomics will revolutionize our knowledge on pathways and enzymes involved in the synthesis of natural products.

REFERENCES

Adachi H, Ehata S, Hayashi T (1996) Anti aging cosmetics containing *Coleus forskohlii* root extracts and antioxidants. Patent-Japan Kokai Tokkyo Koho 08176-57

Adachi H, Hayashi T (1996) Extraction of forskolin from *Coleus forskohlii* roots and tocotrienol from Alzheimer's disease. Patent- Japan Kokai Tokkyo Koho 08217-681

Adzet T, Canigueral S, Iglesias J (1988a) A chromaticgraphic survey of polyphenols from *Salvia* species. *Biochemical Systematics and Ecology* **16**, 29-32

Adzet T, Vila R, Canigurel S (1988b) Chromatographic analysis of polyphenols of some Iberian *Thymus*. *Journal of Ethnopharmacology* **24**,147-154

Agarwal KC, Parks RE Jr. (1982) Synergistic inhibition of platelet regeneration by fraskoin plus PGE or 2- fluoroadenosonie: effects of 2'5'-dideoxyadenosine and 5- methylthioadenosine. *Biochemical Pharmacology* **31**, 3713-3716

Agarwal KC, Parks RE Jr. (1983) Forskolin: a potential antimetastatic agent. *International Journal of Cancer* **32**, 801-804

Alasbahi RH, Safiyeva S, Craker LE (1999) Antimicrobial activity of some Yemeni medicinal plants. *Journal of Herbs*, *Spices and Medicinal Plants* **6**, 75-83

Allemann J, Laurie SM, Thiart S, Vorster HJ (2004) Sustainable production of root and tuber crops (potato, sweet potato, indigenous potato, cassava) in Southern Africa. *South African Journal of Botany* **70**, 60-66

Ammon HP, Muller AB (1985) Forskolin: from an Ayurvedic remedy to a modern agent. *Planta Medica* **6**, 473-477

Anbazhagan K, Kumar NS, Hemavathi V, Sathyanarayana BN (2005) *In vitro* morphogenesis in *Coleus forskohlii*. *Journal of Medicinal and Aromatic Plant Science* **27**, 253-256

Andarwulan N, Shetty K (1999) Influence of acetyl salicylicacid in combination with fish protein hydrolyzates on hyperhydricity reduction and phenolic synthesis in *Origanum vulgare* tissue cultures. *Journal of Food Biochemistry* **23**, 619-635

Asada Y, Li W, Terada T, Yoshikawa T, Sasaki K, Hayashi T, Shimomura K (2000) Biosynthesis of forskolin. In: *Proceedings of the 120th Annual Meeting of the Pharmaceutical Society of Japan*, Gifu, Japan

Asamenew MT, Narayanaswamy P (2000) Induction of callus and plant regeneration in *Coleus forskohlii* Briq. *Journal of Applied Horticulture* **2**, 25-27

Asamenew MT, Narayanaswamy P (2004) *In vitro* shoot multiplication and rooting in *Coleus forskohlii* Briq. *Crop Science Society of Ethopia* **F01**, 126-134

Asolkar IV, Kakkar KK, Chakre OJ (1992) *Second supplement to Glossary of Indian Medicinal Plants with Active Principles. Part I (A-K) (1956-1981)*, Publication and Information Directorate (CSIR), New Delhi, pp 18-20

Baerts M, Lehmann J (1989) Guérisseurs et plantes médicinales de la région des Zaire-Nil au Burundi. Musée Roy. De l'Afrique Centrale Tervuren, Belgique. *Annales des Sciences Economiques* **18**, 214-215

Bajrovic K, Kniewald Z, Vorkapic-Furac J (1993) Stimulation of *Coleus blumei* Benth. callus growth by 1-(6-purinyl)-2,5-dimethylpyrrole. *Pharmazie* **48**, 787-788

Banthorpe DV, Bilyard HJ, Brown GD (1989) Enol esters of caffeic acid in several genera of the Labiateae. *Phytochemistry* **28**, 2109-2113

Baslas RK, Kumar P (1981) Phytochemical studies of the plants of *Coleus* genera. *Herba Hungarica* **20**, 213-221

Bauer N, Leljak-Levanic D, Mihaljevic S, Jelaska S (2002) Genetic transfor-

mation of *Coleus blumei* Benth. using *Agrobacterium*. *Food Technology and Biotechnology* **40**, 163-169

Bauer N, Leljak-Levanic D, Jelaska S (2004) Rosmarinic acid synthesis in transformed callus culture of *Coleus blumei* Benth. *Zeitschrift für Naturforschung C* **59**, 554-560

Berger A, Meinhard J, Petersen B (2006) Rosmarinic acid synthase is a new member of the superfamily of BAHD acyltransferases. *Planta* **224**, 1503-1510

Bejoy M, Vincent KA, Hariharan M (1990) *In vitro* shoot regeneration of *Coleus parviflorus* Benth. *Indian Journal of Plant Physiology* **33**, 175-176

Bentham G (1832-1836) VIII. *Coleus*. In: *Labiatarum Genera et Species*, Ridgeway London, pp 47-59

Bhat SV, Bajwa BS, Dornaueur H, de Souza NJ, Fehlhaber HW (1977) Structures and stereochemistry of new labdane diterpenoids from *Coleus forskohlii* Briq. *Tetrahedron Letters* **19**, 1669-1672

Bhattacharya R, Bhattacharya S (2001) In *vitro* multiplication of *Coleus forskohlii* Briq: an approach towards shortening the protocol. *In Vitro Cellular and Developmental Biology – Plant* **37**, 572-575

Blume KL (1826) *Bijdragen tot de Flora van Nederlandsch Indie*, Lands Drukkerij, Batavia, 730 pp

Boily Y, van Puyvelde L (1986) Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. *Journal of Ethnopharmacology* **16**, 1-13

Bos R, Hendriks H, van Os FH (1983) The composition of the essential oil in the leaves of *Coleus aromaticus* Bentham and their importance as a component of the species antiaphthosae. *Pharmaceutisch Weekblad Scientific Edition* **5**, 129-130

Bouquet A (1969) *Félicheurs et médicines traditionnelles du Congo (Brazzaville)*. Orstom, Paris, 282 pp

Bristow MR, Ginsburg R, Strosberg A, Montgomery W, Minobe W (1984) Pharmacology and inotropic potential of forskolin in the human heart. *The Journal of Clinical Investigation* **74**, 212-223

Brown R (1810) *Prodomus florae novae Hollandiae et Insulae van-Diemen*, Johnson, London, 590 pp

Bruka JF (1983) Effects of selected bronchodilators on antigen and A23187 induced contraction of guinea pig trachea. *The Journal of Pharmacology and Experimental Therapeutics* **225**, 427-435

Bult H, Herman AG, Rampart M (1985) Modification of endotoxin- induced haemodynamic and haematological changes in the rabbit by methylprednisolone, $F(ab')_2$ fragments and rosmarinic acid. *British Journal of Pharmacology* **84**, 317-327

Burkill HM (1995) *The Useful Plants of West Tropical Africa*, Royal Botanic Gardens, Kew, UK, 857 pp

Burstein NR, Sears ML, Mead A (1984) Aqueous flow in human eyes is reduced by forskolin, a potent adenylate cyclase activator. *Experimental Eye Research* **39**, 745-749

Camara CC, Nascimento NR, Macedo-Filho CL, Almeida FB, Fonteles MC (2003) Antispasmodic effect of the essential oil of *Plectranthus barbatus* and some major constituents on the guinea-pig ileum. *Planta Medica* **69**, 1080-1085

Caprioli J, Sears M (1983) Forskolin lowers intraocular pressure in rabbits, monkeys and man. *The Lancet* **30**, 958-960

Caprioli J, Sears M, Bausher L, Gregory D, Mead A (1984) Forskolin lowers intra-ocular pressure by reducing aqueous inflow. *Investigative Ophthalmology and visual Science* **25**, 268-277

Castillo RAM, Gonzalez VP (1999) *Plectranthus amboinicus* (Lour.) Spreng. *Revista Cubana de Plantas Medicinales* **3**, 110-115

Chen H, Chen F (2000a) Effect of yeast elicitor on the secondary metabolism of Ti-transformed *Salvia miltiorrhiza* cell suspension cultures. *Plant Cell Reports* **19**, 710-717

Chen H, Chen F (2000b) Effect of yeast elicitor on the growth and secondary metabolism of a high-tanshinone producing line of the Ti-transformed *Salvia miltiorrhiza* cells in suspension cultures. *Process Biochemistry* **35**, 837-840

Chen H, ChenF, Chiu FCK, Lo CMY (2001) The effect of yeast elicitor on the growth and secondary metabolism of hairy root cultures of *Salvia miltiorrhiza*. *Enzyme and Microbial Technology* **28**, 100-105

Chifundera K (2001) Contribution to the inventory of medicinal plants from the Bushi area, South Kivu Province, Democratic Republic of Congo. *Fitoterapia* **72**, 351-368

Christenson JT, Thulesius O, Nazzal MM (1995) The effect of forskolin on blood flow, platelet metabolism, aggregation and ATP release. *Vasa* **24**, 56-61

Chunekar KC (1960) *Bhav Prakasa Nighantu*, Varanasi: Hindi Translation, 372 pp

Collins JC, Ibrahim KM, Collin HA (1990) *In vitro* selection for salt tolerance in *Coleus blumei*. *Acta Horticulturae* **280**, 431-434

Comer AE, Leonardo L (1981) Phloem differentiation in *Coleus* pith explants. *Physiologia Plantarum* **51**, 130-132

Corey EJ, Jardine PDS, Rohloff JC (1988) Total synthesis of (+/-) – forskolin. *Journal of the American Chemical Society* **110**, 3672-3673

Cos P, Hermans N, de Bruyne T, Apers S, Sindambiwe JB, Berghe DV, Pieters L, Vlietinck AJ (2002) Further evaluation of Rwandan medicinal plant extracts for their antimicrobial and antiviral activities. *Journal of Ethnopharmacology* **79**, 155-163

CSIR (1992) *The Useful Plants of India*, Publication and Information Directorate, Council of Scientific and Industrial Research, New Delhi, 316 pp

d'Amelio FS (1999) *Botanicals: A Phytocosmetic Desk Reference*, CRC Press, London 361 pp

de Eknamkul W, Ellis BE (1984) Rosmarinic acid production and growth characteristic of *Anchusa officinalis* cell suspension cultures. *Planta Medica* **51**, 346-350

de Eknamkul W, Ellis BE (1987) Purification and characterization of tyrosine aminotransferase activities from *Anchusa officinalis* cell cultures. *Archives of Biochemistry and Biophysics* **257**, 430-438

de Eknamkul W, Ellis BE (1988) Rosmarinic acid: production in plant cell cultures. In: Bajaj YPS (Ed) *Biotechnology in Agriculture and Forestry (Vol 4) Medicinal and Aromatic Plants*, Springer-Verlag, Berlin, pp 310-329

de Loureiro J (1790) *Flora Cochinchinensis* (Vol 2), Academiae Ulyssipone, Lisbon, 72 pp

Deena MJ, Sreeranjini K, Thoppil JE (2002) Antimicrobial screening of essential oils of *Coleus aromaticus* and *Coleus zeylanicus*. *The International Journal of Aromatherapy* **12**, 105-107

Deighton N, Glidewell SM, Deans SG, Goodman BA (1993) Identification by EPR spectroscopy of carvacrol and thymol as the major sources of free radicals in the oxidation of plant essential oils. *Journal of the Science of Food and Agriculture* **63**, 221-225

Delpech B, Calvo D, Lett R (1996) Total synthesis of forskolin. Part I. *Tetrahedron Letters* **37**, 1015-1018

de Souza NJ, Shah V (1988) Forskolin – an adenylate cyclase activating drug from an Indian herb. In: Wagner H, Shah V, Farnsworth NR (Eds) *Economic and Medicinal Plant Research* (Vol 2), *Academic Press*, London, pp 1-16

de Souza NJ (1993) Industrial development of traditional drugs: the forskolin example. A mini-review. *Journal of Ethnopharmacology* **38**, 177-180

de Tommasi N, de Simone F, de Feco V, Pizza C (1991) Phenylpropanoid glycosides and rosmarinic acid from *Momordica balsamina*. *Planta Medica* **57**, 201

Dhawan V, Bhojwani SS (1986) Micropropagation in crop plants. *Glimpses in Plant Research* **7**, 1-75

Ding X, Staudinger J (2005) Induction of drug metabolism by forskolin, the role of the pregnane X receptor and the PKA signal transduction pathway. *The Journal of Pharmacology and Experimental Therapeutics* **312**, 849-856

Dohadwalla AN (1986) Biological activities of forskolin. In: Rupp RH, de Souza NJ, Dohadwalla AN (Eds) *Forskolin: its Chemical*, *Biological and Medicinal Potential*, Hoechst India, Bombay, pp 19-30

Dubey CB, Srimal RC, Tandon JS (1997) Clinical evaluation of ethanolic extract of *Coleus forskohlii* in hypertensive patients. *Sachitra Ayurveda* **49**, 931-936

Dubey MP, Srimal RC, Nityanand S, Dhawan BN (1981) Pharmacological study of coleonol, a hypotensive diterpine from *Coleus forskohlii*. *Journal of Ethnopharmacology* **3**, 1-13

Ellis BE (1985) Charcterization of clonal cultures of *Anchusa officinalis* derived from single cells of known productivity. *Journal of Plant Physiology* **119**, 149-158

Ellis BE, Remmen S, Goeree G (1979) Interactions between parallel pathways during biosynthesis of rosmarinic acid in cell suspension cultures of *Coleus blumei*. *Planta* **147**, 163-167

Ellis BE, Towers GHN (1970) Biogenes of rosmarinic acid in Mentha. *Biochemical Journal* **118**, 291-297

Engelberger W, Hadding V, Etschenber E, Graf E, Leyck S, Windelmann J, Parnham MJ (1988) Rosmarinic acid: a new inhibitor of complement C3 convertase with anti-inflammatory activity. *International Journal of Immunopharmacology* **10**, 729-737

Engprasert S, Taura F, Kawamukai, Shoyama Y (2004) Molecular cloning, expression and characterization of recombinant 1-deoxy-D5-phosphate reductoisomerase from *coleus forskohlii* Brig. *Plant Science* **169**, 287-294

Everett TH (1981) *The New York Botanical Garden Illustrated*. *Encyclopedia of Horticulture* (Vol 3), Garland, New York, pp 824-827

Exarchou V, Troganis A, Gerothanassis IP, Tsimidou M, Boskou D (2001) Identification and quantification of caffeic and rosmarinic acid in complex plant extracts by the use of variable-temperature two-dimensional nuclear magnetic resonance spectroscopy. *Journal of Agriculture and Food Chemistry* **49**, 2-8

Fischman LA, Skorupa LA, Souccar C, Lapa AJ (1991) The water extract of *Coleus barbatus* Benth decreases gastric secretion in rats. *Memórias do Instituto Oswaldo Cruz* **2**, 141-143

Frankel EN, Huang SW, Aeschbach R, Prior E (1996) Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *Journal of Agriculture and Food Chemistry* **44**, 131-135

Fukui H, Yazaki K, Tabata M (1984) Two phenolic acids from *Lithospermum erythrosizon* cell suspension cultures. *Phytochemistry* **23**, 2398-2399

Gamborg OL, Eveleigh DE (1968) Culture methods and detection of glucalases in suspension cultures of wheat and barley. *Canadian Journal of Biochemistry* **46**, 417-421

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soyabean root cells. *Experimental Cell Reserch* **50**, 151-158

Ganguli BN (1986) Microbial transformation of forskolin derivatives. In: Rupp

RH, de Souza NJ, Dohadwalla AN (Eds) *Forskolin: its Chemical*, *Biological and Medicinal Potential*, Hoechst India, Bombay, pp 31-38
Gardener A (1985) *Coleus blumei*. *Florsit* **8**, 285-286
Georgiev M, Kuzeva S, Pavlov A, Kovacheva E, Ilieva M (2006) Enhanced Rosmarinic acid production by *Lavandula vera* MM cell suspension culture through elicitation with vanadyl sulfate. *Zeitschrift für Naturforschung* **61**, 241-244
Gertlowski C, Petersen M (1993) Influence of the carbon source on growth and rosmarinic acid production in suspension cultures of *Coleus blumei*. *Plant Cell, Tissue and Organ Culture* **34**, 183-190
Githinji CW, Kokwaro JO (1993) Ethnomedicinal study of major species in the family Labiatae from Kenya. *Journal of Ethnopharmacology* **39**, 197-203
Gracza L, Ruff P (1984) Occurrence and analysis of phenyl propane derivatives in medicinal plants rosmarinic acid in drugs of Pharmacopias and its determination by HPLC. *Archiv der Pharmazie* **317**, 339-345
Gracza L, Koch H, Loffler E (1985) Isolierung von Rosmarinsaure aus Symphytum officinale und ihre anti-inflammatorische Wirksamkeit in einem *in vitro*-Modell. *Archiv der Pharmazie* **318**, 1090-1095
Guedon DJ, Pasquier BP (1994) Analysis and distribution of flavonoid glycosides and rosmarinic acid in 40 *Mentha* × *piperita* clones. *Journal of Agriculture and Food Chemistry* **42**, 679-684
Gupta PP, Srimal RC, Tandon JS (1993) Antiallergic activity of some traditional Indian medicinal plants. *International Journal of Pharmacognosy* **31**, 15-18
Haberlandt G (1902) Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. *Acad Wiss Wien Math Naturwiss* **111**, 69-92
Hamill FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD, (2003) Traditional herbal drugs of Southern Uganda (II). Literature analysis and antimicrobial assays. *Journal of Ethnopharmacology* **84**, 57-78
Han LK, Morimoto C, Yu R H, Okuda H (2005) Effects of *Coleus forskohlii* on fat storage in ovariectomized rats. *Yakugaku Zasshi* **125**, 449-53
Hanefeld M, Herrmann K (1976) Quantitative determination of the caffeic acid esters and catechins by direct measurement on thin layer chromatograms. *Journal of Chromatography* **123**, 391-395
Harsha VH, Hebbar SS, Shripathi V, Hedge GR (2003) Ethnomedicobotany of Uttara Kannada District in Karnataka, India; plants in treatment of skin diseases. *Journal of Ethnopharmacology* **84**, 37-40
Hausler E, Peterson M, Alfermann AW (1991) Hydroxyphenylpyurvate reductase from cell suspension cultures of *Coleus blumei* Benth. *Zeitschrift für Naturforschung* **46**, 371-376
Hausler E, Petersen M, Alfermann AW (1992) Rosmarinsaure. In: In: Haschke HP, Schnarrenberger C (Eds) *Blechnum Spezies*, Botanikertagung, Akademie Verlag, Berlin, 507 pp
Hervey A, Robins WJ (1978) Development of plants from leaf discs of variegated *Coleus* and its relation to patterns of leaf chlorosis. *In Vitro Cellular and Developmental Biology – Plant* **14**, 294-300
Hiller K (1965) Zur Kenntnis der inhaltsstoffe einiger Saniculoideae, 1. Mitteilung: *Sanicula europaea* L. Isolierung quantitative Erfassung von Chlorogen- und Rosmarinsaure. *Pharmazie* **20**, 574-579
Hippolyte I, Martin B, Baccou JC, Jonard R (1991) Influence of maintenance medium and sucrose concentration on the rosmarinic acid production by cell suspension culture of wise (*Salvia officinalis* L.) *Records of the Academy of Sciences* **313**, 365-371
Hippolyte I, Marin B, Baccou JC, Jonard R (1992) Growth and rosmarinic acid production in cell suspension cultures of *Salvia officinalis* L. *Plant Cell Reports* **11**, 109-112
Ho CT, Ferraro T, Chen Q, Rosen RT, Huang MT (1994) Phytochemicals in teas and rosemary and their cancer preventive properties. In: Ho CT, Osawa T, Huang MT, Rosen RT (Eds) *Food Phytochemicals II: Teas*, *Spices*, *and Herbs*, American Chemical Society, Washington, pp 2-19
Holdsworth D (1977) *Medicinal plants of Papua New Guinea*, S. Pacific Commission Technical Paper 175. Noumea, New Caledonia, 123 pp
Hulme MM (1954) *Wild Flowers of Natal*, Shulter & Shooter, Pietermaritzburg, 34 pp
Ibrahim KM, Collins HA, Collin JC (1990) Selection for salt tolerance in regenerating cultures of *coleus blumei*. *Acta Horticulturae* **280**, 425-430
Ibrahim KM, Collins JC, Collin HA (1992) Characterization of progeny of *Coleus blumei* following an *in vitro* selection for salt tolerance. *Plant Cell, Tissue and Organ Culture* **28**, 139-145
Inamdar PK, Khandelwal Y, Garkhedkar M, Rupp RH, de Souza NJ (1989) Identification of microbial transformation products of 1,9 dideoxyforskolin and 7-deacetyl-1, 9-dideoxyforskolin. *Planta Medica* **55**, 386-387
Kansal CM, Srivastava SP, Dube CB, Tandon JS (1978) Clinical evaluation of *Coleus forskohlii* on hypertension. *Nagarjun* **22**, 56-58
Karwatzki B, Peterson M, Alfermann AW (1989) Transient activity of enzymes involved in the biosynthesis of rosmarinic acid in cell suspension cultures of *Coleus blumei*. *Planta Medica* **55**, 663-664
Khory NR, Katrak NN (1999) *Materia Medica of India and their Therapeutics*, BDH Printers, New Delhi, 380 pp
Kim KH, Janiak M, Petersen M (2004) Purification, cloning and functional expression of hydroxyphenylpyruvate reductase involved in rosmarinic acid biosynthesis in cell cultures of *Coleus blumei. Plant Molecular Biology* **54**, 311-323
Kim KH, Oh SR, Lee HK, Huh H (2001) Benzothiadiazole enhances the elicitation of rosmarinic acid production in a suspension culture of *Agastache rugosa* O. Kuntze. *Biotechnology Letters* **23**, 55-60
Kim KH, Petersen M (2002) c-DNA cloning and functional expression of hydroxyphenylpyruvate dioxygenase from cell suspension cultures of *Coleus blumei*. *Plant Science* **163**, 1001-1009
Kintzios S, Makri O, Panagiotopoulos E, Scapeti M (2003) *In vitro* rosmarinic acid accumulation in sweet basil (*Ocimum basilicum* L.). *Biotechnology Letters* **25**, 405-408
Kokwaro JO (1993) *Medicinal Plants of East Africa* (2nd Edn), Kenya Literature Bureau, Nairobi, 118 pp
Kreutner W, Chapman RW, Gulbenkian A, Tozzi S (1985) Bronchodilator and antiallergy activity of forskolin. *European Journal of Pharmacology* **111**, 1-8
Krombholz R, Mersinger R, Kreis W, Reinhard E (1992) Production of forskolin by axenic *Coleus forskohlii* roots cultivated in shake flasks and 20-l glass jar bioreactors. *Planta Medica* **58**, 328-333
Kukreja AK, Garg S (2007) *Agrobacterium rhizogenes* mediated hairy roots in *Coleus forskohlii* – A natural source of labdane diterpenoid (forskolin). In: Trivedi PC (Ed) *Biotechnology – Current Perspectives and Potential Applications*, Avishkar Publications, Distributors, Jaipur, pp 298-346
Kumaran A, Karunakaran RJ (2006) Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chemistry* **97**, 109-114
Kupchan SM, Hemingway RJ, Smith RM (1969) Tumor inhibitors. XLV. Crotepoxide, a novel cyclohexane diepoxide tumor inhibitor from *Croton macrostachys*. *Journal of Organic Chemistry* **34**, 3898-902
Kusumoto IT, Nakabayashi T, Kida H, Miyashiro H, Hattori M, Namba T, Shimotohno K (1995) Screening of various plant extracts used in Ayurvedic medicine for inhibitory effects on Human Immunodeficiency Virus Type 1 (HIV-1) Protease. *Phytotherapy Research* **9**, 180-184
Lamaison JL, Carnat AP, Carnat A (1987) Differentiation of peppermint, *Mentha piperita* Mitcham and Hungary types, grown in Auvergne. *Plant Medicine and Phytotheraphy* **21**, 252-261
Lamaison JL, Petitjean-Freytet C, Carnat A (1990) Rosmarinic acid, total hydroxycinnamic derivatives and antioxidant activity of Apiaceae, Boraginaceae and Lamiaceae medicinals. *Annales Pharmaceutiques Francaises* **48**, 103-108
Lans C (2007) Ethnomedicines used in Trinidad and Tobago for reproductive problems. *Journal of Ethnobiology and Ethnomedicine* **3**, 1-12
Laurenza A, McHugh Sutkowski E, Seamon KB (1989) Forskolin: a specific stimulator of adenylyl cyclase or a diterpene with multiple sites of action? *Trends in Pharmacological Sciences* **10**, 442-447
Lebowitz RJ (1985) The genetics and breeding of *Coleus*. *Plant Breeding Review* **3**, 343-360
Li W, Koike K, Asada Y, Yoshikawa T, Nikaido T (2005) Rosmarinic acid production by *Coleus forskohlii* hairy root cultures. *Plant Cell, Tissue and Organ Culture* **80**, 151-155
Lichey J, Friedrich T, Priesnitz M, Biamino G, Ushinger P, Huckauf H (1984) Effect of forskolin on methacholine-induced bronchoconstriction in extrinsic asthmatics. *Lancet* **21**, 167
Lindner E, Dohadwalla AN, Bhattacharya BK (1978) Positive inotropic and blood pressure lowering activity of a diterpene derivative isolated from *Coleus forskohlii*: Forskolin. *Arzneimittel Forschung* **28**, 284-289
Linnaeus C (1763) *Species Plantarum*, Impensis Direct, Laurentii Salvii, Stockholm, 580 pp
Litvinenko VI, Popova TP, Simonjan AV, Zoz IG, Sokolov VS (1975) "Gerbstoffe" und Oxyzimtsa¨ ureabko¨ mmlinge in Labiaten. *Planta Medica* **27**, 372-380
López-Arnaldos T, López-Serrano M, Rós Barceló A, Calderon AA, Zapata JM (1995) Spectrophotometric determination of rosmarinic acid in plant cell cultures by complexation with Fe^{2+} ions. *Fresen Journal of Analytical Chemistry* **351**, 311-314
Lukhoba CW, Simmonds MS, Paton AJ (2006) Plectranthus: a review of ethnobotanical uses. *Journal of Ethnopharmacology* **103**, 1-24
Madsen HL, Bertelsen G (1995) Spices as Antioxidants. *Trends in Food Science and Technology* **6**, 271-276
Majeed M, Badmaey V, Rajendran R (1998) Method of preparing a forskolin composition from forskolin extract and use of forskolin for promoting lean body mass and treating mood disorders. US Patent No. US5,804,596
Mandler-Henger A (1988) *Somaclonale variation bei Coleus forskohlii*. Doctoral thesis, University of Tubingen, 129 pp
Marcotrigiano M, Boyle TH, Morgan PA, Ambach KL (1990) Leaf color variants from *Coleus* shoot cultures. *Journal of the American Society for Horticultural Science* **115**, 681-686
Martin C (1985) Plant breeding *in vitro*. *Endeavour* **9**, 81-86
Martinez BC, Park CH (1993) Characteristic of batch suspension cultures of preconditioned *Coleus blumei* cells: sucrose effect. *Biotechnology Progress* **9**, 97-100
Mei SX, Jiang B, Niu XM (2002) Abietane diterpenoids from *Coleus xanthanthus*. *Journal of Natural Products* **65**, 633-637
Meinhard EJ (1991) Versuche zur Reinigung der Hydroxyphenylpyruvat-

reduktase aus Zellkulturen von *Coleus blumei*. Diploma Thesis, University of Düsseldorf, Germany, 173 pp

Mersinger R, Dornauer H, Reinhard E (1988) Formation of forskolin by suspension cultures of *Coleus forskohlii*. *Planta Medica* **54**, 200-204

Metzger H, Lindner E (1981) The positive inotropic-acting forskolin, a potent adenylate cyclase activator. *Arzneimittel Forschung* **31**, 1248-1250

Mizukami H, Ellis BE (1991) Rosmarinic acid formation and differential expression of tyrosine aminotransferase isoforms in *Anchusa officinalis* cell suspension cultures. *Plant Cell Reports* **10**, 321-324

Mizukami H, Tabira Y, Ellis BE (1993) Methyl jasmonate-induced rosmarinic acid biosynthesis in *Lithospermum erythrorhizon* cell suspension cultures. *Plant Cell Reports* **12**, 706-709

Mizukami H, Ogawa T, Ohashi H, Ellis BE (1992) Induction of rosmarinic acid biosynthesis in *Lithospermum erythrorhizon* cell suspension cultures by yeast extract. *Plant Cell Reports* **11**, 480-483

Molgaard P, Ravn H (1988) Evolutionary aspects of caffeoyl ester distribution in dicotyledons. *Phytochemistry* **27**, 2411-2421

Morimoto S, Goto Y, Shoyama Y (1994) Production of Lithospermic acid B and Rosmarinic Acid in callus tissue and regenerated plantlets of *Salvia miltiorrhiza*. *Journal of Natural Products* **57**, 817-823

Morton JF (1992) Country borage (*Coleus ambonicus* Lour.): A potent flavoring and medicinal plant. *Journal of Herbs*, *Spices and Medicinal Plants* **1**, 77-90

Motoyama E, Tada H, Shimomura K, Yoshihira K, Ishi-maru K (1996) Caffeic acid ester in tissue cultures of *Haeliotropium pervianum*. *Plant Tissue Culture Letters* **13**, 73-74

Mohagheghzadeh A, Shams-ardakami M, Ghannadi A, Mnaeiam M (2004) Rosmarinic acid from *Zataria multiflora* tops and *in vitro* cultures. *Fitoterapia* **75**, 315-321

Mpiana PT, Tshibanigu DST, Shetonda OM, Ngbolua KN (2007) *In vitro* antidrepanocytary activity (anti sickle cell anemia) of some Congolese plants. *Phytomedicine* **14**, 192-195

Mukherjee S, Ghosh B, Jha S (1996) Forskolin synthesis in *in vitro* cultures of *Coleus forskohlii* Briq. transformed with *Agrobacterium tumefacien*s. *Plant Cell Reports* **15**, 691-694

Mukherjee S, Ghosh B, Jha S (2000) Establishment of forskolin yielding transformed cell suspension cultures of *Coleus forskohlii* as controlled by different factors. *Journal of Biotechnology* **76**, 73-81

Mukherjee S, Ghosh B, Jha S (2003) Higher production of forskolin in genetically transformed cultures of *Coleus forskohlii* Briq. induced by growth regulators. *Journal of Plant Biochemistry and Biotechnol*ogy **12**, 81-85

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Nadkarni SR, Akut PM, Ganguli BN, Khandelwal Y, de Souza NJ, Rupp RH, Fehlhaber H (1986) Microbial transformation of 1,9-dideoxyforskolin to forskolin. *Tetrahedron Letters* **27**, 5265-5268

Narayanan P, Laddha KS, Akamanchi KG (2002) Histochemical localization of forskolin and other terpenoids in *Coleus forskohlii*. *Current Science* **83**, 945-946

Neuwinger HD (2000) *African Traditional Medicine*. *A Dictionary of Plant Use and Applications*, Medpharm Scientific Publishers, Stuttgart, pp 406-408

Pabsch K, Petersen M, Rao NN, Alfermann AW, Wandrey C (1991) Chemoenzymatic synthesis of rosmarinic acid. *Recueil des Travaux Chimiques des Pays-Bas* **110**, 199-205

Park CH, Martinez BC (1992) Enhanced Release of Rosmarinic Acid from *Coleus blumei* cells permeabilized by dimethyl sulfoxide (DMSO) while preserving cell viability and growth. *Biotechnology and Bioengineering* **40**, 459-464

Parnham MJ, Kesselring K (1985) Rosmarinic acid. *Drugs of the Future* **10**, 756-757

Paulus EF (1980a) Molecular and Crystal structure of 1-benzyl-7 bromoisobutyryl forskolin. Absolute configuration of forskolin. *Zeitschrift für Kristallographie* **153**, 43-49

Paulus EF (1980b) Molecular and crystal structure of forskolin. Absolute configuration of forskolin. *Zeitschrift für Kristallographie* **152**, 239-245

Peake PW, Pussel BA, Martyn P, Timmermans V, Charlesworth JA (1991) The inhibitory effect of rosmarinic acid on complement involves the C5 convertase. *International Journal of Immunopharmacology* **13**, 853-857

Petersen M (1991) Characterization of rosmarinic acid synthase from cell cultures of *Coleus blumei*. *Phytochemistry* **30**, 2877-2881

Petersen M (1994) *Coleus* spp: *In vitro* culture and the production of forskolin and rosmarinic acid. In: Bajaj YPS (Ed) *Biotehnology in Agriculture and Forestry (Vol 26) Medicinal and Aromatic Plants VI*, Springer-Verlag, Berlin, pp 69-92

Petersen M (1997) Cytochrome P-450-dependent hydroxylation in the biosynthesis of rosmarinic acid in *Coleus*. *Phytochemistry* **45**, 1165-1172

Petersen M, Alfermann AW (1988) Two new enzymes of rosmarinic acid biosynthesis from cell cultures of *Coleus blumei*: hydroxyphenylpyurvate reductase and rosmarinic acid synthase. *Zeitschrift für Naturforschung – C* **43**, 501-504

Petersen M, Hausler E, Karwatzki B, Meinhard J (1993) Proposed biosynthetic pathway for rosmarinic acid in cell cultures of *Coleus blumei* Benth. *Planta* **189**, 10-14

Petersen M, Hausler, E, Meinhard J, Karwatzk B, Gertlowski C (1994) The biosynthesis of rosmarinic acid in suspension cultures of *Coleus blumei*. *Plant Cell, Tissue and Organ Culture* **38**, 171-179

Petersen M, Simmonds MSJ (2003) Molecules of interest; Rosmarinic acid. *Phytochemistry* **62**, 121-125

Petersen M, Szabo E, Meinhard J, Karwatzki B, Gertlowski C, Kempin B, Fuss E (1995) Biosynthesis and accumulation of rosmarinic acid in suspension cultures of *Coleus blumei*. *Plant Cell, Tissue and Organ Culture* **43**, 89-92

Rajasekharan PE, Ambika SR, Ganeshan S (2005) *In vitro* conservation of *Coleus forskohlii* – an endangered medicinal plant. *Jounal of Plant Biotechnology* **7**, 135-141

Rajendran A, Rao NR, Ravikumar K, Henry AN (1999) Some medicinal and aromatic labiates from the Peninsular India. *Journal of Non-Timber Forest Products* **6**, 26-30

Rady MR, Nazif NM (2005) Rosmarinic acid content and RAPD analysis of *in vitro* regenerated basil (*Ocimum americanum*) plants. *Fitoterapia* **76**, 525-533

Rani G, Talwar D, Nagpal A, Virk GS (2006) Micropropagation of *Coleus blumei* Benth. from nodal segments and shoot tips. *Biologia Plantarum* **50**, 495-500

Rao BSS, Shanbhoge R, Upadhya D, Jagetia GC, Adiga SK, Kumar P, Guruprasad K, Gayathri P (2006) Antioxidant, anticlastogenic and radioprotective effect of *Coleus aromaticus* on Chinese hamster fibroblast cells (V79) exposed to gamma radiation. *Mutagenesis* **21**, 237-242

Ravn H, Pedersen MF, Andary J, Borum C, Anthoni U, Christophersen C, Nielsen PH (1994) Seasonal variation and distribution of two phenolic compounds, rosmarinic acid and caffeic acid, in leaves and roots-rhizomes of eelgrass (*Zostera marina* L.). *Ophelia* **40**, 51-61

Razzaque A, Ellis BE (1977) Rosmarinic acid production in *Coleus* cell cultures. *Planta* **137**, 287-291

Reddy PS, Rodrigues R, Rajasekharan R (2001) Shoot organogenesis and mass propagation of *Coleus forskohlii* from leaf derived callus. *Plant Cell, Tissue and Organ Culture* **66**, 183-188

Reddy PS, Mamatha M, Rudrabhatla P, Rajasekharan R (2007) Importance of threonine residues in the regulation of peanut serine/threonine/tyrosine protein kinase activity. *Plant Science* **172**, 1054-1059

Reuff I, Seitz U, Ulbrich B, Reinhard E (1988) Cryopreservation of *Coleus blumei* suspension and callus cultures. *Journal of Plant Physiology* **133**, 414-418

Reschke A (1983) Capillargaschromatographische bestimmung der Rosmarinsaure in Blattgewurzen. *Zeitschrift für Lebensmitteluntersuchung und –forschung* **176**, 116-119

Rupp RH, de Souza NJ, Dohadwalla AN (Eds) (1986) *Proceedings of the International Symposium on Forskolin*, Bombay, pp 19-30

Rwangabo PC (1993) *La Médicine Traditionnelle au Rwanda*, Edition Karthala, ACCT, 258 pp

Sailo GL, Bagyaraj DJ (2005) Influence of different AM-fungi on the growth, nutrition and forskolin content of *Coleus forskohlii*. *Mycological Research* **109**, 795-798

Sakata R, Shoyama Y, Murakami H (1994) Production of monoclonal antibodies and enzyme immunoassay for typical adenylate cyclase activator, forskolin. *Cytotechnology* **16**, 101-108

Saksena AK, Green MJ, Shue HJ, Wong JK, McPhail AT (1985) Identity of colenol with forskolin: structure revision of a base catalysed rearrangement product. *Tetrahedron Letters* **26**, 551-554

Saleem AM, Dhasan PB, Rafiullah MR (2005) Isolation of forskolin from stem of *Coleus forskohlii*. *Pharmacognosy Magazine* **1**, 89-92

Saleem AM, Dhasan PB, Rafiullah MR (2006) Simple and rapid method for the isolation of forskolin from *Coleus forskohlii* by charcoal column chromatography *Journal of Chromatography* **1101**, 313-314

Sasaki K, Udagawa A, Ishimaru H, Hayashi T, Alfermann AW, Nakanishi F, Shimomura K (1998) High forskolin production in hairy roots of *Coleus forskohlii*. *Plant Cell Reports* **17**, 457-459

Scarmuzzi F, Craca A, Apollonio G (1990) *In vitro* propagation of two *Coleus blumei* Benth. varieties from crops and sub crops of vegetative fragments. *Flortecnica* **14**, 17-26

Scarpati ML, Oriente G (1958) Isolamento e costituzione dell acido rosmarinico (dal *Rosmarinus officinalis*). *Rice Science* **28**, 2329-2333

Scarpati ML, Oriente G (1960) Costituzione sterochimica dell acido β (3, 4-diossifenil) α-lattico dal *Rosmarinus officinalis*. *Rice Science* **30**, 225-229

Schaneberg T, Khan A (2003) Quantitative analysis of forskolin in *Coleus forskohlii* (Lamiaceae) by reversed-phase liquid chromatography. *Journal of AOAC International* **86**, 467-470

Schultze MJ (Ed) (1986) Rudolf Mansfelds Verzeichnis landwritschaftlicher and gartnerischer Kulturpflanzen (ohne Zierpflanzen), Band 3. Akademic-Verlag, Berlin, 272-332

Seamon KB, Daly JW (1986) Forskolin: Its biological and chemical properties. *Advances in Cyclic Nucleotide and Protein Phosphorylation Research* **20**, 1-150

Seamon KB, Padgett W, Daly JW (1981) Forskolin: Unique diterpene activator of adenylate cyclase in membranes and in intact cells. *Proceedings of the National Academy of Sciences USA* **78**, 3363-3367

Sen J, Sharma AK (1991) *In vitro* propagation of *Coleus forskohlii* Briq. for forskolin synthesis. *Plant Cell Reports* **9**, 696-698

Sen J, Sharma AK, Sahu NP, Mahato SB (1992) Prodution of forskolin in *in vitro* cultures of *Coleus forskohlii*. *Planta Medica* **58**, 324-327

Shah V (1998) *Coleus forskohlii* (Willd.) Briq. – an overview. In: Handa SS, Kaul MK (Eds) *Supplement to Cultivation and Utilization of Medicinal Plants*, Vedams Academic Books, New Delhi, pp 20-24

Shah V, Bhat SV, Bajwa BS, Dornauer H, de Souza NJ (1980) The occurrence of forskolin in the Labiateae. *Planta Medica* **39**, 183-185

Shan Y, Xu L, Lu Y, Wang X, Zheng Q, Kong L, Niwa M (2008) Diterpenes from *Coleus forskohlii* (WILLD.) BRIQ. (Labiatae). *Chemical and Pharmaceutical Bulletin* **56**, 52-56

Sharma N, Chandel KPS, Srivastava VK (1991) *In vitro* propagation of *Coleus forskholii* Briq. a threatened medicinal plant. *Plant Cell Reports* **10**, 67-70

Sharma PV (1956) *Dravya Guna Vigyan* (Parts II and III) Varanasi, India, 273 pp

Shen YH, Xu YL (2005) Two new diterpenoids from *Coleus forskohlii*. *The Journal of Asian Natural Products Research* **7**, 811-815

Shetty K, Ohshima M, Murakami T, Oosawa K, Ohashi Y (1997) Transgenic melon (*Cucumis melo* L.) and potential for expression of novel proteins important to food industry. *Food Biotechnology* **11**, 111-128

Shoyama Y (1993) Production and application of monoclonal antibodies against diterpenoids. *Bioscience India*. **51**, 27-29

Siebert A, Vosa A (1896) *Vilmorin's Blumenengartnerei*, Paul Parey, Berlin, 120 p

Singh MP (1986) *Flora of Patna*, International Books and Periodicals Supply Service, New Delhi, 343 pp

Singh S, Painuly P, Tandon JS (1984) Ditrepenes from *Coleus forskohlii*. Stereochemistry from carbonyl chromophore. *Indian Journal of Chemistry* **23**, 952-955

Smith RH (1984) Developmental potential of excised primordial and expanding leaves of *Coleus blumei* Benth. *American Journal of Botany* **71**, 1114-1120

Srinivasan M, Nachiappan V, Rajasekharan R (2006) Potential application of urea-derived herbicides as cytokinins in plant tissue culture. *Journal of Bioscience* **31**, 1-7

Srivastava SK, Chaubey M, Khatoon S, Rawat AKS, Mehrotra S (2002) Pharmacognostic evaluation of *Coleus forskohlii*. *Pharmaceutical Biology* **40**, 129-134

Steward AN (1958) *Vascular Plants of the Lower Yangtze Valley, China*, Oregon State College, Corvallis, OR, 621 pp

Sumaryono W, Proksch P (1993) *Orthosiphon aristatus* (Java tea): *in vitro* culture and elicitation of rosmarinic acid in cell suspensions. In: Bajaj YPS (Ed) *Biotechnology in Agriculture and Forestry (Vol 26) Medicinal and Aromatic Plants* VI. Springer, Berlin, pp 287-299

Sumaryono W, Proksch P, Hartmann T, Nimtz M, Wray V (1991a) Induction of rosmarinic acid accumulation in cell suspension cultures of *Orthosiphon aristatus* after treatment with yeast extract. *Phytochemistry* **30**, 3267-3271

Sumaryono W, Proksch P, Wray V, White L, Hartmann T (1991b) Qualitative and quantitative analysis of the phenolic constituents from *Orthosiphon aristatus*. *Planta Medica* **57**, 176-180

Suryanayanan M, Pai JS (1998) Studies in micropropagation of *Coleus forskohlii*. *Journal of Medicinal and Aromatic Plant Sciences* **20**, 379-382

Szabo E, Thelen A, Petersen M (1999) Fungal elicitor preparations and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei. Plant Cell Reports* **18**, 485-489

Tada H, Murakami Y, Omoto T, Shimomura K, Ishimaru K (1996) Rosmarinic acid and related phenolics in transformed root cultures of *Ocimum basilicum*. *Phytochemistry* **42**, 431-434

Takeda R, Hasegawa J, Sinozaki K (1990) Phenolic compounds from Anthocerotae. In: Zinsmeister HD, Mues R (Eds) *Bryophytes; Their Chemistry and Chemical Taxonomy*. *Proceedings of the Phytochemical Society of Europe* (Vol 29), Oxford Science Publication, Oxford, pp 201-207

Tandon JS, Dhar MM, Ramakumar S, Venkatesan K (1977) Structure of coleonol, a biologically active diterpene from *Coleus forskohlii*. *Indian Journal of Chemistry* **15**, 880-883

Tripathi CKM, Basu SK, Jain S, Tandon JS (1995) Production of coleonol (forskolin) by root callus of plant *Coleus forskohlii*. *Biotechnology Letters* **17**, 423-426

Trivedi A, Mehrotra BN, Tandon R, Jain GK (1982) Estimation of colenol from *Coleus forskohlii* Briq. *Indian Journal of Pharmaceutical Science* **44**, 157-158

Udagawa A, Adachi H, Ishimaru, Hayashi T (1996) Antisenescence agent of *Coleus*. *Japan Kokai Tokkyo Koho*, JP 96,183,792 (CL.CO 7G/100), Appl. 94/340, 577,28, Dec. 1994 7 pp. Chemical Abstract 125, 190958 p

Ulbrich B, Weisner W, Arens H (1985) Large-scale production of rosmarinic acid from plant cell cultures of *Coleus blumei* Benth. In: Neumann KH, Barz W, Reinhard E (Eds) *Primary and Secondary Metabolism of Plant Cell Cultures*, Springer, Berlin, pp 293-303

Uphof JCT (1959) Dictionary of economic plants 191. Wheldon and Whesley, Codicote, 400 pp

Valdes LJ, Mislankar SG, Paul AG (1987) *Coleus barbatus* (*C. forskohlii*) (Lamiaceae) and the potential new drug forskolin (coleonol). *Economic Botany* **41**, 474-483

van Puyvelde L, Ntawukiliyayo JD, Portaels F, Hakizamungu E (1994) *In vitro* inhibition of mycobacteria by Rwandese medicinal plants. *Phytotherapy Research* **8**, 62-69

Vasishta PC (1994) *Embryology and Taxonomy of Angiosperms*, S. Chand & Co. Ltd, 884 pp

Verotta L (1985) Isolation and HPLC determination of the active principle of *Rosmarinus officinalis* and *Gentiana lutea*. *Fitoterapia* **56**, 25-29

Vishwakarma RA, Tyagi BR, Ahmed B, Husain A (1988) Variation in forskolin content in the roots of *Coleus forskohlii*. *Planta Medica* **54**, 471-472

Vogelsang, K, Schneider B, Petersen M (2006) production of rosmarinic acid and a new rosmarinic acid 3′–*O*-β-D-glucoside in suspension cultures of the hornwort *Anthoceros agrestis* Paton. *Planta* **223**, 369-373

Vlietinck AJ, van Hoof L, Totte J, Lasure A, van den Berghe D, Rwangabo PC, Mvukiyumwami J (1995) Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *Journal of Ethnopharmacology* **46**, 31-47

Whitaker RJ, Hashimoto T, Evans DA (1984) Production of the Secondary metabolite, rosmarinic acid by plant cell suspension culture. *Annals of the New York Academy of Sciences* **435**, 364-366

White PR (1963) *The Cultivation of Animal and Plant Cells*, The Ronald Press, New York, pp 57-63

Winterhoff H, Gumbinger HG, Sourgens H (1988) On the antigonadotropic activity of *Lithospermum* and *Lycopus* species and some of their phenolic constituents. *Planta Medica* **54**, 101-106

Wolbling RH, Leonhardt K (1994) Local therapy of herpes simplex with dried extracts from *Melissa officinalis*. *Phytomedicine* **1**, 25-31

Xu LL, Kong LY (2006) Labdane diterpenoids from *Coleus forskohlii* (Wild.) Briq. *Journal of Integrative Plant Biology* **48**, 478-481

Yanagihara H, Sakata R, Shoyama Y, Murakami H (1995) Relationship between the content of forskolin and growth environments in clonally propagated *Coleus forskohlii* Briq. *Biotronics* **24**, 1-6

Yanagihara H, Sakata R, Shoyama Y, Murakami H (1996) Rapid analysis of small samples containing forskolin using monoclonal antibodies. *Planta Medica* **62**, 169-172

Yang QR, Wu HZ, Wang XM, Zou GA, Liu YW (2006) Three new diterpenoids from *Coleus forskohlii* Briq. *The Journal of Asian Natural Products Research* **8**, 355-360

Yang R, Shetty K (1998) Stimulation of rosmarinic acid in shoot cultures of oregano (*Origanum vulgare*) clonal line in response to proline, proline analogue, and proline precursors**.** *Journal of Agriculture and Food Chemistry* **46**, 2888-2893

Yoganarasimhan SN (2000) *Medicinal Plants of India*. In: Srinivasan V, Kosal Ram N (Eds) Tamil Nadu. Cyber Media, Bangalore, p 2

Yokoyama M, Yanagi M (1991) High-level production of arbutin by biotransformation. In: Komamine A, Misawa M, Di Cosmo F (Eds) *Plant Cell Culture in Japan*, CMC Co., Ltd., Tokyo, pp 79-91

Zagrajski N, Leljak-Levanić D, Jelaska S (1997) Organogenesis and callogenesis in nodal, internodal and leaf explants of *Coleus blumei* Benth. *Periodicum Biologorum* **99**, 67-76

Zelnik R, Lavic D, Levy EC, Wang AHJ, Paul IC (1977) Barbatusin and cyclobutatusin. Two novel diterpenoids from *Coleus barbatus*. *Tetrahedron* **33**, 1457-1467

Zenk MH, El-Shagi H, Ulbrich B (1977) Production of rosmarinic acid by cell-suspension cultures of *Coleus blumei*. *Naturwissenschaften* **64**, 585-586

Ziegler FE, Jaynes BH, Saindane MT (1987) A synthetic route to forskolin. *Journal of the American Chemical Society* **109**, 8115- 8116

Zinsmeister HD, Becker H, Eicher T (1991) Moose, eine Quelle biologisch aktiver Naturstoffe. *Angew Chemie* **103**, 134-151