A REVIEW ON ENICOSTEMMA LITTORALE

Abirami.P and M.Gomathinayagam*

Department of Botany, Faculty of Science, Annamalai University
Annamalainagar, Tamilnadu, India

Address for correspondence:
M.Gomathinayagam
Department of Botany
Annamalai University
Annamalainagar – 608 002
Tamilnadu
India
Telephone Number: 09952617379
Email: drmgomathinayagam@gmail.com

Summary

Aim of the review paper is to give an enlightenment on potential phyto-constituents and pharmacology of *Enicostemma littorale* (Gentianaceae). *Enicostemma littorale* is a perennial herb found throughout the greater part of India. The plant is traditionally used in rheumatism, abdominal ulcers, hernia, swelling, itches and insect poisoning. *Enicostemma littorale* Blume is a plant with a number of antioxidative phytochemicals, which include alkaloids, catechins, saponins, sterols, triterpenoids, phenolic acids, flavonoids and xanthones. It also contains minerals like iron, potassium, sodium, calcium, magnesium, silica, phosphate, chloride, sulphate and carbonate. The review gives a bird's eye view mainly on the biological activities, some of the compounds isolated and their pharmacological actions.

Key words: *Enicostemma littorale,* Phytochemicals, Pharmacological activities.

Introduction

Medicinal plants have been used as sources of medicine in virtually all cultures (4). They are of important therapeutic aid for various ailments. During the last decade, the use of traditional medicine has expanded globally and its gaining property. It has continued to be used in not only for primary health care of the poor in developing countries, but also in countries where conventional medicine in predominant in the national health care system (21). According to the WHO, herbal medicines serve the health needs of about 80%of the world's population, especially for millions of people in the vast rural areas of developing countries (41).

Enicostemma littorale (Gentianaceae) is a glabrous perennial herb belonging to the family Gentianaceae (20). It grows throughout India up to 1.5 feet height and more frequently near the sea. It is called as Chota-kirayat or Chota chirayata in Hindi, Mamejavo in Gujarati, Nagajivha in Bengal and Vellarugu or Vallari in Tamil.

Characteristics:

Annual or perennial herbs, Stems often winged, rounded or angular. Leaves sessile, often narrow. Inflorescence axillary, dense clusters or cymes. Flowers 5-merous (rarely 3-, 4-, or 6-merous), sessile, actinomorphic. Calyx narrow, campanulate, divided down halfway to 2/3, thin, with white, thinner margins, persistent in fruit, with colleters. Corolla small, white, tubular to funnel shaped. Stamens inserted in corolla tube, with appendices at filament bases, filaments equal length, anthers erect after anthesis, with sterile apex. (22) Ovary without nectary disk; stigmas capitate, slightly bilobed. Fruit a capsule, obovoid, seeds rounded, not winged. (27)

The plant has number of antioxidative phtochemicals which include alkaloids, catechins, saponins, sterols, triterpinoids, phenolic acid flavonoids and xanthones. It also contains minerals like iron, potassium, calcium, silica, phosphate, chloride sulphate and carbonate (24).

The plant is used in folk medicine to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning (Kirtigar *et al.,)* Its anti-inflammatory (34)hypoglycaemic (17,18) and anticancer(19) activites have been reported.

The taxonomic position of *Enicostemma littorale* is as follows

Subdivision	Angiospermae
Class	Dicotyledonae
Subclass	Gamopetalae
Serius	Bicarpellatae
Order	Gentianales
Genus	Enicostemma
Species	littorale

Phytochemical

Many compounds have been isolated from the plant.Dymock *et al.,* reported that the aerial part of the plant gave 34% of dry alcoholic extract and 15.7% of ash. Qualitative analysis of the ash revealed the presence of minerals like iron, potassium, sodium, calcium, magnesium, silica, phosphate, chloride, sulphate and carbonate. Five alkaloids, two sterols and volatile oil, have been reported by Natarajan and Prasad (25). Monoterpene alkaloids like-enicoflavin and gentiocrucine were also isolated (11,5). The presence of catechins, saponins, steroids and triterpenoids were reported by earlier workers (33). Betulin, a triterpene sapogenin was also isolated by earlier workers (30,7).

A new flavone C-glucoside named as Verticilliside was isolated for the first time this species (9). Swertiamarin was isolated from the green viscous mass obtained from an alcoholic extract of the drug treated with ether followed by ethylacetate. (30,7). Flavonoids and xanthones were found to be present in this plant. Six phenolic acids viz: vanillic acid, syringic acid, p-hydroxy benzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid were also found (6). Seven flavonoids were isolated from alcoholic extract and their structures were identified as apigenin, genkwanin, isovitexin, swertisin, saponarin, 5-O-glucosylswertisin and 5-Oglucosylisoswertisin (10). Methanol extract of *E. littorale* was found to contain different 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, L-Glycine, 2-amino butyric acid and valine (33) .

Pharmacological Activities

Hypoglycemic activity

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. The plant extract 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. Above results suggest the glucose lowering effect of aqueous extract of *E. littorale* (17,18) The aqueous extract of the plant prevented the blood glucose level level as well as insulin level in rat model. It produces an increase in insulin sensitivity, normalizes dyslipidaemia and provides nephroprotection in diabetic rats. (24) In another study showed diabetic rats were having hyperglycemic condition. Rates treated with plant extract for 45 days showed reduction in blood glucose levels (26)

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 *Enicostemma littorale* blume. An active herbal compound SGL-1 was isolated and purified from extract of *Enicostemma littorale* and used to differentiate to modal stem cells showed treamendous 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 charecterization of islet cellular aggregates confirmed significant glucose responsive insulin release. This preliminary data offer exciting possibility of alternate source to increase islet mass which can be used for treatment of diabetic patients. (36)

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 wt.) in alloxan induced diabetic rats. The above dose caused significant decrease in glycosylated haemoglobin, liver glucose-6-phosphatase activity and significant increase in serum insulin levels of the diabetic rats. (23)

The dose dependent effect of three weeks treatment with hot and cold aqueous extract of *Enicostemma littorale* (0.5, 1 and 2 g/kg, po) on streptozotocin induced type 1 diabetic rats (45 mg/kg,iv single dose). *Enicostemma littorale* possess potential antidiabetic activity and improves lipid profile at a small dose of 0.5 g/kg (40)

Antihyperlipiaemic activity

The aerial part of the *Enicostemma 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 (13) A

new study demonstrated new property of swertiamarin as a potent lipid lowering agent and comparable to atorvastatin and it may contribute to its cardioprotective and anti-atherosclerotic role. The isolated swertiamarin and atorvastatin when orally fed also lowered the total serum cholesterol and triglycerides (15)

Enicostemma littorale aqueous extract (1.5 g/100g body weight/day, p.o.) was administered to rats along with hypercholesterolaemic diet for 6 weeks and the hypolipidaemic and antioxidant effect was evaluated. The treatment with the extract showed a decrease in activities of erythrocyte catalase, superoxide dismutase and lipid peroxidation levels, with an increase in reduced glutathione levels as compared to cholesterol fed untreated rats. Liver and kidney cholesterol levels and triglyceride levels were also decreased in *Enicostemma littorale* treated rats. (39)

Antitumour activity

The methanolic extract of *Enicostemma littorale* indirectly inhibited tumour cell growth and it was examined on the peritoneal exudates cells of the normal mice. Methanolic extract of *Enicostemma littorale* found to 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 *Enicostemma littorale* treatment.(19)

Hepato-protective activity

The hepatoprotective activity in rats against paracetamol as hepatotoxin to prove its claims in folklore practice against liver disorders. Paracetamol-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plant extracts and drugs. 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.(12) Swertiamarin isolated from *Enicostemma axillare* 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. (16). The present investigation indicate the ethanolic extract of *E. littorale*, extract exhibited significant hepatomodulation against oxidative stressinduced liver injury by CCl4 in rats through antioxidant potential and free radical scavenging activities alongwith reduction of fat metabolism.(14). The ethanol and ethyl acetate extracts of *Enicostemma littorale* contain pharmacologically active substances with hepatoprotective properties. These attributes provide the rationale for the use of *Enicostemma littorale* in liver disorders by traditional healers in India (31).

Anti-inflammatory activity

The alcohol extract at a concentration of 300 and 600 mg kg-1 p.o., and its ethyl acetate fractions at 25 and 50 mg kg-1 p.o. 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

Enicostemma littorale, and its ethyl acetate fractions, exhibited significant anti-inflammatory activity. (2)

Antinociceptive activity

In vivo antinociceptive activity of swertiamarin isolated from E. axillare 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 (16).

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 (38).

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 (3). 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 (16). 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 (32).

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 (37).

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 (38).

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 (29).

Phytochemical Standardization

Gentianine is a bitter, crystalline monoterpene alkaloid present in *Enicostemma littorale* (1) Swetiamarin is one of the phytoconstituents present in *Enicostemma littorale* Linn. *Enicostemma littorale* is known for its hypoglycemic activity from ancient times. In the present study an attempt has been made to develop a HPTLC method for quantitative estimation of swetiamarin in plants and different marketed antidiabetic polyherbal formulations. This HPTLC method was found to be reproducible, accurate and precise and could detect swetiamarin concentration at nanogram level. The developed HPTLC method would be an important tool in the way of acceptability of quality control method of polyherbal formulations.

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 swetiamarin were found to be about 96.2%, which shows the reliability and suitability of the method. The lowest detectable limit of swetiamarin in different formulations was found upto 50 ng/spot (28).

Swertiamarin is a secoiridoid glucoside present in members of the Gentianaceae family, including Swertia chirata (Wall) Clarke, S. japonica Makino, S. angustifolia Buch.-Ham.ex D. Don, and *Enicostemma littorale* Blume. It has antidepressant and anticholinergic activity and can thus be used as a biomarker. We 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 (%CV), 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). (40)

Conclusion

From the work cited in the article it can be concluded that promising phytochemicals are widely distributed in *Enicostemma littorale* and it has many pharmacological activities. Animal research has thrown light on anti-inflammatory, analgesic, antimalarial, hepatoprotective and hypoglycemic activities of phytochemicals. Swertiamarin should particularly be screened for large scale clinical trials. Antioxidant and hypoglycemic activities are of special interest.

References

1. Amritpal Singh, Phytochemicals of Gentianaceae: A Review of pharmacological Properties, International Journal of Pharmaceutical Sciences and Nanotechnology 2008 ; 1 (1)
2. Arivukkarasu .R, A. Rajasekaran, S. Murugesh, Anti-inflammatory activity of alcoholic extract of *Adenema hyssopifolium* G.Don in acute and chronic experimental models in albino rats, Journal of Applied Biosciences, 2009 ; 19: 1049 -1053
3. Aviram, M, Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases, Free Radic.res., 2000; 33 :85-97
4. Baquar, S.R, The role of traditional medicine in rural environment. In: Issaq, S. (Ed.), Traditional medicine in Africa. East Africa Educational Publishers Ltd., Nairobi, 1995 ;141-142
5. Chaudhuri, R.K., Singh, A.K., Ghosal, S., Chemical constituents of gentianaceae. XVIII. Structure of Enicoflavine. Monoterpene alkaloid from *Enicostemma hyssopifolium*. Chemical Industry (London) 1975; 3:127-128.
6. Daniel, M., Sabnis, S.D. Chemical systematics of family gentianaceae, Current Science, 1978; 47: 109-111.
7. Desai, P.D, Ganguly, A.K, Govindachari, T.R, Joshi, B.S, Kamat, V.N., Manmade, A.H., Mohamed, P.A., Nagle, S.K., Nayak,R.H., Saksena, A.K., Sathe, S.S, Viswanathan, N. Chemical investigation of some Indian Medicinal Plants: Part II. Indian Journal of Chemistry, 1966; 4: 457-459.
8. Dymock W, Warden C.J.H., Hooper, D. Pharmacographia Indica, vol. 2, first ed. Thacker Spink, Calcutta 1893 : 516.
9. Erum Jahan, Shagufta Perveen and Abdul Malik, Journal of Asian Natural Products Research, 2009 ;11 : 257–260
10. Ghosal, S, Jaiswal, D.K., Chemical constituents of Gentianaceae XXVIII: flavonoids of *Enicostemma hyssopifolium* (Willd.) Verd. Journal of Pharmaceutical Sciences 1980; 69 : 53-56
11. Ghosal, S., Singh, A.K., Sharma, P.V., Chaudhuri, R.K. Chemical constituents of Gentianaceae IX: natural occurrence of Erythrocentaurin in *Enicostemma hissopifolium* and Swertia lawii, Journal of Pharmaceutical Sciences, 1974 ;63 :944-945
12. Gite V.N, Pokharkar R.D, Chopade V.V, Takate S.B., Hepato-protective activity of *Enicostemma axillare* in paracetamol induced hepato-toxicity in albino rats, 2010:1:50-53
13. Gopal. R , A. Gnanamani, R.Udayakumar, and S.Sadulla *Enicostemma littorale* Blume- A potential hypolipidemic plant, Natural Product Radiance, 2004 ; 3 :401-405
14. Gupta R.S., and Dharmendra Singh, Hepatomodulatory role of *Enicostemma littorale* Blume against oxidative stress induced liver injury in rats, African Journal of Agricultural Research, 2007 ; 2 :131-138

15. Hitesh Vaidya , Mandapati Rajani , Vasudevan Sudarsanam , Harish Padh , Ramesh Goyal, Swertiamarin: A lead from Enicostemma littorale Blume. for anti-hyperlipidaemic effect, 2009 ; 617:108-112
16. Jaishree Vaijanathappa, Shrisshailappa Badami, and Suresh Bhojraj, InVitro Antioxidant activity of *Enicostemma axillare,* Journal of Health Science, 2008 ; 54 **:** 524-528
17. Jyoti, M., Vasu, V. T. and guptam, S. Dose dependant hypoglycemic effect of aqueous extract of *Enicostemma littorale* blum in allaxon induced diabetic rats. Phytomedicine, 2003; 10: 196-199.
18. Jyoti, M., Vasu, V. T., Ravikumar, A and Sarita, G. Glucose lowering effect of aqueous extract if *Enicostemma littorale* Blume in diabetes a possible mechanism of action. Journal of Ethnopharmacol. 2000; 81: 199-204.
19. Kavimani, S. and Manisenthilkumar, K. T. Effect of methanolic extract of *Enicostemma littorale* on Dalton's aseptic lymphoma. Journal of ethnopharmacol., 2000 ; 71: 349-352.
20. Kirtikar KR, Basu BD Indian medicinal plants. 1935 ;3 :1655–1656
21. Lanfranco, G., Invited review article on traditional medicine, Electronic Journal of Biotechnology ,1999 ;2 :1-3
22. Lindsey, A. A. Floral anatomy in the Gentianaceae , Amer. J. Bot. 1940; 27: 640-652
23. Maroo.J, V. T. Vasu, and S. Gupta Dose dependent hypoglycemic effect of aqueous extract of *Enicostemma littorale* Blume in alloxan induced diabetic rats, Phytomedicine, 2003 10: 196–199,
24. Murali B., Upadhyaya U.M., Goyal R.K. J.Ethnopharmacol. 2002 ; 81 :199
25. Natarajan PN, Prasad S Chemical investigation of *Enicotemma littorale,* Planta Med 1972 ; 22: 42–46
26. Niraj Mukundray Bhatt, Suparna Barua and Sarita Gupta, Protective Effect of *Enicostemma littorale* Blume on Rat Model of Diabetic Neuropathy, American Journal of Infectious Diseases, 2009;5:106-112
27. Padmanabhan, D., D. Regupathy, & S. Pushpa Veni. Gynoecial ontogeny in *Enicostemma littorale* Blume. Proc. Indian Acad. Sci. 1978; 87: 83-92.
28. Patel PM, KN Patel, NM Patel, RK Goyal, A HPTLC method for quantitative estimation of swetiamarin in marketed polyherbal antidiabetic formulations**,** Indian journal of pharmaceutical sciences. 2007 ; 63: 446-448
29. Ponnaian Stanley Mainzen Prince and Mohan Srinivasan, *Enicostemma littorale* blume aqueous extract improves the Antioxidant status in alloxan-induced diabetic rat tissues , Acta Poloniae Pharmaceutica, 2005 ;62 : 363-367
30. Rai, J., Thakar, K.A. Chemical investigation of *E. littorale* Blume. Current Science, 1966; 35:148-149.
31. Rajasekaran. A, R Arivukkarasu and S Murugesh, Hepatoprotective Effect of *Adenema hyssopifolium G.Don* (Gentianaceae) in Carbon Tetrachloride-Induced Hepatotoxicity in Rats, Tropical Journal of Pharmaceutical Research, 2010 ; 9 :157-163
32. Ramamourthy Gopal and Rajangam Udayakumar, Enzymatic and non-enzymatic antioxidant activity in *p*- DAB induced hepatocarcinoma in rats, International Journal of Pharmacology, 2008; 4 : 369-375
33. Retnam, K.R., DeBritto, A.J. Preliminary phytochemical screening of three medicinal plants of tirunelveli hills., Journal of Economy Texas Botany, 1988; 22: 677-681.

34. Sadique, J., Chandra, T., Thenmozhi, V. and Elango, V. The anti-inflammatory activity *Enicostemma littorale* and mullogo cerviana, Biochem Med. Metab. Biol., 1987; 37: 167-176.
35. Santhosh L Vishwakarma, Sonawane Rakesh D, M. Rajani & Ramesh K Goyal, Evaluation of effect of aqueous extract of *Enicostemma littorale* Blume in streptomycin induced type 1 diabetic rats, Indian Journal of Experimental Biology, 2010 , 48, 26-30
36. Sarita Gupta, Nidheesh Dadheech, Anubha Singh, Sanket Soni and R R Bhonde, Enicostemma littorale: A new therapeutic target for islet neogenesis, International Journal of Integrative Biology, 2010; 10: 49-53
37. Sathish kumar. R, P.T.V.Lakshmi and A.Annamalai , Effect of drying treatment on the content of antioxidants in *Enicostemma littorale* Blume. Research Journal of Medicinal plant, 2009; 3 : 93-101
38. Sharata L Derore, S S Khadabadi, Lalita Bhagure and D S Ghorpade, *In vitro* antimicrobial and antioxidant studies on Enicostemma littorale (Lam.) Raynal. Leaves, Natural Product Radiance, 2008 ; 7:409-412
39. Vihas T. Vasu, Hiren Modi, Jyoti V. Thaikoottathil and Sarita Gupta, Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume aqueous extract in cholesterol fed rats, Journal of Ethnopharmacology, 2005 ; 101: 277-282
40. Vishwakarma Santosh L. Bagul Milind S. Rajani M. Goyal Ramesh K. A sensitive HPTLC method for estimation of swertiamarin in *Enicostemma littorale* blume, Journal of planar chromatography, 2004 ; 17 : 128-131
41. World Health Organization, General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO, Geneva, Switzerland 2001; p .1.