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COMPARISON OF ANTIOXIDANT POTENTIAL OF FRESH AND MARKETED SAMPLES OF MEDICINAL PLANT- *ECLIPTA ALBA*

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Keywords:

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ABSTRACT: The present study aimed at comparing the antioxidant potential of three different samples of *Eclipta alba*-fresh plant, marketed leaf powder (packaged in sealed containers), and marketed whole plant (sold in open bags). The presence of alkaloids, saponins, triterpenes, terpenoids, tannins, steroids, flavonoids, glycosides was confirmed in all three samples. Fresh plant samples showed a higher antioxidant activity determined using standard ferric reducing antioxidant power (FRAP) assay (3105.16 ± 202.07 AAE/gm. dry weight) and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay (32.37 ± 5.94%). Fresh plant samples also had a higher content of secondary metabolites such as total phenolics and alkaloids as compared to the other two samples. A high correlation was observed between antioxidant capacity and total phenolic content (R^2 = 0.997). Loss of antioxidant potential in marketed samples indicates that proper processing and storage are important in ensuring the viability of the raw material of medicinal plants.

INTRODUCTION: The Indian subcontinent is known for its rich biodiversity that includes numerous species of medicinal plants. From prehistoric times, medicinal plants have been used extensively in different medicinal systems prevalent in India, *i.e.,* Ayurveda, Unani and Siddha systems of medicine [1]. Medicinal plants have been used in the treatment of diseases like cancer, diabetes, atherosclerosis, cardiovascular diseases and aging. These diseases have been linked to the production of reactive oxygen species or free radicals (ROS) in the human body [2]. Medicinal plant-based anti-oxidants such as polyphenols, carotenoids, flavonoids are highly effective in scavenging these ROS [3].

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Plant-derived antioxidants are essentially of two types: enzymatic and non-enzymatic. Enzymatic antioxidants, *viz.* Catalase and Superoxide Dismutase (SOD), are a group of antioxidants that catalyze the reactions which convert ROS into less harmful products. On the other hand, non-enzymatic antioxidants such as ascorbic acid and salicylic acid interfere with the free radical chain reactions [4].

Medicinally important plant *E. alba*, commonly known as False Daisy is a member of the Asteraceae family. This species grows commonly in warm temperate to tropical areas and is widespread in the Indian subcontinent. Vernacularly known as Bhringraj, this plant holds high value in Ayurvedic medicines for its effectiveness in curing liver ailments, hair issues, and its anti-aging properties [5]. The undeniable benefits of this seemingly small plant have popularized it across the globe. The extract of this plant can be applied topically as well as internally to treat many ailments.

Phytochemical studies on *E. alba* have reported the presence of several classes of secondary metabolites such as alkaloids, caumestans, flavonoids, glycosides, sterols, triterpenoids, volatile oils, *et*c. [6] The leaves of *E. alba* contain caumestan derivatives- wedelolactone, demethyl-wedelolactone, and desmethyl-wedelolactone-7-glucoside; stigmasterol- β-terthienylmethanol *etc*. [7, 8] The roots of *E. alba* contain thiophene acetylenes such as hentriacontanol, 14-heptacosanol, ecliptal (a terthienyl derivative), ecliprostins A–C [9, 10]. The stem of *E. alba* has been reported to contain wedelolactone, wedelic acid, flavonoids like apigenin, luteolin *etc.* [11, 8] The aerial part of *E. alba* has been reported to contain phytosterol, β-amyrin, luteolin-7-glucoside, apigenin [12] and alkaloids like ecliptine, nicotine and bioactive steroidal alkaloids verazine, dehydroverazine [13]. Several saponin triterpene glycosides have been reported from *E. alba* namely eclalbosaponin, ecliptasaponin C and D14, and triterpenesaponin- eclalbatin, α-amyrin, ursolic acid, and oleanoic acid [8, 12]. The presence of large amounts of resins, reducing sugars, stigmasterol, ecliptine *etc.* has also been reported in *E. alba* [6]. Medicinal plants are a rich source of exogenous antioxidants. However, the composition, concentration, and nature of plant extracts are determining factors for their effectiveness [15]. Plant-based medicinal products have to undergo processing, packaging, transportation, storage, etc. before they are made available to consumers, and this may affect the efficacy of the samples. The present study focused on comparing antioxidant potential of different samples of *E. alba* - fresh plants, marketed leaf powder (sold in sealed packs in pharmacy shops), and marketed whole dried plants (sold in open bags in local shops) based on different qualitative and quantitative metrics.

MATERIALS AND METHODS:

Procurement of Samples: Fresh plant samples of *E. alba* were collected from the campus of Maitreyi College, University of Delhi, Chanakyapuri, New Delhi. The samples of the marketed whole (dried) plant and marketed leaf powder of *E. alba* (Bhringraj Leaf Powder, Bio Organic) were procured from a local shop **Fig. 1**. Plant samples were identified and authenticated by Botanist Dr. Monika Heikrujam, Assistant Professor, Maitreyi College, University of Delhi.

FIG. 1: (A) MARKETED LEAF POWDER SAMPLE, (B) MARKETED WHOLE PLANT SAMPLE, (C) FRESH PLANT SAMPLE COLLECTED FROM COLLEGE CAMPUS

Analysis of Samples for Anatomical Details: In order to study the anatomical details of the fresh plant, marketed whole plant and marketed powdered samples of *E. alba*, the different tissues (leaf, stem, roots etc) were subjected to maceration.

The samples were soaked in concentrated Nitric acid for 48 h followed by gentle and thorough washing with water.

Samples were stained with safranin, mounted in glycerin and photographed using a microscope (Nikon E100 LED Micro-scope).

Extract Preparation: The fresh plant samples of *E. alba* collected from campus were air-dried for 48 h. The completely dried material was then crushed into a fine powder using mortar and pestle. The marketed whole plant was already dried, so it was directly crushed into a fine powder using mortar and pestle. For crude extract preparation, methanol was used as a solvent in the ratio of 1:5 (powdered sample: solvent). The mixture was incubated at 37 °C with constant stirring for 24-36 h. The extract thus obtained was filtered through a muslin cloth and stored in air-tight containers at 4°C for further analysis.

Qualitative Phytochemical Analysis: Prepared extracts were tested qualitatively for the presence of alkaloids, saponins, triterpenes, terpenoids, tannins, steroids, flavonoids, glycosides according to standard protocols [16].

Detection of Alkaloids (Hager's Test): To 2 ml of filtrate in a test tube, a few drops of Hager's reagent were added. The formation of yellow colored precipitate indicated the presence of alkaloids.

Detection of Saponins (Foam test): 2 ml of extract and 10 ml distilled water was taken in a test tube and shaken vigorously. The formation of foam indicated the presence of saponin.

Detection of Triterpenes (Salkowski's test): 2 ml of extract evaporated to dryness, and the residue was dissolved in 2 ml of chloroform, to which 2 ml conc. H_2SO_4 was added along the sides of the test tube. The tube was shaken and allowed to stand for 1 min. The formation of the golden yellow layer at the bottom indicated the presence of triterpenes.

Detection of Terpenoids: 2 ml of extract dissolved in 2 ml of chloroform and evaporated to dryness, to which 2 ml of conc. H_2SO_4 was added and heated for 2 min. The development of greyish color indicated the presence of terpenoids.

Detection of Tannins (Ferric Chloride Test): 2 ml of extract and 2 ml of distilled water were taken in a test tube, to which few drops of 5% Ferric chloride solution (in 90% alcohol) was added. The formation of green precipitate indicated the presence of tannins.

Detection of flavonoids (Lead acetate Test): To 1 ml of extract, 1 ml of 10% lead acetate solution was added. The formation of yellow precipitate indicated the presence of flavonoids.

Detection of Sterols (Salkowski Test): 2 ml of extract evaporated to dryness, and the residue was dissolved in 2 ml of chloroform. To this 2 ml conc. H_2SO_4 was added from the sides of the test tube, shaken, and allowed to stand for 1 min. The development of red color in the chloroform layer indicated the presence of sterols.

Detection of Glycosides (Keller- Kiliani Test): To 2 ml of extract in a test tube, 3 ml of glacial acetic acid and 1 drop of 5% Ferric chloride were added. To this, 0.5 ml conc. H_2SO_4 was added carefully along the sides of the test tube. The formation of blue color in the acetic acid layer indicated the presence of glycosides.

Thin Layer Chromatography: For TLC, TLC Silica Gel 60 F254 plates (Merck millipore) were used for loading of the fresh plant, marketed whole plant, and marketed powdered samples of *E. alba*, in the form of circular spots approx. 2 cm above its bottom using fine capillary tubes [17]. The loaded TLC plates were put gently in pre-saturated TLC chambers containing the mobile phase.

The TLC plates were taken out once the mobile phase reached 1/3 of its total length, and the solvent front was marked with a pencil. The plate was dried, the sample spots were observed under UV light, and the separated bands were marked with a pencil. Different solvents (mobile phase) were used for the detection of different phytochemicals. For phenolics, toluene and acetone were taken in 9:1 ratio (Solvent system 1).

For flavonoids, chloroform and methanol were taken in 9:1 ratio (Solvent system 2). For alkaloids, methanol and ammonium hydroxide were taken in a 50:0.75 ratio (Solvent system 3).

Quantitative Tests: Free radical scavenging activity was assessed using ferric reducing antioxidant power (FRAP) assay and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Folin-Ciocalteu method was used for the estimation of total phenolics. All the standard solutions and sample solutions were prepared in triplicates.

FRAP (Ferric Reducing Antioxidant Power) Assay: FRAP assay is based on the reduction of ferric ion (Fe^{3+}) present in Tripyridyltriazine (TPTZ) by antioxidant present in the test sample. To prepare the reaction mixture, 500 µl of each sample was added to 600 µl of distilled water followed by the addition of 3 ml working FRAP reagent at 37° C. After incubation of 30 min; absorbance was measured at 593 nm. A standard curve was obtained using different concentrations of ascorbic acid, and the samples were compared to the standard curve to test the amount of ferric ion reduced.

Antioxidant capacity was expressed in terms of ascorbic acid equivalent/gm dry weight (AAE/ gm. dry weight) [18].

DPPH Free Radical Scavenging Assay: 1, 1Diphenyl-2-picryl hydrazyl (oxidized DPPH) is a stable (in powder form) free radical with red colour which turns yellow when reduced (reduced DPPH-H). The DPPH assay uses this character for free radical scavenging activity. Dilution of each working extract concentration was prepared by adding 50 µl of extract in 1950 µl methanol. This was followed by the addition of 2 ml of DPPH reagent in each sample. For control, 2 ml of DPPH and 2ml of methanol was taken. After incubation of 30 minutes in the dark, absorbance was measured for each sample at 517 nm. A standard curve was obtained using different concentrations of ascorbic acid. As DPPH was reduced by antioxidant, its absorbance decreases; therefore, the degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. The percentage inhibition of the DPPH radical by the samples was calculated using the following equation [19]:

$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where, A_0 is the absorbance of the control sample and A_1 is the absorbance of the test sample.

Total Phenolic Content: The Folin-Ciocalteu reagent (FCR) is a mixture of phosphomolybdate and phosphotungstate. Under alkaline conditions, the phenolic compounds reduce FCR to give blue coloured complex. To prepare the reaction mixture, 1 ml of each sample was added to 5 ml of distilled water.

To this, 0.5 ml of working FCR was added followed by 1.5 ml of 20% Na_2CO_3 after 5 min. The reaction mixture was incubated for 1.5 hours in the dark. The absorbance was measured for all the samples at 750 nm, and the phenolic content was expressed in gallic acid equivalent/gm dry weight (GAE/gm. dry weight). A standard curve was prepared using different concentrations of Gallic acid [20].

Statistical Analysis: All the assays were carried out in triplicate. For each experiment, the mean value and standard deviation (SD) were calculated using standard procedure. Correlation analysis of the total antioxidant capacity and phenolic content of the fresh plant marketed the whole plant and marketed powdered samples of *E. alba* was performed using standard procedure.

RESULTS:

Microscopic Analysis of Macerated Material: The macerated material of fresh plant, marketed the whole plant and marketed powdered samples of *E. alba* showed the presence of trichomes, stone cells, xylem components: tracheid, vessels, and fibers **Fig. 2**.

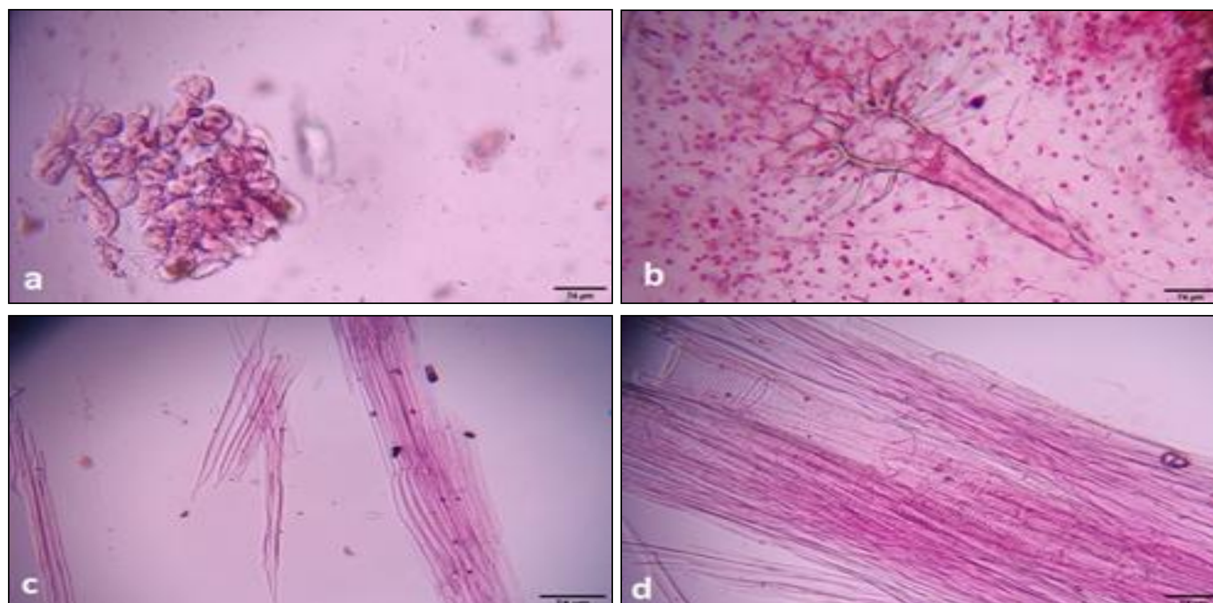

FIG. 2: ANATOMICAL DETAILS OF MACERATED MATERIAL UNDER LIGHT MICROSCOPE. (A) STONE CELLS, (B) MULTICELLULAR TRICHOMES (C) FIBERS AND TRACHEIDS AND (D) VESSELS

Qualitative Phytochemical Analysis: Qualitative analysis indicated the presence of various secondary metabolites in the three samples of *E. alba* **Table 1**. The methanolic extract of the plant collected from the college campus, *i.e.,* fresh plant samples, showed higher numbers of secondary metabolites than marketed whole plant and marketed powdered samples of *E. alba*.

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF DIFFERENT SAMPLES OF *E. ALBA*

Test	Methanolic extracts			Ethanolic extracts			Water extracts		
	Marketed Leaf Powder	Marketed Whole Plant	Fresh Plant	Marketed Leaf Powder	Marketed Whole Plant	Fresh Plant	Marketed Leaf Powder	Marketed Whole Plant	Fresh Plant
Alkaloids	++	++	+++	++	+	+++	+	+	+
Saponins	AB	AB	AB	AB	AB	AB	AB	AB	AB
Triterpenes	+++	+++	AB	++	++	AB	AB	AB	AB
Terpenes	AB	AB	AB	AB	AB	AB	AB	AB	AB
Tannins	++	+	++	+	AB	++	AB	AB	AB
Steroids	++	++	+	AB	AB	+	AB	AB	AB
Flavonoids	++	+	+++	+	++	+++	AB	AB	AB
Glycosides	+++	+	+++	+	AB	+++	AB	AB	AB
Sugars	+++	AB	++	+++	AB	++	AB	AB	AB

Thin Layer Chromatography: On performing TLC, it was observed that the total number of phytochemicals of the fresh plant was higher as compared to marketed whole plant and marketed powdered samples of *E. alba* **Fig. 3**. The fresh sample showed separation of 29 phytochemicals while marketed leaf powder and the whole plant showed 16 phytochemicals **Table 2**.

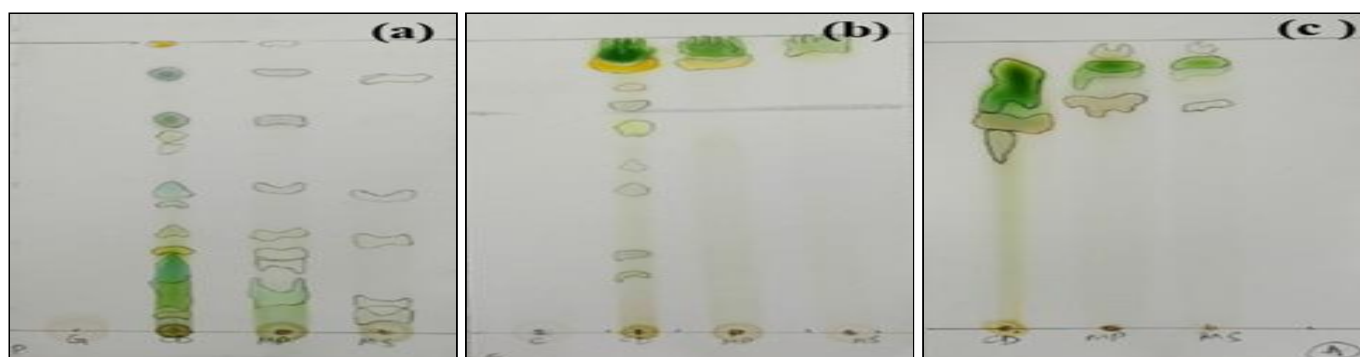

FIG. 3: TLC PLATES SHOWING SEPARATION OF DIFFERENT PHYTOCHEMICALS. (A) SOLVENT SYSTEM 1, (B) SOLVENT SYSTEM 2, AND (C) SOLVENT SYSTEM 3

TABLE 2: RF VALUES OBTAINED USING DIFFERENT TLC SOLVENT SYSTEMS FOR METHANOLIC EXTRACTS OF FRESH PLANT, MARKETED WHOLE PLANT AND MARKETED LEAF POWDER SAMPLES OF *E. ALBA*

Sample		Solvent System 1	Solvent System 2	Solvent System 3
Marketed Leaf Powder	No. of spots	9	3	4
	Rf value	0.261	0.654	0.788
		0.336	0.909	0.899
		0.84	0.963	0.917
		0.117		0.963
		0.134		
		0.168		
		0.521		
		0.798		
		0.915		
Marketed Whole Plant	No. of spots	11	1	4
	Rf value	0.84	0.963	0.77
		0.122		0.798
		0.142		0.917
		0.168		0.963
		0.302		
		0.344		
		0.529		

		0.798		
		0.865		
		0.949		
		0.983		
Fresh Plant	No. of spots	14	11	3
	Rf value	0.05	0.045	0.651
		0.84	0.109	0.724
		0.117	0.2	0.853
		0.009	0.263	
		0.176	0.481	
		0.21	0.563	
		0.243	0.7	
		0.285	0.772	
		0.336	0.845	
		0.504	0.9	
		0.739	0.954	
		0.798		
		0.907		
		0.983		

Quantitative Analysis:

FRAP Assay: The fresh plant sample showed the highest FRAP value (3105.16 ± 202.07 AAE/gm. dry weight) followed by the marketed leaf powder (755.16 ± 105.08 AAE/gm. dry weight). Marketed whole plant showed the least value in FRAP assay (321.83 ± 28.86 AAE/gm. dry weight) as shown in **Table 3** and **Fig. 4**.

TABLE 3: FRAP VALUES EXPRESSED AS AAE/GM. DRY WEIGHT (ASCORBIC ACID EQUIVALENT/GM DRY WT.) OF METHANOLIC EXTRACTS OF FRESH PLANT, MARKETED WHOLE PLANT AND MARKETED LEAF POWDER SAMPLES OF *E. ALBA*

FRAP (AAE/gm. Wt)		
Marketed Leaf Powder	**Marketed Whole Plant**	**Fresh Plant**
755.1667 ± 105.0833	321.8333 ± 28.86751	3105.167 ± 202.0726

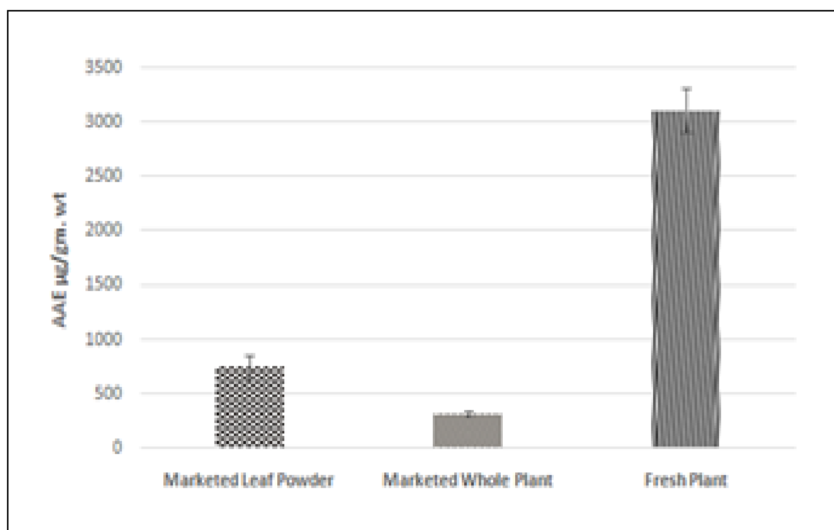

FIG. 4: COMPARISON OF AAE/GM. DRY WEIGHT (ASCORBIC ACID EQUIVALENT/GM DRY WT.) IN FRAP ASSAY OF METHANOLIC EXTRACTS OF FRESH PLANT, MARKETED THE WHOLE PLANT AND MARKETED LEAF POWDER SAMPLES OF *E. ALBA*

Total Phenolic Content: The fresh plant showed the highest total phenolic content, 64.99 ± 6.57 GAE/gm. dry weight followed by the marketed leaf powder (43.19 ± 12.19 GAE/gm. dry weight).

The marketed whole plant showed the least value in the quantitative analysis of total phenolic content (32.77 ± 0.5 GAE/gm. dry weight) as shown in **Table 4** and **Fig. 5.**

TABLE 4: TOTAL PHENOLIC CONTENT EXPRESSED IN GAE/GM. DRY WEIGHT (GALLIC ACID EQUIVALENT/GM DRY WT.) OF METHANOLIC EXTRACTS OF FRESH SAMPLE, MARKETED WHOLE PLANT AND MARKETED LEAF POWDER OF *E. ALBA*

Total Phenolic Content (GAE/gm. Wt)		
Marketed Leaf Powder	**Marketed Whole Plant**	**Fresh Plant**
43.19 ± 12.19	32.77 ± 0.5	64.69 ± 6.57

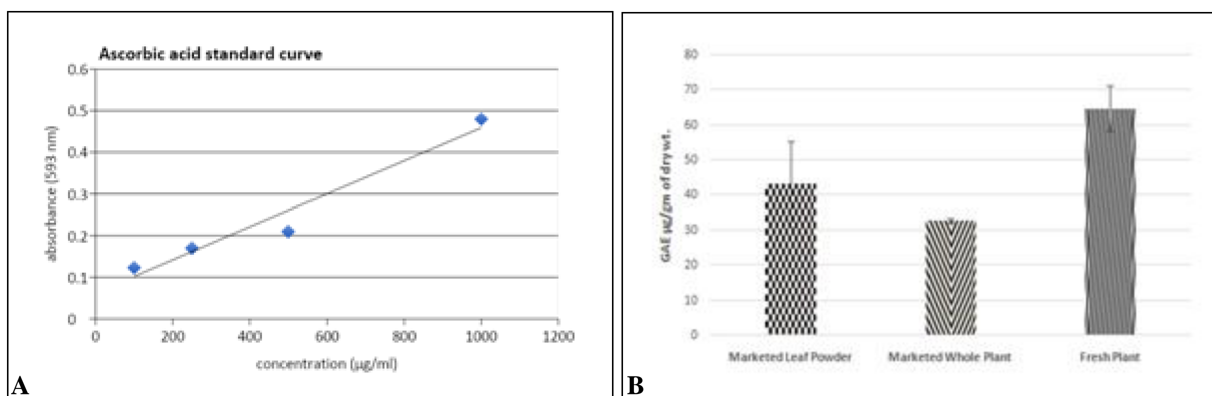

FIG. 5: A. ASCORBIC ACID STANDARD CURVE FOR FRAP; B. COMPARISON OF TOTAL PHENOLIC CONTENT IN GAE/GM. DRY WEIGHT (GALLIC ACID EQUIVALENT/ GM DRY WT.) OF METHANOLIC EXTRACTS OF FRESH SAMPLE, MARKETED WHOLE PLANT AND MARKETED LEAF POWDER OF *E. ALBA*

DPPH Assay: The fresh plant extract showed higher free radical scavenging capacity (32.37 ± 5.94%) followed by the marketed leaf powder (16.37 ± 0.5%). The marketed whole plant showed the least % reduction in DPPH (8.63 ± 0.5%) as shown in **Table 5** and **Fig. 6**.

TABLE 5: PERCENTAGE REDUCTION IN DPPH BY METHANOLIC EXTRACTS OF FRESH SAMPLE, MARKETED WHOLE PLANT AND MARKETED LEAF POWDER OF *E. ALBA*

Percentage reduction in DPPH		
Marketed Leaf Powder	Marketed Whole Plant	Fresh Plant
16.37 ± 0.5	8.63 ± 0.5	32.37 ± 5.94

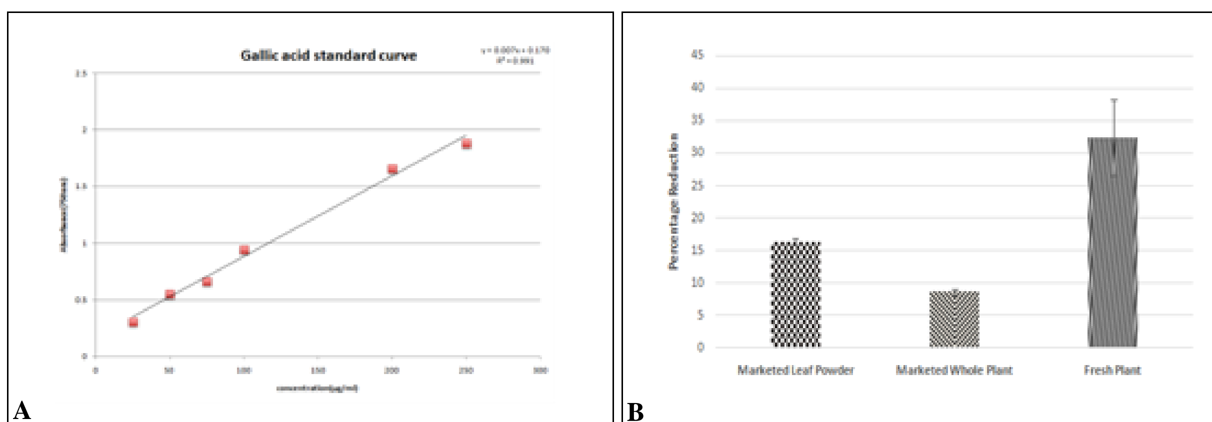

FIG. 6: A. GALLIC ACID STANDARD CURVE; B. COMPARISON OF % REDUCTION IN DPPH BY METHANOLIC EXTRACTS OF FRESH SAMPLE, MARKETED WHOLE PLANT AND MARKETED LEAF POWDER OF *E. ALBA*

Statistical Analysis: To determine the reliability of the two assays used for evaluating the antioxidant capacities of the three samples fresh plant, marketed whole plant and marketed leaf powder of *E. alba*, correlation analysis was performed of the values obtained by DPPH and FRAP assays.

The R^2 values obtained showed that the values of antioxidant capacity obtained by DPPH and FRAP assays were highly correlated (R^2 = 0.983) as shown in **Table 6**. Similarly, a high degree of correlation (R^2 = 0.997) was observed between the antioxidant capacity and total phenolic content (TPC) of the three samples of fresh plant, marketed whole plant and marketed leaf powder of *E. alba*.

TABLE 6: CORRELATION COEFFICIENT AMONG ANTIOXIDANT CAPACITIES AND TOTAL PHENOLIC CONTENT OF FRESH SAMPLE, MARKETED WHOLE PLANT AND MARKETED LEAF POWDER OF *E. ALBA*

	DDPH	TPC
FRAP	0.983	0.984
TPC	0.997	-

DISCUSSION AND CONCLUSION: In the present study, three samples of *E. alba, i.e.,* fresh plant, marketed whole plant and marketed leaf powder, were compared for their antioxidant potential. The samples were subjected to various qualitative and quantitative assays such as qualitative phytochemical analysis, thin layer chromatography, FRAP assay, DPPH assay, and total phenolics. Microscopic examination of the

macerated material revealed the presence of trichomes, tracheids, vessels, fibers, and stone cells in all three samples as also reported earlier by Sharma *et al.* [21] The three samples were found to be anatomically similar to each other. Qualitative analysis showed positive results for various phytochemicals. Saponins and tannins were found to be absent in all three samples of *E. alba*. On performing TLC, it was observed that higher content of total phytochemicals (flavonoids, phenolics and alkaloids) was present in fresh plant samples as compared to the other two samples. The fresh sample showed 25 phytochemicals altogether while the marketed leaf powder and marketed whole plant sample had 16 phytochemicals each. Also, the R_f values of the several bands in three samples were found to be similar to previously reported values for phytochemicals of *E. alba* such as Wedelolactone (R_f: 0.39) and Demethyl-wedelolactone (R_f: 0.28) [22, 23, 24, 25, 26].

The quantitative analysis of antioxidant potential of the three samples of *E. alba* revealed that the fresh plant sample showed higher DPPH free radical scavenging activity, total phenolic content, and reducing power by FRAP assay as compared to the marketed samples. The high positive correlation values obtained between total antioxidant capacities by DPPH and FRAP assays and TPC indicated that phenolic compounds contribute significantly to the antioxidant capacities of the tested samples of *E. alba*. The results clearly showed that freshly harvested samples of *E. alba* have a higher content of antioxidants. Similar studies on *Ocimum basilicum*, *Senna petersiana,* and *Hypoxis hemerocallidea* have shown a reduction in phtyochemical activity occurs due to storage and in a species specific manner [27]. This indicates that storage, handling and drying of plant samples have a significant effect on the antioxidant capacity.

Marketed leaf powder showed higher antioxidant potential as compared to the marketed whole plant as the former contains leaves that are well known to be rich in antioxidants. Additionally, marketed leaf powder was processed and vacuum-sealed under controlled conditions in contrast to the marketed whole plant, which is sold in open bags in shops and therefore had high chances of loss of viability and contamination. Loss of antioxidant potential in processed medicinal plant products as compared to fresh material has also been reported in studies with other medicinal plants such as *Portulaca oleracea*, *Azadirachta indica*, rosemary (*Rosmarinus officinalis*), motherwort (*Leonurus cardiaca*), peppermint (*Mentha piperita*), *etc.* [28, 29, 30, 31].

For providing maximum benefits of medicinal plants to consumers, exploring various methods for their effective commercial harvesting and post-harvest processing techniques is an important aspect to be studied further. It will help to ensure that all the phytochemicals are retained in available medicinal plant products.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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