Research Article

Phytochemical Screening and Evaluation of *In Vitro* Antioxidant Efficacy of *Enicostemma littorale* Blume Leaves Extract

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ABSTRACT

Medicinal plants play a pivotal role in curing various human ailments. Among the series of medicinal plants, *Enicostemma littorale* Blume (*E. littorale*), a perennial herb belongs to the family Gentianaceae is cosmopolitan in occurrence. Various parts of the plant have been reported to possess antimicrobial, antiinflammatory, antiulcer, antioxidant and hypoglycemic properties. The present study was designed to systematically evaluate the free radical scavenging activity of its leaves. Qualitative phytochemical analysis evidenced the presence of alkaloids, flavonoids, saponins, tannins, phytosterols, triterpenoids, glycosides and phenolic compounds. The total phenolic and flavonoid contents in the leaves extract were found to be 16.72 ± 1.13 µg gallic acid equivalents and 10.05 ± 0.16 µg quercetin equivalents, respectively. The carbohydrate content was 4.19± 0.28 mg/g and the protein content was found to be 2.26± 0.32 mg/g of the leaves extract. In addition, the leaves extract is rich in minerals like magnesium, calcium, zinc, iron, sodium, and potassium and phosphorous. The ethanolic leaves extract scavenges DPPH and ABTS radicals in a concentration dependent manner (79.31 % of DPPH radicals and 77.55 % ABTS radicals). The hydroxyl and hydrogen peroxide radical scavenging potential of the leaves extract were found to be 76% and 78%, respectively. The nitric oxide and superoxide scavenging potential of the leaves extract were found to be 75% and 83%, respectively. Likewise, *E. littorale* leaves extract inhibits the generation of FRAP in a dose dependent manner. The percent inhibition using the TBARS assay was ranged from 22.00 to 80.44% for the leaves extract. The results of the present study indicate that *E.littorale* is a good source of biologically active ingredients and minerals. Since, *E. littorale* leaves extract possess significant antioxidant properties, it can be used as an adjunct therapy for the treatment of free radical mediated diseases such as diabetes, cardiovascular, neurodegenerative diseases and cancer.

Keywords: *Enicostemma littorale*; Phytochemicals; Minerals; In vitro antioxidant assays, Antioxidants.

INTRODUCTION

Nature is an indispensable system, which shapes the backdrop for the emergence, evolution and existence of life and is an extraordinary source for wide range of harmless and effective medicines. Plants provide an extraordinary source of natural medicines for various ailments. The secondary metabolites of plant origin serve as an invaluable chemical library for drug discovery in the pharmaceutical industry[1]. Human intervention trials have provided evidence for protective effects of various polyphenol-rich foods against chronic diseases including diabetes mellitus, neurodegenerative disorders and cancer[2].

The demand for medicinal plants is increasing worldwide due to growing recognition of natural products, being non-toxic, and more efficacies, easily available at affordable prices. Throughout the world, many traditional plants have been found successful for the treatment of several primary and secondary health complications. Further, most of the marketed medicines are distillations, combinations, reproductions or variations of substances that exist in nature. Our forefathers recommended some of the natural products, which are abundantly found in nature long before their medicinal values were demonstrated and proved by scientific validation. However, few natural products have received medical scrutiny for their pharmaceutical properties and the World Health Organization (WHO) has consistently recommended that the traditional medicinal plant treatments warrant detailed evaluation for their toxicity and efficacy. Moreover, today it is mandatory to afford scientific evidence as to whether it is justified to use a plant or its active principles for therapeutical applications. *Enicostemma littorale* Blume, is one such medicinal plants widely used in the traditional medicine which lack scientific appraisal for its toxicological, beneficial and pharmacological properties.

Enicostemma littorale Blume (*E. littorale*) plays a vital role in human healthcare. The leaves possess antidiabetic, antioxidant and hepatoprotective properties [3]. *E. littorale* exerts anti-inflammatory, anticancer and antidiabetic properties in rats [4, 5]. The presence of catechins, saponins, steroids, sapogenin, triterpenoids, flavonoids and xanthones and a new flavone C-glucoside named as Verticilliside was reported by Jahan et al 2009 [6]. Monoterpene alkaloids such as enicoflavin, gentiocrucine were isolated from the leaves of *Enicostemma littorale.* Swertiamarin, a secoiridoid glucoside was originally isolated from the leaves of *E. littorale* and is an important constituent of many traditional medicines that are marketed in Japan [7]. Having known the presence and importance of the phytochemicals present in the leaves extract, an attempt has been made to systematically evaluate the free radical scavenging potential of *E.*

littorale Blume leaves extract using an array of *in vitro* methods.

MATERIALS AND METHODS

Plant material- Identification and authentication

Fresh and matured *E. littorale* leaves were collected from Arcot, Vellore District, Tamil Nadu and identified by a plant taxonomist in CAS in Botany, University of Madras where a voucher specimen was deposited in the herbarium.

Preparation of ash

The *E .littorale* leaves were shadow dried and finely powdered using an electrical grinder. One hundred gram of finely powdered leaves were taken in a viterosil crucible and placed over night in an electrical muffle furnace maintaining its temperature between 430-450˚C because the loss of zinc may occur at >450˚ C and loss of potassium occur, if the temperature is too high (>480˚ C). The ash was stored in a vacuum desiccator. The ash content in the powdered leaves was found to be 13.03g/100g.

Total Ash

The ash values, following ignition of plant materials can be determined by different methods, which measures total ash, acid insoluble ash and water-soluble ash [8]. 2g of the ground air-dried material was weighed in a previously ignited and tarred silica crucible. The content was spread in an even layer and ignited by gradually increasing the heat to 450°C until it turns white colour indicating the absence of carbon. The contents were stored in a desiccator.

Determination of water soluble ash

The total ash obtained was boiled with 25 ml of deionized distilled water for 5 minutes. The insoluble matter was collected on an ash less filter –paper, washed with hot water and ignited to constant weight at low temperature. The weight of the insoluble matter was subtracted from the weight of total ash, represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug. The result was calculated with reference to the air dried drug.

Determination of acid insoluble ash

The total ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on tarred silica crucible, washed with hot acidulated water, ignited, cooled and weighed. The percentage acid insoluble ash was calculated with reference to the air dried drug.

Trace element analysis

2g of ash was digested with a triple acid mixture comprising of nitric acid, sulphuric acid and perchloric acid in the ratio of 11:6:3 respectively for the complete removal of organic content. The digested sample was made up to 100 ml using deionized water and this sample is used for the assay of trace elements through atomic absorption spectroscopy (AAS) using hollow cathode lamps.

Instrumentation and analytical procedures

The determination of trace elements in the *E. littorale* leaves was carried out using an atomic absorption spectrometer (GBC-Avanta, Australia).

Preparation of *Enicostemma littorale* leaves extract

E. littorale leaves were washed and dried in a hot air oven at 40°C and subsequently ground into powder in an electrical grinder, which was stored in an airtight brown container at 5°C until further use. The powdered leaves were delipidated with petroleum ether (60-80°C) for overnight. It was then filtered and soxhalation was performed with 95% ethanol. Ethanol was evaporated in a rotary evaporator at 40-50°C under reduced pressure. The yield was around 13.5 % of dry weight.

Preliminary phytochemicals screening

The ethanolic extract of *E. littorale* leaves was subjected to phytochemical screening for the qualitative analysis of various phytoconsituents such as Alkaloids, Flavonoids, Glycosides Saponins, Tannins, Phytosterols, Triterpenoids, Anthraquinones and Phenols [9, 10].

DETERMINATION OF TOTAL PHENOLIC CONTENT

Total phenolic content in the ethanolic extract of *E.littorale* leaves was determined according to the Folin-Ciocalteu colorimetric method [11, 12]. A standard curve was drawn with gallic acid reference solutions. 2 to 10 ml of standard aqueous gallic acid solution (100 µg /ml) was pipetted into a 100 ml volumetric flask containing 70 ml of distilled water. Folin-Ciocalteu reagent (5 ml) and 10 ml of saturated sodium bicarbonate solution were added and the volume was made up to 100 ml with distilled water. The solution was thoroughly mixed. The blank was prepared in the same manner, but without gallic acid. After 1 h of incubation at room temperature, the absorbance was measured at 760 nm. The samples were prepared in triplicates for each analysis and the mean value was calculated. For the determination of total phenolic content of *E. littorale* leaves, aqueous solutions at the final concentration of 20µg/ml were used; proceeding in the same manner described for the reference solutions and the total phenolic content was expressed as mg per gram of gallic acid equivalents.

Determination of Total Flavonoid Content

Total flavonoid content in the ethanolic extract of *E.littorale* leaves was determined according to the method of Quettier- Deleu et al., (2000) with minor modifications [13]. A standard curve was built with quercetin reference solutions. 2 to 8 ml of standard quercetin (50µg/ml) were pipetted into 25 ml volumetric flasks containing 1 ml of 2% aluminum chloride dissolved in ethanol and the volume was made up with ethanol.

The blank was prepared by diluting 1 ml of 2% aluminum chloride dissolved in ethanol in a 25 ml volumetric flask with ethanol. After 1 hour at room temperature, the absorbance was measured at 420 nm. *E. littorale* leaves extract were evaluated at a final concentration of 20µg/ml, proceeding in the same manner described for the reference solutions and the total flavonoid content was calculated as quercetin equivalents from a calibration curve. The experiments were prepared in triplicate for each analysis and the mean value of absorbance was recorded.

Determination of Carbohydrate and Protein Content

The carbohydrate content in the leaves extract was estimated by the 3, 5-dinitrosalicylic acid method [14] and the estimation of proteins was carried out by the Lowry et al. (1951) [15] method.

Free Radical Scavenging Assays

The *in vitro* antioxidant activity was determined by DPPH, ABTS, superoxide radicals, nitric oxide, hydroxyl radicals, H_2O_2 scavenging reducing power, ferric reducing antioxidant power assay (FRAP) and lipid peroxidation inhibition assay. All the analysis was performed in triplicates and average values were taken.

DPPH radical scavenging assay

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by the decrease in absorbance at wavelength 517nm. The free radical scavenging capacity of the ethanolic extract of *E.littorale* leaves was determined using DPPH[16]. DPPH (200µM) solution was prepared in 95% methanol. From the stock leaves extract solution 200, 400, 600, 800 and 1000 µg/ ml were taken in five test tubes. 0.5ml of freshly prepared DPPH solution was incubated with the leaves extract and after 10 minutes, absorbance was taken at 517 nm in a spectrophotometer. Standard ascorbic acid was used as reference.

ABTS assay

ABTS decolourization assay involves the generation of the $ABTS^+$ chromophore by the oxidation of ABTS with ammonium persulphate. The scavenging activity of the leaves extract on ABTS radical cation was measured at 734nm**.** ABTS radical scavenging activity of ethanolic extract of *E. littorale* leaves was determined according to the method of Re et al., 1999[17]. Briefly, ABTS radical cation was produced by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Then, $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.7 at 734 nm. To 3.0 ml of diluted $ABTS^{\bullet+}$ solution, different concentrations (200-1000 µg/ ml) of leaves extract in ethanol was added and after 1 min, the decrease in absorbance was measured at 734 nm spectrophotometrically

Hydroxyl Radical Scavenging Assay

Hydroxyl Radical Scavenging Assay was performed according to the method of Smirnoff and Cumbes (1989) [18]. Hydroxyl radicals were generated from $FeSO_4$ and hydrogen peroxide and detected by their ability to hydroxylate salicylate. The hydroxylated salicylate complex formation was measured at 562nm.The reaction mixture (3ml) contained 1m, $FeSO_4$ (1.5mM), 0.7ml hydrogen peroxide (6mM), 0.3ml sodium salicylate (20mM) and varying concentrations of the leaves extract. After incubation for 1hour at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562nm. The percentage scavenging effect was calculated as,

$$\text{Scavenging activity} = [1-(A_1-A_2)/A_0] \text{ X } 100\%$$

where A_0 was the absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without sodium salicylate.

Hydrogen Peroxide Scavenging Activity

Hydrogen Peroxide Scavenging Activity was performed according to the method of Ruch et al., (1989)[19].The H_2O_2 scavenging activity was measured in terms of decrease in the absorbance at 230nm in spectrophotometrically. A solution of H_2O_2 was prepared in phosphate buffer. Various concentrations of ethanolic leaves extract was added to H_2O_2 and incubated for 10 min. The absorbance at 230nm was determined against a blank containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 by leaves extract and standard compound was calculated using the formula.

% scavenging of H_2O_2 = (A (control) - A (sample) / (A (control)) X 100

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated spectrophotometrically at 546nm. Sodium nitroprusside (5 mM) in phosphate buffer pH 7.7 was incubated with 200-1000 µg/ml concentrations of leaves extract dissolved and the tubes were incubated at 25°C for 120 minutes. At intervals, 0.5ml of incubation solution was removed and diluted with 0.5 ml of Griess reagent. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent N-napthyl ethylenediamine was measured at 546 nm [20].

Superoxide Anion Radical Scavenging Assay

This method is based on the inhibition of production of nitroblue tetrazolium of superoxide ion by the leaves extract and is measured spectrophotometrically at 560nm. The Superoxide radical scavenging activity of *E. littorale* leaves was measured by the method of Fontana et al., (2001)[21]. In this method, the activity is measured by reduction of riboflavin/light/NBT (Nitro blue tetrazolium). The 1 ml of reaction mixture contain phosphate buffer,

NADH, NBT and various concentrations of leaves extract. The method is based on the generation of superoxide radical by autooxidation of riboflavin in presence of light. The superoxide radical reduces NBT to a blue coloured formazon that can be measured at 560 nm.

Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of leaves extract was determined using the ferric reducing ability of plasma FRAP assay as a measure of antioxidant power. FRAP assay measures the change in absorbance at 593nm owing to the formation of a blue coloured Fe II-tripyridyl triazine compound from colourless oxidized Fe III by the action of electron donating antioxidants. The ability to reduce ferric ions was measured using the method[22] described by Benzie and Strain (1996). The FRAP reagent was produced just before use by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM TPTZ (tripyridyl triazine) solution and 20.0 mM $FeCl_3$.6H2O solution in a ratio of 10:1:1 in volume. Leaves extract at different concentrations (200-1000 µg/ ml) were then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37 C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of FeSO4 were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of leaves extract was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of leaves extract. The optical density was read at 593 nm.

RESULTS

The phytochemical analysis of *E. Littorale* leaves extract is presented in Table 1. The ethanolic leaves extract is found to contain alkaloids, flavonoids, saponins, tannins, phytosterol, triterpenoids, glycosides and phenolic compounds.

Phytoconstituents	Inference
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Phytosterols	+
Triterpenoids	+
Glycosides	+
Anthraquinones	-
Phenolic compounds	+

The total phenolic and flavonoid contents were found to be 16.72 ± 1.13 µg gallic acid equivalents and 10.05 ± 0.16 µg quercetin equivalents, respectively. The carbohydrate content was 4.19± 0.28 mg /g and the protein content was found to be 2.26± 0.32 mg /g of the leaves extract (Table 2).

Table 2: Quantitative Analysis of *E. littorale* leaves extract

Constituent	Content
Phenolic Content	16.72 ± 1.13 µg gallic acid equivalents
Flavonoid Content	10.05 ± 0.16 µg quercetin equivalents
Carbohydrate Content	4.19± 0.28 mg /g of the leaves extract
Protein Content	2.26± 0.32 mg /g of the leaves extract

Table 3 represents the mineral content of *E.littorale* leaves extract. It has been found that *E. littorale* leaves extract is rich in minerals like calcium, magnesium, sodium, potassium, iron, zinc and phosphorus.

Table 3: Mineral Composition of *E.littorale* leaves extract

Element	Concentation (µg/g)
Calcium (Ca)	253
Magnesium (Mg)	134
Sodium (Na)	120
Potassium (K)	53
Iron (Fe)	300
Phosphorus (P)	56
Zinc (Zn)	50

The results obtained for the ash values determined by standardized protocols can be used for the quality control purposes for *E. littorale* in various pharmacological interventions. The mean, range and standard error values of ash contents of *E. littorale* that resulted from analyses are summarized in

Table 4: Ash Content

Constituent	Content
Total Ash	11.88 ± 0.20
Water soluble Ash	4.53 ± 0.08
Acid soluble ash	4.00± 0.10

Figure 1 and 2 shows the dose dependent effect of *E. littorale* leaves on the percentage inhibition of DPPH and ABTS radicals present in the reaction mixtures, respectively. The ethanolic extract of *E. littorale* leaves scavenges DPPH and ABTS radical in a concentration dependent manner. The leaves extract of *E. littorale* significantly and concentration dependently reduced DPPH and ABTS radicals. However, at a concentration of 1000µg/ml, the extract significantly scavenged 79.31 % of DPPH radicals and 77.55 % ABTS radicals. The hydroxyl (76% hydroxyl scavenging) and hydrogen peroxide

scavenging potential (78 % hydrogen peroxide radical) of the leaves extract is depicted in figure 3 and 4, respectively. The nitric oxide and superoxide scavenging potential of the leaves extract is depicted in figure 5 and 6, respectively. The leaves extract exhibited a maximum of 75% nitric oxide scavenging potential and 83% superoxide scavenging activity. The radical scavenging activity was compared with standard ascorbic acid.

Fig 1: DPPH RADICAL SCAVENGING ACTIVITY OF *E.littorale* leaves extarct

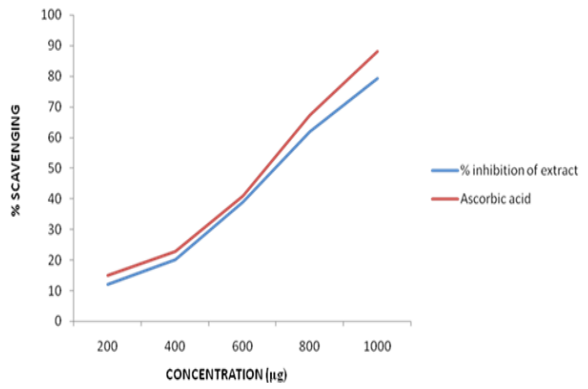


Fig 2: ABTS RADICAL SCAVENGING ACTIVITY OF *E.littorale* leaves extarct

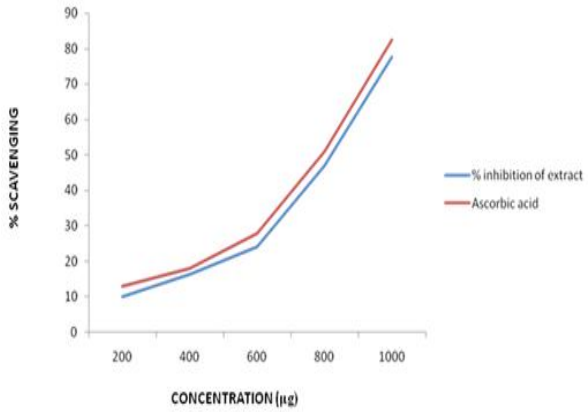


Fig 3: HYDROXYL RADICAL SCAVENGING ACTIVITY OF *E.littorale* leaves extarct

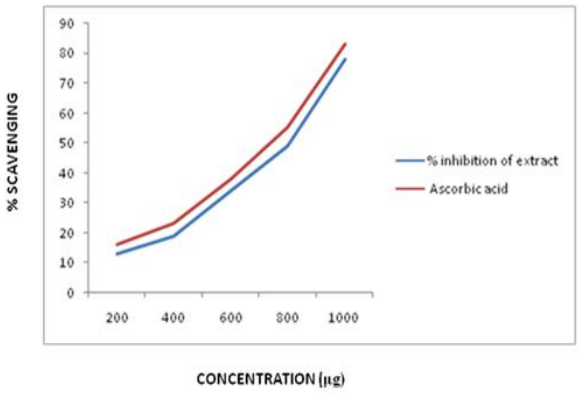


Fig 4: HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF *E.littorale* leaves extarct

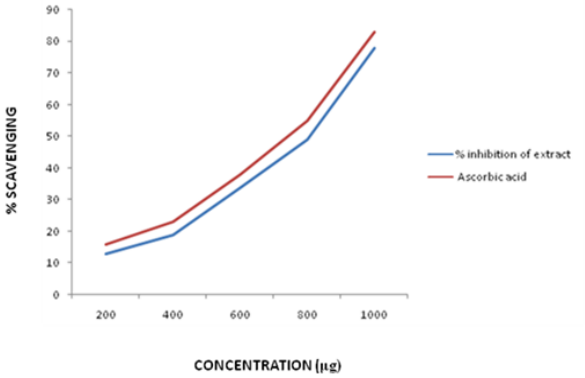


Fig 5: NITRIC OXIDE SCAVENGING ACTIVITY OF *E.littorale* leaves extarct

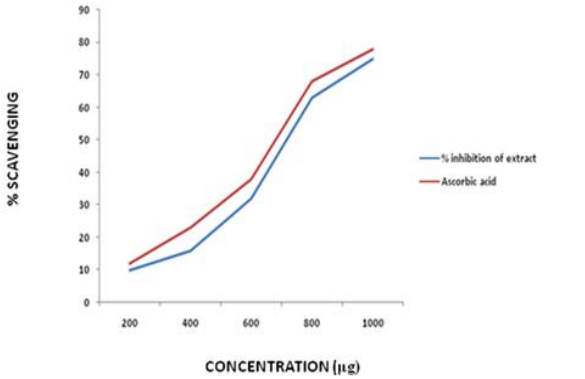
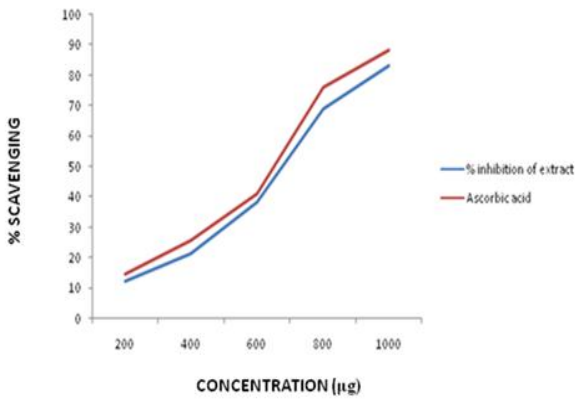


Fig 6: SUPEROXIDE SCAVENGING ACTIVITY OF *E.littorale* leaves extarct



Antioxidant activities of the leaves extracts of *E. littorale* as determined by the FRAP assay. In the FRAP assay the absorbance of *E. littorale* was found to be 0.10, 0.14, 0.20, 0.50 and 1.00 at 200-1000 µg/ml respectively. The percentage inhibition using the TBARS assay ranged from 22.00 to 80.44% for the leaves extract.

DISCUSSION

Medicinal plants are of immense value to the health and communities. The therapeutic value of the medicinal plants lies in some chemical substances that are produced as secondary metabolites with definite physiological

action on the human body. Medicinal plants are the reservoirs of ecologically derived secondary metabolites such as phenolic acids, alkaloids, flavonoids, alkaloids, saponins, anthocyanins and hydroxycinnamic acid derivatives. Among the secondary metabolites, the phenolic classes have gained extensive attention in recent years due of their physiological functions including free radical scavenging, antidiabetic, anticarcinogenic and anti-inflammatory effects [23].

The phytochemical screening of the ethanolic leaves extract of *E.littorale* showed the presence of biologically active secondary metabolites such as alkaloids, flavonoids, glycosides, phytosterols, tannins and phenols. Earlier studies indicated that the leaves were found to contain catechins, saponins, steroids, sapogenin, triterpenoids, flavonoids and xanthones and a new flavone C-glucoside named as verticilliside[6]. Alkaloids such as enicoflavin, gentiocrucine were isolated from the alcoholic extract of *E. littorale*[7].

Carbohydrates are of special importance because they are direct products of photosynthesis and are; therefore, the primary energy storage compound and the basic organic substances from which most other organic compounds found in plants are synthesized. Carbohydrate content of *E. littorale* was found to be 4.19± 0.28 mg /g of the leaves extract. Our results are in accordance with earlier reports [24]. The protein content was found to be 2.26± 0.32 mg /g of the leaves extract. The extract of *E. littorale* was found to be contain amino acids like L-glutamic acid, tryptophan, alanine, serine, aspartic acid, L-proline, L-tyrosine, threonine, phenyl alanine, L-histidine monohydrochloride, methionine, isoleucine, L-arginine monohydrochloride, DOPA, L-Glycine, 2-amino butyric acid and valine indicating that *E. littorale* is a good source of amino acids that are beneficial to the body[25] .

Total, water soluble and acid-insoluble ash contents of the leaves of *E. littorale* are important in determining the purity of an herbal drug. Tanna et al. (2010) reported that the aerial part of the *E. littorale* yield 34% of dry alcoholic extract and 15.7% of ash[26]. Total ash includes physiological ash, which is derived from the plant tissue. Non-physiological ash is derived from environmental contaminations such as sand and soil. *E. littorale*, like other herbal materials, shows a variation in the variety and contents of compounds according to differences in growing conditions, such as soil type, climate which may change the ash content depending upon presence or absence of various contaminants thus becoming an important parameter of quality assessment.

Mineral analysis revealed the presence of magnesium, calcium, sodium, iron, phosphorus, zinc and potassium. Indian Council of Medical Research in its nutritional report suggests that 100 g of fresh leaves of *E. littorale* contains 140 kcal energy, 7 g of protein, 0.7 g of fat, 26.5 g of carbohydrate, 4.2 g of fibre, 8.4 g of minerals, 49.9 mg of iron, 1.641 mg of calcium, 81 mg of phosphorous[27]. Hence, *E. littorale* fresh leaves are recommended for diabetic patients due to their rich nutritious content [28]. Our studies are on par with the earlier findings.

DPPH and ABTS radical assays have been widely used as reliable methods for determining the free radical scavenging efficacy of the lead molecules [29, 30]. The principle of the DPPH free radical scavenging activity assay is based on the reduction of DPPH radicals in methanolic solution. Due to the presence of an odd electron, DPPH gives a strong absorption maximum at 515 nm. It is generally accepted that substances which are able to donate hydrogen or an electron to DPPH, a synthetic nitrogen-centered stable radical, can be considered as antioxidants and therefore free radical scavengers. *E.littorale* leaves extract exhibited a maximum of 79.31% DPPH radical scavenging activity at a concentration of 1000µg/ml.

ABTS radicals are more reactive than DPPH radicals and the reaction with ABTS radicals involve a single electron transfer process. It is applicable for both hydrophilic and lipophilic compounds. ABTS radical assay is the pre-formed radical monocation of ABTS radical which is generated by oxidation of ABTS radicals with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The antioxidant activity of different lead molecules depends on the number and location of hydroxyl and other functional groups such as carboxyl and phenolic ring system [31]. *E. littorale* leaves extract exhibited a maximum of 77.55% ABTS radical scavenging activity at a concentration of 1000µg/ml.

The $O2^{\bullet-}$ scavenging activity was determined by phenazine methosulphate/NADH-NBT system wherein $O2^{\bullet-}$derived from dissolved oxygen by phenazine methosulphate/NADH coupling reaction reduces NBT. The decrease in absorbance at 560 nm in the presence of leaves extract indicates the consumption of superoxide anions in the reaction mixture. *E.littorale* leaves extract exhibited a maximum of 83% superoxide scavenging activity at a concentration of 1000µg/ml. Nitric oxide is synthesized from L-arginine by the enzyme nitric oxide synthase (NOS). It plays an important role in N-methyl-D-aspartate (NMDA) receptor activation and the induction of significant oxidative stress. NO induced oxidative stress causes lipid peroxidation and neuronal cell death by DNA damage [32, 33] . *E.littorale* leaves extract at a concentration of 1000 µg/ml quenched 75% NO radical.

A simple assay measuring the ferric reducing ability of plasma, the FRAP assay, is presented as a novel method for assessing “antioxidant power”. Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. The reducing properties are linked with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom[34]. In the present study, the absorbance of *E. littorale* clearly increased which is due to the formation

of the Fe^{2+} TPTZ complex with increasing concentration as observed. The leaves extract showed a significant antioxidant activity. The high percentage inhibition observed in the TBARS assay indicates the ability of the leaves extract to inhibit linoleic acid peroxidation. The antioxidant activity of the leaves extract may be due to the presence of phenolic compounds with redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and as well as potential metal chelators[35]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in the living cells. Hydroxyl anion is considered to be an important initiator of lipid peroxidation process, abstracting hydrogen atoms from the unsaturated fatty acids[36, 37, 38]. In the present study, the leaves extract scavenged 76% free radicals.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecule but it is an intracellular precursor of hydroxyl radicals which is very toxic to the cell. Although hydrogen peroxide itself is not very reactive it can sometimes cause cytotoxicity by generating excessive hydroxyl radicals in the cell [39, 40]. The efficacy of *E.littorale* leaves extract in the removal of H_2O_2 by neutralization was found to be 78%.

CONCLUSION

The results of the present study has evidenced that the ethanolic extract of *E .littorale* leaves contains biologically active secondary metabolites in addition to significant amounts of essential trace elements. The data obtained through various *in vitro* antioxidant assays clearly established that the leaves of *E. littorale* possess significant antioxidant properties and the leaves may be considered as an important source for the screening of natural lead molecules. The synergistic effect of both the organic and inorganic compounds present in the *E.littorale* leaves can play a chief role in preventing free radical mediated diseases such as diabetes and cancer. Above all, the present study provides a scientific rationale for the use of *E. littorale* leaves in the traditional medicinal system for the treatment of various human ailments.

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