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Evaluation of Phytochemical Constituents and Antioxidant Property of Leaf Acetone Extracts of Five Herbaceous Medicinal Weeds

Pranabesh Ghosh, Swagata Biswas, Alolika Dutta, Maitrayee Biswas,Shaktijit Das, Chandrima Das, Chandreyi Ghosh, Sirshendu Chatterjee*

Department of Biotechnology, Techno India University, West Bengal, EM-4, Salt Lake, Sector- V, Kolkata- 700091, West Bengal, India

Abstract

In the recent era, medicinal weeds are receiving massive attention for their therapeutic efficacy on different oxidative stress-related diseases. In the present study, five common medicinal herbaceous weeds of West Bengal, and India namely; *Heliotropium indicum*, *Tridax procumbens*, *Cleome rutidosperma*, *Commelina benghalensis*, and *Euphorbia hirta* have been chosen from five different families e.g., Boraginaceae, Asteraceae, Cleomaceae, Commelinaceae, and Euphorbiaceae, respectively to decipher their phytochemical, and anti-oxidant properties. The research investigations have concluded that *Euphorbia hirta* possesses a significant amount of phytochemicals, and it exhibits the highest anti-oxidant activities in comparison with the other four medicinal weeds. *Euphorbia hirta* leaves acetone extract showed the highest content of extractive value (11.88±0.21%), polyphenols (191.28±2.42 mg Gallic acid equivalent/g dry tissue), flavonoids (60.80±1.22 mg Quercetin equivalent/g dry tissue), tannins (17.84±0.23 mg Tannic acid equivalent/g dry tissue), and polysaccharides (206.73±1.37 mg Dextrose equivalent/g dry tissue), that supports their anti-oxidant property, i.e., inhibitory concentration (IC%) value for ABTS (58.57±0.29%), DPPH (59.17±0.45%) and H_2O_2 (60.17±0.26%) radical scavenging assays. In *Euphorbia hirta* leaves acetone extract highest amount of phytochemicals were detected by qualitative assays. The bioactive compounds and anti-oxidant activities are well correlated among each other.

Keywords: Medicinal Weeds, Phytochemicals, Qualitative, Quantitative, Anti-oxidants.

INTRODUCTION

Phytomedicines are an essential area of the healthcare system. It is low cost as well as has better compatibility with the human physiology and has minimum side effects too. Plants had been used throughout history in the whole world by different cultures, ethnic groups, and society because of its high pharmaceutical and neutraceutical value as they are the potential source of important primary and secondary metabolites [1, 2].

The current study is focusing on five herbaceous weeds which have immense potential in the herbal medicine based on traditional knowledge. *Heliotropium indicum* Linn. is an annual herbaceous weed and it is commonly known as Indian heliotrope. It has hairy ovate to oblong-ovate leaves and it is native to India. *Cleome rutidosperma* DC. commonly known as Fringed Spider Flower, is an annual herbaceous plant. It is grows up to 100 cm and with trifoliate leaves. It is native to Tropical Africa. *Tridax procumbens* Linn., commonly known as coat buttons. The leaves are toothed and arrowhead-shaped. It is native to tropical Americas. *Commelina benghalensis* Linn., commonly known as Bengal dayflower. It is an annual herbaceous weed native to tropical Asia and Africa. Leaves are ovate to lanceolate. *Euphorbia hirta* Linn., commonly known as asthma-plant. It is actually native to India. The leaves are elliptical, simple and hairy. These weeds are distributed in tropical or temperate parts of the world, and these are readily available in India and used traditionally for ethnomedicine purposes. Extensive literature studies suggested that the various parts of these plants are reported to possess anti-microbial, anti-cancer, anti-diabetic, anti-fertility, hepato-protective, wound healing, anti-oxidant, anti-coagulant and sedative activities [1, 2, 3, 4, 5, 6, 7].

Medicinal properties of the plant are generally attributed to the various bioactive compounds they harbor. Therefore, the need arises to detect and quantify important phytomolecules [8, 9].

The present course of study aims at phytochemical analysis, and deciphering their anti-oxidant activity of these medicinal herbs was done and it is compared with each other. The research investigation was designed to detect and estimate the phytochemical compounds, anti-oxidant activities of acetone extract from the leaves of these plants and correlate with each other.

MATERIALS AND METHODS

Chemicals and Reagents

Analytical (AR) grade chemicals and reagents were used in the experiments. Aluminum chloride, ascorbic acid, and Folin-ciocalteu reagent were obtained from Merck Life Science Private Limited, Mumbai. Sodium nitrite and sodium carbonate were purchased from RFCL Limited, New Delhi. Gallic acid, sodium hydroxide, and hydrogen peroxide were purchased from SD Fine-Chem Limited, Mumbai. Quercetin, DPPH, tannic acid, acetone was obtained from Sisco Research Laboratories Pvt. Ltd.,

Maharashtra, dextrose from Finar limited, Ahmedabad and ABTS from Tokyo Chemical Industry Co. Ltd., Japan.

Collection and Extraction

Fresh leaves of the five plants were collected from surroundings of our University in Salt Lake City, Kolkata, and West Bengal, India and authenticated by Botanical Survey of India, and West Bengal, India. Before shade drying, the leaves were cleaned with distilled water. Leaves made powdered and were weighed and extracted using mortar and pestle by solvent acetone, 50 ml each for 1 g of powder. The solution of the extract was then stored at 4°C and appropriately diluted for further studies.

Extractive Value

The extractive value was determined by the standard method with slight modification using acetone as a solvent [10].

Qualitative Study

Test for Anti-oxidants

To detect the presence of anti-oxidants (rapid screening by dot plot assay) [11], alkaloids (Wagner and Mayer test) [12, 13, 14], flavanoids (alkaline reagent test) [15], volatile oil [16, 17], betacyanin [18], anthocyanin [18], reducing sugars [12, 16], polyphenols (Fecl3 and KMnO4 method) [13, 19], carbohydrate (Molisch's test) [14], steroids (Liebermann-Burchard test) [20], terpenoids (Salkowski's test) [14], amino acids (Ninhydrin test) [21], protein (biuret test) [22], coumarin [23], saponins (Foam test) [24], phlobatannins [18], anthraquinone [25], tannins (FeCl3 test) [16], acids [26], oxalate [20], cardiac glycosides (Keller-Kelliani's test) [20], flavones aglycones [25], fatty acids or fixed oils [27], quinone [28], ketones [29], pentoses [29], resin [12, 30], gums and mucilage [31], xanthoprotein [23], and catechin [23, 32] standard method was used with slight modifications.

Quantitative Assays

Estimation of Total Polyphenols

The total polyphenolic contents were determined by using the Folin-Ciocalteu method with slight modification. Gallic acid was used as a standard. The absorbance was read at 765 nm. The content of polyphenolic compounds was expressed as mg Gallic acid equivalent/g dry tissue [33].

Estimation of Total Flavonoids

Aluminium chloride colorimetric assay was used to determine the total flavonoid content. Quercetin was used as a standard. The OD was recorded at wavelength 510 nm. The unit, mg Quercetin equivalent/g dry tissue was used to express the total flavonoid content [34].

Estimation of Total Tannins

The total tannin content was evaluated using Broadhurst and Jones (1978) method. Tannic acid used as a standard. The absorbance was read at 500 nm. The total tannin content was expressed in terms of mg Tannic acid equivalent/g dry tissue [35].

Estimation of Total Polysaccharides

To estimate the polysaccharide content phenol-H2SO4 method was used. The absorbance was read at 488 nm. Dextrose was used as a standard. The total polysaccharides content was expressed as mg Dextrose equivalent/g dry tissue [36].

Evaluation of Anti-oxidant Activity

ABTS Radical Cation Assay

A slightly modified ABTS radical cation method was used to study the free radical scavenging activity of plant extracts by using a standard protocol. Absorbance was read at 734 nm. Methanol was used as a blank. Ascorbic acid was used as a standard. ABTS radical scavenging activity was expressed in terms of Ascorbic acid equivalent (AAE), as percentage inhibition calculated by the formula: [37]

% Inhibition of ABTS= (OD of control – OD of the sample)/ OD of control*100.

DPPH Radical Scavenging Assay

The free radical scavenging activity was studied by using DPPH by the method of Shen et al. 2010 with slight modifications. OD was recorded at wavelength 517 nm against methanol as blank. Ascorbic acid was used as a standard. DPPH scavenging activity was expressed in terms of Ascorbic acid equivalent (AAE), as the percentage of inhibition calculated by the following formula: [38]

% Inhibition of DPPH= (OD of control – OD of the sample)/ OD of control*100.

H_2O_2 Radical Scavenging Assay

Hydrogen peroxide scavenging ability was measured according to the method of Ruch et al. with slight modification. The absorbance was taken at 230 nm, against phosphate buffer as blank. Gallic acid was used as a standard. H_2O_2 radical scavenging activity was expressed in terms of Gallic acid equivalent (GAE), as percentage inhibition calculated by the formula: [39]

% Inhibition of H_2O_2= (OD of control – OD of the sample)/ OD of control*100.

Statistical Analysis

All the experimental measurements were performed in triplicate and expressed as the average ± standard deviations. The magnitude of the correlation coefficient between two variables, means, standard errors, standard deviations, and one way ANOVA were calculated by using MS Excel Software. $p>0.05$ was categorized as non significant data.

RESULTS AND DISCUSSION

Extractive Value

The present study have showed that the extractive value of EH was maximum (11.88 ± 0.21%) as compared to other plant extracts. The HI showed lowest extractive value (6.01 ± 0.08%). Measurements of extractive value determine the amount of the bioactive constituents in a given amount of plant material when extracted with solvent (Table 1) [10].

TABLE 1: EXTRACTIVE VALUE (%)

Plant Name	Color	EV (%) (Mean±S.D)
HI	Deep Green	6.01±0.08
TP	Light Green	7.42±0.10
CR	Yellowish Green	11.06±0.28
CB	Green	9.22±0.34
EH	Brownish Green	11.88±0.21

TABLE 2: RESULTS OF QUALITATIVE ASSAY

Test Name	Plant Name					Test Name	Plant Name				
	HI	CR	CB	EH	TP		HI	CR	CB	EH	TP
Anti-oxidants	+	+	+	+	+	Phlobatannins	+	+	+	-	-
Alkaloids	+	+	+	+	+	Anthraquinone	-	-	-	+	-
Flavonoids	+	+	+	+	+	Tannin	-	-	-	+	-
Volatile Oil	-	-	-	-	-	Acids	-	+	-	-	-
Betacyanin	-	-	+	+	-	Oxalates	+	+	-	-	-
Anthocyanin	+	+	-	-	-	Cardiac Glycosides	-	+	-	+	-
Reducing Sugar	-	+	-	+	-	Flavones Glycones	+	-	+	-	-
Polyphenols	+	+	+	+	+	Fixed Oil & Fat	-	-	-	-	+
Carbohydrate	-	+	+	-	-	Quinones	+	+	+	+	+
Steroids	-	-	-	+	-	Ketones	+	+	+	-	+
Amino Acid	-	-	+	+	-	Pentoses	-	-	-	+	+
Protein	+	-	+	+	-	Resin	-	-	-	-	-
Coumarin	-	-	+	+	-	Gums & Mucilage	-	-	+	-	-
Terpenoids	+	-	+	-	-	Xanthoprotein	+	+	+	+	-
Saponin	-	+	-	+	+	Catechin/ Catechol	-	-	-	+	-

Where, "+" present, "-" absent.

TABLE 3: CORRELATION BETWEEN STABLE FREE RADICAL ASSAYS AND BIOACTIVE COMPONENTS

Correlation Parameters	Correlation Equation	Correlation Status
Polyphenols & DPPH Assay	$Y=0.146x+31.9$; $R^2=0.935$	Strongly Correlated
Polyphenols & H_2O_2 Assay	$Y=0.119x+37.98$; $R^2=0.588$	Strongly Correlated
Polyphenols & ABTS Assay	$Y=0.350x-6.271$; $R^2=0.893$	Strongly Correlated
Flavonoids & DPPH Assay	$Y=0.393x+34.92$; $R^2=0.945$	Strongly Correlated
Flavonoids & H_2O_2 Assay	$Y=0.324x+40.86$; $R^2=0.609$	Strongly Correlated
Flavonoids & ABTS Assay	$Y=0.961x+1.730$; $R^2=0.944$	Strongly Correlated
Tannin & DPPH Assay	$Y=0.753x+42.75$; $R^2=0.774$	Strongly Correlated
Tannin & H_2O_2 Assay	$Y=0.641x+47.07$; $R^2=0.533$	Strongly Correlated
Tannin & ABTS Assay	$Y=1.997x+19.04$; $R^2=0.913$	Strongly Correlated

Qualitative Assay

Results obtained from qualitative phytochemical screening of leaf acetone extracts of the weeds under study e.g., HI, TP, CR, CB, and EH is presented in Table 2. A total of 30 tests were performed for the detection of various phytomolecules. Among them, 5 of them were present in all the plant extract. These were anti-oxidants, alkaloids, flavonoids, polyphenols, and quinones. Two phytochemicals were absent in all the plants extracts. These were volatile oil and resin. In EH highest amount of phytochemicals were detected. The results indicate that the experimental medicinal weeds have excellent prospects as a source of therapeutically, pharmaceutically and nutritionally important bioactive compounds. Therefore, Estimation of these phytomolecules becomes important [9].

Quantitative Assay

The total polyphenolic content for these medicinal weeds leaf acetone extracts was quantified. The highest amount of polyphenolic compounds was showed in EH extract, and the lowest amount was observed in TP, and it is 191.28±2.42 mg GAE/g dry tissue and 110.98±2.52 mg GAE/g dry tissue, respectively (Figure 1). The p-value<0.05, which showed the significant presence of polyphenols in plant extracts. These findings support previous investigations as well. Polyphenolic compounds are important reactive species

towards oxidation, and it regulates human physiological activity. The oxidation process and free radicals generation lead to diabetes, inflammations, cancer and other related diseases. The activity of polyphenols against oxidative stress-related processes can have therapeutic application in pharmaceutical and nutritional industry. Plants having more polyphenolic content showed significant anti-oxidant activities indicating a linear strong correlation (Table 3) between total polyphenolic content and anti-oxidant activity [40, 41].

The total flavonoids content of leaf acetone extracts was quantified. The highest amount of flavonoids content was showed in EH, and the lowest amount was observed in TP, and it is 60.80±1.22 mg QE/g dry tissue and 31.15±1.47 mg QE/g dry tissue, respectively (Figure 2). The p-value<0.05, which showed the significant presence of flavonoids in plant extracts and findings agree with the previous studies as well. Flavonoids are the class of plant secondary metabolites with prominent anti-oxidant and chelating activities. Anti-oxidant properties of flavonoids depend on the structure and substitution way of hydroxyl groups. The anti-oxidative activities of flavonoids compounds are due to various mechanisms, like scavenging of free radicals, chelation of metal ions (e.g., iron and copper), and inhibition of enzymes which are responsible for a free-radical generation. Depending on their specific structure, flavonoids compounds can inhibit all the possible ROS. Plants having more flavonoids content showed significant anti-oxidant activities indicating a clear strong correlation (Table 3) between total flavonoids content and anti-oxidant activity [40, 42].

The total tannin content of leaf acetone extracts was estimated. The highest amount of tannin content was showed in EH, and the lowest amount was observed in TP, and it is 17.84±0.23 mg TAE/g dry tissue and 5.91±0.07 mg TAE/g dry tissue, respectively (Figure 3). The p-value<0.05, which showed the significant level of presence of tannins in plant extracts. Tannins are generally found in stem and barks of many plants rather than leaves. The higher amount of tannins shows the presence of the potent anti-oxidant, anti-microbial and anti-cancer properties. Tannin is an astringent, bitter plant polymeric compound capable of tanning leather and it precipitates proteins and many other organic substances including amino acids, alkaloids, and nitrogenous substances. The tannin-protein complex can provide persistent anti-oxidant and anti-microbial activity. In the current study, there is a clear strong correlation (Table 3) showed between tannins and anti-oxidant properties [25, 40, 43, 44].

The total polyphenol, flavonoid, and tannin contents are strongly correlated (Table 4) among each other, which signifies a right amount of bioactive components presence the plant samples.

The total polysaccharides content of HI, TP, CR, CB, and EH leaf acetone extract was estimated. The highest amount of polysaccharides content was showed in EH, and the lowest amount was observed in TP, and it is 206.73±1.37 mg DE/g dry tissue and 126.03±1.19 mg DE/g dry tissue, respectively (Figure 4). The p-value<0.05, which showed the significant level of presence of polysaccharides in plant extracts. Polysaccharide exhibits binding, suspending, emulsifying, thickening, stabilizing and water-holding capacities and it can be used for the preparation of pharmaceutical products in the form of tablets, syrups, lotions and for sustained drug release mechanisms. The use of polysaccharides is increased in the preparation of various pharmaceutical drug forms because they are economical, readily available, non-toxic, and capable of chemical modifications and biodegradable. Maximum research on natural polymers in drug delivery mechanisms centered on polysaccharides is also observed [36].

TABLE 4: CORRELATION BETWEEN BIOACTIVE COMPONENTS

Correlation Parameters	Correlation Equation	Correlation Status
Polyphenols & Flavonoids	Y=0.371x-9.308 ; R^2=0.984	Strongly Correlated
Polyphenols & Tannin	Y=0.435x-6.753 ; R^2=0.845	Strongly Correlated
Flavonoids & Tannin	Y=0.152x-9.598 ; R^2=0.743	Strongly Correlated

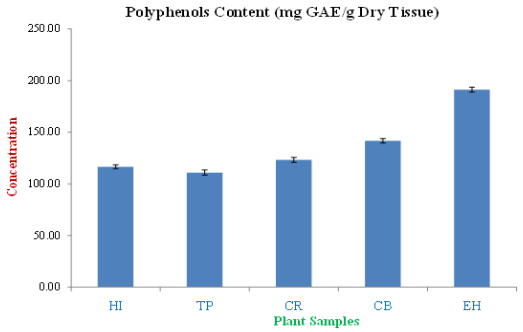

FIGURE 1: POLYPHENOL CONTENT (mg GAE/g DRY TISSUE)

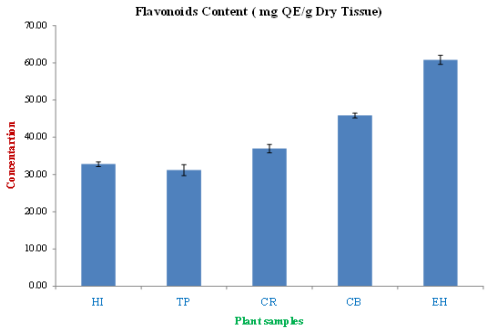

FIGURE 2: FLAVONOIDS CONTENT (mg QE/g DRY TISSUE)

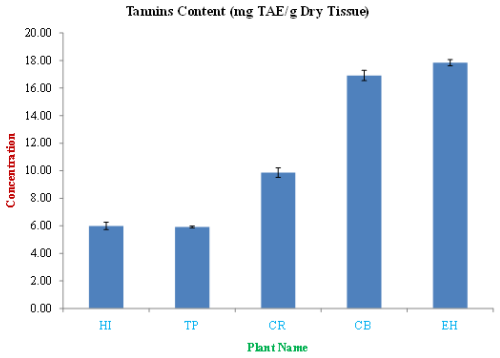

FIGURE 3: TANNIN CONTENT (mg TAE/g DRY TISSUE)

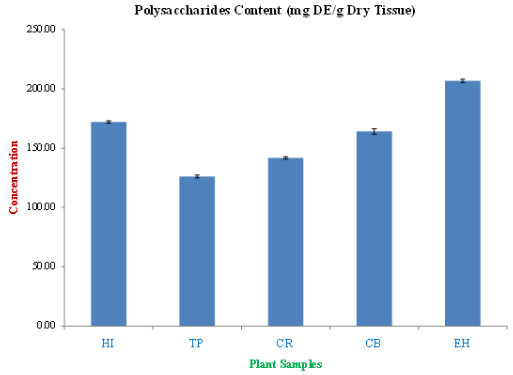

FIGURE 4: POLYSACCHARIDES CONTENT (mg DE/g DRY TISSUE)

Antioxidant Activity

Inhibition percentages and concentration values for different radical scavenging assays were studied in this study. Inhibition concentration is the number of free radicals scavenged in the evaluation of the anti-oxidant property. Bioactive compounds play the role as anti-oxidants by scavenging the free radicals. Three widely used assays have been used to determine the anti-oxidant activity of these medicinal weeds.

ABTS assay is an important free radical scavenging assay and may serve as a significant mechanism for assessing anti-oxidant activity. In the present study the maximum inhibition percentage of ABTS radical scavenging assay was found to be 58.57±0.29 % for EH extract, and the minimum is 31.62±0.68 % for TP extract (Figure 5 and Figure 6), respectively. The p-value<0.05, which showed the significant level of presence of anti-oxidants in plant extracts. It is a radical cation decolorization assay. The ability to scavenge the ABTS cation radical has been compared with ascorbic acid. Potassium persulfate was used to give a stable form of ABTS radical cation. In the current study, there is a strong correlation (Table 3) is found between the bioactive compounds and ABTS cation radical scavenging assay by plant extracts [37, 45, 46].

In the current study the maximum inhibition percentage of DPPH radical scavenging assay was found to be 59.17±0.45 % for EH extract, and the minimum is 46.22±0.91 % for HI extract (Figure 7 and Figure 8), respectively. The p-value<0.05, which showed the significant level of presence of anti-oxidants in plant extracts. DPPH is a stable free radical and is highly utilized to evaluate the radical scavenging activity of anti-oxidant activities. This assay is based on the reduction of DPPH radicals in methanol in the presence of hydrogen–donating anti-oxidant due to the formation of the non-radical shape of DPPH-H. DPPH has the advantage over some factors like it is unaffected by side reactions, such as metal ion chelating and enzyme inhibitions. In the study there is a strong and robust correlation (Table 3) is found between the bioactive compounds and DPPH radical scavenging assay by plant extracts [38, 47].

In the present research investigated the maximum inhibition percentage of H_2O_2 radical scavenging assay was found to be 60.17±0.26% for EH extract and the minimum is 46.91±0.41% for TP extract (Figure 9 and Figure 10), respectively. The p-value<0.05, which showed the significant level of presence of anti-oxidants in plant extracts. H_2O_2 is not very reactive. However, sometimes it is toxic to the cell because it is rapidly decomposed into oxygen and water and gives rise to hydroxyl radical to the cells that can happen lipid per-oxidation and cause DNA damage. So, H_2O_2 removal is necessary for the protection of food. Natural anti-oxidants scavenge hydroxyl radicals. H_2O_2 radical scavenging activity is correlated to the presence of total polyphenol and tannin content. In the present study there is a linear strong correlation (Table 3) is found between the bioactive compounds and H_2O_2 radical scavenging assay by these five medicinal plant extracts [8, 9, 39, 47, 48].

The stable free radicals are well correlated (Table 5) with each other, which signify the availability of anti-oxidants in the leaves of the investigated plant samples. It is concluded that the polyphenolic and other bioactive compounds appear to be responsible for the significant anti-oxidant property of the extracts [9, 11].

TABLE 5: CORRELATION BETWEEN FREE RADICAL SCAVENGING ASSAYS

Correlation Parameters	Correlation Equation	Correlation Status
ABTS & DPPH Assay	Y=0.376x+35.61 ; R^2=0.843	Strongly Correlated
ABTS & H_2O_2 Assay	Y=0.287x+42.35 ; R^2=0.468	Moderately Correlated
DPPH & H_2O_2 Assay	Y=0.746x+16.08 ; R^2=0.528	Strongly Correlated

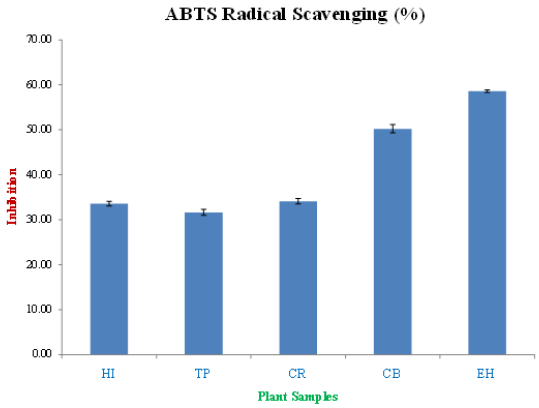

FIGURE 5: ABTS RADICAL SCAVENGING (%)

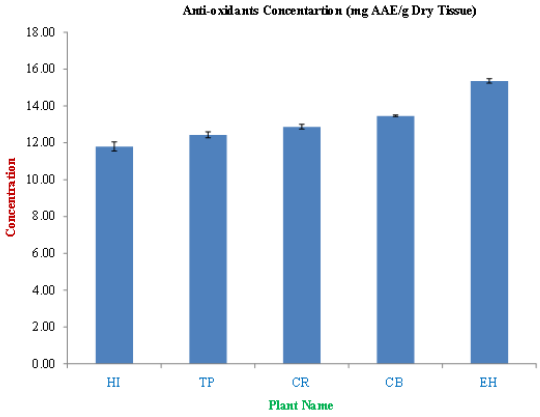

FIGURE 8: DPPH INHIBITION CONCENTRATION (mg AAE/g DRY TISSUE)

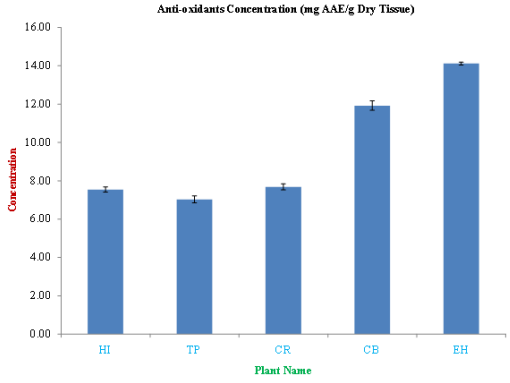

FIGURE 6: ABTS INHIBITION CONCENTRATION (mg AAE/g DRY TISSUE)

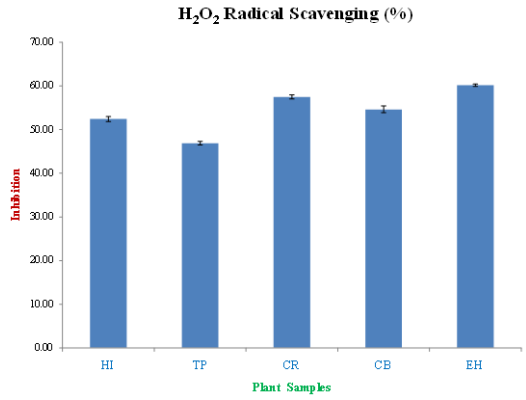

FIGURE 9: H_2O_2 RADICAL SCAVENGING (%)

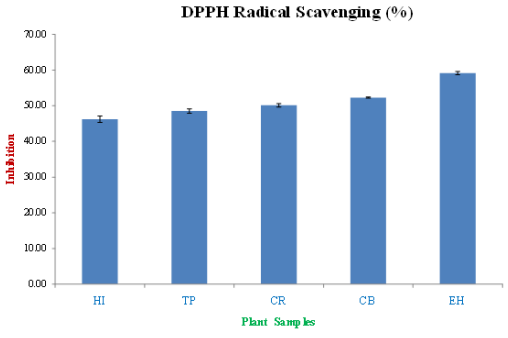

FIGURE 7: DPPH RADICAL SCAVENVING (%)

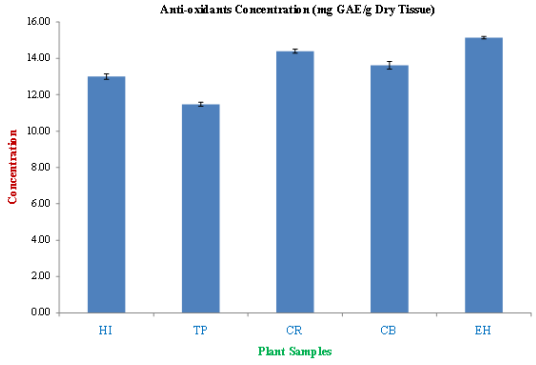

FIGURE 10: H_2O_2 INHIBITION CONCENTRATION (mg GAE/g DRY TISSUE)

CONCLUSION

In the current investigation, the leaf acetone extracts of HI, TP, CR, CB, and EH were found to be rich in secondary metabolites and that possess a significant amount of phytochemicals and anti-oxidant properties. The results concluded the higher content of phytochemical constituents such as polyphenols, tannins, flavonoids and polysaccharides, and higher anti-oxidant activity for ABTS, DPPH and H_2O_2 radical scavenging assay in leaf acetone extracts. The anti-oxidant activities may be attributed to their free radical scavenging properties. The findings of the study support the fact that traditionally used medicinal plants are the primary source of therapeutically used anti-oxidants [48]. Among these five medicinal weeds, EH has highest bioactive component as well as the highest anti-oxidant properties which agree with the previous study of Abu AB et al. 2011 [49]. The reasonable difference between the results of phytochemicals content is may be due to environmental, ecological or biogeochemical factors like pollution, maturity period, climate, rainfalls, location, temperature, precipitation, solar reflectance, fertility, diseases, and pest exposure etc [46, 50, 51, 52]. According to the results obtained in the study, the leaf of these five traditionally used herbaceous medicinal weed is the primary sources of natural anti-oxidants and bioactive molecules, and that could be used as a lead compound to prepare the drug for pharmaceutical and nutritional industry against several diseases and which agrees with the previous studies as well [53, 54, 55].

Acknolwedgement

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Abbreviations

HI= *Heliotropium indicum;* TP= *Tridax procumbens*; CR= *Cleome rutidosperma; CB= Commelina benghalensis;* EH= *Euphorbia hirta;* GAE=Gallic Acid Equivalent; QE=Quercetin Equivalent; TAE=Tannic Acid Equivalent; DE=Dextrose Equivalent; AAE=Ascorbic Acid Equivalent; ROS: Reactive Oxygen Species; OD=Optical Density; ABTS=2, 2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid; DPPH= 1, 1-diphenyl-2-picrylhydrazyl; H_2O_2= Hydrogen Peroxide.

Conflict Of Interest

The author declares no conflict of interest.

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