FT-IR AND SEM-EDS COMPARATIVE ANALYSIS OF MEDICINAL PLANTS, *ECLIPTA ALBA* HASSK AND *ECLIPTA PROSTRATA* LINN

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Abstract. Infrared and energy dispersive X-ray spectra of plant parts (leaf, stem, and root) of the medicinal plants *Eclipta alba* Hassk and *Eclipta prostrata* Linn were recorded. The vibrational assignments, intensities and wave number (cm^{-1}) of dominant peak were obtained from absorption spectra. Probable assignments of the bands were made with respect to the components present in the samples. The microphotograph obtained from scanning electron microscopy (SEM) and weight percentage of specific elemental concentration obtained from energy dispersive X-ray spectrometer attached to SEM are reported and analyzed sample wise and the results are discussed.

Key words: FT-IR spectra, SEM-EDS, *Eclipta alba* Hassk, *Eclipta prostrata* Linn, plant parts.

INTRODUCTION

Plants have been used in traditional medicine for several thousand years. Medicinal plants as a group comprise approximately 8000 species and account for about 50% of all the higher flowering plant species in India. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In a large number of countries, human population depends on medicinal plants for treating various illnesses as well as a source for livelihood. The World Health Organization (WHO) estimated that 80% of populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. The objective of this study was to identify various chemical groups present in the two

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important medicinal plants *Eclipta alba* Hassk *and Eclipta prostrata* Linn available in the studies area using infrared and Energy Dispersive X-ray spectroscopic techniques. *E. prostrata* Linn can be used as environment friendly and sustainable insecticides to control mosquito [8]. The *E. alba* Hassk of the leaf juice is used as tonic for jaundice and leaf paste is applied on the affected area for toothache [14]. It is also good for the diseases of the spleen, stomatitis, toothache, hemicrania, fever, pain in the liver. It is juice in combination with honey that is administered for catarrh [6]. The present result on anti HIV-I in activity provides support for the beneficial effects of using this plant in AIDS treatment [15].

MATERIALS AND METHODS

Two medicinal plants *Eclipta alba* Hassk and *Eclipta prostrata* Linn were collected from C. Kothangudi village, Annamalai Nagar, Chidambaram, Tamil Nadu, India. The leaf, stem and root parts were carefully collected from the plant. These cleaned plant parts were placed separately in polythene bags. Then, these are shade dried in a clean environment to avoid the contamination for 10 days and oven dried at 60 ºC for four hours, to remove the moisture content. The oven dried plant parts were ground into a fine powder by using an agate mortar and the FT-IR spectra were recorded using BRUKER IFS 66 model FT-IR spectrometer in the region 4000–400 cm^{-1} by employing standard KBr pellet technique. The spectra were recorded at the Indian Institute of Technology (IIT), Chennai, and Tamil Nadu, India. The microphotographs of these samples were recorded using SEM JEOL model, JSE-5610 LV available at centralized instrumentation and service laboratory (CISL), Annamalai University, Tamil Nadu, India, with an accelerating voltage of 20 kV, at high vacuum (HV) mode and secondary electron image (SEI). Typically setting at a magnification at ×1000 (10 μm) for a sample at study. The semi quantification elemental analyses to identify the weight percentage of major and minor elements present in the samples were done using OXFORD INCA energy dispersive X-ray spectrometer (EDS).

RESULTS AND DISCUSSION

The FT-IR and EDS spectra of plant parts like leaf, stem and root of medicinal plants *Eclipta alba* Hassk and *Eclipta prostrata* Linn are shown in Figures 1 and 2. The absorption bands, the wave number (cm^{-1}) of dominant peak obtained from absorption spectra are presented in Table 1. The energy value of each peak is matched with X-ray emission wavelength for non-diffractive analysis and the elements presented in Table 2.

The very strong absorption band observed around 3373–3422 cm^{-1} may be due to the presence of bonded N–H/C–H/O–H stretching of amines and amides [11]. The very strong absorption at 3400 cm^{-1} shows the presence of amino acids in the two plants. The very strong absorption band appearing in the region 2933–2922 cm^{-1} for whole plant parts of *Eclipta alba* Hassk and *Eclipta prostrata* Linn is due to N–H stretching

Vibration of NH_3 group shows the presence of primary amines [13]. The C–H asymmetrical stretching methylene group appears near 2926 cm^{-1}. The bands observed at 2853 cm^{-1} ($root^1$) and at 2852 cm^{-1} ($leaf^1$, $stem^1$) represent C–H symmetric stretching of methylene groups in aliphatic compounds [4, 13].

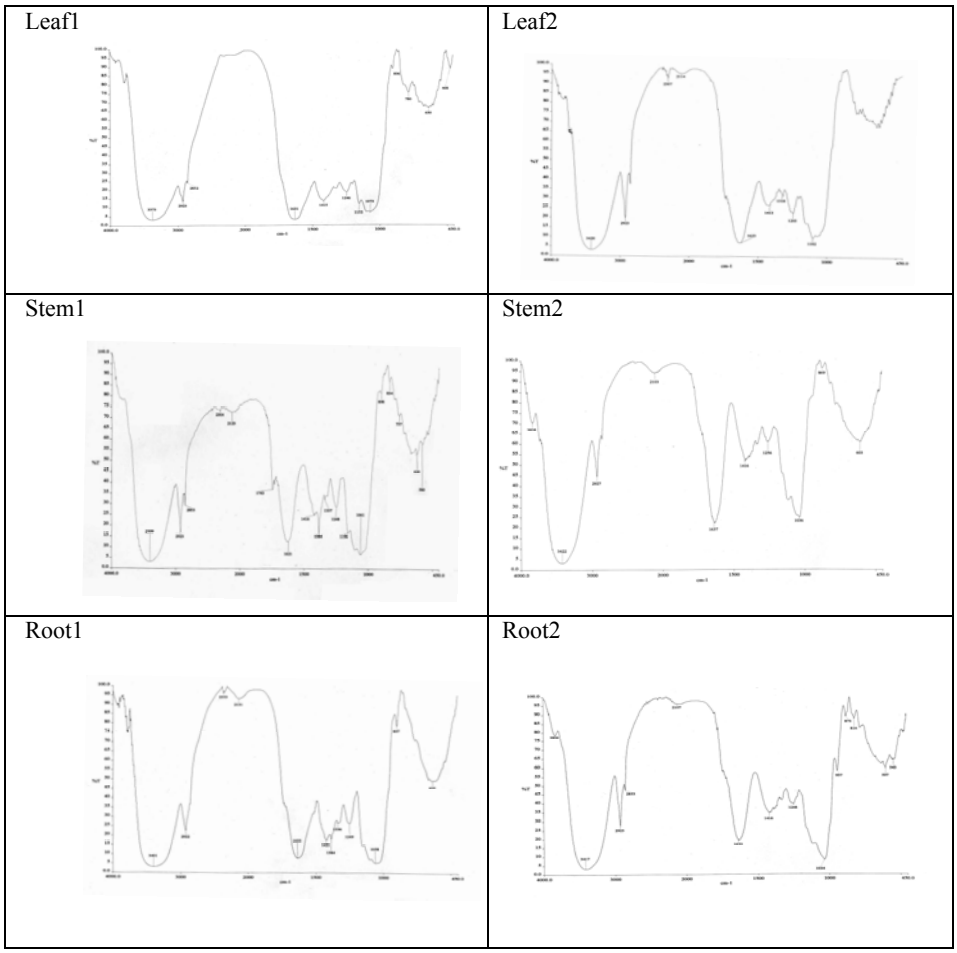

Fig. 1. FT-IR spectrum of vegetative parts of (1) *Eclipta alba* Hassk ($leaf^1$, $stem^1$, $root^1$); (2) *Eclipta prostrata* Linn ($leaf^2$, $stem^2$, $root^2$).

Table 1

Wave number (cm^{-1}) of dominant peak obtained from absorption spectra

Functional groups	*Eclipta alba* Hassk						*Eclipta prostrata* Linn					
	Leaf	V.I	Stem	V.I	Root	V.I	Leaf	V.I	Stem	V.I	Root	V.I
Carboxylic acid												
O–H stretching	2933	s	2933	s	2922	vs	2923	s	2927	M	2925	s
	1246	s	1248	s	1249	s	1243	s	1256	m	1248	s
C–O stretching												
	1415	vs	1416	s	1421	vs	1413	s	1414	m	1416	s
O–H stretching												
Amine												
N–H stretching	3373	vs	3399	vs	3401	vs	3420	vs	3422	vs	3417	vs
N–H bending	1631	vs	1621	vs	1633	vs	1623	vs	1637	vs	1633	vs
C–N stretching	1073	vs	1337	s	1336	s	1318	s	–	–	–	–
Amide												
N–H stretching	3373	vs	3399	vs	3401	vs	3420	vs	3422	vs	3417	vs
C–O stretching	1631	vs	1621	vs	1633	vs	1623	vs	1637	vs	1633	vs
Amino acids												
N–H stretching	2933	s	2933	s	2922	vs	2923	s	2927	M	2925	s
C–O	1415	vs	1416	s	1421	vs	1413	s	1414	m	1416	s
N–H bending	–	–	–	–	–	–	–	–	–	–	–	–
Sulfonyl												
S=O stretching	–	–	1384	vs	1384	vs	–	–	–	–	–	–
Sulfonate												
S=O stretching	–	–	1337	vs	1336	vs	1348	s	–	–	–	–
S–O stretching	896	vw	898	vw	897	w	–	–	–	–	937	m
Polysaccharide												
C–O–C stretching	–	–	1061	vs	1058	vs	–	–	1036	s	1034	vs
Nitrate												
Si–H stretching	896	vw	898	vw	897	w	–	–	869	vw	873	vw
N–H bending	–	–	–	–	–	–	–	–	–	–	816	vw
Chlorate												
C–H stretching	–	–	834	vw	–	–	–	–	–	–	–	–
Carbohydrate												
N–H wagging	780	w	757	w	–	–	–	–	–	–	–	–

V.I. – visible intensity, vs – very strong, s – strong, m – medium, w – weak, vw – very weak.

The lone C–O band due to carboxyl group 2307 cm^{-1} leaf[2], while the C–O band at 2304 cm^{-1} (stem[1]), 2353 cm^{-1} (root[1]) is present as a weak absorption. The weak C≡C stretching band of alkyne molecules normally occurs in the region of 2260–2100 cm^{-1}. In the infrared spectra of monosubstituted alkynes, they appear in the region 2140–2100 cm^{-1} [13]. The corresponding stretching band observed at 2131 cm^{-1} in the case of root[1], 2123 cm^{-1} for stem[1], 2114 cm^{-1} for leaf[2], 2103 cm^{-1} for stem[2] and 2107 cm^{-1} for root[2]. The lone C=O stretching vibration band corresponding to saturated aliphatic ester 1743 cm^{-1} is present only in the stem[1] with a strong absorption.

A symmetrical stretching of NO_2 group results in strong absorption in the region 1660–1625 cm^{-1}. The observed absorption band at1630 cm^{-1} indicates the presence of amines (protein) [7]. This gives the evidence that the plants *E. alba* Hassk and *E. prostrata* Linn are rich in protein. The observed very strong absorption band between 1421 and 1415 cm^{-1} in whole plant parts of *E. alba* Hassk and *E. prostrata* Linn may be due to the presence of bonded C–O/O–H bending. The strong band occurring at 1073 cm^{-1} (leaf[1]) is due to the presence of bonded C–O stretching vibration in secondary alcohol.

The very strong band occurring at 1061 cm^{-1} (stem[1]), 1058 cm^{-1} (root[1]),1034 cm^{-1} (root[2]), and 1036 cm^{-1} (stem[2]) shows the presence of symmetrical C–O–C stretching in vinyl ether. The absorption at 1048 cm^{-1} indicates the presence of polysaccharides. Many C–O–C groups exhibit characteristic bands in the 1150–911 cm^{-1} spectral range and generally the strong band at 1026 cm^{-1} is assigned to the vibration of C–O in alcohol hydroxyl group [5]. The very weak band occurring at 816 cm^{-1} (root[2]) shows N–H stretching vibration, nitrite group. The weak bands occurring at 780 cm^{-1} (leaf[1]), and 757 cm^{-1} (stem[1]) can be attributed to out-of-plane N–H wagging, primary and secondary amide and nitrate group. The absorption band occurring at 723–889 cm^{-1} indicates the carbohydrates in the spectrum. A number of compounds had been isolated from the plant, wedelolactone, chemically described as 7-methoxy-5,11,12-trihydroxy-coumestan, is basically a furanocoumarin, having hepatoprotective activity [12]. The stretching vibrations assigned to the C–S linkage occur in the region at 700–600 cm^{-1}. The weak absorption band of 620 cm^{-1} indicates the presence of sulphate. The medium absorption band occurs at 597 cm^{-1} (root[2]), 580 cm^{-1} (stem[1]) may be due to aliphatic C–Cl absorption and brominate compound. The brominate compound shows an infrared band region 600–500 cm^{-1} [13]. The weak absorption band at 539 cm^{-1} indicates the presence of phosphates in the flower of the plant *Eclipta alba* Hassk. The more intense bands occurring at 3419 cm^{-1}, 2927 cm^{-1}, 2853 cm^{-1}, 1633 cm^{-1}, 1421 cm^{-1}, 1260 cm^{-1}, 1073 cm^{1}, 816 cm^{-1} and 635 cm^{-1} corresponding to O–H/N–H, C–H, C–O and C–Cl/C–S stretching / bending vibrations respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in *Eclipta alba* Hassk and *Eclipta prostrata* Linn.

Trace elements are estimated by determining the percentage abundance (%) of elements Na, Mg, P, S, Cl, K, Ca, Fe, Cu, Zn, Cr, Ni, Co, Se, Al and Cd in the samples collected. The concentrations of such elements, sample wise are reported in Table 2 and Figure 2. Indeed quite a large number of heavy metals are essential to plant and animal (including human) life. These include, naming a few, iron, manganese, copper, nickel, zinc, cobalt, chromium, molybdenum and vanadium. If any of these metals is eliminated from our nutrition we would be suffering from one or the other disease. They are indeed like vitamins and if we are deficient with even one of them, we would suffer from one or other health problem.

It is observed from Table 2 that in 6.43% ($leaf^1$), 12.71% ($root^1$), 6.77% ($stem^1$) and 8.22% ($leaf^2$), 5.43% ($stem^2$), 5.92% ($root^2$) respectively. Sodium was considered to be non-essential for plants. The concentration of magnesium is 6.80% ($leaf^1$), 7.15% ($root^1$), 7.27% ($stem^1$) and 3.26% ($leaf^2$), 1.51% ($stem^2$), 2.57% ($root^2$) respectively. Magnesium is involved in a number of physiological and biochemical functions.

The concentration of silicon is very high in 31.42% ($leaf^1$). Both 7.59% ($root^1$), 5.06% ($stem^1$), 14.64% ($leaf^2$), 10.48% ($stem^2$), 12.84% ($root^2$) show weak absorptions. The value of chloride is high in the whole sample. The concentration of chloride is 8.50% ($leaf^1$), 12.78% ($root^1$), 22.37% ($stem^1$), and 14.11% ($leaf^2$), 21.88% ($stem^2$), 15.90% ($root^2$).

The concentration of potassium is high in 35.74% ($stem^2$), 22.31% ($root^2$), 12.96% ($leaf^2$) and 9.32% ($leaf^1$), 11.57% ($root^1$), 15.96% ($stem^1$). In all plant parts, the concentration of potassium is more than 10%. It is used as ash, manure and fertilizer. In the present investigation, the concentration of calcium is 10.25% ($leaf^1$), 7.05% ($root^1$), 7.18% ($stem^1$), and 10.12% ($leaf^2$), 15.61% ($root^2$). The stem of *Eclipta prostrate* linn shows the absence of calcium. The concentration of calcium is high in $root^2$ compared to other samples. It is used for bone and dental strength.

The concentration of chromium in a small amount is present in 1.03% ($root^1$) and it is absent in all other samples. Chromium has been found in almost all human tissues. The glucose tolerance factor (GTF) to be the only chromium compound which will pass the placental barrier, playing almost the role of vitamin. Manganese concentration is 0.87% ($leaf^1$), 2.34% ($root^1$) and 1.17% ($stem^2$), 0.88% ($root^2$) respectively. It activates many enzyme reactions involved in the metabolism of organic acids, phosphorus and nitrogen [2]. Manganese is an essential micro-nutrient for plants and animals and its deficiency in humans has been implicated with several disease states, including diabetes, nervous instability, convulsions, and disorders of cartilaginous growth in infants and children and rheumatoid arthritis [3].

Table 2

The percentage of trace elements present in the leaf, stem, and root parts of *Eclipta alba* Hassk and *Eclipta prostrata* Linn

Characteristic elements	Elemental percentage (%) *Eclipta alba* Hassk			Elemental percentage (%) *Eclipta prostrata* Linn		
	Leaf	Stem	Root	Leaf	Stem	Root
Na	6.43	6.77	12.71	8.22	5.46	5.92
Mg	6.80	7.27	7.15	3.26	1.51	2.57
Si	31.42	5.06	7.59	14.64	10.48	12.84
Cl	8.50	22.37	12.78	14.11	21.88	15.90
K	9.32	15.96	11.57	12.96	35.74	22.31
Ca	10.25	7.18	7.05	10.12	–	15.61
Cr	–	–	1.03	–	–	–
Mn	0.87	–	2.34	–	1.17	0.88
Fe	1.83	2.09	3.20	0.78	0.41	4.91
Ni	1.32	–	1.31	–	–	–
Cu	13.79	18.59	21.58	17.65	14.34	10.89
Zn	9.46	6.63	11.70	11.00	–	–
Co	–	–	–	0.34	0.46	–
Cd	–	2.12	–	2.61	8.53	2.54
Se	–	2.41	–	–	–	–
Al	–	3.56	–	–	–	–
S	–	–	–	3.01	–	–
Pb	–	–	–	1.30	–	5.62

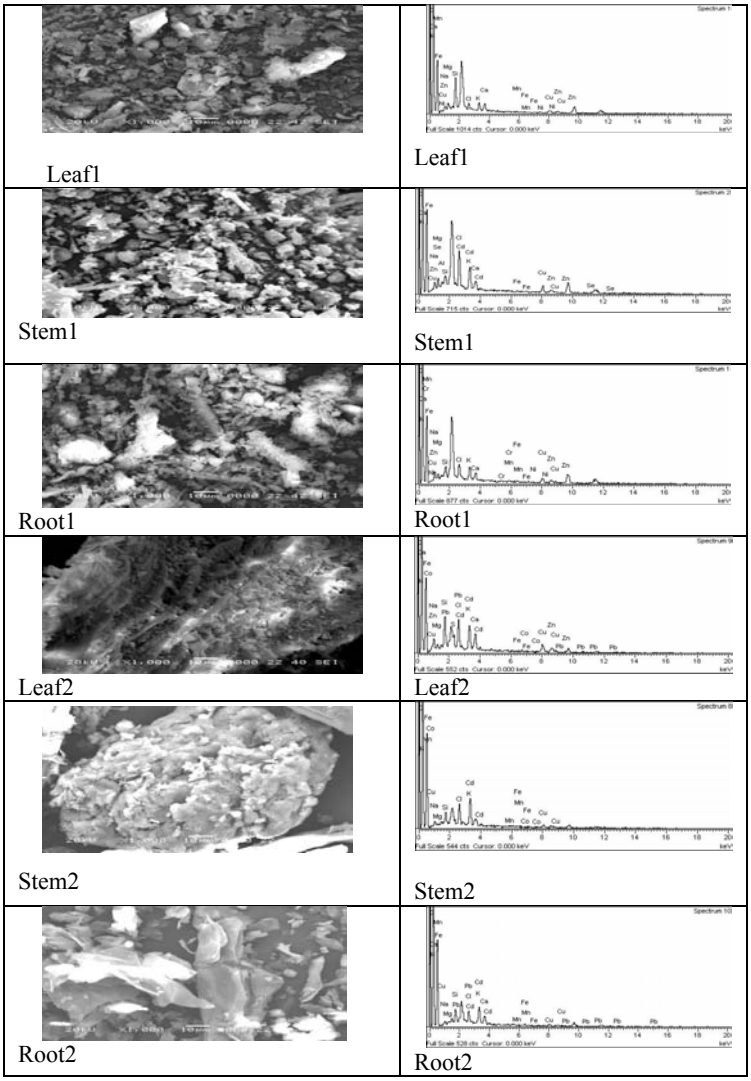

Fig. 2. SEM-EDS spectrum of vegetative parts; (1) *Eclipta alba* Hassk (leaf[1], stem[1], root[1]); (2) *Eclipta prostrata* Linn (leaf[2], stem[2], root[2]**).**

The concentration of iron is in whole plant parts like 4.91% (root[2]), 0.78% (leaf[2]), 0.41% (stem[2]), and 3.20% (root[1]), 1.83% (leaf[1]), 2.09% (stem[1]), respectively.

Nickel is present only in *Eclipta alba* Hassk like 1.32% (leaf[1]) and 1.31% (root[1]). Nickel has been found in a wide variety of plants and animal tissues. Nickel concentrations that are toxic to plants vary in magnitude according to plant species [1]. The concentration of copper in whole plant parts 21.58% (root 1), 18.59% (stem 1), 13.79% (leaf 1) and 17.65% (leaf 2), 14.34% (stem 2), 10.89% (root 2).

Copper provides metabolic control over auxin synthesis and it is involved in protein metabolism. The deficiency of copper has been associated with anemia, neutropenia, diarrhea, demonetization of bones and amyelination of the control nervous system of the newborn [9].

In the present investigation, the concentration of zinc is 11.70% ($root^1$), 9.46% ($leaf^1$), 6.63% ($stem^1$) and 11.00% ($leaf^2$) respectively. Zinc is an essential trace element for the normal functioning of cells including protein synthesis, carbohydrates metabolism cell growth and cell division [10]. Cobalt is only present in *Eclipta prostrata* Linn 0.46% ($stem^2$), 0.34% ($leaf^2$). Cobalt is an essential element for plants, animals including humans, being a consistituent of vitamin B12.

The concentration of Cd records 8.53% ($stem^2$), 2.61% ($leaf^2$), 2.54% ($root^2$) and 2.12% ($stem^1$) respectively. W.H.O. limit for Cd is 0.01 mg. The concentration of selenium is present only in 2.41% ($stem^1$) sample. It is an important element in animal nutrition; it is known to be nonessential for plant growth [2]. The concentration of aluminium is only present in 3.56% ($stem^1$). The sulphur is only present in *Eclipta prostrata* Linn, in a concentration of 3.01% ($leaf^2$).

The concentration of lead is only present in *Eclipta prostrata* Linn plant 5.62% ($root^2$) and 1.30% ($leaf^2$). In human beings the effect of lead on health has been reviewed by WHO (1982). Lead is non-essential for the functioning of human biological systems and for centuries it has been recognized as a cumulative poison. The groups most sensitive to exposure of lead are infants (including the unborn fetus) and pregnant women. Children have a greater ability to absorb ingested lead and also have a higher susceptibility to the metal because of their fast growth rate.

Table 1 shows the group of bands listed under the chemical groups like carboxylic acid, amino derivatives, sulphur derivatives, polysaccharides, nitrates, chlorates, and carbohydrates. From Table 1, it is seen that the main chemical constituent of *Eclipta alba* Hassk and *Eclipta prostrata* Linn is carboxylic acid, which serves as a main pharmaceutical product in curing kapham and vaatam, ulcers, jaundice, headache, stomatitis, hemicrania, fever, pain in liver, wounds in cattle, treatment of edema and rheumatic joint pains. Also both herbs are rich in amines, amides and amino acids, the main groups of protein synthesis and the herbs serve as a herbal oil and hair tonic. Also, they contain sulphur derivatives compounds which are used as disinfectants and dermal cream. The remaining polysaccharides, carbohydrates, chlorates, and nitrates play thus role of disinfectants.

CONCLUSION

The main functional group of these plants is wedelolactone which is confirmed by FT-IR study predicted groups, OH, CH_2, CO, etc. The presence of characteristic functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, nitrates, chlorates, and carbohydrate are responsible for various medicinal properties of both herbal plants. Indeed quite a large number

of heavy metals are essential to plant and animal (including human) life. These include, naming a few, iron, manganese, copper, nickel, zinc, cobalt, chromium, molybdenum and vanadium. If any of these metals is eliminated from our nutrition we would be suffering from one or the other disease. They are indeed like vitamins and if we are deficient with even one of them, we would suffer from one or other health problem. From the comparison of Table 2 for percentage of elements obtained from SEM-EDS, it has been found that *Eclipta alba* Hassk contains a higher percentage of useful elements like Na, Mg, K, Ca, Cu, Zn, and Fe than *Eclipta prostrata* Linn. Also *Eclipta prostrata* Linn contains more toxic elements Cd than *Eclipta alba* Hassk. Cd induces kidney problems. Hence, *Eclipta alba* Hassk is a safer and potent herbal medicine than *Eclipta prostrata* Linn.

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