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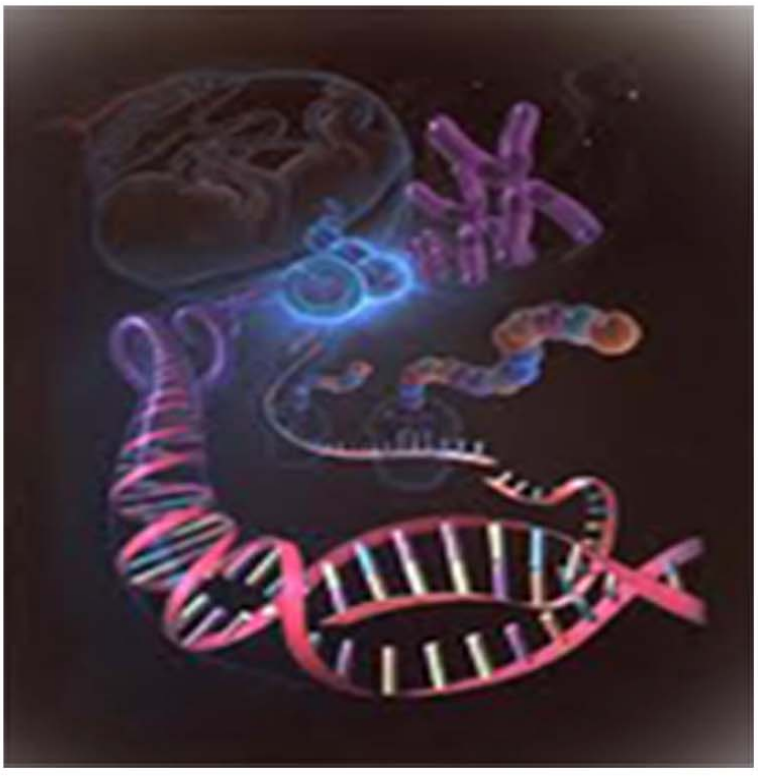

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Research Paper

AN *IN VITRO* CALLUS INDUCTION AND ISOLATION, IDENTIFICATION, VIRTUAL SCREENING AND DOCKING OF DRUG FROM *CONVOLVULUS ALSINOIDES* LINN AGAINST AGING DISEASES

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An in vitro callus induction and *in silico* analysis in *Convolvulus alsinoides*, an ancient Indian traditional medicinal plant can provide evidence in soft callus formation, isolation and virtual screening of medicinal compounds. In this experiment, an investigation was done to find out a suitable and standard protocol for callus induction of an Indian traditional medicinal plant, *Convolvulus alsinoides* Linn. Higher concentrations of 2, 4-D (≥3 mg/l) was more effective for callus induction on terminal buds and flower explants. The experimental results of calli were shown as brownish white, friable and ascribed meristematic nature. Leaving the calli in the medium turned brown and dried within 4 months. Isolation of medicinal compounds from callus is easier as the callus is soft and available throughout the year in lab conditions. Kaempferol, an isolate from callus of *C. alsinoides* predicted good activity in decreasing fat levels, and also have good effect against Alzheimer and Parkinson diseases. Kaempferol predicted least activity against Type 2 Diabetes Mellitus. Various compounds can be isolated from callus cultures and have medicinal usage. The present study can be helpful in callus induction and virtual screening of products from plant species such as *Convolvulus alsinoides*.

Keywords: *Convolvulus alsinoides* Linn, Callus induction, Virtual screening and Docking

INTRODUCTION

Since times immemorial, Medicinal plants have been using virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations in ancient texts such as the Vedas and the Bible were commonly used from traditional herbs and medicinal plants. Traditional medicine has been practicing widely in countries like China, India, Japan, Pakistan, Sri Lanka and Thailand, since ancient times (Lucy and Edgar, 1999; and Kaladhar, 2010a).

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The practice of using medicinal plants for the treatment of various diseases started since the dawn of civilization (Kaladhar *et al*., 2009). Ayurveda is an integral part of Indian culture from Vedic ages (1500-800 BC) mainly employs plant based drugs for the treatment of diseases. Presently there has been an increasing interest for the plant based drugs because of the ready acceptance of local populance, relative inexpensiveness and minimal side effects (Das *et al.,* 1999). About eighty percent of the world population depends on herbal based alternative systems of medicine. An estimated 70,000 plants (including the lower plants) are presently in use in diverse forms for treating ailments. Herbal medicine is now expanding its base at a faster rate due to the great inputs from ethnomedicinal practices being pooled from all over the world (Daniel, 2006).

Plant tissue culture is the science of growing plant cells on artificial media under *in vitro* conditions and is based on the inherent potential of plant cell to regenerate whole plants, if grown aseptically under controlled condition (Mishra, 2009; Edwin *et al*., 2008).

Convolvulus alsinoides (also called as *Evolvulus alsinoides* or *C.alsinoides*) (Common name: Shankapushpi (Andrade *et al*, 2000), Local name: Vishnu krantha) is a perennial herb. Stems are several to numerous, prostrate or ascending, slender, with appressed and spreading hairs. Leaves petiolate or subsessile 0.7-2.5 cm X 5-10 mm. Cymes 1 to few flowered; peduncles filiform, 2.5-3.5 cm; bracts linear-subulate to linear-lanceolate 1.5-4 mm. Sepals lanceolate 3-4 mm, villous. Corolla rotate, 7-10 mm in diameter. Stamens included; filaments filiform, adnate to base of corolla tube. Ovary are glabrous, two Styles and free, Capsule globose and 4-valved, seeds 4 or fewer, black and smooth, Flora and fauna present year round (Fang and George, 1995; and Maurice, 1993).

Ethnomedicinal Uses: nervous exhaustion, memory loss, nootropic agent, general weakness, scrofula, brain tonic, aphrodisiac, digestive and carminative agent, dysentery, chronic bronchitis, asthma, anthelmintic and anti-phlogistic, childhood fever, and the oil stimulates the growth of hair, Nausea and vomiting associated with motion, sickness, sea sickness and pregnancy, jaundice and cold (Kaladhar *et al*., 2009).

Information Technology and Biological sciences are being transformed due to enormous growth of data from laboratories worldwide. The challenge in transformation of information into knowledge will lead to a better understanding of the system processes underlying both health and disease (Andrade *et al.,* 2000; Kaladhar *et al.,* 2010b).

MATERIALS AND METHODS

Collection of Plant Materials: The plant material was largely found in Gajuwaka and Pendurthi region of Visakhapatnam District, India. Whole plant was collected from Visakhapatnam and the experiments were conducted during December 2007 to August 2008 in the Tissue culture Laboratory of the Department of Biochemistry/ Bioinformatics, GITAM University, Visakhapatnam, India.

Preparation of Culture Media: Glass double distilled water was used for the preparation of culture media. After addition of all constituents of MS media, the pH was adjusted to 5.8 using 0.1 N KOH or 0.1 N HCl. Gelling agent (agar-agar) was added as per requirement and the medium was steamed to melt the gelling agent. It was then

dispensed into test tubes (20 ml per tube) or Erlenmeyer flasks (100 ml per 250 ml flask) or screw capped bottles (50 ml per bottle) and was autoclaved at 121°C at a pressure of 15 lbs for 20 min. Heat labile constituents like hormones were filter-sterilized by passing through a Millipore membrane (0.22 µm pore size) ("Millipore Corporation", USA) and added aseptically to the autoclaved medium just before gelling. All the plant growth regulators used during the course of the present work were added before autoclaving the medium. As per the requirements, the medium was also poured in sterile glass petridishes (12 ml per 55 mm dish, 20 ml per 85 mm dish) in a laminar flow hood.

Explants: Healthy explants such as shoot tips and young flowers of *Convolvulus alsinoides* were selected for tissue culture.

Surface Sterilization: Surface sterilization has been conducted on explants for knowing efficient method in removal of microbes.

Method 1: The explants were washed thoroughly under running tap water, followed thrice (3X) with distilled water and submerged for 3 min in 70% ethanol. The explants were again rinsed 3X in sterile, double distilled water and were then submerged in 5% sodium hypochlorite for 10 minutes. Finally the explants were rinsed 3X in sterile, double distilled water.

Method 2: The explants were treated with 0.5% Mercuric chloride for 5 minutes and rinsed 3 times with sterile double distilled water.

After the surface sterilization the explants were cultured on different nutrient media under aseptic conditions.

Control of Phenolic Exudation: To control the phenolic exudation in the cultures of *Convolvulus alsinoides*, activated charcoal (1%) was used in culture media. Periodic subculturing (at weekly intervals) to fresh media with same compositions were also tested to overcome the problem.

Callus Induction: Callus phase of different explants (shoot tips and flower) of *Convolvulus alsinoides* were studied by culturing on MS medium fortified with different combinations of auxins and cytokinins.

Growth and Maintenance of Plant Tissue and Cell Cultures: The callus cultures belonging to the taxa of the present study on solid media were subcultured at regular intervals of 1-2 weeks. Observations were taken at the same time.

Isolation, Identification and Virtual Screening of Ligand: Antioxidant Acylated flavonol glycosides, kaempferol can be isolated from callus cultures of *Convolvulus alsinoides* (Berhow *et al.,* 1994). The structures of new compounds were previously reported in ethanolic extract of *Convolvulus alsinoides* were established by detailed spectroscopic studies, while known compounds were characterized by direct comparison by NMR data (Neeraj, 2009). Two new flavonol-4'-glycoside, kaempferol 4'-O-β-d -glucopyranosyl-(1→2)-β-d-glucopyranoside and kaempferol 4'-O-α-l-rhamnopyranosyl-(1→6)-β-d-glucopyranoside from *C. alsinoides* was previously reported (Guruswamy *et al.,* 1956; Krishnamurthy, 1959; Aulakh *et al.,* 1988; Baveja and Singla, 1969). The isolation of compounds from callus is easier compared with wild plants because callus is softer, grow at lab conditions and available throughout the year. The structure of kaempferol (Neeraj, 2009) is designed and optimized using ChemSketch.

System information: In the present work, we attempted to carry out machine learning evaluation process with the following infrastructure:

Intel (R) Atom (TM) 1.66GHz, 1 GB RAM, Microsoft XP Pro 2002 Service Pack, ACD/ ChemSketch v12.0, Hex 5.1, iGemDOCK v.2.1, HyperChem v8.0.

Docking Studies: Docking studies were done using Hex v5.1 and iGemDOCK v2.1softwares.

Hex: Hex is an interactive molecular graphics program used for protein-ligand docking, assuming the ligand is rigid. It can superimpose pairs of molecules using only knowledge of the 3D shapes of proteins or ligands, for structural comparison.

iGemDOCK: A Generic Evolutionary Method for molecular DOCKing (iGEMDOCK) is a program for computing a ligand conformation and orientation relative to the active site of target protein.

RESULTS

Convolvulus alsinoides is a perennial herb. Fl. and fr. year round. Sandy soil, dry slopes, cultivated areas, maritime areas, grasslands, thickets, roadsides; 0-1800 m. found in Bangladesh, Cambodia, India, Indonesia, Japan (Ryukyu Islands), Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Thailand, Vietnam; Africa, Australia, North America, Pacific Islands, South America (Fang and George, 1995; and Maurice, 1993).

Explants of terminal buds and flower of *Convolvulus alsinoides* were treated with different plant hormones for inducing calli and regeneration. The three surface sterilants (sodium hypochlorite, ethanol and mercuric chloride) were tested. The sterilization was carried out by dipping the plant material into 70% [v/v] ethanol for 2-3 minutes, whereupon it was transferred to a solution of 1% sodium hypochlorite containing 4 drops of Tween-60 for 20 minutes was shown most effective.

Addition of 1% activated charcoal was an effective method to overcome the problem of phenolic exudation but the addition retarded growth in cultures, hence it was not used in subsequent experiments. Weekly subculturing to the medium of same composition produced best results.

Callus Induction

For callus induction, the explants were cultured on MS medium supplemented with IAA, 2, 4-D, BA, Kn either alone or in combinations (Tables 1 and 2)

Effect of IAA: MS medium supplemented with IAA at the range of 0.5 to 3.0 mg/l induced the callus formation in terminal buds within 18 days and flower explants within 20 days. Higher concentrations of IAA (up to 2 mg/l) were effective for callus induction and not have much effect on higher concentrations, beyond 2mg, on terminal buds and flower explants. The calli were brownish white and friable.

Effect of 2, 4-D: MS medium supplemented with 2,4-D at the range of 0.5 to 3.0 mg/l induced the callus formation in terminal buds within 15 days and flower explants within 20 days. Higher concentrations of 2, 4-D (≥3 mg/l) was more effective for callus induction on terminal buds and flower explants. The calli were brownish white and friable. Leaving the calli in the medium turned brown and dried within 4 months.

Table 1: Effect of Growth Regulators (alone) on Callus Induction from Various Explants of *C. alsinoides*

Plant Hormones		Explants	
Growth Regulators	Concentration in mg/l	Terminal Bud	Immature Flower
IAA	0.5	+	+
	1.0	+	+
	2.0	++	++
	3.0	++	++
2,4–D	0.5	+	+
	1.0	++	++
	2.0	+++	++
	3.0	+++	+++
BA	0.5	–	–
	1.0	–	–
	2.0	+	+
	3.0	++	–
Kn	0.5	–	–
	1.0	–	–
	2.0	–	–
	3.0	+	–

Note: – = no callus + = little ++ = Moderate +++ = profuse; Data from 20 replicates in two experiments; Growth period 60 days.

Table 2: Effect of Growth Regulators (Combined) on Callus Induction from Various explants of *C. alsinoides*

Plant Hormones		Explants	
Growth Regulators	Concentration in mg/l	Terminal Bud	Immature Flower
2,4-D+BA	0.5 0.5	+	+
	1.0 0.5	++	+
	0.5 1.0	+	+
	1.0 1.0	+++	+++
2,4-D+kn	0.5 0.5	+	+
	1.0 0.5	++	++
	0.5 1.0	+	+
	1.0 1.0	++	++
IAA+kn	0.5 0.5	-	-
	1.0 0.5	-	-
	0.5 1.0	-	-
	1.0 1.0	+	+

Note: – = no callus + = little ++ = Moderate +++ = profuse; Data from 20 replicates in two experiments; Growth period 60 days.

Effect of BA: MS medium supplemented with BA at the range of 0.5 to 3.0 mg/l was tested for the induction of the callus formation in terminal bud and flower explants. Lesser concentrations of BA upto 1 mg/l in terminal bud and up to 2 mg/l in inflorescence did not induce calli. A little to moderate induction of callus was observed up to 3 mg/l and did not show any progressive change in callus proliferation beyond 3 mg/l on terminal bud and flower explants. The calli were brownish white and friable.

Effect of Kn: MS medium with various concentrations of Kn (0.5-3 mg/l) was found ineffective in inducing the callus formation from terminal buds and flower explants.

Effect of 2, 4-D+BA: Synergistic effects of growth regulators on terminal buds and flowers were cultured with 2, 4-D (1.0 mg/l) and BA (1.0 mg/l) in callus induction of *C.alsinoides* were studied. Profused and higher amount of calli was produced from explants with 1mg/l of 2, 4-D and 1mg/l of BA seem to be optimum concentration for callus induction. The calli were brownish white, friable and meristematic.

Effect of 2, 4-D+Kn: MS media with growth regulators (2, 4-D (0.5-1.0 mg/l) and Kn (0.5-1.0 mg/l)) on terminal buds and flower explants were cultured for callus induction of *C.alsinoides*. Moderate amount of Calli was noticed from explants with 1mg/l of 2, 4-D and 0.5-1mg/l of BA. The calli were brownish white, friable and meristematic.

Effect of IAA+Kn: MS medium containing with combinations of IAA and Kn was found less effect in callus induction in both sources of explants.

Isolation, Identification, Virtual Screening and Docking Studies

Kaempferol (Figure 1) has been selected as ligand in the prediction of active sites and activity against diseased molecules from PDB. The receptors selected were related to Obesity (3LFM.pdb), Diabetes Mellitus (1JB6.pdb), Alzheimer (2V17.pdb) and Parkinson (3IQL.pdb). The surface area and the volume of Kaempferol were predicted as 523.87 A^2 and 276.16 amu based on QSAR results from Hyperchem (Table 3).

Figure 1: Kaempferol {Designed Using ChemSW)

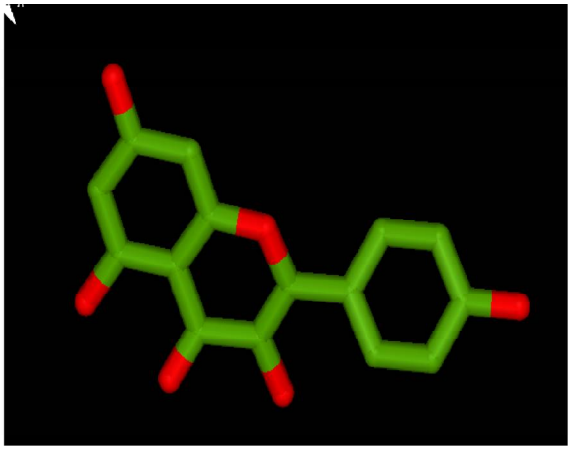


Table 3: QSAR Properties of Kaempferol (from HyperChem)

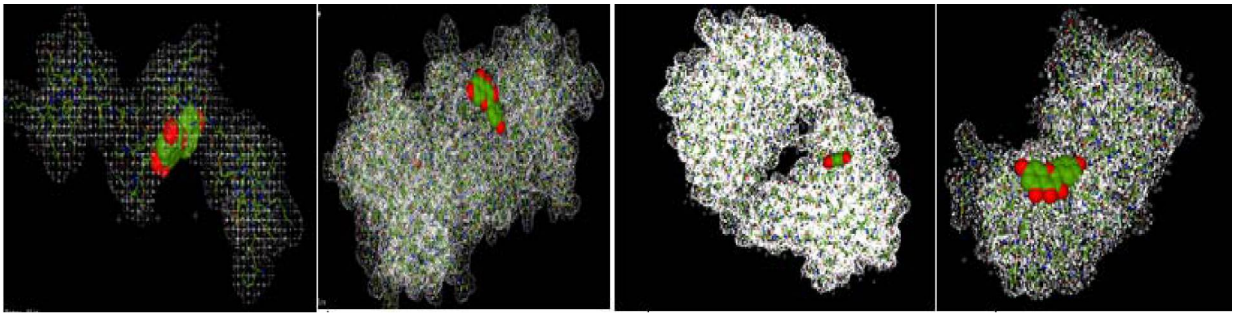
QSAR	Properties
Partial charge	0.00e
Surface area (Approx)	523.87A^2
Surface area (Grid)	447.43 A^2
Volume	721.47A^3
Hydration energy	–10.03 kcal/mol
logP	8.98
Refractivity	37.03A^3
Polarizability	23.35A^3
Mass	276.16 amu

Ligand –Receptor interaction has been predicted well with active sites in iGemDock. As the energies predicted were less for Obesity, the compound may be used for the decrease of fat levels in the multicellular vertebrates such as humans. Kaempferol has predicted good results against aging diseases such as Alzheimer and Parkinson diseases (Table 4). Active sites and the docked images have been shown in Figures 2 and 3.

Table 4: Docking Studies of Kaempferol Against Diseases/Syndromes

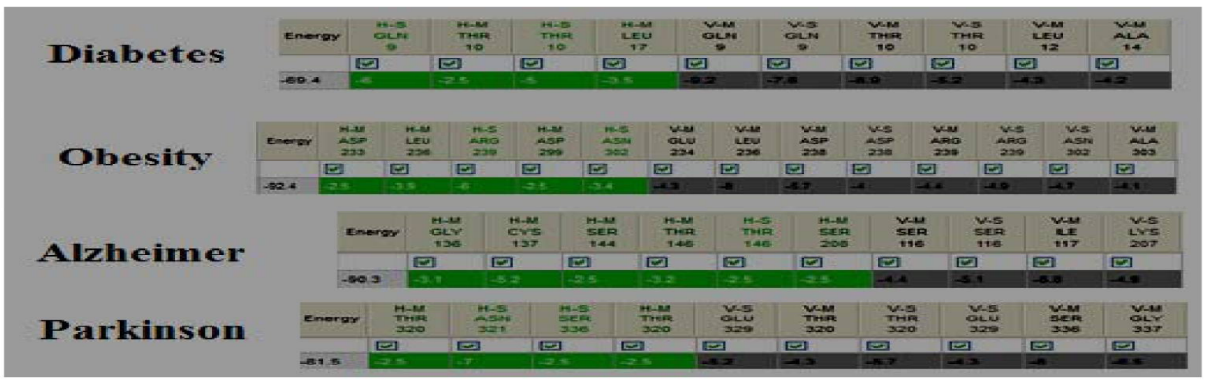
Software	Property	Diabetes	Obesity	Alzheimer	Parkinson
Hex v5.1	Etot	–202.42	–230.48	–228.50	–214.91
	Emax	–113.64	–110.43	–136.06	–102.41
	Emin	–209.45	–230.48	–228.50	–232.13
	Eshape	–202.42	–230.48	–228.50	–214.91
	Distance	2.6	21.8	19.5	13.9
iGEMDOCK v2.1	Energy	–69.44	–92.41	–90.25	–81.52
	VDW	–52.44	–69.58	–67.41	–62.1
	HBond	–17	–22.83	–22.84	–19.42
	Elec	0	0	0	0

Figure 2: Kaempferol Against Diseased Molecules



Diabetes Obesity Alzhiemer Parkinson

Figure 3: Interactive Profile of Kaempferol Against Diseased Molecules



DISCUSSION

To define and describe the future tasks of phytomedicinal research in the new millennium, is not only of the current state of development of phytomedicinal research but also of chemosynthetic pharmaceutical research. An advantage of phytotherapy is the availability of a wide group of medicinal drugs and preparations that have been used over the centuries almost exclusively on the basis of empirical evidence. A reservoir of around 3,00,000 plant species exists, of which only about 30% have been investigated scientifically, inclusively the herbs and preparations of Chinese, Indian, South American and African traditional medicines (Zohara and Uriel, 2005).

Tissue culture methods have been employed as an important aid to conventional methods of plant improvement. The well establishment of good cultural conditions for the removal of external microorganisms and are able to grow readily culture media containing amino acid supplements, growth regulators, organic acids and vitamins (George, 1993).

Convolvulus alsinoides (Latin Name), Vishnukrantha (Sanskrit / Indian Name) is a hairy perennial herb with spreading wiry branches arising from a small woody rootstock. The plant is bitter and is used as a tonic and febrifuge. *Convolvulus alsinoides* (Shankhpushpi) improve memory and help in cases of nervine debility. *Convolvulus alsinoides* (CA) is well known for its memory enhancement, antiepileptic and immunomodulatory properties in the traditional Indian system of medicine, Ayurveda (Siripurapu, 2005).

There is need to cultivate and domesticate some potential medicinal plant such as *Convolvulus alsinoides* used in skin care under social forestry scheme for its sustainable use for human welfare (Singh and Anand, 1996). Tejavathi and Purushothama (2004), developed a protocol on *Convolvulus alsinoides* L. Stem, leaf, pedicel and flower buds were cultured on MS medium supplemented with various growth regulators (including IAA, IBA, NAA, 2, 4-D, kinetin, benzyladenine and isopentenyladenine) either alone or in combination. Callus induction was observed after 6-12 days of culture, when the explants cultured on the medium was supplemented with auxins. The whole plant contains an optically active alkaloid evolvine and optically inactive fatty residue.

Experimentation on three surface sterilants (sodium hypochlorite, ethanol and mercuric chloride) was made to observe the better method of sterilization of the explants. The sterilization was carried out by dipping the explants into 70% [v/v] ethanol for 2-3 minutes, whereupon it was transferred to a solution of 1% sodium hypochlorite containing 4 drops of Tween-60 for 20 minutes was shown most effective. The efficiency of ethanol, sodium hypochlorite, and Tween in surface sterilization was previously reported in some medicinal plants like - *Citrus aurantifolia* (lime) (Jameel and Abdulaziz, 2001), *Polygonum multiflorum* (Li-Chang *et al.,* 2003), *Scrophularia yoshimurae* (Abhay *et al.,* 2001) and *Aframomum corrorima* (Wondyifraw and Surawit, 2004).

Addition of activated charcoal to overcome the problem of phenolic exudation was effective to get best results. The use of activated charcoal to overcome phenolic exudation was previously

reported in *Tinospora cordifolia* (Gururaj *et al.,* 2007), *Gloriosa superba* (Sayeed and Shyamal, 2005) and *Dioscorea bulbifera* (Alka *et al.,* 2003).

The shoot regeneration potential through callus phase from various explants via terminal bud and flower to various concentrations and combinations of growth regulators in MS medium was currently investigated. Previous studies were made on organogenesis from shoot tips and flower of *C.alsinoides* through callus formation on different media supplemented with various hormonal combinations. The selection of terminal bud and flower buds of *Convolvulus alsinoides* was selected as explants were also reported previously (Tejavathi and Purushothama, 2004).

For callus induction, the explants were cultured on MS medium supplemented with IAA, 2, 4-D, BA, Kn either alone or in combinations. Profused and higher amount of calli was produced from explants with 1mg/l of 2, 4-D and 1mg/l of BA. Previous reports has been made with 1mg/l of 2, 4-D and 1mg/l of BA for the regeneration from inflorescence pieces of *Bowiea volubilis* (Afolayan and Adebola, 2004).

Shankhpushpi is considered as Medhya Rasayana in Ayurvedic texts, used for its action on the central nervous system, especially for boosting memory and improving intellect. Evolvine, an alkaloid, and phytochemical, kaempferol isolated by controversial sources from Shankhpuspi, a tropical plant is potent radical scavenger and efficient antioxidants (Hideo *et al.,* 1997).

CONCLUSION

The present study concludes that, various plants were considered as sources of vital energy on this biosphere. The term energy with regards to plants is considered in different angles by different researchers and institutions. The energy yield by these plants depends upon various factors. Our focus with regards to energy yield by these plants is of medicinal importance. *Convolvulus alsinoides* is an important medicinal plant used for the treatment of nervous exhaustion, memory loss, nootropic agent, general weekness, scofula, dysentery, chronic bronchitis, asthma, antihelmintic, antiphlogistic, childhood fever, jaundice, cold and also used in stimulating the growth of hair. The plant was largely used in ayurvedic medicines like Chyawanprash, Mindcare, Migtrim, Peditone, Antiox and Evocen. The present *in silico* analysis predicted good results with diseased proteins such as Alzheimer, Parkinson, Obesity and Diabetes.

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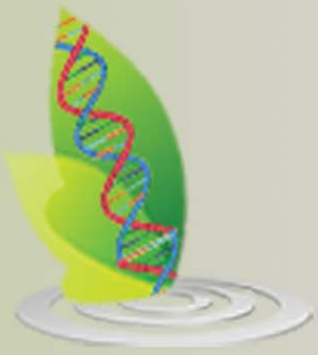

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