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COMPARATIVE ANALYSES OF NON ENZYMATIC AND ENZYMATIC ANTIOXIDANTS OF *ENICOSTEMMA LITTORALE* BLUME

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ABSTRACT

The objective of this study was to characterize the non-enzymatice and enzymatic antioxidants of E. littorale Blume grown under wild condition. Aerial and sub-aerial parts of the plants exposed to different heat stress was analyzed for the non enzymatic antioxidants such as Tocopherol, Phenols, DPPH, FRAP and enzymatic antioxidants such as Polyphenol oxidase, Peroxidase, Catalase, Super Oxide Dismutase, which revealed quite an interesting observation. In the plant, the enzymatic antioxidant activity was found to be significant in the shade dried extracts. This enzymatic antioxidant activity concludes the potential degradation of free radicals. The non-enzymatic antioxidant activity contributes significantly in both fresh and shade dried extracts except the DPPH activity which found to be significant in the sun dried extracts of the plant material

KEYWORDS

Enicostemma littorale Blume, plant parts, Enzymatic, Non-Enzymatic, Aqueous extracts, Antioxidant activity.

INTRODUCTION

Enicostemma littorale (Gentianaceae) also called Chota chirayata in Hindi, Mamejavo in Gujarati, Nagajivha in Bengal and Vellarugu in Tamil is a glabrous perennial herb with sessile lanceolate leaves which grows up to a height of 1500ft is found throughout India [1]. Qualitative analysis of the ash content of aerial parts of the plant have been revealed [2] to exhibit the presence of minerals (iron, potassium, sodium, calcium, magnesium, silica, phosphate, chloride, sulphate and carbonate), Steroids and triterpenoids (Enicoflavin and gentiocrucine, catechinsm saponins, betulin, triterpene, sapogenin and sweriamarin), Flavonoids and xanthones (Apigenin, genkwanin, isovitexin, swerisin, saponarin, 5-O-glucosylswertisin and 5-O-glucosulisoswertisin), Phenolic acids (Vanillic acid. Syringic acid, p-hydroxy benzoic acid, protocatechuic acud, p-coumaric acid and ferulic acid), Aminoacids (L-glutamic acid, trytophane, alanine, serine, aspartic acid, L-proline, L-tyrosine, threonine, phenyl alanine, L-histidine monohydrochloride, methionine, iso-leucine, L-arginine monohydrochloride, DOPA, L-glycine, 2-

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amino butyric acid and valine). Monoterpene alkaloids like, enicoflavin and gentiocrucine along with catechins and saponins have been isolated [3, 4]. Various Ayurvedic formulations containing *E. littorale* as one of the ingredients have produced the antihyperglycemic activity in hyperglycemic rat models [5]. Ethnomedical studies of North Gujarat (India) revealed the use of hot aqueous extract of *E. littorale* by the tribal inhabitants for the treatment of diabetes, fever, stomach pain, dyspepsia and malaria [6]. It is reported for its anticancer [7], hypolipidaemic effect in *p*-dimethylaminobenzene (*p*-DAB) induced hepatotoxic animals [8] and anti-inflammatory [1] properties.

Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide formed *in vivo*, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. They are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase but due to over-production of reactive species, induced by exposure to external oxidant substances or a failure in the defense mechanisms damage cell structures, DNA, lipids and proteins, which increases risk of more than 30 different disease processes [9].

Since, the oxidative damage results in an imbalance between the production of AOS (Active Oxygen Species) and the quenching activity of the antioxidants, when plants are subjected to environmental stresses such as drought, salinity, heat, chilling and mineral deficiency, a variety of toxins, active oxygen species (AOS) such as superoxide (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$) and singlet oxygen ($^1O_2$) are observed [10]. However, the antioxidants help organisms to deal with oxidative stress caused by free radical damage. Perhaps, the main characteristic feature of an antioxidant is its ability to trap free radicals. Antioxidant compounds may be water-soluble, lipid-soluble, insoluble, or bound to cell walls. Highly reactive free radicals and oxygen species present in biological systems form a wide variety of resources, which may oxidize nucleic acids, proteins, lipids or DNA to initiate degenerative diseases, however, antioxidant compounds like phenolic acids, poly phenols and flavonoids scavenge these free radicals and protect the system from the oxidative mechanisms [11]. Flavonoids, a large group of plant phenolics, including anthocyanin and other phenolic compounds present in plant tissues and vegetables, relatively at high concentrations, act as antioxidants by inhibiting the oxidation of lipids, fats, and proteins by donation of a phenolic hydrogen atom to the free radical in cancer and diabetic retinopathy thereby preventing cancer or cardiovascular diseases [12].

The polyphenol oxidases (PPOs) also referred to as catechol oxidase and laccase, are copper-containing enzymes that catalyze the aerobic oxidation of variety of phenolic substrates into *o*-quinones with a concomitant O_2 reduction and are involved in the browning of damaged fruits or vegetables. The so formed *o*-quinones undergo subsequent reactions leading to dark-colored pigments [13]. Hydroperoxidase is an enzyme that converts the hydrogen peroxidase and hydroperoxidase to non radical forms by catalyzing the dehydrogenation of organic compounds such as

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phenols, aromatic amines, hydroquinones, etc, thereby function as natural antioxidants [14]. Super Oxide Dismutase, the metalloenzymes which catalyze the disproportionation of superoxide free radical into oxygen and hydrogen peroxidase, provides an important defense mechanism against superoxide radical toxicity [15]. Catalase (CAT), a tetrahedrical protein constituted by four heme groups in the plant destroys hydrogen peroxide into oxygen and water in high concentration by catalyzing its two-electron dismutation [10].

Tocopherols, the lipid-soluble antioxidants known collectively as vitamin E, occur naturally as tocopherols α, β, γ and δ which differ only in the number and position of methyl substituents on the aromatic ring. α-tocopherol, scavenges lipid peroxyl radicals (LOO) through hydrogen atom transfer in cancer, neurodegenerative diseases and in chronic inflammation[16]. It is a major lipid soluble antioxidant vitamin and free radical scavenger, which protects the cellular membranes from oxidative damage and lipid peroxidation [17]. In addition to their role as antioxidants they stabilize polyunsaturated fatty acids within lipid bilayers by protecting them from lipoxygenase attack. Although all tocopherols are absorbed equally during digestion, only (*R,R,R*)-a-tocopherol is preferentially retained and distributed throughout the body.

Phenolic compounds possess a wide spectrum of biological effects as antioxidant and free radical scavenger [18].They are classified into two groups such as polyphenols and simple phenols [19] and are commonly found in both edible and nonedible plants. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers, in addition to having a metal chelation potential [20].

2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical and is used to measure the radical scavenging activity [21].It spares an electron and delocalizes the electron causing a colour change from deep violet to pale yellow as observed at 520nm [22]. Moreover, the total antioxidant activity could also be measured by ferric reducing antioxidant power (FRAP), which depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH [23] and is measured at 593nm [24]. Therefore, the present study was aimed to bring light the antioxidant properties of *Enicostemma littorale* under different condition such as fresh, sun and shade dry.

MATERIALS AND METHODS

i. CHEMICALS AND REAGENTS

Folin-Ciocalteau Reagent (1N), Ethanol (80%), Sodium Carbonate (20%), Catechol, Methionine, Riboflavin, Ethylene Diamine Tetra Acetic acid (EDTA), Nitroblue Tetrazolium (Nbt) , Xylene, 2,2`-Dipyridyl, Ferric Chloride, Pyrogallol, DL-Alpha-Tocopherols, Hydrogen Peroxide.

ii. PLANT MATERIALS

The wild plant of *Enicostemma littorale* grown at sub-tropical regions of Thirunelveli and Salem districts, Tamilnadu was collected in the month of August and September (2008) at the end of the flowering season by uprooting method. The species identification was examined by comparing its morphological features and microscopic examination of the anatomy as per the standard

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methodologies at Botanical Survey of India, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India (Ref No.BSI/SC/5/23/09-09/tech.-1842). The collected material was brought for investigation to the Phytomatics Laboratory, Department of Bioinformatics, Bharathiar University, Coimbatore, Tamil Nadu, India.

iii. EXPERIMENTAL DESIGN

The Plant materials obtained as separated into two groups; one used fresh and the other was subjected to drying conditions namely, shade and sun dry. However for all the three strategies, approximately five hundred grams of the fresh plant material was washed, drained and used. For shade dry, the pre washed and drained plant material was placed on a filter paper (90 cm × 60 cm) at room temperature (27±1°) for three days. For sun dry, the fresh material was placed in a greenhouse for 3 days. Once the drying process was over, the dry weights were measured to calculate the percentage of water loss and powdered using a laboratory blender and stored for further work. From the storage, approximately fifty milligram of the material was drawn to extract the metabolites under different solvents, which was further used for analyzing the antioxidant contents. However, similar strategy was also adopted for the fresh plant material to concentrate for different solvent extraction and thereafter for antioxidants assay [25].

iv. SAMPLE EXTRACTION

Fifty-milligrams of each treated powder was crushed with 1 mL solvent that varied according to the protocols. It was allowed to stand for 30 min without any disturbance at room temperature and then swirled with a vortex for 5 min after which was centrifuged at 10,000 rpm for 10min to collect the supernatant. This extract was stored at -20°C until further use [25].

v. ASSAY OF POLYPHENOL OXIDASE (PPO)

Poly phenol oxidase was measured according to the method of [26], wherein, the enzyme extract was prepared by grinding 5g of sample in about 20mL reaction medium containing 50mM Tris HCl (pH 7.2), 0.4M sorbitol and 10 mM NaCl respectively. The homogenate mixture was centrifuged at 20,000rpm for 10 minutes and the supernatant was added to the assay mixture containing 2.5mL of 0.1M phosphate buffer and 0.3mL of catechol solution (0.01M) and read at 495 nm at an interval of 30 seconds up to 5 minutes. Hence for the activity of PPO was calculated using the formula,

$$\text{Enzyme unit} = K \times (\Delta/\text{min})$$

Where, K for Catechol oxidase is 0.272, and for Laccase is 0.242.

vi. ACTIVITY OF PEROXIDASE

The activity of peroxidase was assayed according to the method of [27]. 20% homogenate mixture was prepared in 0.1M phosphate buffer (pH 6.5) for all the plant samples of which 0.1 mL enzyme extract was taken and mixed with 3.0mL of Guaiacol solution and the activity was recorded at 436nm for every 30 seconds up to 3 minutes after calibrating with the blank (0.5 mL of 1% H_2O_2).

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$$\text{Guaiacol + Hydrogen peroxide (Donor)} \xrightarrow{\text{Peroxidase}} 2H_20 + \text{purpurogallin (Oxidized donor)}$$

vii. CATALASE

Catalase activity was determined according to [28] by monitoring the decomposition of H_2O_2. The plant sample was homogenized with phosphate buffer (assay buffer diluted 10 times) at 1-4 ° C and centrifuged with in 24hrs. 3 mL of hydrogen peroxide – phosphate buffer was added to the 250 µl of enzyme extract to initiate the H_2O_2 reaction. On decomposition of hydrogen peroxide by catalase, the adsorption decrease with time. The calculation was observed as rate of decrease in absorbance in required time (Δt). The UV light absorption of hydrogen peroxide solution measured between 230 and 250nm. The enzyme activity could be obtained from the decrease in time under 240 nm.

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

Calculation

$$\text{Units/mg} = \frac{\Delta A240/\text{min} \times 1000}{4.36 \times \text{mg enzyme / ml reaction misture}}$$

viii. ACTIVITY OF SUPEROXIDE DISMUTASE (SOD)

The assay of SOD was performed according to [29]. 1g of plant material was homogenized in 10mL ice-cold 50 mM potassium phosphate buffer (pH 7.8) and centrifuged at 10,000 rpm for 10 min at 4° C. The supernatant was used as the enzyme source. To about 50µl of crude enzyme was mixed 3mL reaction cocktail containing 50 mM potassium phosphate buffer (pH 7.8), 13mM methionine, 2 µM riboflavin, 0.1mM EDTA and 75 µM NBT and was exposed to 400 W bulbs for 15 minute. The optical density was absorbed at 560nm and 50% inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity and expressed as unit/mg of protein.

MEASUREMENT OF TOCOPHEROL

Tocopherol [30] was measured by pipetting 1.5mL of each plant extract, standard (α tocopherol) and water respectively in tubes separately. To the test and blank was added 1.5mL ethanol and to the standard was added 1.5mL water and vortexed, to which was added 1.5 mL of xylene and centrifuged at 1000rpm for 10min. About 1.0 mL of xylene layer was transferred into another stopper tube, taking care not to include any ethanol or protein and 0.1 mL of 2, 2`-dipyridyl reagent was added and read at 460 nm for both the extinction of test and standard against the blank. Since, tocopherol is estimated by

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Emmerie- Engel reaction, which indicates the presence of tocopherols by reducing the ferric ions to ferrous ions and also with the change in colour (from colourless to red), 0.33 mL of ferric chloride solution was added and read at 520 nm after 15 min. It was calculated using the formula

$$= \frac{\text{Reading of test at 520nm} - \text{Reading of test at 460nm}}{\text{Reading of standard at 520nm}} \times 0.29 \times 15 \times \frac{\text{Total volume of homogenate}}{\text{Volume used} \times \text{weight of the tissue}}$$

ix. DETERMINATION OF TOTAL PHENOLIC CONTENT

The total phenolic content (TPC) of the plant extracts was determined spectrophotometrically using Folin-Ciocalteau's reagent [31]. 50 µL of the samples in triplicate was added into the test tubes followed by 1.5 mL of 2N Folin-Ciocalteau reagent (diluted 10 times) and 1.2 mL of 20% sodium carbonate. The contents of the tubes were mixed thoroughly and stored at dark for 30 min. Phenols react with phosphomolibdic acid of Folin-Ciocalteau's reagent in alkaline medium and produce blue colored complex, that could be measured at 765 nm and expressed as mg Gallic acid per gm of plant material with Gallic acid as the standard.

x. DETERMINATION OF DPPH (2, 2-DIPHENYL-1-PICRYLHYDRAZYL) RADICAL SCAVENGING ACTIVITY

Free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured [32] in about 50µL of the extract, where 1.5 mL of 0.1 mM DPPH was added and vortexed for 15 to 30 s and allowed to stand without any disturbance for 30 min at room temperature. Indication in the activity of DPPH was observed with a change in the colour from purple to yellow and was measured by reading the absorbance at 517 nm. Ascorbic acid was used as the standard, while the inhibition ratio for DPPH scavenging activity was calculated from the equation:

$$AA\ (\%) = \frac{A_c - A_s}{A_c} \times 100$$

Where,
AA - Ascorbic acid,
A_C- Absorbance of control
A_s - Absorbance of test sample

xi. DETERMINATION OF FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The ferric reducing property of the extracts was determined using the assay described by [33]. To about 50 µL of the extract, 1.5 mL of 0.1 mM FRAP solution (0.2 M Potassium phosphate buffer

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(pH 6.6) and 10mM 2, 4, 6- Tris (2-pyridyl)-S-Triazine) was added and vortexed for 15 to 30 s and incubated at 50°C for 20 min. In order to terminate the reaction, 2.5 mL of 10% trichloroacetic acid and equal volume of ultra pure water was added to the mixture after which was added 0.5 mL of $FeCl_3$ (1 g L^{-1}). The procedure was carried out in triplicate and allowed to stand for 30 min before measuring the absorbance at 593 nm. The absorbance obtained was converted to Gallic acid equivalents in milligrams per gram fresh material (mg g^{-1}) using a Gallic acid standard.

xii. STATISTICAL ANALYSIS

The entire assays were done in triplicate for all the treated plants extracted under different solvents. The data obtained was subjected to statistical One Way Analysis of Variance (ANOVA) and the significant difference among the means were compared with Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ level using the SPSS/PC + Student Ware software (version 17.1.2).

RESULTS

The root, aerial parts (stem, leaf, flower and frits) and whole plant of *E. littorale* extracted as fresh, sun dried and shade dried were observed for the non enzymatic antioxidants the activities of tocopherol, total phenol content, DPPH, FRAP) and enzymatic antioxidants polyphenol oxidase, peroxidase, catalase, Superoxide Dismutase(SOD), and compared with each other.

1. ENZYMATIC ANTIOXIDANTS

The polyphenol oxidase (PPO) comprising of catechol oxidase and laccase is an enzyme that catalyzes the aerobic oxidation of variety of phenolic substrates in the plant material. The catechol activity was affected by sun drying. The fresh and the shade dried plant material produced significant activity in the flower followed by the fruits and leaves, however least activity was observed in the roots. The extracts of flowers from freshly harvested plant material quantified to 0.0072 ± 0.0020 unit /min which was about 43.03 % higher compared to the roots and the same was also revealed to be 1.02 % higher compared to the plant material harvested under shade dry condition. Similarly, the activity of laccase was also revealed to be significant (0.0064 ± 0.0020) unit /min in the freshly harvested flowers of the plant compared to the roots which differed by 43.8 % and the same was also revealed to be 1.02 % higher against the flower extract of shade dried material.

Unlike the observations of PPO, Peroxidase was quantified higher in plant parts subjected to shade drying, which produced the significant value of 37.971 ± 0.1572 units/lit in the floral organs. Perhaps, it was 35.19 % and 24.25 % higher compared to fresh (13.363 ± 0.0735 unit/min) and sun dried (9.209 ± 0.1389 unit/lit) flower extracts respectively. The fruits and leaves ranked second while the roots responded very meagerly in its expression under all strategies examined.

Relative to the activity of Peroxidase, catalase activity was also observed to be high in plant parts extracted from shade dried condition, however, was found to be significant in leaves with the value of 0.0431 ± 0.0474 unit/mg followed by the flowers

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(0.0238 ± 0.0313unit/mg) and root (0.0196 ± 0.0101unit/mg) respectively. It was about 55.22% and 45.48% higher compared to fresh sample and 50.35 % and 17.17 % higher respectively compared to the sun dried extracts.

The activity of Super Oxide Dismutase in different parts of the plant was observed as unit/mg of protein. SOD catalyzes the reaction of superoxide radical converting into water and oxygen. Unlike the earlier enzyme activities detected, SOD activity was observed to be high in the whole plant material, which expressed 0.0129 ± 0.005 unit/mg under the shade dry condition. The leaves and the fruits of the shade dried extract followed the second and third order in expression level which yielded 0.0097 ± 0.008 unit/mg and 0.0059 ± 0.008 unit/mg respectively. Although, fresh plant parts also yielded comparative quantity of SOD, sun drying weekly influenced it by distracting the activity through heat mass.

2. *NON-ENZYMATIC ANTIOXIDANTS*

In concomitance to the enzymatic antioxidants the non-enzymatic compound tocopherol also contributed significantly in the extracts of both fresh and shade dried conditions. However, unlike that of flowers in enzymatic radicals, here the whole plant parts exhibited higher activity of 150.25 ± 0.12µg/g under fresh condition followed by the leaves 72.14 ± 0.068 µg/g which revealed to be (48.01 %) less compared to whole plant. Perhaps expressing the plant parts to the sun drastically affected the tocopherol content.

In conjunction to the tocopherol content, the total phenol content was also revealed to be high in the extract of freshly harvested plants. Phenols are the aromatic compounds with hydroxyl group included to form an array of compounds like tannins, flavonols, etc. Fresh extract of the leaves and flowers showed a significant content of 3.02 ± 0.0020 mg/g and 3.00 ± 0.0041 mg/g respectively, which the same declined in the sun dried plant parts indicating the depletion of phenolic substrates during the drying process.

Retrospective to the activities of Polyphenol oxidase and peroxidases, the DPPH radical scavenging activity of *E.littorale* was altered in both fresh and shade dry extracts. However the significant activity was measured in the sun dried extracts especially in whole plant material, which expressed the activity of 76.57 ± 0.38 mg AA/g exhibiting a like by 26.8 % and 49.4 % compared to the whole plant of fresh extract (20.52 ± 0.84 mg AA/g) and shade dried extract (64.46 ± 0.41 mg AA/g) . Within the sun dried extract, the leaves and fruits showed the scavenging activity of 59.87 ± 0.99 mg AA/g and 43.73 ± 0.71 mg AA/g respectively.

With effective measure of tocopherols, the FRAP activity measured was also found to be higher in the fresh extracts especially in the leaves that expressed 1.225 ± 0.0019mg/g, which was found to be 62.85 % and 89.79 % higher compared to the leaf extract of shade and sundry conditions respectively. Sun drying negatively influenced the ferric reducing activity and yielded very less in the flowers and fruits.

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Table 1

Effect of drying treatments on Enzymatic anti oxidants activity of *E. littorale*

Anti oxidant contents	Treatment strategies	Enzymatic anti oxidants					
		Root	Stem	Leaves	Flowers	Fruits	whole plant
Polyphenol oxidase (Catechol) (Enzyme activity unite /min)	Fresh	0.0031 ± 0.0008	0.0044 ± 0.0011	0.0050 ± 0.0013	0.0072 ± 0.0020*	0.0053 ± 0.0013	0.0054 ± 0.0015
	Shade Dry	0.0021 ± 0.0005	0.0031 ± 0.0008	0.0044 ± 0.0012	0.0070 ± 0.0019	0.0045 ± 0.0012	0.0050 ± 0.0013
	Sun dry	0.0015 ± 0.0003	0.0023 ± 0.0004	0.0042 ± 0.0011	0.0041 ± 0.0009	0.0041 ± 0.0011	0.0022 ± 0.0005
Polyphenol oxidase (Laccase) (Enzyme activity unite /min)	Fresh	0.0028 ± 0.0008	0.0039 ± 0.0011	0.0045 ± 0.0013	0.0064 ± 0.0020*	0.0040 ± 0.0012	0.0048 ± 0.0014*
	Shade Dry	0.0019 ± 0.0005	0.0027 ± 0.0008	0.0040 ± 0.0012	0.0063 ± 0.0019	0.0037 ± 0.0011	0.0045 ± 0.0013
	Sun dry	0.0014 ± 0.0003	0.0020 ± 0.0004	0.0038 ± 0.0011	0.0036 ± 0.0009	0.0047 ± 0.0013	0.0020 ± 0.0005
Peroxidase (Enzyme activity unite /lit)	Fresh	6.505 ± 0.2736	4.702 ± 0.0879	20.847 ± 0.0786	13.363 ± 0.0735	7.406 ± 0.1146	14.029 ± 0.1002
	Shade Dry	8.660 ± 0.1905	12.187 ± 0.1905	21.043 ± 0.2023	37.971 ± 0.1572*	23.982 ± 0.0786	21.121 ± 0.1146
	Sun dry	1.998 ± 0.1690	2.351 ± 0.1389	3.135 ± 0.3779	9.209 ± 0.1389	7.249 ± 0.1389	4.232 ± 0.2023
Catalase (Enzyme activity unite /mg)	Fresh	0.0127 ± 0.0067	0.0166 ± 0.0121	0.0217 ± 0.0201	0.015 ± 0.0213	0.0132 ± 0.0160	0.0108 ± 0.0173
	Shade Dry	0.0196 ± 0.0101	0.0175 ± 0.0153	0.0431 ± 0.0474*	0.0238 ± 0.0313	0.0134 ± 0.0211	0.0166 ± 0.0153
	Sun dry	0.0024 ± 0.0023	0.0069 ± 0.0109	0.0074 ± 0.0195	0.0045 ± 0.0080	0.0095 ± 0.0049	0.0102 ± 0.0075
Superoxide dismutase (Enzyme activity unit /mg)	Fresh	0.0010 ± 0.006	0.0013 ± 0.010	0.0031 ± 0.006	0.0064 ± 0.018	0.0023 ± 0.006	0.0028 ± 0.007
	Shade Dry	0.0036 ± 0.008	0.0015 ± 0.009	0.0097 ± 0.008	0.0051 ± 0.005	0.0059 ± 0.008	0.0129 ± 0.005*
	Sun dry	0.0008 ± 0.009	0.0019 ± 0.004	0.0020 ± 0.007	0.0043 ± 0.004	0.0022 ± 0.009	0.0014 ± 0.006

Data expressed as mean ± SEM of triplicates and values in * indicates the significant difference (P≤0.05).

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Table 2

Effect of drying treatments on Non Enzymatic anti oxidants activity of *E. littorale*

Anti oxidant contents	Treatment strategies	Non Enzymatic anti oxidants					
		Root	Stem	Leaves	Flowers	Fruits	whole plant
Tocopherol (µg / g)	Fresh	33.08 ± 0.015	68.51 ± 0.05	72.14 ± 0.07	64.62 ± 0.06	57.61 ± 0.05	150.25 ± 0.12
	Shade Dry	23.69 ± 0.05	50.60 ± 0.07	58.778 ± 0.078	34.64 ± 0.07	29.20 ± 0.07	131.96 ± 0.19
	Sun dry	1.16 ± 0.03	8.17 ± 0.030	10.12 ± 0.032	2.72 ± 0.026	2.34 ± 0.026	28.34 ± 0.12
Total Phenol (mg/ g)	Fresh	1.90 ± 0.002	1.50 ± 0.0012	3.02 ± 0.002*	3.00 ± 0.004	2.65 ± 0.002	2.85 ± 0.001
	Shade Dry	0.80 ±0.002	0.90 ± 0.004	2.05 ± 0.0025	1.85 ± 0.002	2.40 ± 0.003	2.70 ± 0.002
	Sun dry	0.45 ± 0.002	0.80 ± 0.003	1.49 ± 0.0008	1.34 ± 0.001	1.02 ± 0.0032	1.67 ± 0.003
DPPH activity (mg AA /g)	Fresh	10.96 ± 0.36	19.69 ± 0.92	32.04 ± 1.026	25.661 ± 1.06	29.66 ± 0.69	20.52 ± 0.83
	Shade Dry	12.43 ± 1.07	23.31 ± 0.56	42.24 ± 0.99	27.719 ± 0.59	34.921 ± 0.71	64.462 ± 0.41
	Sun dry	15.491 ± 0.82	32.128 ± 0.55	59.877 ± 0.99	33.039 ± 0.98	43.739 ± 0.7136	76.57 ± 0.382*
FRAP activity (mg/g)	Fresh	0.34 ± 0.0018	0.585 ± 0.0023	1.22 ± 0.002*	1.185 ± 0.001	0.955 ± 0.003	1.05 ± 0.0007
	Shade Dry	0.205 ± 0.0015	0.300 ± 0.002	0.775 ± 0.002	0.745 ± 0.002	0.55 ± 0.002	0.96 ± 0.002
	Sun dry	0.135 ± 0.002	0.225 ± 0.004	0.110 ± 0.0025	0.050 ± 0.003	0.05 ± 0.0012	0.200 ± 0.002

Data expressed as mean ± SEM of triplicates and values in * indicates the significant difference (P≤0.05)

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DISCUSSION

The body posses defense mechanisms against free radical-induced oxidative stress, which involve preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) etc., while non-enzymatic antioxidants are ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids and etc. All these act by one or more of the mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants [34].

India is one of the richest countries in the world as regards to resource of medicinal and aromatic plants and constitutes 11% of total known world flora having medicinal property [35]. Ayurveda has been in practice in India for more than 3500 years and the first recorded book on Ayurvedic medicine was Charaka Samhita which dates back to 600 BC. The plants form secondary metabolites for protection against pests, as colouring, scent, or attractants and boost the immune system, protect the body from free radicals and kill pathogenic germs and much more. The secondary metabolites include carotenoids, phytosterols, saponins, glucosinolates, flavonoids, etc. Flavonoids are organic and water soluble compounds which give plants a red, violet or blue colour. The traditional healers have used this resource since time immemorial for the benefit of mankind because, it inhibits the growth of bacteria and viruses, protect the cell against the free radicals, protect against cancers and heart attacks, have a repressive effect against inflammations and influence blood coagulation. Thus a diet which is rich in plant foods contains a variety of secondary metabolites and contributes to protecting the body against cancer and cardiovascular illnesses [36]. Since some plants with antioxidant activities have been reviewed earlier [25] this study has become essential to investigate some in *Enicostemma littorale* in a significant way to be adapted in future to cure most of the diseases.

The Polyphenol oxidase activity in *E.littorale* was found to be in considerable quantity and was correlated to the degree of browning of fruits. However, the phenolic compound was found high in shade dried extracts which revealed the fact that the phenolic substrates are fixed in the plant parts and that even an evaporation of water facilitates the enzyme activity [37] and was also depended on the amounts of phenolic compounds [38].

Reactive oxygen species (ROS) get special attention due to many factors such as drought, cold, heat, herbicides and heavy metals, because they harm the cell by raising the oxidative level through loss of cellular structure and function [37], hence demands the detoxification agents like enzymes such as SOD, catalase and peroxidase [39] and non enzymatic antioxidants such as flavones, anthocyanins, carotenoids and ascorbic acid [40]. The activity of peroxidases, catalases and SODs screened was observed to be high in the floral parts, leaves and in whole plants respectively of plants extracted under shade dry conditions and it implied *E.littorale* to contain potential detoxifying agents. Peroxidases are heme containing enzymes that oxidizes organic and inorganic compounds by hydrogen peroxide which are again scavenged by catalases that function as cellular sinks [41].

Tocoperol's chemopreventive efficacy and the mode of action [42] reveal its antioxidant

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properties, where the oxidation of L-ascorbic acid (AA) to L-dehydroascorbic acid (DHA) has been evidenced by the stabilized production of tocopherol that was found to be significantly high in the plant as a whole under fresh condition which declined during the drying process especially under sun dry.

There are claims that phenolics compounds and its derivatives are secondary plant metabolites that have an important role in providing flavour and colour characteristics to the fruits and strongly correlate with antioxidant activities [43]. Apart from antioxidant property, phenols also regulate nitric oxide, decrease leukocyte immobilization, involve in apoptosis by inhibiting cell proliferation and angiogenesis, and exhibit phytoestrogenic activity [44]. Perhaps, its quality and quantity in vegetables are influenced by different processing strategies. Microwave heating and stir-frying to cook vegetables has reported [45] to affect the components such as vitamin C, irons and ß-carotenes and has also decreased the amount of total phenolics and total flavonoids respectively [46, 47]. Since, increase in temperature during the sun drying depletes the phenolic compounds, a highest phenolic compound was found in the shade dried extracts of the plant and was relatively in concordance to the principle that their concentration could rapidly decreases during fruit development [19].

Since, the proton radical-scavenging ability is known to be one of the various mechanisms for antioxidation, as indicated by a reduction process of hydrogen- or electron donation [48, 43, 12] is recommended by many scientists because of its reproducibility and accuracy. Hence, the results obtained from DPPH free radical scavenging assay and FRAP assays were reliable and yielded significantly higher under sundried conditions relatively disproving its performance with that of [49] who found that scavenging ability of ethanolic extracts of purple yam decreased with increase in temperature and time.

It is reasonable to expect high antioxidant foods to have greater potential to reducing free radicals in the body than do low antioxidant foods. Perhaps, in the present study even after sun and shade drying process, the plant showed significant antioxidant activity in reducing the ferric compounds and scavenging DPPH free radicals as evidenced by [50, 51]. When the samples reacted with FRAP solution, the appearance of dark blue color indicated the formation of ferrous tripyridyltriazine complex [52], which could be detected at 593 nm and here the extracts exhibited higher activity of FRAP in leaves of sun dried materials. In fact, apart from drying, other factors like the kind of solvents used etc., could also affect the expression of difference in the pattern of antioxidants.Perhaps, increased level of both TPC and and (FRAP) Ferric reducing activity in fresh material compared to other shade and sun dried extracts may have been due to the denaturing properties of the polyphenols oxidases under heat treatment [25].

There has been always a strong correlation between the DPPH assay and FRAP assays because of their potential reducing properties [53]. Infact, their ability has been reported to remain constant even after different cooking strategies as reported in broccoli samples and in *Tamarindus indica* seeds respectively. This could also be attributed to the organic and volatile solvent used for extraction, which efficiently degraded the plant cell wall leaching out more amounts of endocellular materials than water [54]. The percentages for DPPH free radical scavenging activity was higher than that of FRAP and the probable reason could be attributed to the strength in the compounds of reducing power [12].

In this study, the activity of Peroxidase, Poly Phenol Oxidase (PPO), Super Oxide Dismutase (SOD),

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Tocopherol were clearly studied in *Enicostemma littorale* making the plant as a highly potential one. In general, plant phenolic compounds and their antioxidants present at different binding status would depend on plant species. Perhaps, data on the effects of drying and solvent usage on the antioxidant properties of herbs and vegetables, although are conflicting due to several factors may show variation in their activities in response to the mode of extraction [54]. With all the data obtained on non enzymatic and enzymatic antioxidant activities in aqueous extract of differently treated *E.littorale,* it is suggested for further investigation on biophysical dimension to explore the highly available compound for structural information.

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