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## EVALUATION OF ORGANOLEPTIC, PROXIMATE PARAMETERS AND ANALYSIS OF NUTRITIONAL COMPOSITION OF FIVE WILD WEEDS: A SEARCH FOR LOW-COST NUTRACEUTICALS

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### Keywords:

*Heliotropium indicum, Tridax procumbens, Cleome rutidosperma, Commelina benghalensis, Euphorbia hirta*, FT-IR, Nutraceuticals, AAS

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**ABSTRACT:** Medicinal plants are the predominant source of phytochemicals and nutraceuticals, and it is used for the treatment of many diseases for its high effectiveness, low cost, and wide availability. In the current research study five, commonly used ethnomedicinal weeds of West Bengal, and India namely; *Heliotropium indicum, Tridax procumbens, Cleome rutidosperma, Commelina benghalensis*, and *Euphorbia hirta* were used for analyzing their nutritional potentiality measurements. Different solvents extracts of leaves of the plants were used for detecting and quantifying the essential phytochemicals and nutraceuticals. For the quantitative study, UV-Vis spectrophotometer and titration method was used. Mineral content analysis was done by atomic absorption spectrophotometer followed by acid digestion method. To the best of our knowledge, the comparative evaluations of organoleptic, proximate characteristics, and nutritional components of these herbs are reported first. FT-IR study of leaves aqueous extracts reveals different types of functional groups. Among the studied plants, EH leaves extracts showed the highest amount of presence of polysaccharides, amino acids, ascorbic acids, thiamine, and riboflavin. CR leaves extracts showed the maximum amount of anthocyanin and lipid presence. All the leaves showed a good amount of mineral presence. The investigation concluded that *Euphorbia hirta* possesses significant content of phytochemicals and nutraceuticals in comparison with the other four plants.

**INTRODUCTION:** The wild medicinal and edible plants have been the primary source of food and medicines for ethnic groups throughout human history. These plants have huge nutritional and medicinal values and importance. The livelihoods of rural or tribal people of underdevelopment and a developing country do not depend only on the agricultural products, but also on natural resources, too.

Functional food is not only used for nutritional purposes but also it can be utilized for the prevention and treatment of various disorders. The functional foods are also termed nutraceuticals (nutrition + pharmaceuticals). It is inexpensive, easy to cook, and huge sources of various kinds of nutrients<sup>1</sup>. All human beings need a number of complex organic substances as added caloric requirements for their regular body activities. Due to the presence of bioactive substances in medicinal plants, they are used for healing different diseases and provide necessary nutrients to human health<sup>2</sup>. As the population growth is increasing and scarcity of conventional foods is arises, as well as due to malnutrition, various disorders are also the regular phenomena of the under develop and developing countries.

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For that reason, alternative food sources would be the most favorable endeavor wherein wild plants getting its priorities<sup>1,3</sup>.

A nutritional analysis is necessary to identify the new source of bioactive substances having prosperous medicinal significance and to make the positive use of available natural resources. Among the huge phytodiversity of India, we have chosen five wild medicinal weeds, and those are very less characterized and available with reported various biological activities, and sometimes these herbs leaves are traditionally used as food as well<sup>4,5,6</sup>.

*Heliotropium indicum* Linn. (Family: Boraginaceae) is an annual herb and commonly known as Indian heliotrope, and it is native to India<sup>7</sup>. *Tridax procumbens* Linn. (Family: Asteraceae) is annual herb and commonly known as coat buttons; and it is native to tropical America<sup>8</sup>. *Cleome rutidosperma* DC. (Family: Cleomaceae) is an annual herbaceous weed and commonly known as Fringed Spider Flower; it is native to Tropical Africa<sup>9</sup>. *Commelina benghalensis* Linn. (Family: Commelinaceae) is an annual herbaceous weed native to tropical Asia and Africa, commonly known as Bengal dayflower<sup>10</sup>. *Euphorbia hirta* Linn. (Family: Euphorbiaceae) is an annual herb, commonly known as an asthma plant, and it is native to India and Central America<sup>11</sup>.

The studied weeds are growing in almost similar types of habitat though they belong to different families. A huge number of plants are accepted as weeds, but those are grown in gardens and other settled areas; these are termed as beneficial weeds<sup>4-6</sup>. The current study focuses on searching for those widely available medicinal weeds which have enormous future prospects for pharmaceutical and nutritional industries.

The dietary excellence of the wild plants can be determined by proximate, phytochemical and nutritional compositional assessments<sup>2,3</sup>. Therefore, the need arises to detect and estimate the phytochemical and nutritional components of these five medicinal plant leaves, which are ethnomedicinally used for curing several physiological disorders and complications in different parts of the world. The current study was designed to analyze the organoleptic and proximate

characteristics of the five wild weeds and to detect and estimate the phytochemical and nutraceutical constituents from the leaves extracts of these medicinal plants through several biochemical assays by using standard methods, and it is compared with each other.

## MATERIALS AND METHODS:

**Collection, Identification, and Extraction:** The five medicinal weeds were collected from Salt Lake City, Kolkata, West Bengal, and India. Then plants were authenticated (specimen no. PG-01, PG-02, PG-03, PG-04 and PG-05 dated 21-01-2019) by the Botanical Survey of India, West Bengal, and India. The leaves of the weeds were washed and dried at room temperature for 30 days under shade. Then dried leaves were made powdered and extracted by different solvents for various biochemical assays.

**Chemicals and Reagents:** The chemicals and reagents utilized in the experiments were of analytical (AR) grade. Ascorbic acid, chloroform, methanol, ethanol, hydrochloric acid and sulphuric acid were purchased from Merck (Mumbai, India). Bovine serum albumin was purchased from HiMedia (Mumbai, India). Sodium hydroxide was obtained from SD Fine-Chem (Mumbai, India). Riboflavin, thiamine, and Bradford reagent was obtained from SRL (Maharashtra, India), Dextrose from Finar Ltd. (Ahmadabad, India). Phenol from Sigma-Aldrich Co. (St. Louis, USA) and Lysine from Lobal Chemie (Mumbai, India). For quantitative assays, Systronics 117 model spectrophotometer was used to determine the specific optical density.

**Organoleptic Characteristics:** To observe the organoleptic parameters of the leaf samples, standard methods were used<sup>12,13</sup>.

## Proximate Analysis:

**Determination of Extractive Values:** Standard protocol by Khandelwal, KR (2002) was used to determine the extractive values (EV). The percent extractive value was calculated using the following formula:<sup>14</sup>

Extractive value (%) = Weight of dried extract/ Weight of plant material × 100

**Determination of Total Moisture Content:** To evaluate the total moisture content (MC) of the leaves, the standard method was used. From the following formula, the total moisture content of the leaves was calculated<sup>15, 16</sup>.

$$MC_{wb} = W_i - W_f / W_i \times 100$$

Where MC = Moisture Content,  $W_i$  = Initial weight of Samples,  $W_f$  = Final weight of samples

**Determination of Relative Water Content:** The Relative water content (RWC) of the leaves was determined with the standard method of Singh (1997). The RWC is calculated as<sup>17</sup>

$$RWC = (FW-DW) / (TW-DW) \times 100$$

Where, FW = Fresh weight, TW = Turgid weight, DW = Dry weight

**Determination of pH of the Leaves Extracts:** To determine the pH of the leaves aqueous extracts standard method of Aremu *et al.*, 2010 was used with proper adjustments and modifications<sup>18</sup>.

**Determination of Conductivity of the Extracts:** 1 g of the fresh leaves was pestle in 10 ml double distilled water. This was filtered, and the conductivity of the leaf extract determined after calibrating the conductivity meter with a buffer solution of pH 7 and double distilled water at the 25 °C temperature.

**Determination of Aqueous Solubility and Insolubility of Leaves:** 100 mg of fresh plant materials were homogenized with double distilled water and filtered. Before that weight of the filter paper ( $W_1$ ) was taken. After filtration, again the weight of the filter paper ( $W_2$ ) was taken. Water-soluble and insoluble part of the leaves was determined using the following formula:

$$\text{Insoluble portion } (W_i) = W_2 - W_1$$

$$\text{Soluble portion } (W_s) = 100 - W_i$$

Where,  $W_s$  = soluble portion,  $W_i$  = insoluble portion

**Phytochemical Screening:** For qualitative assays, aqueous and 70% ethanolic extracts of the leaves were used. To detect antioxidants<sup>19</sup>, alkaloids<sup>19, 20</sup>, flavonoids, betacyanin, carbohydrate, terpenoids, steroids, amino acids, protein, saponins, phlobatannins, oxalate, cardiac glycosides, fatty

acids or fixed oils, quinine<sup>20</sup>, volatile oil, tannins, resin, reducing sugars<sup>21</sup>, anthocyanin<sup>22</sup>, polyphenols<sup>19, 22</sup>, coumarin, xanthoprotein, catechin<sup>23</sup>, anthraquinone, flavones aglycones<sup>24</sup>, acids<sup>25</sup>, ketones, pentoses<sup>26</sup> and gums and mucilage<sup>27</sup> standard method was used with necessary modifications.

**UV-Vis Spectroscopic Screening:** For UV-Vis absorption spectrophotometer peak analysis of the aqueous and 70% ethanolic leaves extracts standard protocol was used with proper adjustments. The extracts were centrifuged and filtered and were diluted to a 1:10 ratio. The extracts were scanned in the wavelength ranging from 200-800 nm, and the characteristic peak was recorded<sup>28-30</sup>.

**Fourier Transform Infrared (FT-IR) Spectroscopic Analysis:** FT-IR analysis of the aqueous extracts was done by using FT-IR RX1-Perkin Elmer spectrophotometer system with a transmittance in the range of 4000 to 450  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ , which was used to detect the characteristic peaks and their corresponding functional groups present in the extracts. The peak values were recorded<sup>28-31</sup>.

**Nutritional Analysis:**

**Estimation of Polysaccharides Content:** To estimate the polysaccharides content of aqueous and 70% ethanolic extracts phenol- $\text{H}_2\text{SO}_4$  method was used. Absorbance was read at 488 nm. Dextrose was used as a standard. The polysaccharides content was expressed as mg Dextrose Equivalent/g dry weight<sup>32</sup>.

**Extraction and Estimation of Total Lipid Content:** The total lipid content was estimated by using the standard method of Bligh and Dyer (1959). The total lipids were extracted with chloroform: methanol: water (2:2:1.8 ratio) mixture (10 ml mixture per 1g sample) by cold extraction method. The total lipid content (%) of the sample is calculated by using the following formula and were expressed in mg/100 mg dry weight:<sup>33</sup>

$$\text{Total Lipid Contents (\%)} = (\text{lipid weight in aliquot} \times \text{chloroform layer volume}) / \text{aliquot volume}$$

**Extraction and Estimation of Total Protein Content:** The total protein content was measured by the Bradford method using bovine serum albumin as a standard. Fresh plant samples were

extracted with phosphate buffer (pH 7.4). The samples and reagents were used in 1:1 ratio. Absorbance was taken at 595 nm. Total protein content was expressed as mg Bovine Serum Albumin Equivalent/g fresh weight<sup>34</sup>.

#### **Extraction and Estimation of Amino Acids**

**Content:** The total free amino acid content of 80% ethanolic extracts of the leaves were determined by the method of sircelj *et al.*, (2005). Absorbance was measured at 570 nm. Lysine was used as a standard. Total free amino acid contents were expressed in mg Amino Acid Equivalent/g dry weight<sup>35</sup>.

#### **Extraction and Estimation of Ascorbic Acid**

**Content:** Ascorbic acid content was determined by the iodine titration method with proper adjustments. Vitamin C standard solution was prepared by L-ascorbic acid. Aqueous extracts of the leaves were prepared for estimating the ascorbic acid contents. The calculation was carried out with the help of the equation:  $V_1S_1=V_2S_2$ . The ascorbic acid content was expressed as mg Ascorbic Acid Equivalent (AAE)/g fresh weight<sup>36</sup>.

#### **Extraction and Estimation of Thiamine**

**Content:** Extraction and estimation of thiamine content of ethanolic sodium hydroxide (20%) extracts of the leaves were done by the standard method of Poornima *et al.*, (2009) with adjustments. The absorbance was recorded at 360 nm. The results were expressed as mg Thiamine Equivalent/g fresh weight<sup>37-39</sup>.

#### **Extraction and Estimation of Riboflavin**

**Content:** Extraction and estimation of riboflavin content of 50% ethanolic extracts of the leaves were done by standard method of Poornima *et al.*, (2009) with slight modifications. The absorbance was read at 510 nm. The results were expressed as mg Riboflavin Equivalent/g fresh weight<sup>37-39</sup>.

#### **Extraction and Estimation of Total Anthocyanin**

**Content:** Total anthocyanin content of acidic methanol extracts of the leaves was determined by using the modified protocol of Ruohe Yin *et al.*, (2012). Absorbance was determined at 530 nm and 657 nm. Quantification of anthocyanins was performed by using the following equation:

$$\text{Anthocyanins} = (A_{530} - 0.25 \times A_{657}) \times (M-1)$$

Where,  $A_{530}$  and  $A_{657}$  are the absorbances at the mentioned wavelengths, and M is the weight of the

fresh plant material used for extraction (g). The total anthocyanin content was expressed as mg/g of fresh weight<sup>40</sup>.

**Mineral Analysis:** The standardized dry ashing method was used for mineral composition estimation of the 5 g dry leaf samples. The powdered sample was placed into a crucible for 1 hr in a muffle furnace; the temperature was maintained at 500 °C. Ashing was carried out to destroy all of the organic substances present in the plant samples. The ash was digested with a mixture of HCl and HNO<sub>3</sub> in the ratio 1:3. The digested samples were dissolved in 50 ml of double-distilled water and used for the analysis of mineral elements through atomic absorption spectrophotometer. The mineral contents results are expressed in mg/kg<sup>31</sup>.

**Statistical Analysis:** All the experiments were performed in triplicates (except FT-IR and mineral analysis) and expressed as the average of the three analyses  $\pm$  standard deviations (SD). The means, standard deviations, standard errors, standard curve, and one way ANOVA followed by Bonferroni post hoc test were calculated by using MS Excel 2007 Software (Microsoft Corporation, Redmond, WA, USA). Figures are prepared in Origin Pro 8 Software (Northampton, MA, USA). A P-value <0.05 was considered as statistically significant. In the figures, different lower case letters (a, b, c, d, e, x, y, z, w, v) in the bars indicate significant differences among means (P<0.05).

## **RESULTS:**

**Organoleptic Parameters:** In the present study, different organoleptic characteristics such as shape, size (length and width), odor, color, and texture of the five medicinal weeds leaves have been investigated and represented in **Table 1**.

**Proximate Analysis:** The proximate analysis was investigated and represented in **Table 2**. The current research investigations observed that the extractive value of EH aqueous (15.91 $\pm$ 0.08%) and CR 70% ethanolic (17.23 $\pm$ 0.25%) extracts were maximum as compared to other extracts. The HI aqueous (11.64 $\pm$ 0.10%) and TP 70% ethanolic (8.43 $\pm$ 0.12%) extracts showed the lowest extractive values compared with other extracts. The color of the aqueous and 70% ethanolic extractive residues was dark brown and green, respectively. The moisture content is in between (72.42 $\pm$ 1.39%) to



(84.44±1.11%). The relative water content was observed highest in CB (94.57±1.28%) and lowest in CR (53.08±0.53%). Result highlighted that the pH of all the plant aqueous extracts was acidic, except HI extract was alkaline in nature. Results showed that the highest and lowest pH containing extract is HI (7.52±0.03) and CB (6.60±0.02),

respectively. The highest and lowest conductivity was showed in the EH (8.60±0.04 mS/cm) and TP (5.17±0.03 mS/cm) extracts. The highest and lowest solubility in water medium was shown by the leaf samples of TP (85.33±3.06%), and HI (63.33±1.53%), respectively **Table 2**.

**TABLE 1: ORGANOLEPTIC CHARACTERISTICS OF LEAF SAMPLES**

Plant Name	Shape	Size		Odor	Color	Texture
		Length (cm)	Width (cm)			
<i>Heliotropium indicum</i>	Ovate or Serrulate	4.3-6	1.9-2.9	Stringent	Dark Green	Stiff
<i>Tridax procumbens</i>	Hastate	3.4-6.4	1-5.1	Normal Leafy	Green	Rough
<i>Cleome rutidosperma</i>	Lanceolate or Ovate	1.4-3.4	0.7-1.3	Stringent	Green	Smooth
<i>Commelina benghalensis</i>	Obovate	4.4-7.2	2.6-3.8	Bitter	Light Green	Glabrous
<i>Euphorbia hirta</i>	Lanceolate or Elliptical	2.1-2.7	1.1-1.4	Vegetative	Dark Green	Rough

**TABLE 2: PROXIMATE PARAMETERS ARE SHOWN IN TABLE**

Plant Name	Extractive values		Moisture Content (%)	Relative Water Content (%)	pH Values	Conductivity (mS/cm)	Water Soluble and Insoluble (%)	
	Water	70% Ethanol					Soluble (%)	Insoluble (%)
HI	11.64±0.10	14.38±0.09	72.42±1.39	82.77±0.75	7.52±0.03	5.93±0.71	63.33±1.53	36.67±1.53
TP	14.88±0.11	8.43±0.12	82.80±1.73	76.65±0.24	6.84±0.03	5.17±0.25	85.33±3.06	14.67±3.06
CR	15.47±0.17	17.23±0.25	78.57±1.43	53.08±0.53	6.89±0.02	7.23±0.21	74±1.73	26±1.73
CB	13.74±0.36	9.73±0.08	84.44±1.11	94.57±1.28	6.60±0.02	7.50±0.62	79±1.73	21±1.73
EH	15.91±0.08	12.67±0.07	75.69±1.80	71.91±1.25	6.68±0.03	8.60±0.36	70.33±2.08	29.67±2.08

In a table, ± signifies the standard deviation from mean

**TABLE 3: RESULTS OF QUALITATIVE ASSAYS**

Plant Name	HI		TP		CR		CB		EH	
	W	E	W	E	W	E	W	E	W	E
<b>Solvent Name</b>										
<b>Test Name</b>										
Anti-oxidant	+	+	+	+	+	+	+	+	+	+
Alkaloids	-	+	+	-	-	-	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Volatile Oil	-	+	+	+	+	-	-	-	+	+
Betacyanin	-	-	-	-	+	-	-	-	-	+
Anthocyanin	+	+	+	+	-	+	+	+	+	-
Reducing Sugar	+	-	-	+	+	-	+	-	+	+
Polyphenols	+	+	+	+	+	+	+	+	+	+
Carbohydrate	-	+	-	+	-	+	-	+	-	+
Steroids	-	+	+	+	+	+	+	+	-	+
Terpenoids	-	-	-	-	-	+	-	-	+	+
Amino Acid	+	+	-	+	+	-	+	+	+	+
Protein	+	+	+	+	+	+	+	+	+	+
Coumarin	-	+	-	+	+	-	+	-	+	+
Saponin	-	+	-	+	-	+	+	+	+	+
Phlobatannins	-	+	-	-	+	-	-	-	+	+
Anthraquinone	+	-	+	-	+	-	+	-	+	-
Tannin	-	+	-	-	+	+	-	-	+	+
Acids	-	-	-	-	-	-	-	-	-	-
Oxalates	-	-	-	-	-	-	-	-	-	-
Cardiac Glycosides	-	+	-	+	+	-	+	+	+	+
Flavones Aglycones	-	+	-	-	+	-	-	+	-	+
Fixed Oil & Fatty Acids	-	-	-	-	+	-	-	-	+	+
Quinones	+	-	-	+	+	+	+	+	+	+
Ketones	-	-	+	-	-	-	+	-	+	+
Pentoses	-	-	-	+	-	-	+	-	+	+
Resin	-	-	-	-	-	-	-	-	-	-
Gums & Mucilage	-	-	+	+	+	-	+	+	+	+
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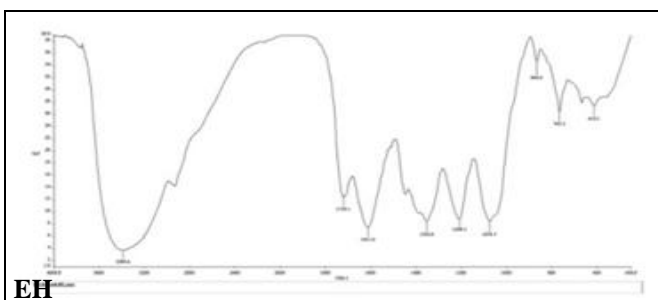
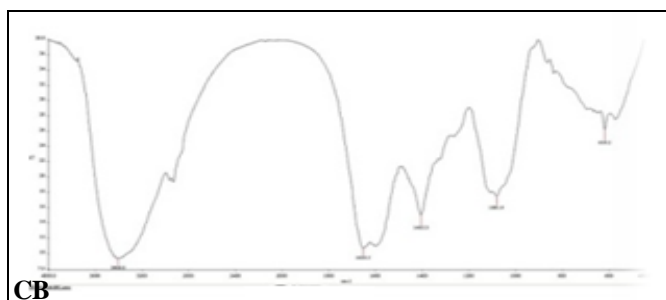
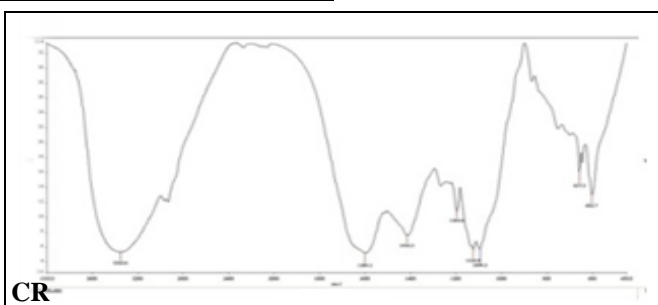
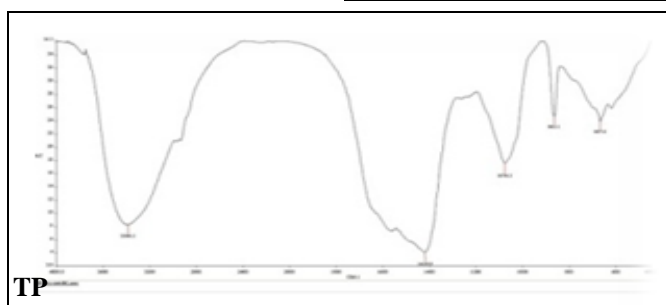
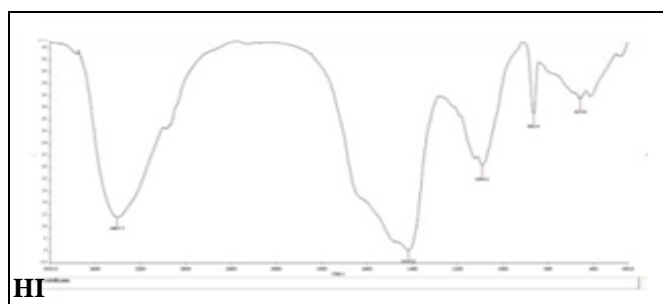
Where, “+” Present, “-” Absent

**Phytochemical Screening:** Results obtained from qualitative assays of leaf aqueous and 70% ethanolic extracts of HI, TP, CR, CB, and EH is presented in **Table 3**. A total of 30 tests were carried out to detect different bioactive constituents (primary and secondary metabolites). Among those, 3 (antioxidants, flavonoids, and polyphenols) were present in all the extracts. Three bioactive compounds (resin, acids, and oxalates) were absent in all the extracts. EH showed the highest amount of phytochemicals presence.

**UV-Vis Absorption Spectrum Profile:** The UV-Vis absorption spectrum profile of aqueous and 70% ethanolic extracts was determined on the basis of sharp peak values and proper baselines. UV spectrum profile of aqueous extracts showed the peaks at the UV range in between 212 nm to 233 nm as well as the 70% ethanolic extracts showed the peaks at UV and Visible range both in between of 219 nm to 672 nm **Table 4**.

**TABLE 4: UV-VIS SPECTRUM PEAK VALUES (nm) AND ABSORBANCE OF EXTRACTS**

Plant Name	Aqueous Extracts		70% Ethanolic Extracts	
	Peak (nm)	Absorbance	Peak (nm)	Absorbance
HI	212.6	2.667	219.8 669.8	2.932 0.243
TP	216.2	2.579	227 671.6	3.000 0.149
CR	227	2.812	228.8 671.6	2.906 0.151
CB	218	2.617	227 669.8	3.000 0.156
EH	232.4	2.714	320.6 228.8 671.6	1.312 2.963 0.059



**FIG. 1: FT-IR SPECTRUM ANALYSIS OF HI, TP, CR, CB, AND EH AQUEOUS EXTRACT.** In the figure, X-axis denotes the transmittance range (cm<sup>-1</sup>), and Y-axis denotes the wavenumbers (%T)

**FT-IR Analysis:** The results of FT-IR analysis of crude leaves aqueous extracts detected various functional groups, and it was given in **Table 5**, and the graphical profile was represented in **Fig. 1**. FT-IR spectrum profile confirmed the presence of different functional groups including alcohols,

phenols, carboxylic acid, alkanes, alkynes, alkyl halides, esters, aldehydes, ketones, polysaccharides, aromatics, nitro compounds, proteins, and amines. The highest number of functional groups present is observed in EH (9), and the lowest number is in TP and HI (5) aqueous extracts, respectively.

**TABLE 5: FT-IR PEAK VALUES (WAVE NUMBERS) AND FUNCTIONAL GROUPS IN AQUEOUS EXTRACTS**

Plant Name	Peaks at (cm <sup>-1</sup> )	Functional Groups
HI	3407.7	Hydroxyl compound, Bonded N-H/C-H/O-H stretching of Amines and Amides
	1419.2	Aromatic ring, Alkane, Carboxylic acids
	1093.1	Secondary alcohol, Alkenes, Alkyl Amine, C-O-C group
	865.4	Alkene, Alkyl halides (C-Cl stretching), Alcohol hydroxyl groups
	657.8	C-S linkage, Aromatic substitution types, Halo compounds
TP	3388.5	Hydroxyl compound, Bonded N-H/C-H/O-H stretching of Amines and Amides
	1419.9	Aromatic ring, Alkane, Carboxylic acids
	1076.5	Primary alcohol, Polysaccharides, Alkenes, Alkyl Amine, C-O-C group
	865.1	Alkene, Alkyl halides (C-Cl stretching), Alcohol hydroxyl groups
	667.6	C-S linkage, Aromatic substitution types, C-Br stretching
CR	3352.6	Alcohol, stretching of Amines and Amides
	1599.5	Diketones
	1416.2	Aromatic ring, Alkane, Carboxylic acids, S=O sulphate
	1195.6	C-O group, Ester carbonyl, Alkyl amine, Proteins
	1124.9	C-O-C group, Alkyl amine, Proteins
	1094.2	Secondary alcohol, Alkenes, Alkyl Amine, C-O-C group, Aliphatic ether
	657.2	C-S linkage, Aromatic substitution types, Halo compounds
	602.7	C-S linkage, Halo compound, P-S stretching
	CB	3406.0
1653.5		Quinolines, C=C ring skeletal stretching, Aromatic ketones, Amide, Primary Amine
1405.3		Aromatic ring, Alkane, Carboxylic acids
1081.9		Primary and Secondary alcohol, Aldehyde, Aliphatic Ether, Polysaccharides
618.2		Halo compounds (C-Br/ C-I), C-S linkage, Alkene, P-S stretching
EH	3389.6	Hydroxyl compound, Bonded N-H/C-H/O-H stretching of Amines and Amides, Alcohol
	1719.1	Ketones (C=O stretching vibration of carbonyl), Aldehyde
	1611.6	Conjugated alkene (C=C) ring skeletal stretching vibration of aromatic, $\alpha$ , $\beta$ unsaturated ketone or Diketones, Amine
	1352.0	C-O (Carbonyl group), Isopropyl group, methyl group
	1208.5	Alkyl Ketone, Alkyl amine, Ester carbonyl, Carboxylic acid, P=O phosphonate
	1074.7	Alkyl amine, Polysaccharides, Primary alcohol
	866.8	Vibration of C-O in alcohol hydroxyl group, C-Cl stretching of alkyl halides, Aromatic compounds, Alkene
	765.5	Chloro compound, Aromatic Compounds, N-H wagging
	612.1	Halo compounds (C-Br/ C-I), C-S linkage, P-S stretching

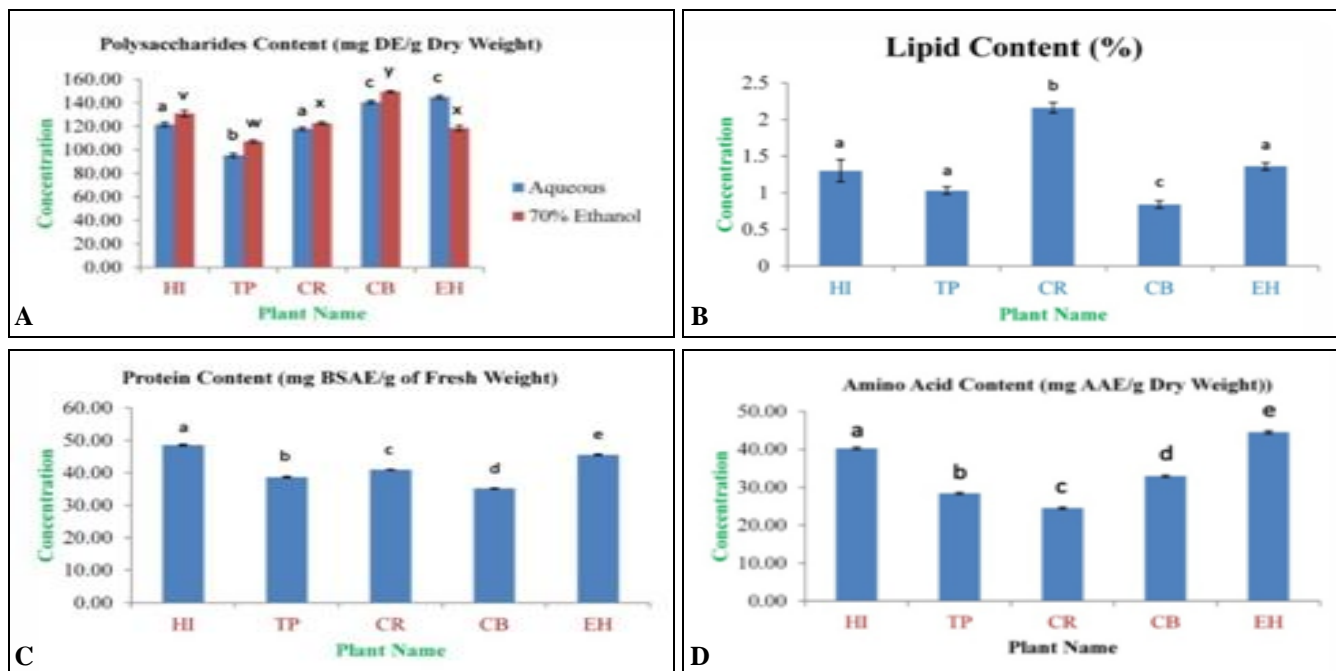
**Nutritional Analysis:** The highest amount of polysaccharides content was showed in EH aqueous and CB 70% ethanolic extracts, it is 145.03±1.39 and 149.50±0.79 mg DE/g dry weight, respectively. The lowest amount was observed in TP aqueous and 70% ethanolic extracts, it is 95.35±1.76 and 107.14±1.24 mg DE/g dry weight, respectively **Fig. 2a**. The highest and lowest amount of lipid contents was showed in CR and CB extracts; it is 2.16±0.07% and 0.84±0.05%, respectively **Fig. 2b**. The highest and lowest amount of protein contents was showed in HI and CB phosphate buffer extract; it is 48.58±0.19 and

35.19±0.15 mg BSAE/g fresh weight, respectively **Fig. 2c**. The highest and lowest amount of free amino acid content was showed in EH and CR 80% ethanolic extracts; it is 44.48±0.42 and 24.50±0.26 mg AAE/g dry weight, respectively **Fig. 2d**.

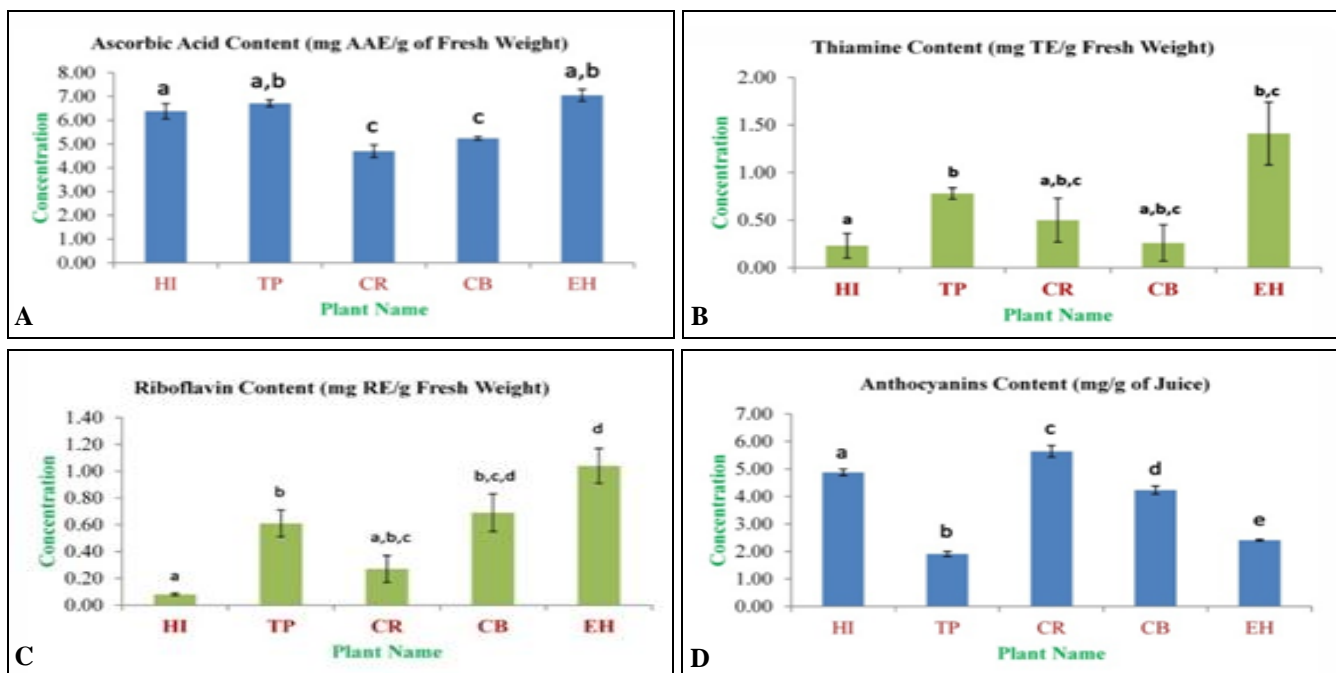
The highest and lowest amount of ascorbic acid content was showed in EH and CR aqueous extracts; it is 7.05±0.25 and 4.70±0.26 mg AAE/g fresh weight, respectively **Fig. 3a**. The maximum and minimum amount of thiamine contents was showed in EH and HI crude 20% ethanolic sodium hydroxide extracts; it is 1.41±0.33 and 0.23±0.13

mg TE/g fresh weight, respectively **Fig. 3b**. The highest and lowest amount of riboflavin contents was showed in EH and HI crude 50% ethanolic extracts; it is  $1.04 \pm 0.13$  and  $0.08 \pm 0.01$  mg RE/g fresh weight, respectively **Fig. 3c**. The highest and

lowest amount of anthocyanins content was showed in CR, and TP acidified methanol extracts; it is  $5.64 \pm 0.21$  and  $1.91 \pm 0.09$  mg/g of juice, respectively **Fig. 3d**.



**FIG. 2:** A. TOTAL POLYSACCHARIDES CONTENT (mg DE/g DRY WEIGHT); B. TOTAL LIPID CONTENTS (%); C. TOTAL PROTEIN CONTENT (mg BSAE/g FRESH WEIGHT); D. TOTAL AMINO ACID CONTENT (mg AAE/g DRY WEIGHT); X-AXIS DENOTES THE PLANT NAME, AND Y-AXIS DENOTES THE CONCENTRATIONS OF NUTRITIONAL COMPONENTS. DIFFERENT LETTERS (a, b, c, d, e, x, y, z, w, v) ABOVE BARS INDICATE SIGNIFICANT DIFFERENCES AMONG MEANS (P<0.05)



**FIG. 3:** A. ASCORBIC ACID CONTENT (mg AAE/g FRESH WEIGHT); B. TOTAL THIAMINE CONTENT (mg TE/g FRESH WEIGHT); C. TOTAL RIBOFLAVIN CONTENT (mg RE/g FRESH WEIGHT); D. TOTAL ANTHOCYANINS CONTENT (mg/g of JUICE); X-AXIS DENOTES THE PLANT NAME AND Y-AXIS DENOTES THE CONCENTRATIONS OF NUTRITIONAL COMPONENTS. DIFFERENT LETTERS (a, b, c, d, e, x, y, z, w, v) ABOVE BARS INDICATE SIGNIFICANT DIFFERENCES AMONG MEANS (P<0.05)



**Mineral Analysis:** In the research study, medicinal weeds leaves were analyzed for eight important minerals such as potassium, sodium, iron, calcium, zinc, copper, boron, and magnesium. The study concludes that the leaves had higher potassium and

calcium presence and a lower amount of iron, copper, and zinc presence, whereas the sodium, magnesium, and boron content are moderate in **Table 6**.

**TABLE 6: AMOUNT OF MINERALS PRESENT IN THE PLANT LEAF SAMPLES (mg/kg)**

Sample Name	Minerals Presence (mg/kg)							
	Ca	Mg	Fe	Cu	Zn	Na	K	B
HI	36143.80	9070.67	169.05	28.10	55.08	388.23	27899.65	910.65
TP	25662.25	4398.54	286.73	32.45	60.37	4942.57	30430.45	1248.20
CR	18577.55	2321.65	254.18	31.05	63.88	2334.83	37649.85	1610.85
CB	26057.43	8462.12	291.08	21.68	69.81	519.67	65225.68	857.56
EH	16598.75	2264.77	181.33	35.65	83.00	200.15	31157.63	1492.15

**DISCUSSION:** Proximate analyses are used to determine the biological or physical parameters of plant materials or extracts such as extractive values, moisture content, relative water content, pH, conductivity, and aqueous solubility and insolubility. These parameters are highly necessary to evaluate the nutritional aspects of the food contents. Measurements of extractive value determine the amount of the bioactive constituents in a given amount of plant material when extracted with solvents<sup>14</sup>. Moisture content is one of the most widely used parameters for the preservation and storage of foods and drugs. Moisture content results suggested that the shelf life of these samples at fresh condition is low and long storage lead to spoilage due to its susceptibility of microbial attacks<sup>15, 16</sup>. Except for HI, all plant leaves pH values are within the range of values that will not affect the germination process; the previously evaluated range was 3.0 to 7.0<sup>41</sup>. Biophysically, the conductivity is the measurement of dissolved plant materials in an aqueous medium; the higher the dissolved phyto-compounds in the aqueous medium, the higher will be the electrical conductivity of that plant sample will be shown<sup>42</sup>. Qualitative screening has shown that these five medicinal plants are the potential source for therapeutically, pharmaceutically, and nutritionally important bioactive compounds<sup>20, 21</sup>.

UV-Vis absorption spectrum study of both the extracts showed a range of variations in wavelengths. The reason behind the variation in wavelengths is due to the surface plasmon resonance of the particle present in the extracts<sup>28-30</sup>.

FT-IR spectrum is a rapid, sensitive, noninvasive, high-resolution analytical screening method for

detecting different kinds of chemical bonds and structures in a particular molecule by giving an IR (infrared) spectrum profile. It has been reported that the FT-IR study can give an informative idea about the metabolic compositional profile of a tissue extract at a particular period<sup>28, 31</sup>. The intense peak at 3407.7, 3406.0, 3389.6, 3388.5, and 3352.6  $\text{cm}^{-1}$ , attributed to O-H stretching (carboxylic acid) vibrations, N-H stretching of amines, amides, and aldehydes C-H stretching. Several absorption bands between 1500.0  $\text{cm}^{-1}$  and 700.00  $\text{cm}^{-1}$  as assigned to amide functional groups. The earlier study had reported the closest amide bands of proteins at peak 1653.5, 1611.6, 1599.5 and 1416.2, 1405.3  $\text{cm}^{-1}$ , which is quite similar to the current investigations as well. A similar identification of such groups has also been reported previously<sup>28, 31</sup>.

Polysaccharides exhibit binding, suspending, emulsifying, thickening, stabilizing, and water-holding properties, and it can be used for the production of pharmaceutical formulations in the form of tablets, syrups, lotions, and for sustained drug release processes. They can easily be oxidized to yield instant energy, polymers act as energy storage molecules, and their derivatives are found in a number of biological molecules including coenzymes and the nucleic acids<sup>32</sup>. Lipid is vital metabolites for normal physiological processes and for healing different biological disorders, and it is the basic nutrient for growth and reproduction<sup>33</sup>. Protein is the essential nutrient and primary metabolite, which is highly essential for physiological functions<sup>34</sup>. Basically, the bio-molecules amino acids are the building blocks of proteins, and it is necessary to cure various physiological disorders<sup>35</sup>.

Ascorbic acids are natural bioactive molecules broadly distributed in plants that have been reported to exert multiple biological effects, including antioxidant activity. Scientists proved that Vitamin C has been important for our immune system<sup>36</sup>. Thiamine is an essential nutrient for human health, and it also has necessary for different oxidative stress-related diseases. Riboflavin is a necessary vitamin for physiological functions, and it also has essential for oxidative phosphorylation<sup>37-39</sup>.

The anthocyanins are water-soluble colored plant pigments found in flower and fruits. Anthocyanin research investigations showed monoamine oxidase inhibitor properties that are connected to the functions in various oxidative stress-related disorders<sup>40, 43</sup>.

Minerals are essential to plant nutrients and directly related to organic substances synthesized by the plants. The presences of these nutritional compounds are very important and essential for the effective functioning of a biological and free radical scavenging system, especially iron, copper, and zinc. Nutrient profile causes increasing interest in such leaves as food ingredients in the form of gluten-free products<sup>3, 16, 31</sup>.

**CONCLUSION:** In the context of present study results, it is concluded that the experimental wild medicinal weeds are the vast sources of nutrients, and it can be used for the healing of various physiological disorders. The leaf extracts of HI, TP, CR, CB, and EH were found to be rich in different types of primary and secondary plant metabolites, which are basically known as phytonutrients. The variations between the presence of the phytochemical may be due to leaves maturity period, fertility, pest exposure, moisture content, relative water content, pH, solubility, solvents polarity, pollution, solar reflectance, rainfalls, precipitation, geographical location and temperature<sup>6, 44-47</sup>.

In conclusion, results suggested that among these five medicinal weeds, EH has shown the maximum phytochemicals, nutraceuticals, and mineral presence, which is in agreement with the earlier studies, too<sup>48, 49</sup>. Another study showed that EH showed significant cytoprotective in aqueous and 70% ethanolic extracts<sup>50</sup>. So in the future,

*Euphorbia hirta* Linn. can be used as an important medicinal weed to isolate and identify the active phytochemicals for the preparation of the Low-cost nutraceutical for therapeutic purposes effectively.

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