A Compendious Review of *Enicostemma littorale* Blume. Panacea to Several Maladies

Vigneswaran M, Prem Kumar G, Sivakumar S, Siva G, Nandakumaran T,
Lakshmi Prabha A and Jayabalan N

Abstract - Medicinal plants are playing a significant role in curing various diseases from ancient times. Among them, *Enicostemma littorale* Blume. is one of the perennial herb of the family Gentianaceae is cosmopolitan species occurrence in India. The bittery natural plant which is important indigenous medicine for treatments various ailments like laxative, tooth decay, obesity, curing fever, skin diseases, rheumatism, abdominal disorders, snake bite and helps to regulate blood sugar levels. *E. littorale* possesses valuable bioactive compounds including alkaloids, saponins, catechins, sterols, phenolic acids, triterpenoids, flavonoids and xanthones. *In vitro* studies and *in vivo* models have provided a simple bio-scientific justification for its various ethanopharmacological uses. The plant also contains minerals like calcium, iron, potassium, phosphate, carbonate, chloride sulphate and silica. Moreover, they also posses significant antimicrobial, anti inflammatory, hepatoprotective hypolipidaemic and hypoglycaemic properties. This review article encompasses botanical description, ethanopharmacological uses, phytochemistry and biological activities of *E. littorale*, for the period up to 2017 and provides a bird's eye view about its geographical distribution, physicochemical parameters, phytoconstituents and pharmacological properties. Possible trends and perspectives for future research on this plant are discussed, as well. Meticulous phytochemical studies and sustainable conservation of *E. littorale* could yield more reliable compounds of pharmacological significance for better healthcare.

Keywords - *Enicostemma littorale*, Swertiamarin, Antidiabetic, Anticancer

1 Introduction

For improved "quality of life" there is significant increase in demand of herbal medicines for primary health care, because of their effectiveness, safety and minimal or no side effects. The synthetic drugs although effective against various health related disorders, produce some severe side effects, which culminate in deterioration of human health [46,110]. In order to overcome these side effects, scientists have focused their research on medicinal herbs [96]. Moreover, the herbal formulations also offer remedy for age-related disorders like osteoporosis, immune disorders, memory loss, etc., for which very few modern medicines are available [57]. The medicinal plants are a major source of biodynamic compounds of therapeutic value and have been known for their health benefits in Ayurveda, Unani and traditional system of medicines. Herbal molecules are safe and have the potential to overcome the resistance produced by the pathogens, as they exist in a pooled form of more than one molecule in the protoplasm of the cell [67, 133]. Nowadays, almost all the dreadful diseases including cancer, AIDS, kidney damage, cardiovascular diseases and many more are curable by the use of medicinal herbs [24, 61, 136].

There are several plant genus which are reputedly known for their contribution to traditional as well as modern medicines. The plant *E. littorale* is one among them and it plays a vital role in human healthcare. The plant leaves possess antioxidant, hypoglycemic, hepatomodulatory and hepatoprotective properties and helps in reducing obesity. Medicinal compounds derived from this plant were considered to be very effective since these were less toxic, palatable, eco-friendly, long shelf life and free from side effects. According to the World Health Organization [151], herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the enormous rural areas of developing countries.

The plant has number of antioxidative phtochemicals which include alkaloids, saponins, sterols, catechins, xanthones, phenolic acid flavonoids and triterpinoids [81]. The plant is used in folk medicine to treat diabetes mellitus, abdominal ulcers, rheumatism, hernia, insect poisoning, itching, swelling anti-inflammatory [109] hypoglycemic [74,75] and anticancer [60] activities.

2. Clear Description of The Plant

The plant is native to tropical Africa, Southeast Asia, India and Malaysia; Africa to Lesser Sunda Islands.

2.1. Nomenclature: The word *Enicostemma* is probably formed from the three words, "en" means inside, "icos" means 20 and "stemma" means wreath or circle due to the many flowers arranged in circles in the leaf axils along the stem.

2.2. Latin names: *Enicostemma littorale* Blume, *Enicostema axillare* (Lam.) A. Raynal, Family: Gentianaceae (Gentian Family).

2.3. Common names: Ayurvedic medical: Mamejava, Mamejav, Mamejavo [2], Mamejva, Mamjjak; Hindi/Hindustani: Kariyatu, Chota-chiretta, Chota-kirayat, Chota-chirayata; Bombay: Manucha, Kada-vinayi; Tamil: Vellarugu, Telugu: Nela-gulimidi, Nela-guli; Additional names: Gormadi koora (In the UK product description for Glucostat, Maharishi Ayurveda Products [71], incorrectly refers to *E. littorale* as Indian Gentian; this English common name generally refers to Swertia spp. Whitehead is a common name [82].

- *Vigneswaran M, Prem Kumar G, Sivakumar S, Siva G, Lakshmi Prabha A and Jayabalan N, Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu - 620 024.*
- *Nandakumaran T, Department of Botany, Kandaswami Kandar's College, Namakkal, Tamil Nadu - 637 207.*

2.4. Detailed description of the plant.

Fig. 1. Enicostemma littorale with white flowers

Erect perennial herb, 5–90 cm tall, simple or branched at the base. Stem cylindric, glabrous with a decurrent ridge below each leaf. Leaves sessile (sometimes narrowed into a petiole-like base), longer than the internodes; lamina (1)5–8 x 0.3–1 cm, linear to lanceolate or narrowly oblong, entire, obtuse and mucronate at the apex, somewhat narrowing towards the base, 3-nerved from the base, glabrous. Inflorescence in many flowered axillary clusters, numerous in the axils of each pair of leaves. Flowers white with green lines, drying yellowish, sessile or subsessile; bracts long, shorter than the calyx, lanceolate-acuminate, carinate. Calyx tube 1–2 mm long; lobes usually unequal, 0.7-1.5(2) x 0.4-0.7 mm, triangular to lanceolate, acute at the apex and narrowly scarious at the margin, or obovate to subcircular, obtuse and mucronate at the apex, with wide scarious margin. Corolla tube 3.5-6 mm long; lobes 1.5-2 x 0.7-1 mm, ovate and abruptly narrowing to an acute or mucronate apex. Stamens inserted below the sinuses, just above the middle of the tube; filaments 1.5-2.3 mm long, with a double hood at the insertion point; anthers. 1 mm long, erect, shortly apiculate. Ovary 5-6 x 1 mm, ovoid; style 2-2.5 mm long, subulate; stigma subcapitate. Capsule 3-4.5 x 2-2.5 mm, obovoid. Seeds 0.4-0.5 mm in diam., subglobose, reticulate faveolate [88]. The gynoecial development of the plant. The marginal meristem continues to be active and proliferates into a much folded structure and the two carpels fuse by their margins and form the unilocular syncarpous gynoecium with parietal placentation [87].

3. Growing Environment

The crop grows well in areas having 700-800 mm rainfall with warm climate, temperature ranging 25-35° C during summer months. In winter months, temperature should not go below 2-5° C. States of Gujarat, Maharashtra, Rajasthan, and Madhya Pradesh are suitable for its cultivation. However, it can also be grown in some areas of Andhra Pradesh, Karnataka and Tamil Nadu having above mentioned climatic conditions [2].

4. Nutritional Information

According to a nutritional analysis of E. littorale by the National Institute of Nutrition, Indian Council of Medical Research, 100g fresh E. littorale greens contain 140 Kcal energy, 7g protein, 0.7g fat, 26.5g carbohydrates, 4.2g fiber, 8.4g minerals, 49.9mg iron, 1,641mg calcium, 81mg phosphorous, and 53.2g moisture [28]. Based on this analysis, 100g of fresh *E. littorale* daily would be highly nutritious and recommended. *E.littorale* is an uncultivated leafy green eaten in southern India as a source of calcium and iron [28]. Greens (quelites) are important supplemental sources of nutrients such as calcium, iron, magnesium, vitamin C, B vitamins, betacaroten, in traditional societies [30]. *E. littorale*, locally known as gorumadi koora or gorumadi, is eaten as a curry with pulses or other greens [28]. In a clinical trial with 84 diabetic patients who ingested 2g of *E. littorale* per day for 3 months, no adverse side effects were reported [137].

5. Traditional Medical Use

E. littorale plays very important role in human healthcare. *E. littorale* is traditionally used in India as the plant parts such as leaves and roots were used in traditional practice for treating several ailments like malaria, shin diseases, diabetes, leprosy, stomachic, bitter tonic, laxative, carminative [82], arthritis, back pain [143], to reduce fever and as a "tonic" for appetite loss [56]. It is traditionally used as urinary astringent, antidiabetic, anthelmintic, antiperiodic, laxative, anti-inflammatory, and carminative. It possesses antimicrobial, antioxidant, antitumor, antiedematologinic activities [26]. The leaf possesses antioxidant, hypoglycemic, hepatomodulatory and hepatoprotective properties and helps in reducing obesity. Many other genera in the gentian family have similar traditional uses worldwide [56,150]. In Ayurvedic (India) medicine, *E. littorale* is taken in combination with other herbs, especially for diabetes [137]. Medicinal use or other non-nutrition purposes of the ingredients - Clinical trial *E. littorale* was administered in Ayurvedic pill form (known as ghavantis) at a daily dosage of 2000 mg for three months to 84 patients with Type 2 Diabetes. *E. littorale* reduced blood glucose and serum insulin levels, and significantly improved kidney function, lipid profile, systolic and diastolic blood pressure and pulse rate [137]. The plant is traditionally used in abdominal ulcers, rheumatism, hernia, itches, insect poisoning and swelling [3]. It used for liver disorder by traditional healers in India and no side effects were reported in this study [101].

6. Pharmacological Activities

6.1. Known pharmacological effects and mechanisms of Pharmacological activity

E. littorale contains a number of antioxidative phytochemicals, which include catechins, alkaloids, sterols, saponins, triterpenoids, flavonoids, phenolic acids and xanthones. It also contains minerals like calcium, iron, magnesium, potassium, sodium, silica, chloride, phosphate, carbonate and sulphate [3]. *E. littorale* is known to have antibacterial [90], antiinflammatory [74, 109], anti-cancer [60, 74] and antidiabetic activity [74] found that *E. littorale* enhances glucose-dependent insulin release. Swertiamarin, a secondary compound present in *E. littorale* [15], has antispasmodic and anticholinergic activity [149]. Outcome of a human clinical study with *E. littorale* demonstrated significant hypotensive, hypoglycemic, and hypolipidemic effects [137]. Swertiamarin has demonstrated antibacterial activity *in vitro* [65] (Table 1).

6.2. Hypoglycemic activity

The plant extract of *E. littorale* has the potential to enhance glucose-induced insulin release at 11.1 mM glucose from isolated rat pancreatic islets and was partially able to reverse the effect of diazoxide (0.25 mM). Incubation with Ca^{2+} chelator and Ca^{2+} channel blocker (nimodipine) did not affect the glucose-induced insulin release augmented by the extract. A single dose of aqueous extract of *E. littorale* (15 g dry plant equivalent extract per kg) had shown significant increase in the serum insulin levels in alloxan-induced diabetic rats at 8 h. The insulinotropic action of aqueous extract of *E. littorale* was further investigated using rat pancreatic islets. Above results suggest the glucose lowering effect of aqueous extract of *E. littorale* [74, 75]. The aqueous extract of the plant prevented the blood glucose level as well as insulin level in rat model. It normalizes dyslipidaemia, provides nephroprotection in diabetic rats and increase in insulin sensitivity [81]. In another study showed diabetic rats were having hyperglycemic condition. Rats treated with plant extract for 45 days showed reduction in blood glucose levels [19]. The multipotent differentiation property of stem cells opens up new arena for the treatment of the diabetic patients. Many chemical and biochemical compounds make stem cells get differentiate into insulin producing cells. The study highlighted islet neogenic property of the plant *E. littorale*. An active herbal compound SGL-1 was isolated and purified from extract of *E. littorale* and used to differentiate modal stem cells which showed tremendous islet neogenic potential and significant islet yield compared to control serum free medium. Morphological, molecular and immunological characterization of newly generated islet like cellular aggregates proved them differentiated and positive for islet hormones. Functional characterization of islet cellular aggregates confirmed significant glucose responsive insulin release. Another study suggested that *E. littorale* is a potent antidiabetic agent without any toxic effect at this particular dose (1.5 g dry plant equivalent extract/100 g body weight.) in alloxan induced diabetic rats. The above dose caused significant decrease in liver glucose-6-phosphatase activity, glycosylated haemoglobin and significant increase in serum insulin levels of the diabetic rats [76]. The dose dependent effect of three weeks treatment with cold and hot aqueous extract of *E. littorale* (0.5, 1 and 2 g/kg,) on streptozotocin induced type 1 diabetic rats (45 mg/kg, iv single dose). *E. littorale* possesses potential antidiabetic activity and improves lipid profile at a small dose of 0.5 g/kg (40) (Table 2).

6.3. Antihyperlipidemic activity

A new study demonstrated new property of swertiamarin as a potent lipid lowering agent and comparable to atorvastatin and it may contribute to its antiatherosclerotic and cardioprotective role. The isolated atorvastatin and swertiamarin when orally fed also lowered triglycerides and the total serum cholesterol [138]. *E. littorale* aqueous extract (1.5 g/100g body weight/day,) was administered to rats along with hypercholesterolaemic diet for 6 weeks and the antioxidant and hypolipidemic effect was evaluated. The treatment with the extract showed a decrease in activities of superoxide dismutase, lipid peroxidation levels and erythrocyte catalase, with an increase in reduced glutathione levels as compared to cholesterol fed untreated rats. Kidney and Liver cholesterol levels and triglyceride levels were also decreased in *Enicostemma littorale* treated rats [141]. The shoot part of the *E. littorale* reduces the serum cholesterol level in hepatoma-bearing rats hepatoma induces hypercholesrestolemia, a component of plant enhances cholesterol acyl transfereras by esterification of free cholesterol in the HDL [44] (Table 2).

6.4. Antitumor activity

The methanolic extract of *E. littorale* indirectly inhibited tumor cell growth and it was examined on the peritoneal exudates cells of the normal mice and enhance potential cell counts. These results demonstrated the indirect effect on the cells, probably mediated through enhancement and activation of macrophages or through some cytokine product inside the peritoneal cavity produced by methanolic extract of *E. littorale* treatment [60] (Table 2).

6.5.Hepato-protective activity

Paracetamol-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plant extracts and drugs. The hepatoprotective activity of *E. littorale* in rats against paracetamol as hepatotoxin to prove its claims in folklore practice against liver disorders. The extent of hepatic damage is assessed by histological evaluation and the level of various biochemical parameters in circulation. Highly reactive trichloro free radical formation, which attacks polyunsaturated fatty acids of the endoplasmic reticulum, is responsible for the hepatotoxicity of paracetamol. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals. The study was evident that the extract was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication [40]. Swertiamarin isolated from *E. littorale* possesses significant antioxidant and hepatoprotective properties against D-GalN induced hepatotoxicity given at 100 and 200 mg/kg body weight orally for 8 days, which might be due to its *in vitro* antioxidant activity [52]. Ethanolic extract of *E. littorale*, exhibited significant hepatomodulation against oxidative stress induced liver injury by CCl_4 in rats through antioxidant potential and free radical scavenging activities along with

reduction of fat metabolism. [44]. The ethanol and ethyl acetate extracts of *E. littorale* contain pharmacologically active substances with hepatoprotective properties. These attributes provide the rationale for the use of *E. littorale* in liver disorders by traditional healers in India [101] (Table 2).

6.6. Anti-inflammatory activity

The alcohol extract at a concentration of 300 and 600 mg kg-1, and its ethyl acetate fractions at 25 and 50 mg kg-1 showed a significant dose dependent anti-inflammatory activity in carrageen induced rat hind paw edema as well as formalin induced rat hind paw edema chronic model in rats. The study showed that the alcohol extract of *E. littorale* and its ethyl acetate fractions, exhibited significant anti-inflammatory activity [16] (Table 2).

6.7. Antinociceptive activity

In vivo antinociceptive activity of swertiamarin isolated from *E. littorale* was carried out using three different methods in mice. In the hot plate method, a significant increase in the latency period was observed for the treatment with swertiamarin at 100 and 200 mg/kg after 30 and 45 min. The percent protection observed after 45min was 109.42, 147.42 and 157.14, respectively, for the standard paracetamol and swertiamarin at 100 and 200 mg/kg bw treatments. A significant increase in the tail withdrawal reflex was observed for the swertiamarin treatment at both the doses with percent protections of 150 and 200, respectively. In both these methods, swertiamarin produced potent activity than that of standard paracetamol [52] (Table 2).

6.8. Antimicrobial

The *in vitro* antimicrobial activity of aqueous, hydro alcoholic, methanolic, chloroform and ethyl acetate extract of leaves of this plant has been evaluated. The antimicrobial activity against *Bacillus subtilis,* Staphyloccous *aureus, Escheichia coli, Shigella sonni, Pseudomaonas aeruginosa, Proteus vulgaris, Aspergillus niger and Candida albicans* by well diffusion method. It was observed that chloroform, ethyl acetate and hydrochloric extract showed prominent antimicrobial against all microorganisms [33] (Table 2).

6.9. Antioxidant activity

The free radical reactive oxygen species are well known inducers of cellular and tissue pathogenesis leading to several human diseases, such as cancer, inflammatory disorders and diabetes mellitus, as well as aging process [17]. Potent antioxitant activity was observed using many methods for all extracts of *Enicostemma littorale*. Among the extracts, the petroleum ether, chloroform and ethyl acetate extracts exhibited potent activities [52]. The crude powder form of the aerial part of the *Enicostemma littorale* showed enzymatic and non-enzymatic antioxidant activity in *p*- DAB induced hepatocarcinoma in rats [43]. The antioxidant activity of *Enicostemma littorale* showed a strong free radical scavenging activity and ferric reducing property indicating it to be a good source of natural antioxidants to prevent free radical mediated oxidative damages [113]. The *in vitro* antioxidant activity of aqueous, hydro alcoholic, methanolic, chloroform and ethyl acetate extract of leaves of this plant has been evaluated. The possible mechanism involved was investigated by using different model covering nitric oxide and DPPH method. The result indicated efficacy of extracts for antioxidant activity in following sequence: methanol > hydro alcoholic > aqueous > chloroform [33]. The effect of oral administration of an aqueous *Enicostemma littorale* whole plant extract on antioxidant defense in alloxan-induced diabetes in rats. A significant increase in blood glucose and increased concentration of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) in liver, kidney and pancreas were observed in alloxan diabetic rats. Decreased concentration of reduced glutathione (GSH) and decreased activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) were also observed in these tissues of diabetic rats. Oral administration of aqueous *E. littorale* whole plant extract (1 and 2 g/kg) to diabetic rats daily for 45 days significantly decreased blood glucose, TBARS, HP and increased GSH, SOD, catalase and GPx. *E. littorale* extract at the dose of 2 g/kg was more effective than 1 g/kg. Insulin (6 units/kg) administration to diabetic rats for 45 days brought back all the parameters to near normal status [100] (Table 2).

7. PHYTOCHEMICAL STANDARDIZATION

E. littorale is known for its hypoglycemic activity from ancient times. It contains Gentianine, a bitter, crystalline monoterpene alkaloid [13] and Swertiamarin as important phytoconstituents. An attempt to develop a HPTLC method for quantitative estimation of swertiamarin in plants and different marketed antidiabetic polyherbal formulations has been reported [91]which was found to be reproducible, accurate and precise and could detect swertiamarin concentration at nanogram level. The proposed HPTLC method is rapid, simple and accurate for quantitative estimation of swetiamarin in different marketed polyherbal formulations and small fruits, big fruits and fresh fruits variety of *E. littorale*. The recovery values of swertiamarin were found to be about 96.2%, which shows the reliability and suitability of the method. The lowest detectable limit of swertiamarin in different formulations was found upto 50 ng/spot [91] (Table 2).

Swertiamarin is a secoiridoid glucoside present in members of the Gentianaceae family, including *Spiraea japonica* Makino, *Swertia chirata* (Wall) Clarke, *Sphaeralcea angustifolia* Buch.-Ham.ex D. Don and *Enicostemma littorale*. It has antidepressant and anticholinergic activity and can thus be used as a biomarker. Vishwakarma et al [146] have developed a simple HPTLC method for quantification of swertiamarin which can be used for analysis of plant materials and formulations to determine swertiamarin content. The method was validated for precision, repeatability, and accuracy and found to be precise; intra- and inter-day relative standard deviations (RSD) were in the ranges 0.68 to 0.85 and 0.71 to 1.03, respectively, for two different concentrations. Instrumental precision and repeatability of the method were 0.95 and 0.69 (%), respectively. The accuracy of the method was checked by determination of recovery at two different

levels and the average recovery was found to be 100.13%. The method was used for estimation of the swertiamarin content of whole plants of *E. littorale* and *S. chirata* and of herbal formulations containing *E. littorale* as an ingredient. The method requires no clean-up of sample extracts before TLC and swertiamarin was well resolved from other components of the extracts. The method is simple, precise, specific, sensitive, and accurate and can be used for routine quality control of raw materials and herbal formulations. The method is suitable for quantification of swertiamarin in samples containing amounts ranging from 0.15 to 7.7% (w/w). *E. littorale* whole plant was found to be a rich source of swertiamarin (7.7% w/w). [146] (Table 2).

7.1. Chemical substances which belong to the product include:

This plant contains secoiridoid compounds, C-glucosides [56] erythrocentaurin [39] cited in [56] swertiamarin [15]. Swertiamarin exhibited significant general toxicity in brine shrimp lethality bioassay and the LD50 value was 8.0 µg/ml, whereas that of the positive control podophyllotoxin, a well known cytotoxic lignan, was 2.79 mg/ml [65]. Swertiamarin is metabolized by human intestinal bacteria. Erythrocentaurin is one of the swertiamarin intestinal metabolites (Table 1).

8. PHYTOCHEMICALS

Many compounds have been isolated from *E. littorale*. Dymock et al [36] reported that the aerial part of the plant gave 15.7% of ash and 34% of dry alcoholic extract. Qualitative analysis of the ash revealed the presence of minerals like potassium, iron, sodium, magnesium, calcium, silica, chloride, phosphate, carbonate and sulphate. Two sterols, five alkaloids and volatile oil, have been reported by [86]. Monoterpene alkaloids like gentiocrucine and enicoflavin were also isolated [25, 39]. The presence of saponins, catechins, triterpenoids and steroids were reported by earlier workers [106]. Betulin, a triterpene sapogenin was also isolated by earlier workers [34]. A new flavone C-glucoside named as Verticilliside was isolated for the first time in this species [49]. Swertiamarin was isolated from the green viscous mass obtained from an ethylacetate extract of the drug treated with ether followed by alcohol. [34]. Flavonoids and xanthones were found to be present in this plant. Six phenolic acids viz: syringic acid, vanillic acid, p-hydroxy benzoic acid, p-coumaric acid, ferulic acid and protocatechuic acid were also found [29]. Seven flavonoids were isolated from alcoholic extract and their structures were identified as genkwanin, apigenin, swertisin, isovitexin, saponarin, 5-Oglucosylisoswertisin and 5-Oglucosylswertisin [38]. Methanol extract of *E. littorale* was found to contain different amino acids like tryptophane, L-glutamic acid, alanine, aspartic acid, serine, L-proline, threonine, L-tyrosine, L-histidine monohydrochloride, phenyl alanine, iso leucine, methionine, DOPA, L-arginine monohydrochloride, lglycine, valine and 2-amino butyric acid [106, 111] (Table 1).

9. ANIMAL TRIALS

E. littorale has demonstrated antiinflammatory activity [109] and tumor inhibition [60] in rats. Swertiamarin may have a CNS depressant effect in rats [23]. There are no reported symptoms of overdose (Table 2).

10. TISSUE CULTURE STUDIES

Plant cell and tissue culture conservation of many threatened medicinal plants has become a prerequisite to produce active compounds for herbal and pharmaceutical industries. In recent years, there has been an increased in *in vitro* techniques which offer a viable tool for mass multiplication and germplasm conservation of rare and endangered medicinal and aromatic plants. *Enicostemma littorale* is one of the herbaceous medicinal plants and a reservoir of various bioactive compounds. The seed setting of this plant is very high, but germination frequency is very poor under natural conditions. However, micropropagation can be an effective alternative for mass production of *E. littorale* clones within a reasonable time period. Although earlier attempts have been made for the propagation of *E. littorale* through tissue culture but, considerable effort is still required to make it more practicable. There is a need to develop an efficient *in vitro* method for conservation and sustainable production of *E. littorale* through different explants, followed by successful transfer of the micropropagated plants into field conditions. There are limited reports on the *in vitro* regeneration of *E. littorale* and have been carried out using shoot tip, leaf, node and root explants, as shown in table 3 [18, 62, 66, 83, 84, 85, 112, 120].

In a report by Sasidharan and Jayachitra [112] various *in vitro* cultures were performed for multiple shoot regeneration from *E. littorale.* Shoot tip, shoot buds and shoots were used as explants and cultured on MS media supplemented with BAP and KIN for multiple shoot induction and shoot bud regeneration and ½ MS media supplemented with IBA for root formation (Table 3).

In another study, leaves of *E. littorale* were used as explants for multiple shoot regeneration. For effective multiple shoot induction, MS media supplemented with BAP (15 µM), KIN (5 µM) and IAA (2 µM) was usedand for rooting, NAA (2 µM) was adequate [85] (Table 3).

In a report by Kutty et al [66] on *E. littorale* leaf explants were used for epiphyllous buds induction. Epiphyllous buds induction from leaves was obtained on MS media supplemented with BAP (2 mg/l) and basal MS medium (Table 3).

Kousalya and Narmatha Bai [62] worked on *E. littorale* nodal explants for multiple shoot induction. The nodal explants of *E. littorale* were used for multiple shoot initiation and cultured on MS media supplemented with BAP (2.0 mg/l) and KIN (0.5 mg/l) and ½ MS media supplemented with IAA (0.5 mg/l) for root formation (Table 3).

In a report by Nalini and Velayutham [84], multiple shoots were obtained from shoot tip segments of *E. littorale*, on MS media supplemented with KIN (15 µM/l) and BAP (15 µM/l) and the shoots were transferred to MS media supplemented with IBA (2 µM/l) for shoot elongation. The

elongated shoot were transferred to MS media supplemented with IBA (2 μM/l) and NAA (2 μM/l) for rooting (Table 3).

In a report by Nagarathnamma and Sudarshana [83], multiple shoot induction was achieved from leaves and nodal explants of *E. littorale* on MS media supplemented with BAP (3.0 mg/l) and NAA (1.0 mg/l) and the regenerated shoots were elongated on MS media supplemented with GA_3 (1.0 – 1.5 mg/l), roots were formed on MS media supplemented with IAA (1.0 mg/l) (Table 3).

An efficient regeneration protocol was developed by Seetharam et al [120], where multiple shoots were obtained when leaf segments were cultured on MS media supplemented with BAP (1.5 mg/l) and IAA (0.5 mg/l), nodal explants were cultured on MS media supplemented with BAP (1.5 mg/l) and IAA (1.5 mg/l). Regenerated shoot were cultured on MS media supplemented with KIN (1.0 mg/l and BAP 0.5 and 1.0 mg/l). Rooting was achieved on MS media supplemented with IAA (1.0 mg/l) (Table 3).

Table 1. Bioactive Compounds and Medicinal Properties of *Enicostemma littorale* Blume.

Medicinal property and phytochemical analysis	Part used	Chemical constituents	References
Witches' broom diseases	Whole plant	Swertiamarin	[1]
Rich nutrient and antibiotics	Whole plant	Total phenols, protein carbohydrate Vitamin E and C	[6]
Traditionally used in rheumatism, abdominal ulcers, hernia, swelling, itches and insect poisoning	Whole plant	Alkaloids, catechins, saponins, sterols, triterpenoids, phenolic acids, flavonoids and xanthones. Minerals like iron, potassium, sodium, calcium, magnesium, silica, phosphate, chloride, sulphate and carbonate	[3]
		Iron, potassium, sodium, calcium, magnesium, silica, phosphate, chloride, sulphate, carbonate, alkaloids sterols and volatile oil	[86]
		Monoterpene alkaloids like-enicoflavin and gentiocrucine	[25, 39]
		Catechins, saponins, steroids and triterpenoids	[106]
		Betulin, triterpene sapogenin	[34]
		A new flavone C- glucoside named as Verticilliside was isolated for the first time this species	[49]
	Alcoholic extract of the drug treated with ether followed by ethyacetate	Swertiamarin	[34]
		Flavonoids and xanthones .Six phenolic acids viz: vanillic acid, syringic acid, p-hydroxy benzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid	[29]
	Alcoholic extract of whole plant	Seven flavonoids identified as apigenin, genkwanin, isovitexin, swertisin, saponarin, 5-Oglucosylswertisin and 5-Oglucosylisoswertisin	[38]
	Methanol extract of whole plant	Aminoacids like L-glutamic acid, tryptophane, alanine, serine, aspartic acid, L-proline, L-tyrosine, threonine, phenyl alanine, L-histidine monohydrochloride, methionine, iso leucine, L-arginine monohydrochloride, DOPA, LGlycine, 2-amino butyric acid and valine.	[106]
Reduce the total serum cholesterol and triglycerides	Aqueous extract	Swertiamarin and atorvastatin	[139]
Produced potent activity than that of standard paracetamol	Whole plant	Swertiamarin	[52]
		Gentianine, crystalline monoterpene alkaloid	[13]
Hypoglycemic activity from ancient times and anti-diabetic polyherbal formulation	Fruits	Swetiamarin	[91]
	Methanol extract	Swertiamarin	[10]
Antifungal agents	Methanol extract of whole plant	*Laminaribiitol (79.93%), 12-hydroxy-9-octadecenoic acid (9.546%). Myricetin (4.7519%), 3.3-Methylenebis (4-hydroxycoumarin) (2.811%), catechin (2.002%). By* GC-MS method	[12]
		enicoflavine	[25]
Various traditional medicines for diabetic activity.	Hexane extract of stem	Palmitic acid, unsaturated and saturated hydrocarbons	[26]

IJSER

	Chloroform extract of stem	Long chain hydrocarbons, acids, carbonyl compounds etc	
	Alcohol extract of stem	Hydroxy compounds, carbonyl compounds, benzo-pyrone etc.	
	Hexane extract of flower	Palmitic acid, oleic acid, phytosterol etc	
	Chloroform extract of flower	Long chain hydrocarbons, acids, carbonyl compound etc	
	Alcohol extract of flower	Hydroxy compounds, carbonyl compounds, benzo-pyrone etc.	
	Hexane extract of leaf	Unsaturated and saturated hydrocarbons	
	Chloroform extract of leaf	Myristic acid, palmitic acid, carbonyl compounds etc.	
	Alcohol extract of leaf	Ribose, glucopyranose Hydroxy compounds, carbonyl compounds, etc	
Antipyretic and antacid activities	Aqueous extract of the aerial parts	Alkaloids, carbohydrates, steroids, glycosides, saponins and flavonoids	[38]
	Whole plant	flavonoids: apigenin (I), genkwanin (II), isovitexin (III), swertisin (IV), saponarin (V), 5*O*glucosylswertisin (VI), and 5*O*glucosylisoswertisin (VII).	
	Whole plant	Erythrocentaurin	[39]
Phytochemical Analysis	Chloroform extract	Alkaloids	[40]
	Ether and chloroform extract	Fats and oils	
	Methanol and water extract	Tannins and phenols, saponins, flavonoids and carbohydrates	
	Chloroform, methanol and water extracts	Proteins, amino acids	
	Ethyl acetate extract	Flavone C-glucoside, 5,7,40-Trihydroxyflavone 8-C-b-D-glucopyranoside and isoorientin 30-O-methyl ether.	[49]
Hepatomodulatory response	Ethanol extract	Oxidative stress-induced liver injury by carbon tetrachloride (CCl_4) in albino wistar male rats	[44]
Involved in pharmacokinetic and Biotransformation activites	Ethyl acetate	Erythrocentaurin	[47]
Antimicrobial activity	Ethanol, petroleum ether, chloroform, water and acetone extracts	Terpenoid	[48]
New secoiridoids	Aerial part of the plant	Enicostemin A (I) and B (II), gentiocrucine and rutin	[50]
New compound	Aerial part of the plant	Littoralmine (dihydropyran2, 4dione with secoiridoid skeleton)	[51]
Antinociceptive activity	Whole plant	Swertiamarin	[53]
Compound isolation	Ethyl acetate extract of the plant	Swertiamarin isolated and determined by high performance thin layer chromatography (HPTLC)	[55]
Phytochemical analysis	Petroleum ether	Steroids and triterpenoids	[52]
	Chloroform extract	Alkaloids, flavonoids phenols and triterpenoids	
	Ethyl acetate extract	Flavonoids, phenols, saponins, iridoid glycosides and tannins	
	Methanol extract	Alkaloids, flavonoids, phenols iridoid glycosides and tannins	

IJSER

Anti-diabetic compound	Methanol extract of whole plant	Swertiamarin by HPLC-UV method	[7]
Anti-diabetic drug	Methanol extract of the whole plant	Swertiamarin by HPLC and UV method	[22]
Quantitative analysis	Whole plant power	Isoswertisin, 5-βdglucoside, swertiamarin, and swertisin by HPTLC method	[118]
Quantification	Whole plant power	Isovitexin by HPTLC method	[119]
Phytochemical analysis	Methanol extract of whole plant	Alkaloids, Glycosides, Tannins, Carbohydrates, Proteins and amino acids, Saponins and Flavonoids by TLC	[69]
Phytochemical analysis	Petroleum ether, ethanol, methanol water-soluble extracts	Protein, glycosides, alkaloids, tannins, phenolic compound, steroid reducing sugars and saponin glycosides	[78]
Aldose Reductase Inhibitory Activity	Methanol extract	Swertisin, Cglycosidic Flavonoids	[92]
Post prandial hyperglycemia (PPHG) activity		Swertiamarin isolated by normal phase column chromatography	[79]
Phytochemical analysis	Ethyl acetate fraction	Secoiridoid glycoside and flavonoids by HPLC-LC/MS/MS	[89]
Quantitative analysis	Aerial part of the plant	Swertisin by RPHPLC and HPTLC with UV detection (HPLC method were better than that in HPTLC method)	[94]
Phytochemical Analysis	Ethanolic leaf extract	Phenol, tannins, flavanoids, steroids, alkaloids, saponins, carbohydrates, and rutin by Thin-Layer Chromatography and High-Performance Thin Liquid Chromatography Analysis	[128]
Anti-depressant and anti-cholinergic activity	Whole plant	Swertiamarin by HPTLC method	[147]
Compound isolation	Methanol extract of the Whole plant	Swertiamarin separated by column chromatography over silica gel and confirmed through infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR), HPTLC and differential scanning calorimetry.	[146]
Anti-diabetic activity	Hot aqueous extract of the plant	Swertiamarin by HPTLC method	[148]
Biomass and Phytoconstituent analysis	Fresh leaves extracts of the plant	Chlorophyll and protein	[115]
	Fresh fruits extracts of the plant	Flavonoids	
Anti-diabetic, anti-inflammatory, anti-cancer and antioxidant activity	Methanolic extracts of the plant	Isovitexin by HPTLC	[116]
Pharmacognostical studies	Whole plants	TLC finger printing	[117]
Phytochemical analysis	Leaf extract of the plant	Terpenoids, flavonoids, phenols, tannins, steroids, quinones, saponins, cardiac glycosides and alkaloids by qualitative methods. Total phenol by Folin-Ciocalteau method and terpenoid, Swertiamarin by HPLC method.	[121]
Total phenolic contents	Ethanol extract of the plant	Ferric chloride test	[127]
Total flavonoids contents		The Shinoda test	
Phyto, physicochemical standardization	whole plant	Organoleptic characters and physicochemical parameters(loss on drying, pH, ash valies, extractability in water and ethanol and preliminary	[144]

IJSER

		Phytochemical screening	
Preliminary phytochemical screening	Hot and cool ethanol, methanol and aqueous extra	Alkaloids, saponins, flavonoids, steroids, tannins, proteins, reducing sugar coumarins and quinones and absence of anthraquinones	[143]
	Cold and hot water extracts	Fat and fixed oil	
Insulin resistance in type II Diabetes	Aqueous extract of the whole plant	Swertiamarin	[95]
Phytochemical screening	Aqueous extract of the whole plant	Alkaloids, saponins, steroids, flavonoids, glycosides and triterpenoids	[134]

Table 2. Summary of Known Pharmacological Activities of *Enicostemma littorale*.

Pharmacological activities	Tested substance	Study model/ methodology	Reference(s)
Anti-apoptotic and cytoprotective effects	Methanolic extract of whole plant	Tryphan blue dye exclusion assay and Comet assay	[131]
Agricultural practices	Whole plant	Nursery technique	[2, 77]
Anti-oxidant activity	Leave, flower, stem and root.	Ascorbic acid, total phenols, α- tocopherol, Reduced glutathione, Glutathione-S-transferase, Superoxide dismutase, Catalase, Peroxidase	[4]
Anti-bacterial activity	Chloroform, methanol and acetone extracts of leaf, stem and root	Disc diffusion method	[6]
Hypoglycemic activity	Aqueous extract	Increase in the serum insulin levels in alloxan-induced diabetic rats at 8 h	[74, 75]
		Produces an increase in insulin sensitivity, normalizes dyslipidaemia and provides nephroprotection in diabetic rats	[81]
		Reduce in blood glucose levels in hyperglycemic rats.	[19]
	Methanol extract of the aerial part	Aldose reductase (AR) inhibition, α glucosidase inhibition, effect on gluconeogenesis in rat hepatoma, cytoprotection against streptozotocin (STZ)induced toxicity on RINm5F cells, normalization of glycemic control in acute hyperglycemic rat model, and insulin releasing effect both *in vitro* and *in vivo*	[93]
	Aqueous extract of the whole plant	Normoglycemic, hyperglycemic and Alloxan induced diabetes mellitus rats	[105]
Anti-diabetic activity	Aqueous extract	Increase islet mass, which used for treatment of diabetic patients.	[45]
		Significant decrease in glycosylated haemoglobin, liver glucose-6-phosphatase activity and significant increase in serum insulin levels of the diabetic rats.	[74]
	Hot and cold aqueous extract	In streptozotocin induced type 1 diabetic rats potential antidiabetic activity and improves lipid profile at a small dose of 0.5 g/kg	[145]
Anti-hyperlipidemic activity	Aqueous extract of the aerial part	Reduces the serum cholesterol level and enhances cholesterol acyl transfereras by esterification of free cholesterol in the HDL	[42]
Hypolipidemic and antioxidant effect	Aqueous extract	Reduce liver and kidney cholesterol levels and triglyceride levels	[141]
Anti-tumor activity	Methanolic extract	Activation of macrophages or produced some cytokine product inside the peritoneal cavity.	[60]
Antioxidant and hepatoprotective		Induced hepatotoxicity	[52]
Antioxidant and free redical scavenging	Ethanolic extravt	Exhibited significant hepatomodulation against oxidative stress induced liver injury by CCl4 in rats	[44]

IJSER

activities			
Hepatoprotective properties	Ethanol and ethyl acetate extracts	Used for liver disorder by traditional healer in India	[101]
Anti-inflammatory activity	alcohol extract	Rat hind paw edema chronic model in rats	[16]
Anti-nociceptive activity	Isolated compound - swertiamarin	Hot plate method	[52]
Anti-microbial	Aqueous, hydro alcoholic, methanolic, chloroform and ethyl acetate extract of leaves	Well diffusion method	[33]
Anti-oxidant activity	Petroleum ether, chloroform and Ethyl acetate extract	All anti-oxidant methods	[33]
	The crude powder form of the aerial part	Enzymatic and non-enzymatic antioxidant activity	[43]
		Free radical scavenging activity and ferric reducing property	[33]
	Aqueous, hydro alcoholic, methanolic, chloroform and ethyl acetate extract of leaves	Covering nitric oxide and DPPH method	[33]
	Aqueous extract of whole plant	Antioxidant defense in alloxan-induced diabetes in rats	[100]
Hypoglycemic and anti-diabetic activity	Fruits	Swertiamarin isolated by HPTLC method	[91]
Antidepressant and anticholinergic activity	Whole plant	HPTLC method	[146]
	Methanol extract	HPTLC method	[10]
Anti-cancer activity	Methanol extract of whole plant	Chemotherapy, radiation therapy, surgery, immunotherapy, monoclonal antibody therapy and other methods.	[97]
Anti-diabetic activity - streptozoticin induced male rats.	Ethanol extract of whole plant	Emulsification, cross linking with calcium chloride and solvent removal. photon correlation spectroscopy (PCS), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR)	[31]
Control and prevention of type 2 diabetes mellitus	Whole plant	Paired student t-test	[11]
Anti-pyretic activity	Aqueous extract of the aerial parts	Lipopolysaccharide (LPS) induced pyrexia using mice as the animal model	[37]
Anti-lipase and anti-obesity activity	Aqueous and ethanol extracts	(High fat diet) HFD induced obesity rats	[14]
Restoration of liver function	Aerial part	p-dimethylaminoazobenzene induced animals	[42]
Isolation of erythrocentaurin	ethyl acetate	Medium pressure liquid chromatography and high pressure thin layer chromatography	[47]
Anti-oxidant activity	Methanolic, distilled and boiled distilled water extract	Dried methods	[8]
Anti-hyperlipidemic activity	Swertiamarin isolated from whole plant	High cholesterol fed rats	[138]
Anti-hyperlipidemic activity	Swertiamarin isolated from whole plant	Poloxamer 407 (P407) Induced hyperlipidemic rats	[139]
Anti-microbial activity	Ethanol, petroleum ether, chloroform,	Disc diffusion method	[48]

IJSER

Anti-nociceptive activity		Hot plate method	[53]
Anti-oxidant and hepatoprotective activity	Ethyl acetate extract of the plant	D-galactosamine induced acute liver damage in rats	[55]
	Ethyl acetate extract of the plant	CCl_4-induced liver injury in rats	[54]
Anti-oxidant activity	Chloroform, Petroleum ether, ethyl acetate, Methanol extracts of the whole plant	ABTS, H_2O_2, nitric oxide, hydroxyl radical, deoxyribose, p-nitroso dimethyl aniline (p-NDA), phosphomolybdenum, hydrogen peroxide, Superoxide radical and DPPH methods	[52]
Anti-diabetic activity	Methanol extract of the whole plant	HPLC and UV analysis methods	[22]
	Methanol extract of the whole plant	Alloxan induced Diabetic Rats	[76]
Glucose lowering effect	Aqueous extract of whole plant	Alloxan induced diabetic rats	[74]
Anti-arthritic activity	Methanol, chloroform and aqueous extracts of whole plant	Inhibition of protein denaturation, effect of membrane stabilization, and Proteinase inhibitory action	[124]
Biodegradable less toxic inhibitor	5% HCl and H_2SO_4 acid extract	Low (LPR) polarizations and high (TI) polarizations	[58]
Anti-tumor activity	methanolic extract	Dalton's ascitic lymphoma (DAL) in swiss albino mice	[60]
Anti-oxidant activity	Methanol and water extract	DPPH assay	[62]
Anti-cancer activity	Methanolic extract of Whole plant	HeLa cell line by Tryphan blue dye exclusion method MTT Assay	[64]
Anti-Oxidant activity	Ethanolic extract	$ABTS^+$, DPPH, FRAP, SOS and NOS Radical Scavenging activity	[63]
	Methanolic extract	TRAP assay	
	Petroleum ether extract	Lesser than the other two extracts	
Genetic diversity analysis at molecular level	Sathyamangalam, Coimbatore and Erode	RAPD markers	[68]
Anti-microbial activity	Methanol extract of whole plant	Disk diffusion method	[69]
In vitro Anti-Inflammatory Activity	Methanol extract of whole plant	Albumin denaturation assay, proteinase inhibitory activity, membrane stabilization, and anti-lipoxygenase activity	[70]
Antihepatotoxic activity against CCl_4induced hepatic damage in rats	Alcohol extract of the whole plant	Serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase, total bilirubin, and γ.glutamate transpeptidase.	[122]
Dose dependent hypoglycemic effect	Aqueous extract of the whole plant	Alloxan induced diabetic rats	[75]
Aldose Reductase Inhibitory Activity	Methanol extract	Polyol accumulation in rat lenses	[92]
Post prandial hyperglycemia (PPHG) activity	Swertiamarin isolated by normal phase column chromatography	*In vitro* α amylase and α glucosidase inhibitory activity and *in vivo* antihyperglycemic studies in starch and sucrose fed mice	[79]
Analgesic activity	Methanolic extract of the whole plant	Hot plate and tail immersion method	[123]
Anti-inflammatory and antioxidant activities		Complete Freund's adjuvant induced arthritic model	
Anti-inflammatory activity	The methanol, chloroform and aqueous extract of the plant	Carrageenan induced hind paw edema and Egg albumin induced hind paw edema in albino wistar rats	[125]
Effect of chronic treatment	Aqueous extract of the plant	Non insulin dependent diabetic (NIDDM) rats	[81]

IJSER

Treatment of type 2 diabetic patients	Aqueous extract of the plant	Anti-diabetic; lipid tolerance test; comet assay; prevents DNA damage	[73]
Treatment in diabetes	Isolated swertisin	Differentiates NIH3T3 Cells into Islet-Like Clusters and Restores Normoglycemia upon Transplantation in Diabetic Balb/c Mice	[27]
Nephrotoxicity	Methanolic extract of the whole plant	An antioxidant therapy to counteract mitochondrial and post mitochondrial oxidative stress generated in kidney upon GM treatment	[20]
Cardioprotective and antihypertensive effects	Aqueous extract of the whole plant	High fructose (HF) fed rats induced hypertensive rats	[21]
Antioxidant capacity and Acetylcholinesterase inhibitory activity	Alcoholic, aqueous, ethyl acetate, petroleum ether extracts of shoots	DPPH free radical scavenging activity, ABTS free radical scavenging activity, FRAP and AChEi activity. Total phenolic content was determined by Folin–Ciocalteu reagent	[66]
Antimicrobial activity	Chloroform, methanol and acetone extracts of leaf, stem and root of the plant	Disc diffusion method	[5]
Antioxidant activity	Aqueous extract of the whole plant	Alloxan-induced diabetic rat tissues	[100]
Anti-microbial Activity	Organic extract of the plant	Agar diffusion method	[98]
	Chloroform, Ethyl acetate, Methanol, Petroleum ether extract of whole plant	Minimum Inhibitory Concentration by Micro-titre plate method	[99]
The estimation of swertiamarin	60% Methanolic extract of the plant	HPLC-UV Method	[9]
Antioxidant activity	Aerial part of the plant	DPPH method	[59]
Acute oral toxicity	Aqueous extract of the plant	Mice	[104]
The subacute toxicity		Male and female rats	
The chemopreventive potential activity	Ethanolic extract of the leaves	7,12dimethylbenz(a)anthracene (DMBA)induced hamster buccal pouch carcinogenesis	[102]
Type 1 diabetic nephropathy	Aqueous extract of the plant	Serum urea, creatinine, lipid profile and water intake levels in SD rats	[129]
Antifertility activity	Ethanolic extracts of leaves of the plant	Adult male Wistar albino rats	[35]
Enzymatic and non-enzymatic anti-oxidant activity	Aerial parts of the plant	Plasma and liver in p-Dimethylaminoazobenzene (p-DAP)-induced hepatocarcinoma in rats	[43]
Antimicrobial Evaluation	Petroleum ether, chloroform, *n*-Butanol, ethanol and aqueous extracts of the whole plant	Agar gel diffusion susceptibility test and The MIC (Minimum inhibitory concentration), MBC (Minimum Bactericidal Concentration) and MFC (Minimum Fungicidal Concentration) Micro broth dilution technique	[128]
Antioxidant Activity	Ethanolic leaf extract	Phosphomolybdenum method, Reducing power by Potassium Ferricyanide Method, free radical scavenging activity Using 1,1-diphenyl-2-picryl-hydrazil Method and hydrogen peroxide scavenging activity.	
Antiulcer and Anti inflammatory Activity	Aerial part of the plant	Aspirin, ethanol, and pyloric ligationinduced ulcers in rats and bovine serum albumin denaturation	[107]
In vitro anthelmintic activity	Hexane, chloroform, ethyl acetate, ethanol, and water extracts of the whole plant	Dose dependent activity was observed in all extracts	[142]
Anti-inflammatory activity	Alcoholic extract of the plant	Carrageenan induced inflammation and cotton pellet granuloma method in rats	[109]

IJSER

Diabetes mellitus	Water and alcohol soluble extracts	The physico-chemical parameters, qualitative test for various functional groups, quantitative estimation of total alkaloids, HPTLC, heavy metal analysis and microbial overload	[132]
Anti-diabetic activity	Aqueous extract of the plant	Streptozotocin induced NIDDM rats	[140, 145]
Islet neogenic property	Methanolic extract of the plant	stem cell lines PANC-1 and NIH3T3	[45]
Anti-diabetic activity	Hot and cool aqueous extract of the plant	Streptozotocin-induced type 1 diabetic rats	[148]
Non-enzymatic activity	Aqueous extracts of an	Tocopherol, Phenols, DPPH, FRAP	[114]
Enzymatic anti-oxidant activity	Aerial and sub aerial parts of the plants	Polyphenol oxidase, Peroxidase, Catalase, Super Oxide Dismutase	
The Total Phenolic Content and anti-oxidant activity	Methanolic extracts of Microwave treated plants	Folin ciocalteau method, DPPH, Free radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) assays	[113]
In vitro antioxidant activity	Petroleum ether, chloroform, acetone, aqueous and methanol extracts of the leaf of the plant	1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity using the standard procedure	[121]
Anti-tumor initiating potential (Oral cancer) activity	Leaf extract of the plant	By analyzing the expression pattern of apoptotic (p53, Bcl2 and Bcl2 associated Xprotein), cellproliferative (cyclin D1 and proliferating cell nuclear antigen), angiogenic (vascular endothelial growth factor), invasive (matrix metalloproteinase2 and 9), and inflammatory (NFκB and cyclooxygenase2) markers during 7, 12dimethylbenz (a) anthracene (DMBA) induced hamster buccal pouch carcinogenesis	[72]
Anti-bacterial activity	Chloroform, methanol and aqueous extract of the leaves	Disk diffusion method and MIC using the cylinder agar diffusion method against wound pathogens	[126]
In vitro anti-microbial	Aqueous, hydro alcoholic, methanolic, chloroform and ethyl acetate extract of the leaves	Well diffusion method	[33]
In vitro anti-oxidant activity	Aqueous, hydro alcoholic, methanolic and chloroform extract of the leaves	Nitric oxide and DPPH method	
In-vitro anthelmintic activity	Petroleum ether and ethanolic extract of aerial parts of the plant	Adult Indian earthworms, Pheretima postuma	[80]
Carbohydrate metabolic enzymes, lipid peroxides and anti-oxidant activity	Aqueous extract of the plant	Alloxan-induced diabetic rats	[130]
Anti-tumour activity	Methanolic extract of the plant	Dalton's Ascitic Lymphoma (DAL) in Swiss albino mice	[108]
Hypoglycemic and anti-oxidant activity	Aqueous extract of the whole plant	Cholesterol fed rats	[141
Anti-diabetic activity	Aqueous extract of the whole plant	Newly diagnosed non-insulin-dependent mellitus patients (NIDDM)	[140]
Anti-diabetic activity	Whole plants	Type two diabetic patients	[137]
Anti-microbial activity	Aqueous extracts of Leaves, stems and roots of the plant	Agar well diffusion method	[32]

IJSER

Endophytic fungal isolation	Root	*Cladisporium* sp., *Penicillium* sp., *Asprgillus* sp., *Phomopsis* sp.	
	Stem	*Eurotium* sp., *Aspergillus flavus*, *Aspergillus fumigates*, *Asprgillus* sp., *Sartorya* sp.,	
	Leaves	*Asprgillus* sp.,	
Anti-diabetic activity	Aqueous extract of the whole plant	Noninsulin dependent diabetes mellitus	[95]
Hypolipidemic and anti-oxidant activity	Aqueous leaf extract of the plant	Ethanol induced hepatic injury in albino rats	[135]
Anti-microbial activity	Aqueous leaf extract of the whole plant	The cut well diffusion and agar dilution methods	[134]
The protective activity	Leaves	7,12-dimethylbenz(a) anthracene (DMBA) induced hamster buccal pouch carcinogenesis	[103]

Table 3. Tissue Culture Reports on *Enicostemma littorale*.

Explant	Media composition (mg/l or µM or %)	Response	Reference(s)
Shoot tip	MS media+BAP (1 mg/l)+KIN (0.2mg/l)	Multiple shoot bud induction	[112]
Shoot buds	MS media+BAP (1 mg/l)+IBA (0.5mg/l)	Multiple shoot bud regeneration (15.12±2.12 shoots/explants)	
Shoots	½ MS media+IBA (1.5 mg/l)	Roots	
Leaf	MS media+BAP (15 µM)+KIN (5 µM)+IAA(2 µM)	Multiple shoot (16.3)	[85]
Isolated shoots	½ MS media+NAA (2 µM)	Root induction (15.2±1.12) and root length (6.16±0.89 cm)	
Leaf	MS media + NAA (1mg/l)+BAP (0.2mg/l)	Callus formation in 18 days, shoot Initiation from callus after 45 days	[66]
Root	MS media + NAA (1mg/l)+BAP (0.2mg/l)	Callus initiation after 20 days	
Nodal	MS media + NAA (1mg/l)+BAP (0.2mg/l)	Callus initiation after 24 days	
Apical meristem	MS media+BAP (1 mg/l)+KIN (0.1mg/l)	Direct organogenesis within 30 days	
Shoots	½ MS media+IBA (1mg/l)	Shoots and roots	
Leaves	MS media +BA (2 mg/l)	Epiphyllous buds induction	[18]
	MS media (hormone free)	Epiphyllous buds formation	
Nodal explants	MS media + BAP (2mg/l)+KIN (0.5 mg/l)	Multiple shoot	[62]
Shoots	½ MS media +IAA (0.5 mg/l)	Rooting	
Shoot tip	MS media + KIN (15 µM/l)	Multiple shoot (28.3±1.15)	[84]
	MS media + BAP (15 µM/l)	Multiple shoot (29.2±1.19)	
Isolated shoots	MS media + IBA (2 µM/l)	Root induction (7.1±3.9) and developed (2.65±0.14 cm)	
	MS media + IBA+NAA (2 µM/l)	Root induction (8.1±0.33) and developed (3.01±0.09 cm)	
Leaf	MS media+ BAP (3mg/l)+NAA (1mg/l)	Multiple shoot induction (52±2.83)	[83]
		Shoot bud regeneration (%) (86.8±3.9)	
Nodal	MS media+ BAP (3mg/l)+NAA (1mg/l)	Multiple shoot induction (50±1.63)	
	MS media+ Kn (3mg/l)+NAA (0.5mg/l)	Shoot bud regeneration (%) (51.4±3.2)	
Regenerated shoots	MS media + GA_3 (1.0 – 1.5mg/l)	Shoot elongation (8-10cm)	
Elongated shoots	MS media + IAA (1.0 mg/l)	Root induction	
Leaf	MS media+BAP (1.5 mg/l)+IAA (0.5 mg/l)	Multiple shoot bud induction	[120]

IJSER

Shoot buds	MS media+KN (1.0 mg/l)+BAP (1.0 mg/l)	Shoot proliferation and elongation
Nodal explants	MS media+BAP (1.0 mg/l)+IAA (1.5 mg/l)	Multiple shoot bud induction
Shoot buds	MS media+KN (1.0 mg/l)+BAP (0.5 mg/l)	Shoot proliferation and elongation
Shoots	½ MS media+IAA (1.0 mg/l)	Roots

11. Conclusion

Medicinal plants are crucial source of the life saving drugs for majority of the world's population. The biotechnological tools play a vital role to select, multiply and conserve the critically endangered genotypes of medicinal plants. *In vitro* regeneration procedures hold tremendous potential for the production of high quality plant based medicines. The demands for the use of herbal medicines has tremendously grown in developed countries. Interestingly, 40% of the compounds used in pharmaceutical industry are directly or indirectly derived from plants because the chemical synthesis of such compounds is either not possible and/or is expensive. Therefore, a large number of plant species (especially medicinal) are under threat of extinction because of their over exploitation to meet the growing demands of the pharmaceutical industries.

Enicostemma littorale is one of the herbaceous medicinal plant. The plant is difficult to propagate because of poor seed-germination. The plant has immense potential to cure various diseases and disorders and the most important among these is anti-diabetic, anti-inflammatory, anti-cancer and anti-oxidant activities. The efficiency and efficacy of the bioactive compounds present in the plant has been evaluated from time to time. Now, since there is awareness complemented with scientific proof regarding the medicinal benefits of *E. littorale*, its mass cultivation and extraction of bioactive compounds should be the objective of further research. The commercialization of pharmaceutical drugs containing *E. littorale* as a sole or a part of the ingredients shall bring relief to millions of suffering people, in a natural way. To ensure sustained production availability and benefit of *E. littorale* products, we must ensure its mass cultivation through conventional as well as tissue culture techniques. The conservation efforts would ensure continuous and sustainable supply of the bioactive compounds like swertiamarin, swertisin, isoswertisin, gentianine, atorvastatin and etc., to the concerned pharmaceutical industry. Although, tissue culture or rapid regeneration protocols for *E. littorale* is not yet developed but, the same can ensure enhancement of bioactive compound production in the near future, through suspension culture or transgenic technology. These techniques may also simplify the path for further research on the bioactive principles and for the discovery of new compounds with novel properties in future. To conclude, *E. littorale* has immense potential to act as panacea to several health related maladies and so its conservation before excessive exploitation should be a prerequisite.

12. Abbreviations

ABA: Abscisic acid; **BAP**: 6-benzyl amino purine; **BVN**: Bavistin; **CH**: Casein hydrolysate; **2, 4-D**: 2, 4-Dichlorophenoxyacetic acid; **IAA**: Indole 3-acetic acid; **IBA**: Indole butyric acid; **KIN**: Kinetin; **MS**: Murashige and Skoog; **NAA**: α-naphthalene acetic acid; :Thin-layer chromatography; **HPLC**: High performance liquid chromatography; **HPTLC**: High performance thin layer chromatography; **2**, **4-D**: 2, 4-dichlorophenoxyacetic acid; **2ip** : N^6-(2-isopentenyl) adenine; **PGR**: Plant growth regulator. **cm**: Centimeter; **Mm**: Millimeter; **m**: Meter; **CNS**: Central Nervous System; **LD50**: Lethal Dose; **Ml**: Milliliter; **µg**: Microgram; **g**: Gram; **mg**: Milligram; **kg**: Kilogram; **°C**: Celsius; **RSD**: Relative Standard Deviations; **TLCDPPH** : 1,1-Diphenyl-2-Picryl-Hydrazyl; **p-DAB**: p-Dimethyl aminoazo benzene; **TBARS** :Thiobarbituric Acid Reactive substances; **HP**: hydroperoxides; **GSH**: Glutathione; **SOD**: Superoxide Dismutase; **GPx**: glutathione peroxidase; **RSD**: relative standard deviations; **CCl_4**: Carbon Tetrachloride; **HDL**: High Density Lipoprotein; **D-GalN**: d-Galactosamine; **bw**: Body Weight; **MS medium**: Murashige and Skoog medium; **WHO**: World Health Organization.

13. References

[1] Abirami K, Sharma A, Samanta JNV, Rana S, Mandal K. Morphological, anatomical and molecular investigation into witches' broom disease of mamejvo (*Enicostemma axillare*). *Phytoparasitica* 2012; **40**: 445-450.

[2] Abirami K, Rana VS, Geetha KA, Satyabrata Maiti. Good Agricultural Practices of *Enicostemma axillare*. *OAJMAP* 2014; **5**: 23-25.

[3] Abirami P, Gomathinayagam M. A review on *Enicostemma littorale. Pharmacologyonline* 2011; **1**: 75-83.

[4] Abirami P, Gomathinayagam M, Panneerselvam R. Antioxidant activity of the medicinal plant *Enicostemma littorale* Blume. *IJGP* 2011; **5**: 342-345.

[5] Abirami P, Gomathinayagam M, Panneerselvam R. Preliminary study on the antimicrobial activity of *Enicostemma littorale* using different solvents. *APJTM* 2012; 552-555.

[6] Abirami P, Gomathinayagam M. Biochemical composition of *Enicostemma littorale* Blume. extracts. *Bangladesh J Bot* 2013; **42**: 367-369.

[7] Ahamad J, Amin S, Mir SR. Development and validation of HPLC-UV method for estimation swertiamarin in *Enicostema littorale. J Pharm Bio Sci* 2014; **1**: 9-16.

[8] Akhtar H. Antioxidant Potential of Dried *Enicostemma littorale. Pak J Biol Sci* 2011; **14**: 956-957.

[9] Alam P, Ali M, Singh R, Shakeel F. Estimation of Swertiamarin in Enicostemma Littorale and Marketed Formulations Using HPLC-UV Method. *J Bioanal Biomed* 2009; **1**: 22-27.

[10] Alam P, Ali M, Singh R, Shakeel F. A new HPTLC densitometric method for analysis of swertiamarin in *Enicostemma littorale* and commercial formulations. *Natural product Research* 2011; **25**: 17-25.

[11] Amarnath Shukla, Preliminary study of the clinical hypoglycemic effects of *Enicostemma littorale* Blume. In Type 2 Diabetic Patients. *WJPPS* 2015; **4**: 872-880.

[12] Ambikapathy V, Mahalingam R, Panneerselvam A. GC-MS Determination of Bioactive compounds of *Enicostemma littorale* (Blume). *Asian J Plant Sci Res* 2011; **1**: 56-60.

[13] Amritpal Singh, Phytochemicals of Gentianaceae: A Review of pharmacological Properties. *IJPSN* 2008; **1**: 33-36.

[14] Ankur G, Singh R. Antiobesity activity off aqueous and ethanol extracts of *Enicostemma littorale* in high fat diet induced obese rats. *Int J of Phytomed* 2014; **6**: 433-443.

[15] Anwar M, Ahmad M, Aslam M, Aftab K. *Enicostema littorale*: a new source of swertiamarin. *Pak J Biol Sci* 1996; **9**: 29-35.

[16] Arivukkarasu R, Rajasekaran A, Murugesh S. Anti-inflammatory activity of alcoholic extract of *Adenema hyssopifolium* G.Don in acute and chronic experimental models in albino rats. *J Appl Biosci* 2009; **19**: 1049 -1053.

[17] Aviram M. Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. *Free Radical Res* 2000; **33**: 85-97.

[18] Aravind Barad, Malashetty VB, Seetharam YN. *In vitro* epiphyllous buds formation in *Enicostemma hyssopifolium* (Willd.) ver. induction and cellular origin. *Int J Herb Med* 2014; **2**: 50-56.

[19] Bhatt NM, Barua S, Gupta S. Protective Effect of *Enicostemma littorale* Blume on Rat Model of Diabetic Neuropathy. *American J Infect Dis* 2009; **5**: 99-105.

[20] Bhatt NM, Chauhan K, Gupta S, Pillai P, Pandya C, Thaikoottathil JV, Gupta SS. Protective effect of *Enicostemma littorale* Blume Methanolic Extract on Gentamicin-Induced Nephrotoxicity in Rats. *American J Infect Dis* 2011; **7**: 83-90.

[21] Bhatt NM, Chavda M, Desai D, Zalawadia R, Patel VB, Burade V, Sharma AK, Singal PK, Gupta S. Cardioprotective and antihypertensive effects of *Enicostemma littorale* Blume extract in fructose-fed rats. *Can J Physiol Pharmacol* 2012; 90.

[22] Bhatt AJ, Rathod R, Jairaj V, Goyal RK. Development and validation of analytical method for estimation of Swertiamarin in marketed preparation containing *Enicostemma littorale*. *WJPPS* 2016; **5**: 1467-1478.

[23] Bhattacharya SK, Reddy PK, Ghosal S, Singh AK, Sharma PV. Chemical constituents of Gentianaceae XIX: CNS-depressant effects of swertiamarin. *J Pharm Sci* 1976; **65**: 1547-1549.

[24] Ceylan-Isik AF, Fliethman RM, Wold LE, Ren J. Herbal and traditional Chinese medicine for the treatment of cardiovascular complications in diabetes mellitus. *Curr Diabetes Rev* 2008; **4**: 320–328.

[25] Chaudhuri RK, Singh AK, Ghosal S. Chemical constituents of gentianaceae. XVIII. Structure of Enicoflavine. Monoterpene alkaloid from *Enicostemma hyssopifolium*. *Chemical Industry (London)* 1975; **3**: 127-128.

[26] Clarina T, Maheswari M, Nalini Mabel GD, Rama V. Pharmocognosy and Phytochemical study of *Enicostemma littorale* Blume. *AJPTR* 2012; **2**: 1-6.

[27] Dadheech N, Soni S, Srivastava A, Dadheech A, Gupta S, Gopurappilly R, Bhonde RR, Gupta S. A Small Molecule Swertisin from *Enicostemma littorale* Differentiates NIH3T3 Cells into Islet-Like Clusters and Restores Normoglycemia upon Transplantation in Diabetic Balb/c Mice *ECAM* 2013; 1-20.

[28] Dalit Database, Community Food System Data Table #200. Quebec, Canada: McGill University Centre for Indigenous Nutrition and Environment (CINE) 2006.

[29] Daniel M, Sabnis SD. Chemical systematics of family gentianaceae. *Curr Sci* 1978; **47**: 109 -111.

[30] Deccan Development Society, Uncultivated foods and the poor. India Together 2002. (http://www.indiatogether.org/agriculture/dds/uncultivated.htm (Retrieved November 8, 2006))

[31] Deepa V, Sridhar R, Neelakanta Reddy P, Balakrishna Murthy P. Development, characterization, efficacy and repeated dose toxicity of nanoemulsified ethanolic extract of *Enicostemma littorale* in Streptozotocin-induced diabetes rats. *Inter J Phytomed* 2012; **4**: 70-89.

[32] Deepake US, Das Y, Algunde S, Gyananath G. Preliminary screening of endophytic fungi from *Enicostemma axillare* (Lam.) Raynal. for antimicrobial activity. *Curr Bot* 2012; **3**: 23-29.

[33] Derore SL, Khadabadi SS, Bhagure L, Ghorpade DS. *In vitro* antimicrobial and antioxidant studies on *Enicostemma axillare* (Lam.) Raynal. Leaves. *NPR* 2008; **7**: 409-412.

[34] Desai PD, Ganguly AK, Govindachari TR, Joshi BS, Kamat VN, et al. Chemical investigation of some Indian Medicinal Plants: Part II. *IJC* 1966; **4**: 457-459.

[35] Dhanapal R, Ratna JV, Gupta M, Sarathchandran I. Preliminary study on antifertility activity of *Enicostemma axillare* leaves and Urena lobata root used in Indian traditional folk medicine. *APJTM* 2012; **5**: 616-622.

[36] Dymock W, Warden CJH, Hooper D. Pharmacographia Indica. Thacker Spink, *Calcutta* 1893; **2**: 516.

[37] Garad MC, Upadhya MA, Kokare DM, Itankar PR. Aerial parts of Enicostemma littorale Blume serve as antipyretic and antacid: in vivo and in vitro evaluations. *Phcog Commn* 2012; **2**: 42-45.

IJSER

[38] Ghosal S, Jaiswal DK. Chemical constituents of gentianaceae XXVIII: Flavonoids of *Enicostemma hyssopifolium* (Willd.) verd. *J Pharm Sci* 1980; **69**: 53–56.

[39] Ghosal S, Singh AK, Sharma PV, Chaudhuri RK. Chemical constituents of gentianaceae IX: Natural occurrence of erythrocentaurin in *Enicostemma hyssopifolium* and Swertia lawii. *J Pharm Sci* 1974; **63**: 944–945.

[40] Gite VN, Pokharkar RD, Chopade VV, Takate SB. Evalution of Physicochemical Standardization Parameters of *Enicostemma axillare*. *J Biosci Tech* 2010; **1**: 187-190.

[41] Gite VN, Pokharkar RD, Chopade VV, Takate SB. Hepato-protective activity of *Enicostemma axillare* in paracetamol induced hepato-toxicity in albino rats. *IJPLS* 2010; **1**: 50-53.

[42] Gopal R, Gnanamani A, Udayakumar R, Sadulla S. *Enicostemma littorale* Blume – A potential hypolipidemic plant. *Natural product Radiance* 2004; **3**: 401-405.

[43] Gopal R, Udayakumar R. Enzymatic and Non-Enzymatic Antioxidant Activities of *Enicostemma littorale* in p-DAB Induced Hepatocarcinoma in Rats. *Int J Pharmacol* 2008; **4**: 369-375.

[44]. Gupta RS, Singh D. Hepatomodulatory role of *Enicostemma littorale* Blume against oxidative stress induced liver injury in rats. *Afr J Agric Res* 2007; **2**: 131-138.

[45] Gupta S, Dadheech N, Singh A, Soni S, Bhonde RR. *Enicostemma littorale*: A new therapeutic target for islet neogenesis. *IJIB* 2010; **9**: 49-53.

[46] Harvey AL. Natural products in drug discovery. *Drug Discov Today* 2008; **13**: 894–901.

[47] Hassan N, Javed A, Saima A, Mohd M, Mir SR. Rapid preparative isolation of erythrocentaurin from *Enicostemma littorale* by medium-pressure liquid chromatography, its estimation by high-pressure thin-layer chromatography, and its α-amylase inhibitory activity. *J Sep Sci* 2015; **38**: 592-598.

[48] Indumathi C, Durgadevi G, Nithyavani S, Gayathri PK. Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorrale*. *Int J ChemTech Res (USA)* 2014; **6**: 4264-4267.

[49] Jahan E, Perveen S, Malik A. Verticilliside, a new flavone Cglucoside from *Enicostemma verticillatum*. *JANPR* 2009; **11**: 257-260.

[50] Jahan E, Fatima I, Malik A. Enicostemins A and B, New Secoiridoids from *Enicostemma verticillatum.* Zeitschrift für Naturforschung B, A *J Chem Sci* 2011; **66**: 749-751.

[51] Jain UK, Dixit VK. A new secoiridoid from *Enicostemma littorale* Blume. *Hamdard Medicus* 2006; **49**: 5-7.

[52] Jaishree V, Badami S, Bhojraj S. *In vitro* Antioxidant Activity of *Enicostemma axillare*. *JHSci* 2008; **54**: 524-528.

[53] Jaishree V, Badami S, Rupesh Kumar M, Tamizhmani T. Antinociceptive activity of swertiamarin isolated from *Enicostemma axillare*. *Phytomed* 2009; **16**: 227-232.

[54] Jaishree V, Badami S, Krishnamurthy T. Antioxidant and hepatoprotective effect of the ethyl acetate extract of *Enicostemma axillare* (Lam.) Raynal against CCl_4-induced liver injury in rats. *Indian J Exp Biol* 2010; **48**: 896-904.

[55] Jaishree V, Badami S. Antioxidant and hepatoprotective effect of swertiamarin from *Enicostemma axillare* against D-galactosamine induced acute liver damage in rats. *J Ethnopharmacol* 2010; **130**: 103-106.

[56] Jensen SR, Schripsema J. Chemotaxonomy and pharmacology of Gentianaceae. In Gentianaceae: Systematics and Natural History, Struwe, L. & Albert V. Eds. Cambridge, UK: *Cambridge University Press* 2002.

[57] Kamboj VP. Herbal medicine, *Curr Sci* 2000; **78**: 35-39.

[58] Kalpana M, Mehta GN. Evaluation of *Enicostemma axillare* as biodegradable less toxic inhibitor for acid corrosion of mild steel. *B Electrochem* 2003; **19**: 381-384.

[59] Kaskoos RA, Hagop AB, Faraj AM, Ahamad J. Comparative antioxidant activity of Gymnema sylvestre, *Enicostemma littoral*, Momordica charantia and their composite extract. *J Pharm Phytochem* 2015; **4**: 95-98.

[60] Kavimani S, Manisenthikumar KT Effect of methanolic extract of *Enicostemma littorale* on Dalton's ascetic lymphoma. *J Ethnopharmacol* 2000; **71**: 349-352.

[61] Kolasani A, Xu H, Millikan M. Determination and comparison of mineral elements in traditional Chinese herbal formulae at different decoction times used to improve kidney function - Chemometric approach. *Afr J Tradit Complement Altern Med* 2011; **8**: 191–197.

[62] Kousalya L, Narmatha Bai V. High frequency in vitro plantlet regeneration and antioxidant activity of *Enicostema axillare* (Lam.) Raynal ssp. *littoralis* (Blume) Raynal: An important medicinal plant. *APJR* 2014; **3**: 241-248.

[63] Krishnaveni A, Mohandass S. Anti-Oxidant Activity of *Enicostemma axillare* (*in vitro* studies). *JPR* 2012; **5**: 3954-3956.

[64] Krishnaveni A, Mohandass S. *In-vitro* Cytotoxic Activity of *Enicostemma axillare* Extract against Hela Cell Line. *Int J Pharmacognosy and Phytochem Res* 2014; **6**: 320-323.

[65] Kumarasamy Y, Nahar L, Cox PJ, Jaspars M, Sarker SD. Bioactivity of secoiridoid glycosides from Centaurium erythraea. *Phytomed* 2003; **10**: 344-7.

[66] Kutty NN, Gaikwad NK, Moon UR, Kumar M, Kshirsagar SR, Mitra A. Evaluation of antioxidant capacity and acetylcholinesterase inhibitory activity of field-grown plants and in vitro plantlets of *Enicostemma littorale*. *Free Radicals and Antioxidants* 2014; **4**: 21-26.

[67] Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. *Curr Med Chem* 2004; 11: 1451-1460.

[68] Lakshmi PTV, Annamalai A, Ramya C. A study on the genetic diversity analysis of a medicinally potential

herb- *Enicostemma littorale* Blume. (Gentianaceae). *IJPBS* 2011; **2**: 438-445.

[69] Leelaprakash G, Mohan Das S. Antimicrobial Activity and Phytochemical Screening of Methanol Extract of *Enicostemma axillare*. *Int J Pharm Pharm Sci* 2012; **4**: 342-348.

[70] Leelaprakash G, Mohan Dass S. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare. Int J Drug Dev and Res* 2011; **3**: 189-196.

[71] Maharishi Ayurveda Products. Ayurveda Ltd. Lancashire, UK. 2006. http://www.maharishi.co.uk/productinfo/91407.pdf (Retrieved November 7, 2006)

[72] Manoharan S, Duraisamy R, Murugaraj MP, Sekar K, Asokan M. Modulating Effect of *Enicostemma littorale* on the Expression Pattern of Apoptotic, Cell Proliferative, Inflammatory and Angiogenic Markers During 7, 12 Dimethylbenz (a) Anthracene Induced Hamster Buccal Pouch Carcinogenesis. *Toxicol Int* 2015; **22**: 130-140.

[73] Mansuri MM, Goyal BR, Upadhyay UM, Sheth J, Goyal RK. Effect of long term treatment of aqueous extract of *Enicostemma littorale* in Type 2 diabetic patients. *Orient Pharm Exp Med* 2009; **9**: 39-48.

[74] Maroo J, Vasu VT, Aalinkeel R, Gupta S. Glucose lowering effect of aqueous extract of *Enicostemma littorale* Blume in diabetes: a possible mechanism of action. *J Ethnopharmacol* 2002; **81**: 317-320.

[75] Maroo J, Vasu VT, Gupta S. Dose dependent hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume in alloxan induced diabetic rats. *Phytomedicine* 2003; **10**: 196-199.

[76] Maroo J, Ghosh A, Mathur R, Vasu VT, Gupta S. Antidiabetic Efficacy of *Enicostemma littorale* Methanol Extract in Alloxan-Induced Diabetic Rats. *Pharm Biol* 2003; **41**: 388-391.

[77] Mathur R. Phytochemical and Antimicrobial Evaluation of Plant Extracts of *Enicostemma hyssopifolium. J Pharma and Phytochem* 2013; **2**: 30-36.

[78] Rathod CM, Dhale DA. Pharmacognostic characterization of *Enicostemma littorale* Blume. *Int J Res Ayurveda Pharm* 2013; **4**: 893-898.

[79] Mir SR, Ahamad J, Hassan N, Amin S. Management of post prandial hyperglycemia with swertiamarin isolated from *Enicostemma littorale* Blume. *Planta Medica* 2014; **80**: 13.

[80] Mishra S, Mishra A, Shukla P, Shukla P. *In Vitro* Anthelmintic Activity of *Enicostemma littorale* Blume. *IJPSR* 2011; **2**: 1193-1196.

[81] Murali B, Upadhyaya UM, Goyal RK. Effect of chronic treatment with *Enicostemma littorale* in noninsulin dependent diabetic (NIDDM) rats. *J Ethnopharmacol* 2002; **81**: 199-204.

[82] Nadkarni AK. Dr. K. M. Nadkarni's Indian Materia Medica, Vol. 1. Bombay: *Popular Prakashan Private Ltd* 1976.

[83] Nagarathnamma M, Sudarshana MS, Niranjan MH. Pandurangamurthy, Rapid regeneration of *Enicostemma littorale* Blume from leaf and stem cultures. *J Plant Interactions* 2010; **5**: 69-73.

[84] Nalini P, Velayutham P. *In vitro* mass propagation of *Enicostemma littorale* Blume. from shoot tip explants. *J Bio Agri Healthcare* 2013; **3**: 72-79.

[85] Nalini P, Velayutham P, Karthi C. Plant Regeneration from the Leaf Explants of *Enicostemma littorale* Blume through Direct Organogenesis. *Int J Sci Res* 2016; **5**: 1809-1814.

[86] Natarajan PN, Prasad S. Chemical investigation of *Enicotemma littorale. Planta Medica* 1972; 22: 42-46.

[87] Padmanabhan D, Regupathy D, Pushpa Veni S. Gynoecial ontogeny in *Enicostemma littorale* Blume. *Proceedings of the Indian Academy of Sciences* 1978; **87**: 83-92.

[88] Paivera J, Nogueira I. Gentianaceae. Flora Zambesiaca, *Kew UK: Royal Botanical Gardens* 1990; **7** (4).

[89] Pandita N, Prabhakar B, Sawant L. HPLC-LC/MS/MS of secoiridoid glycosides and flavonoids in *Enicostemma littorale. Planta Medica* 2010; **76**: SL-45.

[90] Patel RP, Trivedi BM. The *in vitro* antibacterial activity of extracts from *Enicostemma littorale. Indian J Med Sci* 1957; **11**: 887-91.

[91] Patel PM, Patel KN, Patel NM, Goyal RK. A HPTLC method for quantitative estimation of swetiamarin in marketed polyherbal antidiabetic formulations. *Indian j pharma sci* 2007; **63**: 446-448.

[92] Patel MB, Mishra M. Aldose Reductase Inhibitory Activity of a C-glycosidic Flavonoid Derived from *Enicostemma hyssopifolium*. *J Complemen Integrative Med* 2009; **6**.

[93] Patel MB, Mishra SH. Hypoglycemic activity of C-glycosyl flavonoid from *Enicostemma hyssopifolium*. *Pharm Biol* 2011; **49**: 383-391.

[94] Patel MB, Mishra SH. Quantitative analysis of marker constituent swertisin in *Enicostemma hyssopifolium* verdoon by RP-HPLC and HPTLC. *Acta Chromatographica* 2012; **24**: 85-95.

[95] Patel TP, Soni S, Parikh P, Gosai J, Chruvattil R, Gupta S. Swertiamarin: An Active Lead from *Enicostemma littorale* Regulates Hepatic and Adipose Tissue Gene Expression by Targeting PPAR-γ and Improves Insulin Sensitivity in Experimental NIDDM Rat Model. *Evid Based Complement Alternat Med* 2013; 1-11.

[96] Petrovska BB. Historical review of medicinal plants' usage. Pharmacognosy Review 2012; **6**: 1-5.

[97] Pradip D, Karan S, Chatterjee TK. *In vivo* anticancer activity of methanol extract of the whole medicinal plant *Enicostemma littorale* in swiss albino mice. *Pharma online* 2010; **3**: 637-643.

[98] Prakash P, Milan S. Antimicrobial activity of Enicostemma littorale. *J Pharma Res Regulatory Affair* 2016; **1**: 66-67.

IJSER

[99] Praveena P, Sudarsanam D. *In vitro* antimicrobial activity studies on *Enicostema littorale* (Lam), Raynal. whole plants. *Int J Curr Res* 2011; **3**: 123-124.

[100] Prince PS, Srinivasan M. *Enicostemma littorale* Blume aqueous extract improves the antioxidant status in alloxan-induced diabetic rat tissues. *Acta Pol Pharm* 2005; **62**: 363-367.

[101] Rajasekaran A, Arivukkarasu R, Murugesh S. Hepatoprotective Effect of *Adenema hyssopifolium G.Don* (Gentianaceae) in Carbon Tetrachloride-Induced Hepatotoxicity in Rats. *Trop J Pharm Res* 2010; **9**: 157-163.

[102] Rajasekaran D, Kowsalya R, Selvasundaram R, Manoharan S. *Enicostemma littorale* protects cell surface abnormalities during dmba-induced hamster buccal pouch carcinogenesis. *Int J Pharma Bio Sci* 2015; **6**: 104-109.

[103] Rajasekaran D, Manoharan S, Prabhakar MM, Manimaran A. *Enicostemma littorale* prevents tumor formation in 7,12 dimethylbenz(a) anthracene-induced hamster buccal pouch carcinogenesis. *Hum Exp Toxicol* 2015; **34**: 911-921.

[104] Rajasekaran A, Arivukkarasu R. Acute and Subacute Toxicity Study of Aqueous Extracts of *Enicostemma axillare* (Lam.) Raynal in Animal models, *Pharmacogn J* 2016; **8**: 10-19.

[105] Ravi V, Kumar M, Gupta S. Hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume (Chhota chirayata) on alloxan induced diabetes mellitus in rats. *Indian J Exp Biol* 2000; **38**: 781-784.

[106] Retnam KR, DeBritto AJ. Preliminary phytochemical screening of three medicinal plants of Tirunelveli hills. *J Economy Texas Bot* 1988; **22**: 677-681.

[107] Roy PS, Niranjan CM, Jyothi TM, Shankrayya MM, Vishawanath KM, Prabhu K, Gouda VA, Setty RS. Antiulcer and Antiinflammatory Activity of Aerial Parts *Enicostemma littorale* Blume. *J Y Pharm* 2010; **2**: 369-373.

[108] Ruckmani K, Kavimani S, Sundari SKK. Anti-tumour activity of methanolic extracts of *Enicostemma littorale* and *Aristolochia bracteolata* on Dalton's Ascitic Lymphoma. *Hamdard medicus* 2003. 46.

[109] Sadique J, Chandra T, Thenmozhi V, Elango V. The anti-inflammatory activity of *Enicostemma littorale* and mullogo cerviana. *Biochem Med Metab Biol* 1987; **37**: 167-176.

[110] Samuelsson G. Drugs of natural origin: a textbook of pharmacognosy. *Swedish Pharmaceutical Press, Stockholm* 2004.

[111] Saranya R, Thirumalai T, Hemalatha M, Balaji R, David E. Pharmacognosy of *Enicostemma littorale*: A review. *Asian Pac J Trop Biomed* 2013; **3**: 79-84.

[112] Sasidharan P, Jayachitra A. Direct shoot bud regeneration from shoot tip explants of *Enicostema axillare*: an important medicinal plant. *Agroforest Syst* 2016; 1-7.

[113] Sathishkumar R, Lakshmi PTV, Annamalai A. Effect of drying treatment on the content of antioxidants in *Enicostemma littorale* Blume. *Res J Med plant* 2009; **3**: 93-101.

[114] Sathishkumar R, Lakshmi PTV, Annamalai A. Comparative analyses of non enzymatic and enzymatic antioxidants of *Enicostemma littorale* Blume. *IJPBS* 2010; **1**: 1-16.

[115] Sathishkumar R, Lakshmi PTV, Annamalai A. Estimation of biomass contents and phytoconstituent analysis of *Enicostemma littorale* blume. *Int J Pharm Bio Sci* 2012; **3**: 506 – 522.

[116] Sawant L, Prabhakar B, Pandita N. HPTLC method development and validation for estimation of isovitexin from methanolic extract of *Enicostemma littorale* Blume. *Planta Med* 2010; **76**: SL_43.

[117] Sawant L, Prabhakar B, Kachwala Y, Pandita N. Pharmacognostical standardization of *Enicostemma littorale* Blume. *Phcog J* 2010; **2**: 15-23.

[118] Sawant L, Prabhakar B, Pandita N. A validated quantitative HPLE method for analysis of biomarkers in *Enicostemma littorale* Blume. *JPC* 2011; **24**: 497-502.

[119] Sawant L, Prabhakar B, Pandita N. HPTLC method for quantification of isovitexin in the whole plant powder of *Enicostemma littorale* Blume. *JPC* 2011; **24**: 301-305.

[120] Seetharam YN, Aravind B, Gururaj C, Jyothishwaran G, Ghanti KS, Bhakri V. *In vitro* Shoot Regeneration from leaf and nodal explants of *Enicostemma hyssopifolium* (Willd.) Verd. –A vulnerable medicinal plant. *Indian J Biotechnol* 2002; **1**: 401-404.

[121] Selvaraj S, Chittibabu CV, Janarthanam B. Studies on phytochemical screening, antioxidant activity and extraction of active compound (Swertiamarin) from leaf extract of *Enicostemma littorale*. *Asian J Pharm Clin Res* 2014; 7: 240-244.

[122] Mani Senthilkumar KT, Rajkapoor B, Kavimani S. Protective Effect of *Enicostemma littorale*. against CCl4Induced Hepatic Damage in Rats. *Pharm Biol* 2008; **43**: 485-487.

[123] Shabi MM, Uthrapathy S, David Raj C, Krishnamoorthy G, Ravindhran D, Joseph J, Rajamanickam VG. Analgesic and Anti-Arthritic Effect of *Enicostemma littorale* Blume. *Advan Biosci Biotech* 2014; **5**: 1018-1024.

[124] Mohammed SKAS, Senthamarai R, Reeta Vijaya RK, Sri Rekha B. *In vitro* anti- arthritic activity of whole plant Of *Enicostemma axillare* linn. *Advan Pharm Toxicol* 2012; **13**: 7-11.

[125] Mohammed SKAS, Senthamarai R, Kumara Sundari SK, Sri Rekha B. Evaluaion of anti-inflammatory activity of whole plant of *Enicostemma axillare* in albino wistar rats. *Int J Pharmacol Bio Sci* 2012: **6**: 29-34.

[126] Shanmugapriya ON, Priya S. Antibacterial Activity of *Enicostemma Axillare* Against Wound Pathogens. *RRJBT* 2014; **4**: 1-3.

[127] Shantha Sheela V, Selvakumar S, Shanthi G. Phytochemical analysis of *Cayartia pedata, Enicostemma*

axillare and *Terminalia chebula. WJPPS* 2015; **4**: 1102-1111.

[128] Sharma R, Joshi A, Pandey D, Hemalatha S. Pharmacognostical Standardization, Antioxidant Activity, and Phytochemical Analysis of Leaves from *Enicostemma verticillatum*. *J Herbs Spi Med Plants* 2015; **21**: 182-192.

[129] Sonawane RD, Vishwakarma SL, Lakshmi S, Rajani M, Padh H, Goyal RK. Amelioration of STZ-induced type 1 diabetic nephropathy by aqueous extract of *Enicostemma littorale* Blume and swertiamarin in rats. *Mol Cell Biochem* 2010; **340**: 1-6.

[130] Srinivasan M, Padmanabhan M, Prince PS. Effect of aqueous *Enicostemma littorale* Blume extract on key carbohydrate metabolic enzymes, lipid peroxides and antioxidants in alloxaninduced diabetic rats. *J Pharm Pharmacol* 2005; **57**: 497–503.

[131] Srivastava A, Bhatt NM, Patel TP, Dadheech N, Singh A, Gupta S. Anti-apoptotic and cytoprotective effect of *Enicostemma littorale* against oxidative stress in Islets of Langerhans. *Pharm Biol* 2016; 1-12.

[132] Tanna SP, Patgiri B, Shukla VJ, Prajapati PK. Pharmaceutical standardization of Mamajjaka (*Enicostemma littorale* Auct. non Bl) Ghana. *AYU* 2012; **33**: 294–298.

[133] Tapsell LC, Hemphill I, Cobiac L, Patch CS, Sullivan DR, Fenech M, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA, Inge KE. Health benefits of herbs and spices: The past, the present, the future. *Med J Aust* 2006; **185**: 4-24.

[134] Thayalini T, Thevanesam V, Kathirgamanathar S, Gamage TM. Antibacterial Activity and Preliminary Screening of Phytochemicals of Whole Plant of *Enicostemma littorale*. *Trop Agric Res* 2012; **24**: 99-105.

[135] Thirumalai T, Viviyan Therasa S, Elumalai EK, David E. Hypolipidemic and antioxidant effect of *Enicostemma littorale* Blume. *Asian Pac J Trop Biomed* 2011; **1**: 381-385.

[136] Turchi G, Alagona G, Lubrano V. Protective activity of plicatin B against human LDL oxidation induced in metal ion-dependent and -independent processes. Experimental and theoretical studies. *Phytomed* 2009; **16**: 1014–1026.

[137] Upadhyay UM, Goyal RK. Efficacy of *Enicostemma littorale* in type 2 diabetic patients. *Phytother Res* 2004; **18**: 233-235.

[138] Vaidya H, Rajani M, Sudarsanam V, Padh H, Goyal R. Antihyperlipidaemic activity of swertiamarin, a secoiridoid glycoside in poloxamer-407 induced hyperlipidaemic rats. *J Nat Med* 2009; **63**: 437-442.

[139] Vaidya H, Rajani M, Sudarsanam V, Padh H, Goyal R. Swertiamarin: A lead from *Enicostemma littorale* Blume. for anti-hyperlipidaemic effect. *Eur J Pharmacol* 2009; **617**: 108-112.

[140] Vasu VT, Ashwinikumar C, Jyoti M, Gupta S, Gupta S. Antidiabetic effect of *Enicostemma littorale* Blume aqueous extract in newly diagnosed non-insulin-dependent diabetes mellitus patients (NIDDM): A preliminary investigation. *OPEM* 2003; **3**: 84-89.

[141] Vasu VT, Modi H, Thaikoottathil JV, Gupta S. Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume aqueous extract in cholesterol fed rats. *J Ethnopharmacol* 2005; **101**: 277-282.

[142] Vidyadhar S, Saidulu M, Gopal TK, Chamundeeswari D, Umamaheswara rao, David Banji. *In vitro* anthelmintic activity of the whole plant of *Enicostemma littorale* by using various extracts. *IJABPT* 2010; **1**: 1119-1125.

[143] Vinotha S, Thabrew I, Sri Ranjani S. Preliminary Phytochemical Screening of Different Extracts of Whole Plant of *Enicostemma littorale* Blume. *IJSBAR* 2013; **11**: 99-104.

[144] Vinotha S, Ira T, Sri Ranjani S. Phyto, Physicochemical Standardization of Medicinal Plant *Enicostemma Littorale,* Blume. *IOSR J Pharmacy* 2013; **3**: 52-58.

[145] Vishwakarma SL, Rajani M, Goyal RK. Comparative antidiabetic activity of different fractions of *Enicostemma littorale* Blume in streptozotocin induced NIDDM rats. *OPEM* 2003; **3**: 196-204.

[146] Vishwakarma SL, Rajani M, Bagul M, Goyal RK. A Rapid Method for the Isolation of Swertiamarin from *Enicostemma littorale*. *Pharm Biol* 2004; **42**: 400-403.

[147] Vishwakarma SL, Bagul M, Rajani M, Goyal RK. A sensitive HPTLC method for estimation of swertiamarin in *Enicostemma littorale* Blume, Swertia chirata (Wall) Clarke, and in formulations containing *E. littorale*. *JPC* 2007; 17: 128-131.

[148] Vishwakarma SL, Sonawane RD, Rajani M, Goyal RK. Evaluation of effect of aqueous extract of *Enicostemma littorale* Blume in streptomycin induced type 1 diabetic rats, *IJEB* 2010; **48**: 26-30.

[149] Yamahara J, Kobayashi M, Matsuda H, Aoki S. Anticholinergic action of Swertia japonica and an active constituent. *J Ethnopharmacol* 1991; **33**: 31-35.

[150] Weiss RF. Herbal Medicine. Gothenburg, Sweden: AB Arcanum 1988.

[151] World Health Organization, General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO, Geneva, Switzerland 2001.

IJSER