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MEDICINAL USES, CHEMICAL CONSTITUENTS AND MICRO PROPAGATION OF THREE POTENTIAL MEDICINAL PLANTS

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ABSTRACT

In recent years herbal medicines and extracts have gained renewed interest for several reasons; affordability, low pricing, no side effects, solutions for chronic diseases and disorders, time tested remedies, preventive approaches, etc. Enhanced market demands have posed threats to phytoresources due to unscrupulous mode of collections. There is an urgent need to conserve genetic diversity of medicinal plant resources by developing protocols for micropropagation. Plant cell, tissue, organ culture techniques offer an integrated approach for rapid multiplication and production of material with dependable active ingredients. Phytopharmacutical properties and protocols for the mass production of three medicinal plants form the bases for the present study. These are *Cissus quadrangularis* (Vitaceae), *Tridax procumbens* and *Verbesina encelioides* (Asteraceae).

Key Words: Regeneration, micropropagation, phytopharmaceuticals, *C. quadrangularis, T. procumbens, V. encelioides,* medicinal plant species

1. INTRODUCTION

Since times immemorial human beings have endeavored for good health and immortality.
Yoga (techniques to elevate the physical and mental status of an individual), and Ayurveda (use of medicaments to maintain health, longevity and vitality) have played a pivotal role. Plants have been used as an important source of medicine since ancient times and their products are being used for different purposes such as medicine, food, health care, agriculture, agrochemicals, pharmaceutical, etc.

Initially practiced as ethnomedicine then transformed into organized systems e.g. Ayurveda, Unani, Siddha, etc. (Natesh, 2001). Plants were used in crude form or even essence. With the advances made in phytochemistry and pharmacology, umpteen active principles of various medicinal plants were isolated and used as valuable drugs in contemporary medicine (Trivedi, 2004).

According to World Health Organization (WHO), nearly 80 per cent of the people in developing countries consume traditional medicines for sustaining health and vitality. According to one estimate 20,000 to 35,000 species of plants are used as medicines, pharmaceuticals, cosmetics and neutraceuticals by different ethnic groups the entire world over (Trivedi, 2006). The medicinal properties of different plant species have contributed to the origin and evolution of many traditional herbal therapies. Attempts have been made to categorize them as plants used in organized systems of medicine e.g. Unani, Sidha, Ayurveda (plant sp of codified knowledge) and include nearly 1500 sp. As many as three thousand species are used as ethnomedicine (plants of empirical knowledge) and nearly 700 species are researched pharmacologically and chemically. In most of these species active principles are exploited in modern

medicines and are referred to as plants of scientific knowledge.

Medicinal plants are an integral component of research and development in the pharmaceutical industry. They constitute nearly 70 % of the basis of modern pharmaceutical products including 25 % of drugs derived from different plants and many others are synthetic analogues built on prototype compounds isolated from them. A large number of drugs of plant's origin, used in western medicine are Digitoxin, L-Dopa, Quinine, vincaleukoblastin, digoxin, ajmalicine, codeine, reserpine, pilocarpine, atropine, morphine, etc. WHO estimated that approximately one fourth of the 500 million prescriptions written in US each year contain a mention of leafy plant extracts or active ingredients obtained from or modeled on plant substances. The most popular analgesic, aspirin, was originally derived from species of *Salix* and *Spiraea* and some of the most valuable anti-cancer agents such as paclitaxel and vinblastine are derived solely from plant sources (Katzung et al.1995. Pezuto et al.1996). Table 1 mentions some important plant species with their mode of action.

Table 1. ***Some plant species with their mode of action.***

Sr. no.	Plant species	Action
1.	*Abrus precatorius*	Aphrodisiac
2.	*Achyranthes aspera*	Bronchial infection
3.	*Adhatoda vasica*	Spasmolytic
4.	*Agrimonia eupatoria*	Anthelmintic
5.	*Aloe barbedensis*	Purgative
6.	*Ammi visnaga*	Antiasthmatic
7.	*Anabasis aphylla*	Antismoking, myorelaxant
8.	*Angelica polymorpha*	Sedative
9.	*Artemisia absinthium*	Vermicide
10.	*Artemisia annua*	Ascaricide
11.	*Aspidosperma quebr.* Blanco	Respirostimulant
12.	*Atropa belladonna*	Blocking of cholinergic
13.	*Baccharis sp.*	Anti-inflammatoric
14.	*Boerhaavia diffusa*	Diuretic
15.	*Caesalpinia crista*	Antipyretic
16.	*Calotropis procera*	Diaphoretic
17.	*Cassia fistula*	Laxative
18.	*Centella asiatica*	Diuretic
19.	*Closemma amosata*	Flavouring
20.	*Daphne genkwa*	ecbolic, antitumor
21.	*Datura stramonium*	hallucinogenic
22.	*Drimys winteri*	Fungicide
23.	*Drosera sp.*	Proteolytic
24.	*Eclipta alba*	hepatoprotective
25.	*Euphorbia antiquorum*	Anthelmintic
26.	*Gnmaphalium sp.*	Anti-inflammatoric
27.	*Marsdenia condurango*	Bitter substances
28.	*Neurolaena lobata*	Anti-malarial
29.	*Petoveria alliaceae*	Immunostimulant
30.	*Phyllanthus niruri*	Antiseptic
31.	*Psidium guajava*	Spasmolytic

32.	*Siphonichilus natalensis*	Anti-malarial
33.	*Urginea sp.*	Cardioactive
34.	*Heliotropium indicum*	antitumor, hypotensive
35.	*Hemsleya amabilis*	anti dysenteric, antipyretic
36.	*Holarrhena anlidysenterica*	amebicide, anesthetic, hypotensive, vasodilator
37.	*Mentha spicata*	rubefacient anesthetic
38.	*Montanoa* spp	Contraceptive
39.	*Phyllanthus* spp.	Antitumor
40.	*Platycodon grandiflorum*	Amtlgesic, antitussive
41.	*Salix alba*	Analgesic
42.	*Taxus brevi/olia*	Antitumor

2. GLOBAL SALES OF MEDICINAL PLANTS

Estimates of volume and value of herbal products, which include medicines, health supplements, herbal beauty and toiletry products vary widely globally. According to Phytopharm Consulting the global sales of herbal products in 1996 was US $ 14 billion and demand for herbal products has been growing at the rate of 10-15 % per annum. It touched around US $ 62 billion. The global herbal product markets are mainly in Europe, Japan, France, Asia and North America which together accounted for 63% of the world market. Europe annually, imports about 400,000 tones of medicinal plants with an average market value of US $ 1 billion from Africa and Asia. China has attained a top position in the export of traditional medicine to the world market. A report by the Export-Impost Bank of India has estimated that Global market of medicinal plants trade was around US $ 60 billion and grew annually at the rate of 7 % (Natesh, 2001). According to the Secretariat of the convention on Biological Diversity, global sales of herbal products have gone up to an estimated US $ 60,000 million in 2002. The largest global markets of medicinal and aromatic plants are China, France, Japan, Germany, Italy, US, UK and Spain. Japan has the highest *per capita* consumption of botanical medicines in the world. Though Germany dominates the European trade it ranks third after Hong Kong as a world consumer. In Germany plant-based medicines are referred to as phytomedicines. It is rather difficult to derive accurate assessment of the volume and value of herbal trade in India but one estimate mentions that the medicinal plants related trade in India could be approximately Rs. 5.5 billion. Over the years herbal medicines have gained up ward trend for consumption especially with the development and standardization of herbal medicines (Malik et al. 2008).

There are several reasons why people globally are showing added interest for herbal therapies and these are low pricing, no side effects, solutions for chronic diseases and disorders, time tested remedies, preventive approaches etc.

3. SECONDARY METABOLITES FROM MEDICINAL PLANTS

Plants play a dominant role in the introduction of new therapeutic agents and also drugs from the higher plants continue to occupy a pivotal niche in modern medicine. With deforestation, medicinal wealth is rapidly being lost, such that many valuable plants are threatened with extinction. Pharmaceutical companies depend largely upon materials procured from naturally occurring stands that are being rapidly depleted because of the use of parts like roots, bark, wood, stem and the whole plant in the case of herbs. This poses a specific threat to the genetic stocks and to the diversity of medicinal plants.

Plant species like *Origanum vulgare, Saussurea obvallata, Ocimum sanctum, Cedrus deodara, Aegle marmelos, Zanthoxylum armatum, Ficus religiosa, Nardostachys grandiflora* and *Juniperus communis* are some of the instances of medicinal plants of immense importance propagated through tissue culture. The conventional cultivation of some of the medicinal plants is relatively expensive, and production of medicinal compounds can be elicited

in vitro. *In vitro* production of secondary metabolites can be exploited through plant cell culture. Which have the potential to produce and accumulate chemicals similar to the parent plant species from which they are derived**.** Secondary metabolites are compounds produced in plants and in general not involved in metabolic activity and these include alkaloids, phenolics, lignins, essential oils, tannins, steroids etc.

There are numerous reports describing the production of diverse secondary metabolites like anthocyanins, alkaloids, carotenoids, coumarins, steroidal alkaloids, terpenoids, flavones, tannins, saponins, sterols and several others. Growth of differentiated cells, elicitation and biotransformation are various approaches which have been attempted to increase secondary product activity in the cells. The process of *in vitro* culture of cells for the large scale production of secondary metabolites is complex and involves some important aspects like selection of high yielding cell lines, large scale cultivation of plant cells, elicitor induced production of secondary metabolites, biotransformation using cell cultures, secondary metabolites release and analysis. Successful establishment of cell lines capable of producing high yields of secondary compounds in cell suspension cultures have been reported (Zenk et al. 1978).

Establishment of hairy root cultures by genetic transformation of plant tissues by bacterium *Agrobacterium rhizogenes* is one of the most recent organ culture systems employed for large scale production of secondary metabolites. Transformed roots accumulate secondary products. The roots exhibited high degree of genetic stability and show biosynthetic activity for extended period. A number of reports have appeared on the successful production of secondary metabolites. Recent review paper summarized the molecular biology of hairy root induction from a range of species and reported on the production of specific secondary metabolites (Malik, 2007; Kaur and Malik,2009).

Table 2. ***Instances of Phytochemicals Produced by Hairy Roots.***

Sr.No.	Compound	Culture
1	Ajmalicine; Catharanthine	*Catharanthus roseus*
2	Hyoscyamine	*Datura stramonium*
3	Nicotine	*Nicotiana tabacum*
4	Saponin	*Panax ginseng*
5	Shikonins	*Listhospermum erythrhizon*
6	Quinine	*Cinchona ledgeriana*
7	Thiophenes	*Tagetes patula*
8	Arabasine;scopolamine	*Atropa belladonna*
9	Serotonin	*Peganum harmala*

4. DANGERS OF EXPANDING HERBAL MEDICINE MARKETS AND DEPLETION OF RESOURCE BASED SPECIES

Interestingly the major markets for medicinal plants are situated in the geographic zones where the producing countries are located though the established markets are situated elsewhere. Expansions of markets for medicinal plants are providing better opportunities for the herbal industry and increased utilization of resources. With enhanced demands by the consumer, industry there is unprecedented threats to the plant specific resources to cater to the increasing demands of the industry. Very few medicinal plant species are being cultivated and many plant species are harvested from their phyto-diversity rich areas from wild population. The job is enacted by the unskilled workers who resort to over harvesting, unscientific collections. In many instances whole plant species is collected even though leaves, flowers, fruits or seeds are used for plant based drugs. In some instances even immature plants are being harvested. Additionally several other factors e.g. dwindling forests, extension of land for agricultural purposes,

grazing, over exploitation of resource sp have exerted extraordinary demands on wild population of important medicinal plant species. Consequently there is a threat to biodiversity and the very existence of potent plant species.

Threat to medicinal plants is in many ways e.g. over collection of fruits (*Momordica dioica, Annona squamosa, Emblica officinalis* etc.) unripe fruit collection (*Annona squamosa, Jatropha curcas, Momordica dioica*), over collection of seeds (*Sterculia urens*), harvesting underground parts (*Chlorophytum borivillianum, Gloriosa superba,Plumbago zelanica, Urginea indica* etc.), selective extraction (*Bombax ceiba, Pterocarpus marsupianum*) and poor fruit setting (*Sterculia urens, Oroxylum indicum*) and low seed germination (*Asparagus racemosus, Celastrus peniculatus, Tinospora cordifolia*) and habitat destruction (*Tribulus terrestris, Argemone mexicana, A.ochroleuca, V. encelioides, T. procumbens* etc.)

Both in China and India, serious attempts are being made to cultivate medicinal herbs and the projects are being supported by large pharmaceutical companies. Even then very few species are harnessed after cultivation and these include *Plantago ovata, Atropa belladonna*, *Papaver somnifera*, *Catharanthus roseus*, *Cassia angustifolia*, *Digitalispurpurea, Hyocyamus muticus*. For many plant species Package of Practices is also available. Several of CSIR and ICAR institutions have made available agro-technology Packages for many medicinal plants. Ensured and successful cultivation of medicinal plant species will lessen pressure on wild resources improve quality of resource material and sustain supply of properly identified raw material.

Biotechnological tools are important for multiplication and genetic enhancement of the medicinal plants by adopting techniques such as *in vitro* regeneration and genetic transformations. They can also be exploited for the production of secondary metabolites using plants as bioreactors. In this paper we review the achievements and advances in the application of tissue culture and *in vitro* regeneration of medicinal plants from various explants.

5. MICROPROPAGATION

Most of the plants raised through seeds are highly heterozygous and show great variations in growth, habit and yield may have to be discarded because of poor quality of flowers and fruits for their commercial release. Likewise majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Moreover many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items. Due to extensive utilization of medicinal plants for medicine and scientific research, many of them are facing extinction. Therefore, it is imperative to adopt alternative methods for rapid multiplication.

In the recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions. *in vitro* propagation also called micropropagation is in fact the miniature version of conventional propagation, which is carried out under aseptic conditions.

Micropropagation provides a fast and dependable method for production of a large number of uniform plantlets in a short time. Moreover, the plant multiplication can continue throughout the year irrespective of season and the stocks of germplasm can be maintained for many years (Malik, 2007). Singh (2004) has listed improved cultivars of various medicinal and aromatic plants which have been developed/ identified or raised through superior selection (table3).

Table. 3 ***Improved cultivars identified, developed superior selections in medicinal plants and aromatic species (BP Singh , 2004).***

Sr. No.	Local Name	Botanical Name	Improved Cultivars/Superior Selection
1.	Isabgol	*Plantago ovata*	Gujrat Isabgol-1, Gujrat Isabgol-2
2.	Opium poppy	*Papaver somniferum*	Jawahar Aphium-16 (JA-16), Trishna (I.C.402), Shaktiman, Kirtiman, Chetak=Aphium, E.C. 179777, E.C.196430 (Finland), E.C. 196433 (Hungary), Sujata (Opium less and alkaloid free poppy)
3.	Senna	*Cassia angustifolia*	ALFT 2
4.	Periwinkle	*Catharanthus roseus*	E.C.120837 (Russia), I.C. 49581
5.	Liquorice	*Glycyrrhiza glabra*	E.C. 114304 (Russia), E.C. 41911 E.C. 128587 (Pakistan)
6.	Asgandh	*Withania somnifera*	R.S.I. Jawahar Asgand-20 (WS-20)
7.	Jasmine	*Jasminum grandiflorum*	Pitchi (Co.)
8.	Palmarosa	*Cymbopogon martini var. motia*	I.W. 31245
9.	Vetiver/khus	*Vetiveria zizanioides*	For root yield: Hyb.9, Hyb.8, Hyb.26, NC.66403, NC 66404, NC 66415. For oil yield:Hyb.8, NC 66403, NC 66416
10.	Lemon grass	*Cymbopogan flexuosus*	O.D. 15, O.D. 19, Pragati
11.	Rose geranium	*Pelargonium graveolens*	NIC 23414- an Algerian type
12.	Patchouli	*Pogostemon patchouli*	
Introductory Crops			
13.	Henbane	*Hyoscymus muticus,* *H. niger*	E.C.93928 I.C. 66
14.	Chamomilla	*Matricaria chamomilla*	E.C. 217012 (Romania)
15.	Sage	*Salvia officinalis*	E.C.314321
16.	Basil/ Tulsi	*Ocimum basilicum*	I.C. 75730, E.C.176934 (France)
17.	Lavender	*Lavendula officinalis*	E.C. 120176 (Portugal), E.C. 16543, E.C.15023 (U.K.)
18.	Rosemary	*Rosemarinus officinalis* *Melissa officinalis*	E.C. 273873 E.C. 273873
19.	Mints	*Mentha* sp.	*M.piperita:* E.C. 41911 (Russia), E.C. 41912, Siwalik *M. longifolia:* E.C.390182 (U.S.A.), *M.spicata*- PBVPBY
Native Species			
20.	Mucuna/kawanch	*Mucuna pruriens*	
21.	Gwarpatha/Aloe	*Aloe barbedensis*	

22.	Giloe	*Tinospora cordifolia*	I.C. 281970, I.C. 281959, I.C. 281872
23.	Celastrus	*Celastrus paniculatus*	
24.	Piplamool	*Piper longum*	Cheemathippali
25.	Satwari	*Asparagus racemosus*	
26.	Guggal	*Commiphora wightii*	
27.	Babchi/Psoralea	*Psoralea cordifolia*	I.C. 111249, I.C. 111238, I.C.11246
28.	Safed musli	*Chlorophytum Borivillianum*	
29.	Annis	*Pimpinella anisum*	E.C. 22091
30.	Sarpagandha	*Rauwolfia serpentina*	R.S. 1
31.	Steroidal yarns	*Dioscorea floribunda*	PB(c) 1, Arka Upkar
32.	Khasi kateli	*Solanum viarum*	Arka Sanjeevani
33.	*Kangaroo Keali*	*Solanum laciniatum*	E.C. 113465

5.1 Advantages of Micropropagation:

1. Micropropagation can be used as an alternative to conventional methods of vegetative propagation with the objective of enhancing the rate of multiplication.
2. Through *in vitro* clonal propagation, a large number of plants can be raised from even small sized explant within a short span of time.
3. Micropropagation provides reliable and economical method of maintaining pathogen free plants in a state that can allow rapid multiplication and also facilitate exchange of germplasm and transportation.
4. Plant multiplication can continue throughout the year irrespective of season.
5. Stocks of germplasm can be maintained for many years.

5.2 Micropropagation of Selected Medicinal Plants

Basically tissue culture implies *in vitro* growth of plantlets sampled from any part of the plant sp in defined, suitable nutritive culture medium. It is also referred to as micropropagation. The usual methods of regeneration through tissue culture are callus mediated organogenesis and somatic embryogenesis. Media used in plant tissue culture are composed of several components e.g. salts, vitamins, amino acids, sugars, growth regulators, gelatin or agar and water. All these compounds in variable combinations fulfill one or more functions for *in vitro* growth of plants. The minerals are both micro- and macronutrients and they aid in synthesis of basic blocks comprising plant body. Some of these act as catalysts for mediating several enzyme reactions. Nitrogen, phosphorus, sulphur are components of proteins and nucleic acids. Magnesium is important part of chlorophyll and some of the micro elements form essential part of some enzymes. Calcium and boron are chiefly located in cell wall and calcium helps in stabilizing biomembranes. Potassium and chloride play a pivotal role in osmotic regulation to maintain electrochemical potential and for the activation of many enzymes. Plant growth regulators added to plant tissue culture media are taken up and enhance the endogenous level of plant hormones. In different combinations, PGRs induce cell division, cell growth, tissue differentiation and organ formation. In most instances, PGR/hormones are inactivated after uptake. Usually only minute quantities of exogenously supplied PGRs remain in the free form and are utilized.

Based on different combinations of these components umpteen media are formulated e.g Anderson's Rhododendron medium, Che'e' and Pool medium, Chu medium, Gamborg B5 medium, McCown woody plant medium, Murashige and Skoog medium,Nitsch medium,etc. Of these Murashige and Skoog medium has been variously modified and extensively used for micropropagation of several plant species very successfully.

Agrotechnology or protocol for the micropropagation of several medicinal plants is available (Debnath, 2007; Malik, 2007; Purohit et al. 1994; Rao et al. 2010; Kaur and Malik, 2009).

We have developed micro propagation protocols successfully for three important medicinal species

such as *C. quadrangularis, T. procumbens* and *V. encelioides.*

6 CISSUS QUADRANGULARIS L

C.quadrangularis Linn. belongs to the family Vitaceae commonly known as bone setter due to its bone fracture healing property . It is frequently used as a common food item in southern India and Sri Lanka where the green stems are fried or curried before consumption (Sivarajan et al. 1994). It is also found on the lower slopes of the Western Ghats and is widespread across drier areas of Arabia, Africa, India, Sri lanka, Malaysia and Thialand (Udupa et al.1970). Three morpho-variants of *Cissus quadrangularis* with square-stemmed, round-stemmed and flat-stemmed are available. They are differentiated as variant I, II and III respectively (Kannan et al. 1999, Anoop et al. 2004). It requires warm tropical climate and are mainly propagated by stem cuttings in months of June and July. The plant is useful for the treatment of bone fracture, diarrhea, skin disorders, irregular menstruation, piles, tumors, wounds and scurvy (Kritikar et al. 2000). Pharmacological studies have revealed the bone fracture healing property (Deka et al. 1994) and antiosteoporotic effect of this plant. Shirwaikar et al. 2003 found that 750 mg/kg of body-weight of ethanolic extract given to rats was effective in ovariectomy-induced osteoporosis. The plant has been documented in ayurveda for its medicinal uses in gout, syphilis, veneral disease, piles, leucorrhoea, dysentery and kapham (Shirwaikar et al. 2003). The entire *Cissus* plant is of medicinal value, and is considered to be an alterative, anthelmintic, aphrodisiac, and antiasthmatic. *C. quadrangularis* is used for the treatment of pain & inflammation associated with hemorrhoid as well as reducing the size of hemorrhoids (Lans et al. 2006). The extract of *C. quadrangularis* shows the gastroprotective effect. The finding suggests that the extract of *C. quadrangularis* promotes ulcer protection by the decrease in ulcer index, gastric secretions and increase in the glycoprotein level, gastric mucin content and NPSH concentration. The extract of *C. quadrangularis* may protect the gastric mucosa against ulceration by its antisecretory and cytoprotective property (Jainu et al. 2006). It is useful in treating gastrointestinal disorders such as colic and dyspepsia (Williamson et al. 2002) Recent preliminary research studies have revealed that *Cissus* may have the potential to act in the management of metabolic syndrome, particularly reduces the body weight by inhibiting the oxidation of LDL cholesterol and by lowering the blood glucose in obese patients and central obesity (Oben et al. 2006). The oral administration of *C. quadrangularis* extract (CQR-300) to human subjects resulted in significant reductions in weight and blood glucose levels, while decreasing serum lipids (Oben et al. 2007). The plant extract of *C. quadrangularis* shows antioxidant activity & antimicrobial activity. The antioxidant activity of methanol extract and aqueous extract were comparatively less significant than that of ethyl acetate extract and n-hexane extract showed the least activity. The ethyl acetate extract and methanol extract of both fresh and dry stems further exhibited antimicrobial activity against Gram-positive bacteria, including *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus,* and *Streptococcus species* (Chidambara et al. 2003).The methanolic extract of the plant has promoted the free radical scavenging activity (Jainu et al. 2005).

The Petroleum ether extract of *C. quadrangularis* enhances bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis and can be used as preventive/alternative natural medicine for bone diseases such as osteoporosis and it might be a potential candidate for prevention and treatment of postmenopausal osteoporosis (Potu et al. 2009). The active constituents of CQ extract, such as phytosteroids, enhanced the human mesenchymal stem cells (hMSCs) proliferation and promoted osteogenic differentiation and biomineralization process. The Alg/O-CMC/CQ-E scaffold (Alg, alginate; O-CMC, O-carboxymethyl chitosan; CQ-E, *C. quadrangularis* extract) have excellent osteoinductive property which make it an ideal candidate for bone tissue engineering (Singh et al. 2011). Muthusami et al. (2011) explains for the first time the molecular mechanism behind fracture healing properties of *C. quadrangularis*.

6.1 Bone healing activity

Clinical trials and animal studies have shown that treatment with *Cissus* facilitates the remodeling process of the healing bone, speeding the restoration of bone tensile strength. In clinical trials, *Cissus* shortened fracture healing time between 33 % and 55 %.The stem extract of this plant contains a high percentage of calcium ions and phosphorus, both essential for bone growth (Enechi et al. 2003). Thus the plant *C. quadrangularis* appears to be very useful in treating diseases involving deficiency in the bone formation and fracture healing. The calcium ions, phosphorous and phytoestrogens present in this plant extract may contribute in the process of ossification and fracture healing. A phytosterol fraction isolated from *Cissus* demonstrated significant bone-healing activity. *Cissus* is an ingredient in the Ayurvedic preparation, *Laksha Gogglu*, which has proven highly effective in relieving pain, reducing swelling and promoting healing of simple fractures, as well as in curing various disorders associated with fractures.

6.2 Anti-inflammatory, analgesic and antipyretic activity

The ethanolic extract of *C. quadrangularis* shows analgesic, anti-inflammatory and antipyretic activity. *C. quadrangularis* possessed analgesic properties comparable to aspirin and anti-inflammatory drugs like ibuprofen. Its effects on antipyretic activity were also appreciable it significantly reduces fever at higher doses within 2 hrs.

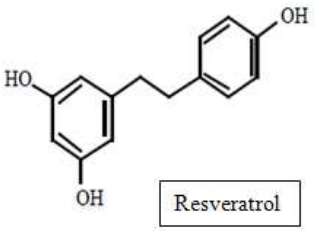
6.3 Antiviral activity

Partially purified methanolic extract of *C. quadrangularis* showed antiviral activity.
HSV1 and HSV2 showed more sensitivity against the partially purified compound.

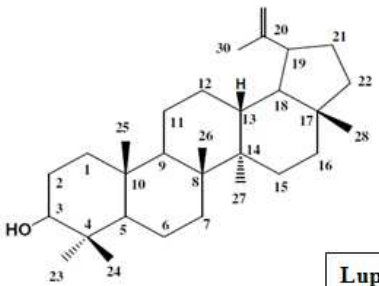
6.4 Ethno veterinary usage

Cissus quadrangularis is fed to cattle as a galatologue to induce flow of milk. The whole plant is used in cases of fractures, sprains, rheumatism, irregular growth of teeth, broken horn, anthrax, haematuria, elephantiasis, dislocation of hip, various wounds and cracked tail.

Chemical analysis of *C. quadrangularis'* stem has revealed unique stilbene derivatives, which are termed quadrangularins A, B and C. The alkaloids resveratrol, piceatannol, pallidol and parthenocissin are also found in *Cissus*' stem (Adesanya et al. 1999). Resveratrol is an effective anticancer agent of natural chemicals from *C. quadrangularis* that can trigger the human tumor cells, CD 95 signaling dependent cell death (Clement et al. 1998). Resveratrol (3, 4, 5 tri-hydroxystilbene) is a phytoalexin produced in huge amount in grapevine skin in response to infection by *Bothrytis cinerea*. This production of resveratrol blocks the proliferation of the pathogen, thereby acting as a natural antibiotic. Is also able to activate apoptosis, to arrest the cell cycle or to inhibit kinase pathways. Resveratrol acts on the process of carcinogenesis by affecting the three phases: tumor initiation, promotion and progression phases and suppresses the final steps of carcinogenesis, i.e. angiogenesis and metastasis. Interestingly, resveratrol does not present any cytotoxicity in animal models (Delmas et al. 2006). For chemical configurations please see below:



Resveratrol



Lupeol

Chemical structures of resveratrol and Lupeol .

Phytosterols identified in the plant are sitosterols, stigmasterol and campesterol. A phytochemical investigation of *C. quadrangularis* leaves yielded five additional known compounds including ecosyl eicosanoate, tetratriacontanol, tetratriacontanoic acid, α-amyrin and β-sitosterol (Jainu et al. 2009).

Characterization of the chemical constituents of *C. quadrangularis* has revealed the presence of calcium and polyphenols such as quercetin, daidzein and genistein (Singh et al. 2007).

The stem of the plant contains two asymmetric tetracyclic triterpenoids, onocer – 7 ene 3 α, 21 β diol and onocer - 7 ene – 3 β, 21 α diol and two steroidal principles (Bhutani et al. 1984). The presence of β-sitosterol, δ-amyrin, δ-amyrone, and flavonoids (quercetin) has also been reported (Jakikasem et al. 2000).

The aerial parts of *C. quadrangularis* are found to contain a new asymmetric tetracyclic triterpenoid, 7-Oxo-Onocer-8-ene-3 β 21 α diol. Thakur et al. (2009) reported the presence of three compounds namely triterpene δ-amyrin acetate, lipid constituent hexadecanoic acid and stilbene glucoside trans-resveratrol-3-O-glucoside (Piceid). Rao et al. (2011) reported for the first time the presence of lupeol compound, which shows the melanin promotion activity in the cell lines (B16F10 melanoma).

C. quadrangularis is rich in vitamin C and beta-carotene. Analysis showed that *Cissus* contained Ascorbic acid at a concentration of 479 mg, and carotene 267 units per 100g of freshly prepared paste, in addition to calcium oxalate (Chidambara et al. 2003). Propagation through seeds is unreliable because of no seed viability or plants will not come true from seeds. The traditional propagation method by 'cuttings' limits the number of propagules. Therefore, it is imperative to adopt alternative methods for rapid multiplication. Rapid *in vitro* multiplication of shoots from nodal explants has yielded encouraging results in *C. quadrangularis.* Shoot multiplication was through axillary bud sprouting which is a preferred mode of propagation to ensure stability of regeneration. Sharma et al. (2011) also attempted to micro propagate this species but succeeded in raising callus only with MS medium supplemented with various growth regulators.

Multiple shoot regeneration from nodal explant was observed on Murashige and Skoog (MS) medium supplemented with different concentration and combinations of plant growth regulators either individually or in combination such as BAP alone or in combination with KN and Zeatin alone or in combination with BAP and 2, 4-D alone. BAP was used from .5 mg L^{-1} to 8 mg L^{-1}, Zeatin was used from .5 mg L^{-1} to 2 mg L^{-1}, KN was used from .5 mg L^{-1} to 8 mg L^{-1} and 2, 4-D was used from 1 mg L^{-1} to 4 mg L^{-1}.

Maximum break down of axillary buds was accomplished in nearly three weeks in almost all the aseptic cultures on MS medium supplemented with 2 mg L^{-1} BAP(87 %) (Fig.1 c) About 38 % bud break response was observed with 1 mg L^{-1} KN. Several studies on BAP supplementation have been published in many medicinal plants (Purohit et al. 1994; Rao et al. 2010).

All the concentration of BAP (0.5 mg L^{-1} to 8 mg L^{-1}), Zeatin (0.5 mg L^{-1} to 2 mg L^{-1}) and 2, 4-D (1 mg L^{-1} to 4 mg L^{-1}) alone facilitated shoot induction from the nodal explant. The results showed that nodal segments cultured on MS basal medium supplemented with various concentration of BAP in combination with KN were very effective for shoot proliferation than KN alone. Shoot proliferation was induced from nodal segment after 7-10 days of inoculation on MS medium supplemented with BAP (2 mg L^{-1}) in combination with KN (2 mg L^{-1}) (Fig.-1 b). Callus induction was observed within 10 days from nodal explants cultured on MS medium supplemented with NAA (2 mg L^{-1}) in combination with KN (0.5 mg L^{-1}) but the shoot proliferation was not observed and the callus turned brown after 25-30 days of inoculation.

Nodal explant cultured on MS supplemented with BAP (4 mg L^{-1}) in combination with Adenine sulphate (8 mg L^{-1}) also induces shoot proliferation (Fig-1 a). Medium fortified with 2, 4-D -1 mg L^{-1} induced multiple shoots from 75 % of the cultured nodal explant within 15-20 days of inoculation (Fig-1 d). These shoots were then transferred to BMS medium supplemented with or without auxins (IBA, IAA) for root induction. The optimal rooting efficiency for shoots (80 %) as well as the highest

root length (6.13 cm) were obtained on BMS media without auxins, where rooting was initiated after 12 days of culture. (Fig.-1 e) Debnath et al. (2006) reported rooting from explants using different concentrations of auxins supplemented to ½ MS medium. The rooted plantlets with well developed shoots and roots were transferred to pots for acclimatization (Fig – 1 f).

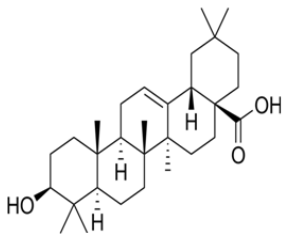
7. TRIDAX PROCUMBENS

T. procumbens L. belongs to family asteraceae commonly known as 'Ghamra' and in English popularly called 'coat buttons' because of the appearance of its flowers. The plant is native of tropical America and naturalized in tropical Africa, Asia, Australia and India. It is a wild herb distributed throughout India especially in Maharashtra, Madhya Pradesh and Chhattisgarh region as weed. Its widespread distribution and importance as a weed are due to its spreading stems and abundant seed production. It is hardy, perennial, procumbent herb (Chauhan et al. 2008). *T. procumbens* has been extensively used in Indian traditional medicine for wound healing, as anticoagulant, antifungal and insect repellent; in diarrhoea and dysentery (Ali et al. 2001). Pathak et al. investigated hair growth promoting activity of *T. procumbens* (Pathak et al. 1991).

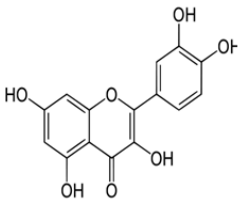
Pharmacological studies have revealed the Antimicrobial activity against both gram-positive and gram-negative bacteria, Hypotensive activity, Anti septic, Insecticidal, Parasiticidal and hepatoprotective activity (Mahato et al. 2005, Salahdeen et al. 2004, Saxena et al. 2005, Vilwanathan et al. 2005). The plant is used for the treatment of diarrhea, malaria and stomachache (Burkill et al. 1984). The leaf juice of *T.* possesses antidiabetic property. Aqueous and alcoholic extract of leaves of *Tridax* showed a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats (Bhagwat et al. 2008). Ethanolic extracts of leaves of *Tridax* have immunomodulatory effect on Albino rats dosed with *Pseudomonas aeruginosa* also inhibits proliferation of same (Oladunmoye et al. 2006).

Earlier workers have already reported the presence of dexamethasone luteolin, glucoluteolin, β-sitosterol and quercetin in this plant (Subramanian et al. 1968; Reddy et al. 2006).

The phytochemical screening of the leaves of *T. procumbens* revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), saponins and tannins. The proximate profile shows that the plant is rich in sodium, potassium and calcium. *T. procumbens* can serve as a good source of plant protein and potassium supplement, as well as being potential source of provitamin A (carotenoids) (Jude et al. 2009). *T. procumbens* has very high saponin content. Saponins are known to reduce the uptake of certain nutrients like glucose and cholesterol so may help in lessening the metabolic burden that would have been placed on the liver (Price et al. 1987). Carotenoids provide many brilliant animal colors, as in the flamingo, starfish, lobster and sea urchin and are precursors of vitamin A (Chaney, 2006b). Carotene is used as a food colorant. Oleanolic acid was obtained in good amounts from *Tridax* and found to be a potential antidiabetic agent when tested against a-glucosidase (Ali et al. 2001). Chemical analysis showed that *Tridax* contained crude proteins 26 %, crude fiber 17 % soluble carbohydrates 39 % calcium oxide 5 %, Luteolin, glucoluteolin, quercetin and isoquercetin, fumaric acid and fl-sitosterol (Verma et al. 1988). For chemical configurations please see below:



Oleanolic acid



Quercetin

T. procumbens is widely exploited and its distribution has been declining over the years. Propagation through seeds is likely to cause variations. Therefore attempts were made to propagate the species by *in vitro* method. Modified Murashige and Skoog (1962) medium
(MS) was used for shoot proliferation. The medium was supplemented with 0.5 - 4.0 mg L-1 KN/BAP + mesoinositol (100 mg L^{-1}, w/v) + sucrose (3 % w/v). Among the different explants tried (young leaves, nodal (with and without axillary buds), apical bud and internodal segments), only nodal explants with single dormant axillary bud shows positive response after 10 days of inoculation. Rapid *in vitro* multiplication of shoots from nodal explants with single dormant axillary bud was observed on modified MS medium supplemented with different concentration and combinations of plant growth regulators. Highest percentage of shoot development, maximum shoot length and higher number of shoots per explant was achieved with 2 mg L^{-1} KN. Callus induction was observed from nodal explants cultured on MS medium supplemented with BAP (1 mg L^{-1}) (Fig.-2 a), later leaflets arose from the callus cultured on BAP + 100 mg L^{-1} mesoinositol (Fig.-2 b). Among the two cytokinins tested BAP + GA_3 was found more effective than KN+GA_3 for producing multiple shoots from nodal explant. Of the various concentrations tried, BAP was found to be most effective at 1 mg L^{-1}. When the concentration of BAP was increased beyond optimal level, there was suppression of sprouting. The promotory effect of BAP added to MS medium on bud break and multiple shoot formation in *T. procumbens* is comparable to the reports published in other medicinal plants (Debnath et al. 2006; Bhat et al. 1995).

When the explants were collected during July-September, there was high bud break (85 %) and produced maximum number of shoots (4.9). This was rainy season in northern India. Whereas during December it was below 50 %. These shoots were then transferred to BMS medium supplemented with or without auxins (IBA, IAA and NAA: 0.5, 1.0, 2.0 mg L^{-1}) for root induction. The optimal rooting efficiency for shoots (85 %) was obtained on MS+ IBA 1mg L^{-1} (Fig.-2 e) With NAA, no roots were induced but instead callus-like nodules were produced. Plantlets having 4-5 leaves and well developed roots were hardened in growth chamber and then gradually shifted to outside environments (Fig- 2 f).

8. VERBESINA ENCELIOIDES

V. encelioides (Cav.)Benth. & Hook. f.ex A. Gray (fam.: Asteraceae.) is commonly known as "Golden crown beard" and can be grown as an ornamental in the gardens (Fig. 3). It is native to the Southwestern United states the Mexican Plateau and other parts of Tropical America. *V. encelioides* is an erect annual spp. (Wagner, 1990) commonly seen up to the heights of 0.3 to 1.66 meters (Feenstra et al. 2008). Its common names are Golden Crown beard, Crown beard, Wild sunflower, Girasolcito, Yellow top del Muerto.
Seeds of *V. encelioides* germinate in autumn or early spring. Seeds can survive drought and high temperatures; long periods of seed dormancy and high germination rates are reported. The plant is self-and cross-pollinated and reproduces by seeds. A single flower head produces 300 to 350 seeds and each plant can produce 2 to 6 flowers leading to the production of 600 to 2100 seeds per plant. *V. encelioides* does not require large amounts of water (Al-Farraj, 1990) and is considered a drought tolerant plant (AZ Dept. of Water Resources website). It is propagates by seeds, which have shown to be able to survive under drought and high temperatures (Kaul and Mangal, 1987).

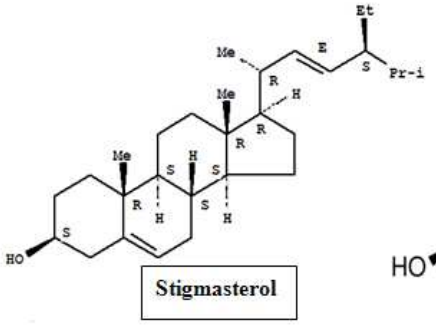
Though not widely treated ornamentally, there are a few companies in the Southwestern United States (specifically in New Mexico and Texas) that promote the planting of *V. encelioides* for its fast growing abilities, its bright colors, and the plant's drought resistant qualities. Additionally, the Arizona Department of Water Resources has the species listed on its "Official Regulatory List of Low Water Use and Drought Tolerant Plants" as a recommended plant for landscaping usage due to its low water requirements (AZ Department of Water Resources website).

Medicinal uses of *Verbesina* are many, used with both gum sores and as well as in a hemorrhoid treatment. It is analgesic, emetic, febrifuge, insecticide and anti-inflammatory.

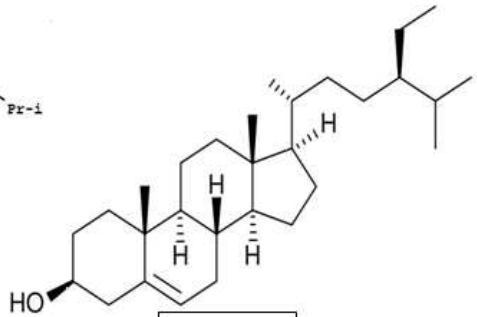
Earlier medicinal uses are thought to have been practiced by the North Dakota Hopi Indian tribe, utilizing *V. encelioides* for the treatment of spider bite. A hot cup of *Verbesina* tea is reported to bring down fever induces copious sweating, relaxation and a mild laxative effect.

The phytochemical analysis of *V. encelioides* revealed the presence of primary metabolites (Jain et al. 1988) sesquiterpenes (Joshi et al. 1983), flavonoids (Glennie et al. 1980), galegine (Oelrichs et al. 1981) and triterpenoids (Tiwari et al. 1978)

Various compounds viz. friedelin, epifriedelin, lupeol, α-, β-amyrin, stigmasterol, betulin and β-sitosterol have been isolated and identified using spectral studies in cell culture (Jain et al. 2008). For chemical configurations please see below:



Stigmasterol



Sitosterol

V. encelioides is one of the most common weeds in Northern India, germinating after the rainy season and invading crop fields. Attempts were made to propagate the material by *in vitro* method. Axillary buds, hypocotyls, immature leaves of 7 days old seedlings and nodal segments were used as explants. *In vitro* regeneration seemed to be more successful by axillary bud regeneration than by indirect organogenesis. The explants were cultured on MS salts supplemented with macro-elements, micro-elements and 3 % sucrose. Production of callus tissue was achieved from leaf segment by culturing on nutrient medium supplemented with various concentrations of BAP (0.4, 0.5, 1.0, 2.0 and 3.0).Only at high concentration calli formation was high. The leaf calli expanded but failed to undergo embryogenesis. The callus was nodular, green and compact. It started rooting after three weeks of culturing. Fig. - 4 a shows green callus formations with middle (M) segment of leaf on MS + BAP without any auxin. Shoot regeneration was occurred from leaf callus on MS+BAP+NAA (1mg L^{-1}) after 15 days of inoculation (Fig – 4 b). Multiple shoots were proliferated from hypocotyls explant on MS medium supplemented with BAP+ NAA (1mg L^{-1}) after 30 days of inoculation (Fig.- 4 c).

Nodal explants exhibited positive morphogenetic response in MS basal medium supplemented with different concentrations of BAP, where maximum response was observed in 3 mg / L. Multiple shoot regeneration from axillary bud was observed on MS medium supplemented with BAP-0.5 mg L^{-1} (Fig. 4 d). Some workers obtained multiple shoots on MS medium containing BAP (1-60 mg L^{-1}) though efficient shoot regeneration from axillary buds was obtained on MS medium with low levels of BAP than on media supplemented with higher concentrations of BAP.

Callus induction occurred from Middle segment of leaf on MS medium supplemented with BAP but the shoot proliferation was not observed and the callus turned brown after 25-30 days of inoculation.

For root induction *in vitro* developed shoots were transferred to full strength MS medium supplemented with various concentrations of IBA (1.0 to 5.0 mg L^{-1}). Maximum rhizogenesis was observed on IBA (1 mg L^{-1}) and the number enhanced with the increase in concentration of IBA (5 mg L^{-1}). Root formation from the basal cut portion of the shoot was observed 4 days after transfer to the rooting medium (MS media). With 1.5 mg L^{-1} of IBA root induction was earlier and maximum.

For different species different phytohormones are reported to induce rhizogenesis e.g. IAA in groundnut (Palanivel et al. 2009), IBA for *Alnus nepalensis* (Thakur et al. 2001). The rooted plantlets were hardened and transferred to plastic cups having sterile soil. The survival percentage was more than 65 %.

Cissus quadrangularis L

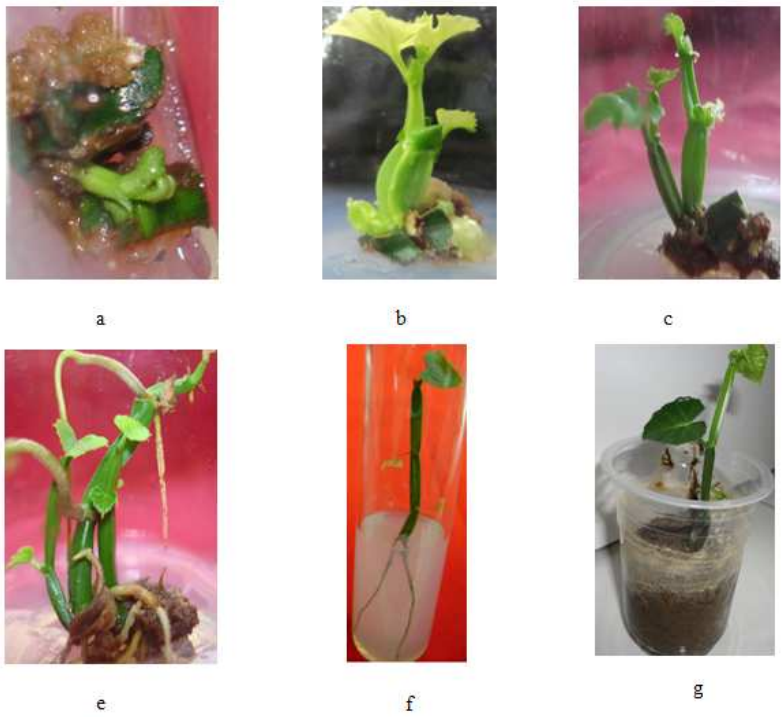


Figure 1 ***C. quadrangularis* L.** ***(a) Single shoot formation from nodal segment on BAP-4 mg L^{-1} with adenine sulphate-8 mg L^{-1} after 15 days of inoculation (b) Regeneration of shoots from nodal segment on BAP-2 mg L^{-1} with KN-2 mg L^{-1} after 35 days (c) Regeneration of shoots from nodal segment on BAP-2 mg L^{-1} after 60days of inoculation. (d) Multiple shoot regeneration on 2,4-D- 1 mg L^{-1} after 90 days of inoculation (e) rooted shoots 25days after inoculation (f) acclimatized plants after 40 days.***

Tridax procumbens

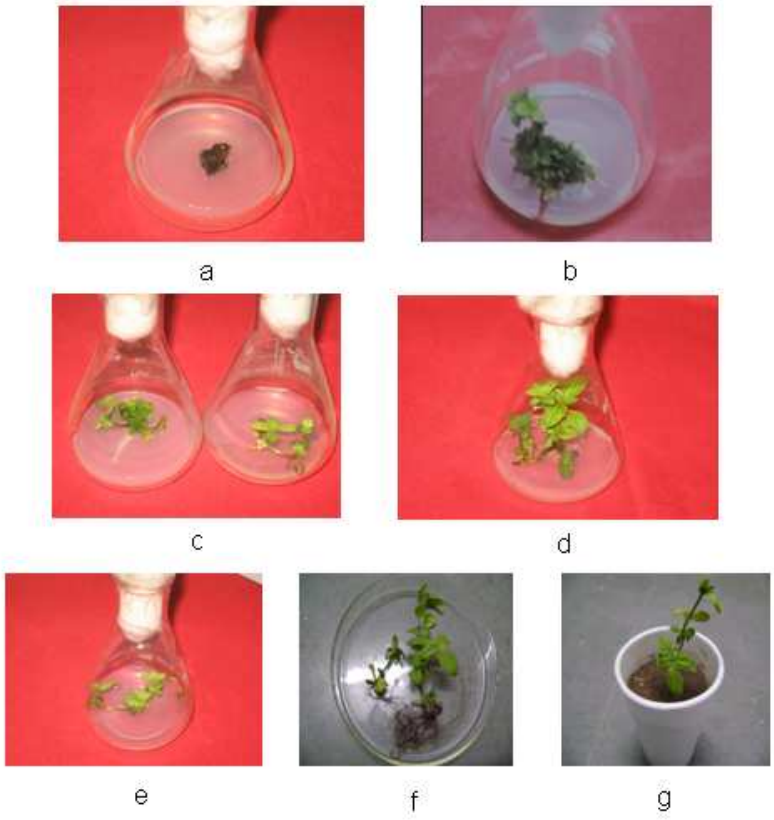


Fig. 2 a-g. ***In vitro multiplication in Tridax procumbens (a) Induction of callus from nodal explant, on MS medium supplemented with BAP (2 mg L^{-1}+ mesoinositol 100 mg L^{-1}) (b) callus with shoot primordial (c) shoot tip with leaves and roots after three weeks (d) shoots (e) A flowering shoot (f) Flowering shoot with copious roots (g) Transfer of plantlets to soil.***

Verbesina encelioides

Figure. 3. ***Verbesina encelioides plants growing in nature***

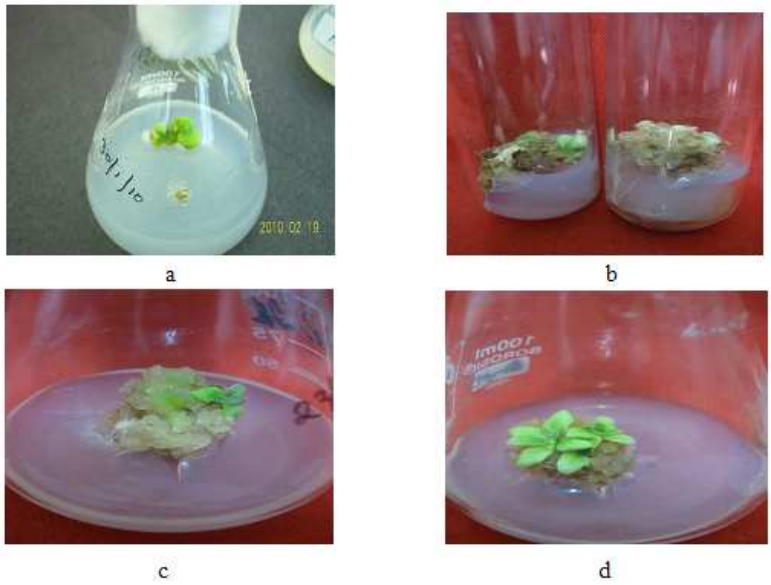


Figure. 4. ***Different types of callus. Callus formation from young leaves and multiple shoot induction from nodal explants. a. Callus formation from young leaves when plated on BAP (3 mg L^{-1}). b. Rooting in callus derived from middle portion of leaf. c. Shoot bud induction on MS medium (BAP 3 mg L^{-1}). d. Proliferation of shoot buds on medium containing BAP (3 mg L^{-1}).***

9. CONCLUSION

Micro propagation has great potential as a tool for rising elite plants which provide raw material for traditional medicines. Phytochemicals obtain from plant species constitute most diverse groups of plant's secondary metabolites. Recent advances in bioinformatics tools, genetic engineering and cell culture, in many instances, have made it possible to modify biosynthetic compounds. Based on availability of data on secondary metabolites and the pertinent enzymes, in some cases, have made it possible to identify relevant plant gene (s) from the plant species for the production of high quality phytochemicals. Protocols have been developed for the multiplication of elite plant species for medicinal and aromatic uses in several labs all over the country. In fact tissue culture protocols have been developed for several species of plants which are used by pharmaceutical industries over the years. Biotechnology tools have been increasingly exploited to raise phyto resources. Hence, advantages in tissue culture (micro propagation, genetic engineering, cell culture, transformation technology) have opened several vista foe the bulk production of pharmaceutical and neutracutics and various economically important metabolites. In fact manipulation of genome is leading to the production of enormous compounds with different qualities in various plant species. Unarguably, micro propagation and genetic engineering hold great promise for the production and supply of phytochemicals independent of plant availability.

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