# Evaluation of antioxidant capacity and acetylcholinesterase inhibitory activity of field-grown plants and *in vitro* plantlets of *Enicostemma littorale*

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## ABSTRACT

**Background:** *Enicostemma littorale* is an ethno-medicinally important plant belonging to *Gentianaceae* family. **Objective:** The main aim of this study was to establish tissue culture of *E. littorale* and compare the antioxidant capacities and acetylcholinesterase inhibitory (AChEi) properties of *in vitro* and field-grown *E. littorale* plants. **Materials and Methods:** Antioxidant capacities for various *E. littorale* extracts were studied in terms of 1,1-diphenyl-2-picrylhydrazyl and 2,2′-azinobis-3 ethylbenzothiazoline-6-sulfonic acid free-radical scavenging along with ferric reducing antioxidant power and total phenolic content was also determined. AChEi properties were also compared between field-grown and *in vitro E. littorale* extracts. **Results:** *In vitro* plantlets of *E. littorale* was established in Murashige and Skoog (MS) medium with three different hormonal combinations, viz. naphthalene acetic acid (1 mg/l) and 6-benzylaminopurine (BAP)(0.2 mg/l) or $N_1B_{0.2}$, BAP (1 mg/l) and kinetin (0.1 mg/l) or $B_1K_{0.1}$ and indole butyric acid (IBA) (1 mg/l) or $IBA_1$. Alcoholic extracts of shoots developed in MS medium supplemented with $B_1K_{0.1}$ showed highest antioxidant potential than other extracts. Alcoholic extracts of plantlets grown in MS medium supplemented with $IBA_1$ showed both antioxidant and AChEi potential equivalently to field-grown extracts. **Conclusion:** *In vitro* plantlets grown in MS medium half strength supplemented with $IBA_1$ can be considered as most suitable for rapid proliferation because it offers advantage of equivalent potential of bioactivity in terms of antioxidant capacity and AChEi when compared to field-grown plants. Further studies can lead to better utilization of therapeutic potential of *E. littorale*.

**Keywords:** Acetylcholinesterase inhibition, antioxidant, *Enicostemma littorale*, secoiridoids

## INTRODUCTION

The *Gentianaceae* is a medicinally important family; several genera are known to produce bioactive compounds, including xanthones, iridoids and C-glucoflavones.[1] Members of this family such as, *Canscora decussata, Swertia chirata* are considered as potent source of acetylcholinesterase inhibitors (AChEi).[2,3] Acetylcholine is a neurotransmitter that can stimulate nicotinic receptors associated with memory and cognitive process. Short life span of this compound is due to the hydrolysis of the compound by AChE enzyme leading to the acceleration of Alzheimer's disease. Alzheimer's disease is a slow progressive neurodegenerative disorder affecting the people worldwide. Thus inhibiting the AChE enzyme has been a strategy for treatment of such diseases.[4] Along with AChE inhibitors, antioxidants are also used for treatment of disease since it can minimize or prevent cell damage caused by free radicals in disease state.

*Enicostemma littorale*, locally known as "chota chirata" is a short, erect, perennial herb belonging to *Gentianaceae* family. The plant is widely distributed in South America, Africa and Asia, and can also grow in diverse environments

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ranging from savannas, grasslands, and forests to beaches.[5] The plant, when used in combination with other herbs, is said to cure diabetes and often patients are advised to eat fresh leaves of this genus.[6] The aqueous or alcoholic extract of this plant also showed a range of pharmacological properties, including antioxidant, anti-inflammatory, antitumor, hypoglycemic and hepatoprotective.[7-11] Different phytochemicals like, monoterpene alkaloids, xanthones, catechins, C-glycosides, flavanoids, etc. have been isolated from *E. littorale*.[12,13] This plant is also considered as a potent source of swertiamarin, a secoiridoid compound.[14] Swertiamarin isolated from *Gentiana* species showed potent AChEi activity.[15] However, no attempt was made to evaluate AChEi activity from *E. littorale* extract till date. Although *E. littorale* is distributed in a few agro-climatic zones of India, it has a low frequency of germination.[16] Furthermore recent urbanization has led to the destruction of plant's natural habitat. Thus, there is a need for the establishment of *in vitro* regeneration system for conservation and sustainable utilization of *E. littorale* bioresources. A few reports are available on *in vitro* regeneration of this plant using leaf, shoot tip and nodal explants.[16,17] However no initiatives have been made to evaluate the efficiency of *in vitro* grown plantlets for bioactive properties. Therefore, the aim of this study has been to compare and evaluate the antioxidant and AChEi properties of field-grown and *in vitro* plantlets of *E. littorale*.

## MATERIALS AND METHODS

### Plant material

*E. littorale* plants were collected from the botanical garden of Dr. PR Ghogrey Science College, Dhule, Maharashtra (India). Explants (leaf, node, apical meristem and root) were surface sterilized and used for establishing tissue culture immediately and rest was stored at −80°C until further use.

### Reagents and chemicals

Acetylthiocholine iodide (ATCI), AChE type VI-S from electric eel, 5,5'-dithiobis (2-nitrobenzoic acid) DTNB, eserine salicylate, ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ) and bovine serum albumin were purchased from sigma. Murashige and Skoog (MS) medium was purchased from HiMedia, India.

### Establishment of *in vitro* cultures

*E. littorale* plant collected from Dhule (Maharashtra) was surface sterilized (Figure 1). Leaf, root and nodes were inoculated in MS medium supplemented with naphthalene acetic acid (1 mg/l) and 6-benzylaminopurine (BAP) (0.2 mg/l) or $N_1B_{0.2}$.[18] Apical meristems were inoculated in MS medium supplemented with BAP (1 mg/l) and kinetin (0.1 mg/l) or $B_1K_{0.1}$. Liquid cultures were established by inoculating shoots obtained in MS medium supplemented with $N_1B_{0.2}$ in MS (half strength) supplemented with indolebutyric acid (IBA) (1 mg/l) or $IBA_1$. All the culture tubes were maintained in the culture room at 24 ± 1°C with photoperiod of 16 h dark and 8 h light under white cool fluorescent light. Mean growth response at 30 days was calculated (fresh biomass weight [g]). Once established, cultures were regularly sub-cultured in MS medium with all three different hormone combinations: $N_1B_{0.2}$, $B_1K_{0.1}$ and $IBA_1$ for maintenance.

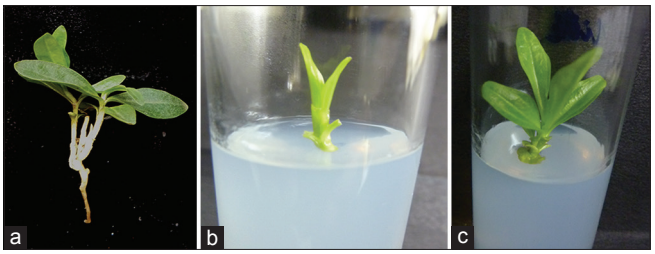


**Figure 1:** (a) Field-grown *Enicostemma littorale* plant, (b) apical meristem showing shoot initiation, (c) and differentiation after 30 days in Murashige and Skoog medium supplemented with $B_1K_{0.1}$

### Sample extraction

*In vitro* and field-grown plant material was shade dried at room temperature. Dried plant material was crushed in four different solvents: ethanol, water, ethyl acetate and petroleum ether using sterile mortar and pestle. Crushed plant material was ultrasonicated for 30 min and kept dipped overnight in respective solvents. The sample was evaporated under vacuum (concentrator plus) and concentration of the extract was calculated. Dry powder of extract of known concentration was redisolved in respective solvents and used for all the phytochemical assays. 10-15% recovery was observed in all four different solvent extractions for both *in vitro* plantlets and field-grown *E. littorale*.

### Antioxidant studies

#### *DPPH free radical scavenging activity*

DPPH free radical scavenging activity was determined quantitatively according to previously described method with slight modification.[19] Extracts (0.1 ml) were mixed with 0.9 ml ethanolic solution of DPPH (0.1 mM). The mixture was vortexed and kept in the dark for 30 min at room temperature. The decrease in absorbance was

monitored spectrophotometrically (Shimadzu UV 1800) at 517 nm. The percentage inhibition was calculated using the equation:

$$\text{Inhibition } \% = 1 - (A_{sample}/A_{control}) \times 100 \quad (1)$$

Where, $A_{sample}$ is the absorbance of the sample extracts, and $A_{control}$ is the absorbance of the blank (solvent only). Inhibitory concentration ($IC_{50}$) values were calculated by performing regression analysis of percentage inhibition and extract concentration.

### *ABTS free radical scavenging assay*

ABTS assay was performed according to the modified method of Re *et al.* (1999).[20] ABTS free radical solution (7 mM) was prepared having 2.45 mM potassium persulfate and incubated in the dark for 12-16 h. For working solution, absorbance of ABTS solution was adjusted to 0.700 ± 0.020 (at 734 nm) by diluting it with ethanol. The reaction mixture consisted of working solution (995 µl) and crude extract (5 µl). The mixture was incubated for 6 min at room temperature and decrease in absorbance was monitored at 734 nm. The percentage inhibition was calculated using the equation (1). Further, activity in terms of mM concentration of ascorbic acid equivalent per gram dry weight was calculated by using the standard curve of different concentrations of ascorbic acid.

### Ferric reducing antioxidant power (FRAP) assay

The reducing power of the extract was determined by modified method of FRAP assay.[21] Stock solutions included TPTZ solution (10 mM) which was prepared in HCl (40 mM), $FeCl_3.6H_2O$ (20 mM) and acetate buffer (300 mM, pH 3.6). The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $FeCl_3.6H_2O$. The extracts (3 µl) were allowed to react with FRAP working solution (997 µl). The mixture was then incubated for 6 min at 37°C and absorbance was recorded at 593 nm. $FeSO_4$ equivalent per gram dry weight was calculated by using the standard curve.

### Determination of total phenolic content

Total phenolic content was determined using Folin–Ciocalteu reagent.[22] Extract (100 µl) was mixed with 10% Folin–Ciocalteu reagent (200 µl) and 800 µl of $Na_2CO_3$ (700 mM) and vortexed. The tubes were incubated for 2 h at room temperature. Increase in absorbance was measured at 765 nm. Total phenolic content was obtained by comparing the absorbance change of solution containing the extract at 765 nm with that of the calibration plot of gallic acid and was expressed as mg gallic acid equivalent per gram dry mass.

### Acetylcholinesterase inhibitory (AChEi) studies

### *Determination of AChEi activity*

The AChEi activity of the extracts was determined by the modified Ellman's spectrophotometric method, using acetylthiocholine as substrate.[23] The reaction mixture consisted of 125 µl of 3 mM DTNB, 23 µl of 15 mM ATCI, 178 µl of 50 mM Tris-HCl buffer (pH 8.0), sample extract and 50 µl 0.28 U/ml AChE. The reaction mixture was monitored at 405 nm for 5 min. Eserine salicylate (1 mg/ml) was used as the positive inhibitor, while respective solvents were used as control to ensure that there was no inhibition of AChE. The percentage inhibition was calculated using the equation (1). $IC_{50}$ values were calculated by performing regression analysis of percentage inhibition and extract.

### Statistical analysis

All the experiments were done in triplicate and results obtained were analyzed using analysis of variance (ANOVA). Duncan's multiple range test ($P < 0.05$) was performed using the software MSTATC (developed by Crop and Soil Sciences Department of Michigan State University, United States).

## RESULTS

### Establishment of *in vitro* cultures

Leaf explants inoculated in MS medium with $N_1B_{0.2}$ showed callus formation after 18 days following which shoot initiation from callus was observed after 45 days. While root and nodal explant given in MS medium with $N_1B_{0.2}$, which showed callus initiation after 20 and 24 days respectively. Apical meristem was given in MS medium with $B_1K_{0.1}$ medium showed direct organogenesis of the plant within 30 days (Figure 1). In all cases, 100% survival rate was observed. Shoots developed in MS medium supplemented with $N_1B_{0.2}$ showed both shoot and root proliferation when given in liquid MS medium (half strength) supplemented with $IBA_1$ within 30 days. Effect of different hormones on average growth of plantlets in terms of mean growth after 30 days is given in Table 1.

### Antioxidant studies

### *DPPH free radical scavenging capacity*

DPPH free radical scavenging capacity was studied for field-grown and *in vitro* plantlet extracts. DPPH is a stable

free radical having an odd electron contributing to its deep violet colour which when scavenged causes the colour change from violet to pale yellow. This can be measured spectrophotometrically and is relatively proportional to scavenging ability. The $IC_{50}$ value or concentration at which 50% inhibition occurred (mg/ml) was calculated after regression analysis and Duncan's multiple range test was performed after ANOVA ($P < 0.05$) (Table 2). Alcoholic extract ($IC_{50} = 0.38 \pm 0.015$) showed highest DPPH free radical scavenging capacity than other solvent extracts of field-grown plant. Similarly, alcoholic extract of *in vitro* plantlet grown in MS medium supplemented with $IBA_1$ ($IC_{50} = 0.24 \pm 0.014$) and $B_1K_{0.1}$ ($IC_{50} = 0.29 \pm 0.015$) showed the highest capacity among other *in vitro* plantlet extracts. However, aqueous extract of field-grown plant showed higher antioxidant capacity than aqueous extracts of *in vitro* plantlets grown in different media. Extracts of *in vitro* plantlets grown in MS medium supplemented with $N_1B_{0.2}$ showed relatively lower capacity than other *in vitro* extracts.

**Table 1: Effect of different hormones on growth of *in vitro* plantlets of *E. littorale* observed after 30 days of subculture**

| Hormone | Growth response | Growth (g fresh biomass)[#] |
|---|---|---|
| $N_1B_{0.2}$ | S (++) R (++) | 0.980±0.01 |
| $B_1K_{0.1}$ | S (+++) R (+) | 1.188±0.02 |
| $IBA_1$ | S (+++) R (+++) | 3.38±0.37 |

[#]Values are mean±SE of at least three independent determinations. S: Shooting, R: Rooting, (+): Poor/absent, (++): Moderate, (+++): Good, *E. littorale: Enicostemma littorale*, IBA: Indole butyric acid, SE: Standard error

### *ABTS free radical scavenging activity*

This assay was performed to test the ABTS free radical scavenging capacity of field-grown and *in vitro* plant of *E. littorale*. ABTS free radical on incubation with sodium persulfate forms ABTS cation, which is deep blue in color and is highly reactive towards antioxidants. The rapid reaction between them causes decrease in color that can be monitored at 734 nm. The activity was calculated as equivalent mM concentration of ascorbic acid per gram dry biomass and Duncan's multiple range test was performed after ANOVA ($P < 0.05$)(Table 2). Alcoholic extract of *in vitro* plantlets supplemented with $B_1K_{0.1}$ showed highest ABTS free radical scavenging capacity (ABTS value = 0.438 ± 0.015). Among ethyl acetate extracts, $B_1K_{0.1}$ had shown the highest antioxidant capacity, whereas field-grown and $IBA_1$ extracts had shown equal ABTS scavenging potentials. Aqueous extract of field-grown *E. littorale* showed highest antioxidant capacity (ABTS value = 0.297 ± 0.01) than all *in vitro* aqueous extracts.

### FRAP assay

FRAP was estimated for field-grown and *in vitro* plantlet extracts of *E. littorale*. This assay helps in understanding the reducing capability of the antioxidant by measuring the formation of blue colored reduced form of TPTZ that could be monitored at 593 nm. The reducing power was expressed as mM concentration of $FeSO_4$ per gram dry biomass and Duncan's multiple range test was performed after ANOVA ($P < 0.05$)(Table 2). Highest reducing

**Table 2: Antioxidant capacity and acetylcholinesterase inhibitory activity of various *E. littorale* extracts**

| Assay | Sample | Activity[#] | | | |
|---|---|---|---|---|---|
| | | FG | $IBA_1$ | $N_1B_{0.2}$ | $B_1K_{0.1}$ |
| DPPH free radical scavenging activity | Alcoholic | $0.38\pm0.01^{ab}$ | $0.24\pm0.01^{a}$ | $0.41\pm0.04^{b}$ | $0.29\pm0.01^{ab}$ |
| | Aqueous | $0.63\pm0.02^{a}$ | $0.87\pm0.01^{c}$ | $0.73\pm0.006^{b}$ | $1.16\pm0.02^{d}$ |
| | Ethyl acetate | $0.45\pm0.03^{a}$ | $0.43\pm0.01^{a}$ | $0.96\pm0.03^{b}$ | $0.45\pm0.03^{a}$ |
| | Petroleum ether | $0.98\pm0.01^{c}$ | $0.38\pm0.04^{a}$ | $0.55\pm0.01^{ab}$ | $0.56\pm0.03^{b}$ |
| ABTS free radical scavenging activity | Alcoholic | $0.333\pm0.008^{b}$ | $0.376\pm0.015^{b}$ | $0.238\pm0.06^{c}$ | $0.438\pm0.015^{a}$ |
| | Aqueous | $0.297\pm0.01^{a}$ | $0.135\pm0.007^{c}$ | $0.163\pm0.03^{b}$ | $0.154\pm0.013^{bc}$ |
| | Ethyl acetate | $0.284\pm0.02^{b}$ | $0.295\pm0.009^{b}$ | $0.177\pm0.017^{c}$ | $0.316\pm0.011^{a}$ |
| | Petroleum ether | $0.0465\pm0.014^{b}$ | $0.068\pm0.068^{ab}$ | $0.084\pm0.023^{ab}$ | $0.130\pm0.031^{a}$ |
| FRAP | Alcoholic | $7.76\pm0.98^{c}$ | $8.14\pm0.25^{c}$ | $9.38\pm0.24^{b}$ | $11.94\pm0.94^{a}$ |
| | Aqueous | $3.35\pm0.24^{a}$ | $2.03\pm0.09^{b}$ | $1.5\pm0.44^{c}$ | $1.18\pm0.14^{c}$ |
| | Ethyl acetate | $4.19\pm0.14^{b}$ | $4.61\pm0.69^{b}$ | $1.82\pm0.14^{c}$ | $7.52\pm0.92^{a}$ |
| | Petroleum ether | $1.53\pm0.68^{a}$ | $0.86\pm0.43^{ab}$ | $0.71\pm0.09^{b}$ | $1.04\pm0.17^{ab}$ |
| AChEi activity | Alcoholic | $0.013\pm0.003^{a}$ | $0.015\pm0.015^{ab}$ | $0.029\pm0.004^{b}$ | $0.049\pm0.001^{c}$ |
| | Aqueous | $0.027\pm0.015^{a}$ | $0.062\pm0.001^{c}$ | $0.071\pm0.017^{c}$ | $0.037\pm0.002^{b}$ |
| | Ethyl acetate | $0.043\pm0.005^{a}$ | $0.072\pm0.015^{b}$ | $0.075\pm0.007^{b}$ | $0.029\pm0.013^{a}$ |
| | Petroleum ether | ND | ND | ND | ND |

[#]Activity of FG and *in vitro* ($IBA_1$, $B_1K_{0.1}$ and $N_1B_{0.2}$) extracts of *E. littorale* measured in terms of $IC_{50}$ values (mg/ml) for DPPH free radical scavenging activity and AChEi activity, mM concentration of $FeSO_4$/g dry biomass for FRAP and mM concentration of ascorbic acid equivalent/g dry biomass for ABTS free radical scavenging assay. All the values are mean±SE for triplicate readings. Values with the same superscript letter along the same row are not significantly different ($P<0.05$). ND: Not detectable, FG: Field-grown extract of *E. littorale*, IBA: Indole butyric acid, DPPH: 1,1-diphenyl-2-picrylhydrazyl, ABTS: 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid, FRAP: Ferric reducing antioxidant power, AChEi: Acetylcholinesterase inhibitory, *E. littorale: Enicostemma littorale*

power (FRAP value = 11.94 ± 0.94) was observed in alcoholic extracts of *in vitro* plantlets grown in MS medium supplemented with $B_1K_{0.1}$. Both alcoholic and ethyl acetate extracts of *in vitro* plantlet grown in MS medium supplemented with $IBA_1$ and field-grown *E. littorale* had shown relatively equal reducing power. However, aqueous extract of field-grown plant showed higher reducing power than all aqueous *in vitro* extracts.

### Total phenolic content

Total phenolic content was determined by Folin–Ciocalteu reagent for all the extracts of field-grown and *in vitro* plantlets. The phenolic content obtained was expressed in terms of gallic acid equivalent (mg) per gram dry biomass, and Duncan's multiple range test was performed after ANOVA ($P < 0.05$) (Table 3). Highest phenolic content was observed in an alcoholic extract (0.562 ± 0.007 mg/g dry biomass gallic acid equivalent) of *in vitro* plantlets grown in MS medium with $B_1K_{0.1}$. Phenolic contents of alcoholic extracts of field-grown and *in vitro* plantlet grown in MS medium supplemented with $IBA_1$ were relatively same. However, aqueous extract of field-grown plant showed higher phenolic content than *in vitro* aqueous extracts. Petroleum ether extracts had the least phenolic content.

### AChEi studies

Quantitative AChEi activity of *E. littorale* was studied using Ellman's method.[24] The $IC_{50}$ value (mg/ml) was calculated, and Duncan's multiple range test was performed after ANOVA ($P < 0.05$)(Table 2). Eserine salicylate was used as the standard inhibitor of AChE ($IC_{50}$ = 0.0013 ± 0.02). Alcoholic extracts of field-grown plant ($IC_{50}$ = 0.013 ± 0.003) and *in vitro* plantlet grown in MS medium supplemented with $IBA_1$ ($IC_{50}$ = 0.015 ± 0.015) showed maximum AChEi potential. Alcoholic extract of *in vitro* plantlet grown in MS medium supplemented with $N_1B_{0.2}$ ($IC_{50}$ = 0.029 ± 0.004) also showed relatively equal AChEi activity with that of $IBA_1$. However, aqueous extracts of field-grown plants showed higher AChEi and antioxidant activity than *in vitro* plantlets.

**Table 3: Total phenolic content of various *E. littorale* extracts**

| Sample | Concentration of Gallic acid equivalent in mg/g dry biomass[#] | | | |
|---|---|---|---|---|
| | Field-grown | *In vitro* | | |
| | | $IBA_1$ | $N_1B_{0.2}$ | $B_1K_{0.1}$ |
| Alcoholic | 0.447±0.011[b] | 0.431±0.029[b] | 0.484±0.056[b] | 0.562±0.007[a] |
| Aqueous | 0.159±0.015[a] | 0.127±0.005[b] | 0.114±0.01[bc] | 0.103±0.011[c] |
| Ethyl acetate | 0.399±0.014[b] | 0.4±0.01[b] | 0.282±0.015[c] | 0.464±0.013[a] |
| Petroleum ether | 0.092±0.013[a] | 0.111±0.014[a] | 0.101±0.013[a] | 0.112±0.023[a] |

[#]All values are mean±SE for triplicate readings. Values with the same superscript letter along the same row are not significantly different ($P$<0.05), *E. littorale: Enicostemma littorale*, IBA: Indole butyric acid, SE: Standard error

## DISCUSSION

*E.* littorale plantlets were developed via indirect (using leaf explants given in MS medium supplemented with $N_1B_{0.2}$), direct organogenesis (using apical meristem given in MS medium with $B_1K_{0.1}$) and also in liquid MS medium (half-strength supplemented with $IBA_1$). Our results were in correlation with the earlier reports of tissue culture establishment of *E. littorale*.[16,17]

Antioxidant studies showed that highest antioxidant capacity was observed in an alcoholic extract of *in vitro* plantlets grown in MS medium supplemented with $B_1K_{0.1}$, which also correlated with its phenolic content. The higher antioxidant power in alcoholic and ethyl acetate extracts of *in vitro E. littorale* may be due to the different hormonal treatments used in media for optimal growth. Similar results were reported in tissue cultured *Cassis siamea* where total phenolics and antioxidant power were higher than field-grown plant.[25] The activities are shown by different extracts also correlated with total phenolic contents thus suggesting the presence of bioactive phenolic compounds. Alcoholic extracts of field-grown plant and *in vitro* plantlet grown in MS medium supplemented with $IBA_1$ showed highest AChEi potential. Higher activities in these extracts could be due to the presence of both roots and shoot metabolites since the media supported both root and shoot development. Swertiamarin, a prominent compound of *E. littorale* when isolated from other *Gentianaceae* species showed potent AChEi activity.[14,15] Earlier studies have also reported that gentiopicricin and swertiamarin are dominant compounds present in roots of *Centaurium erythraea*, a *Gentianaceae* plant.[24]

Extracts of *in vitro* grown *E. littorale* showed potent antioxidant and AChEi capacities. Since cholinergic deficit is one of the most early detected symptoms in neurodegenerative diseases like Alzheimer's and Parkinson's diseases and thus, inhibition of AChE is a viable therapeutic strategy.[4] In recent times, huperzine A (*Huperzia serrata*) a *Lycopodium* alkaloid was tested through clinical trials for Alzheimer's disease. In USA it is marketed as a nutraceutical for the same.[26] Isolation and identification of AChEi like swertiamarin, xanthones and other phenolic compounds from the crude extracts may help in the development of new plant-based drugs for the treatment. *In vitro* cholinesterase inhibitory and antioxidant activities of five *Gentiana* species and *Gentianella caucasea*

has been reported in recent times where presence of compounds such as gentiopicroside, swertiamarin, isoorientin, isovitexin and vitexin have been detected thus suggesting their therapeutic potential.[15] No reports on human studies of these compounds are found. Oxidative stress often aggravates disease condition thus antioxidant potential of crude extracts may also abate the disease progressions.

## CONCLUSION

This study has led to the development of the protocol for *in vitro* propagation of *E. littorale*. MS medium (liquid; half strength) supplemented with IBA (0.1 mg/l) was found to be optimal for rapid proliferation of plant. Antioxidant capacity and AChEi activity of plantlets grown in MS medium supplemented with IBA (0.1 mg/l) showed equal potential to that of field-grown plant of *E. littorale*.

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