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Characterization of *Eclipta prostrata* (L.) L. leaves by FTIR spectroscopy method, CHNS and ICP-MS analysis techniques

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Abstract

Eclipta prostrata (L.) L. is a medicinal herb which has wide application in the indigenous medicinal system. Chemical constituents play important role in any therapeutic activity. CHNS and ICP-MS techniques were used for quantitative determination of chemical composition of Eclipta prostrata (L.) L. leaves. Various functional groups were determined by using FTIR spectroscopy method. Elemental analysis of Eclipta prostrata (L.) L. leaves confirmed presence of pharmaceutically essential elements such as B, Na, Mg, P, K, Ca, Cr, Mn, Fe, Se, Mo, Co, Ni, Cu and Zn. Some traces of heavy metals also have been observed. The FTIR spectroscopy is important to obtain information of different characteristic peak values with various functional groups. The aqueous, methanolic and acetone extracts of Eclipta prostrata (L.) L. leaves were used for FTIR analysis which successfully revealed presence of medicinally and biologically active functional groups. The present investigation has proved that Eclipta prostrata (L.) L. has some bioactive compounds and may be useful for isolation of pharmacologically important components. It would help to find new drug formulations.

Keywords: *Eclipta prostrata* (L.) L., Therapeutic activity, CHNS, ICP-MS, FTIR, Elemental analysis.

Introduction

In India many indigenous plants are used to cure several diseases. Most popular renewed indigenous medicinal systems such as Ayurveda, Unani and Siddha are originated in India. Many medicinal plants are the part of the folk and traditional systems of medicines. Single plant or its various parts are used to prepare medicines in all medicinal systems. Efficacy depends on the presence of quantity and nature of primary and secondary metabolites in raw drugs. Quantity and nature of metabolites are correlated with the concentration of elements and presence of functional groups. Certain Medicinal properties of plants are directly or indirectly dependant on the elemental concentrations of that plant. Elemental concentrations of medicinal plants prove the curative ability of certain diseases[1].

Hence it is important to know the elemental concentration and functional groups of medicinal plants. Medicinal plants contain vital elements which are widely used as a precursor for regulating pharmaceutical products[2].

Eclipta prostrata (L.) L. is well known traditional medicinal plant and used in several formulations of herbal drugs. *Eclipta prostrata* (family-Asteraceae) is an annual herb, native of tropical and subtropical regions of the world. It has various synonyms, well known is *Eclipta alba*. It is commonly used as a hair tonic for nourishment, blackening and strengthening of hairs. Datta *et.al* proved potential of extract of *Eclipta alba* for promoting hair growth[3]. Leaf extract of *Eclipta prostrata* is good livertonic and rejuvenative according to Ayurveda. Traditionally it is applied externally on eczema and dermatitis. In Charak samhita and Chikista stan *Eclipta* is reported to cure raktpitta (Haemorrhage), kasa (Cough), palitya (Whitening of hairs) and vaman (Vomiting). In Sushruta samhita *Eclipta is* used on Vrana (Inflammation), jwar (fever), swas rog (asthma) etc. It is used as a folk medicine on balya (tonic and deobstruent), netrabhishy and (new born suffering from catarrh), conjunctivitis, constipation, antiseptic etc.[4] Several pharmacological properties of *Eclipta prostrata* have been reported by earlier researchers such as anti-inflammatory, antimicrobial[5], antihyperglycemic[6], antioxidant, cytotoxic activity,[7] antihepatotoxic activity[8], analgesic activity[9] and memory enhancing activity[10].

A literature survey of *Eclipta prostrata* revealed that sufficient work was not done so far in elemental analysis and FTIR spectroscopy. Hence, an attempt has been made in present investigation to characterise *Eclipta prostrata* by using ICP-MS, CHNS analyser and FTIR spectroscopy techniques.

Material and methods

Plant material collection and authentication: *Eclipta prostrata* has been collected from surrounding area of Pune district. The plant has been identified morphologically and authenticated by Botanical survey of India (BSI), Pune, Maharashtra, India. Here a voucher specimen no. VMK-07 has been deposited.

ICP-MS and CHNS analysis: Dry leaves powder of *Eclipta prostrata* was digested and elemental analysis was conducted on ICP-MS spectrometer. Quantification and analysis of CHNS were carried out at Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay.

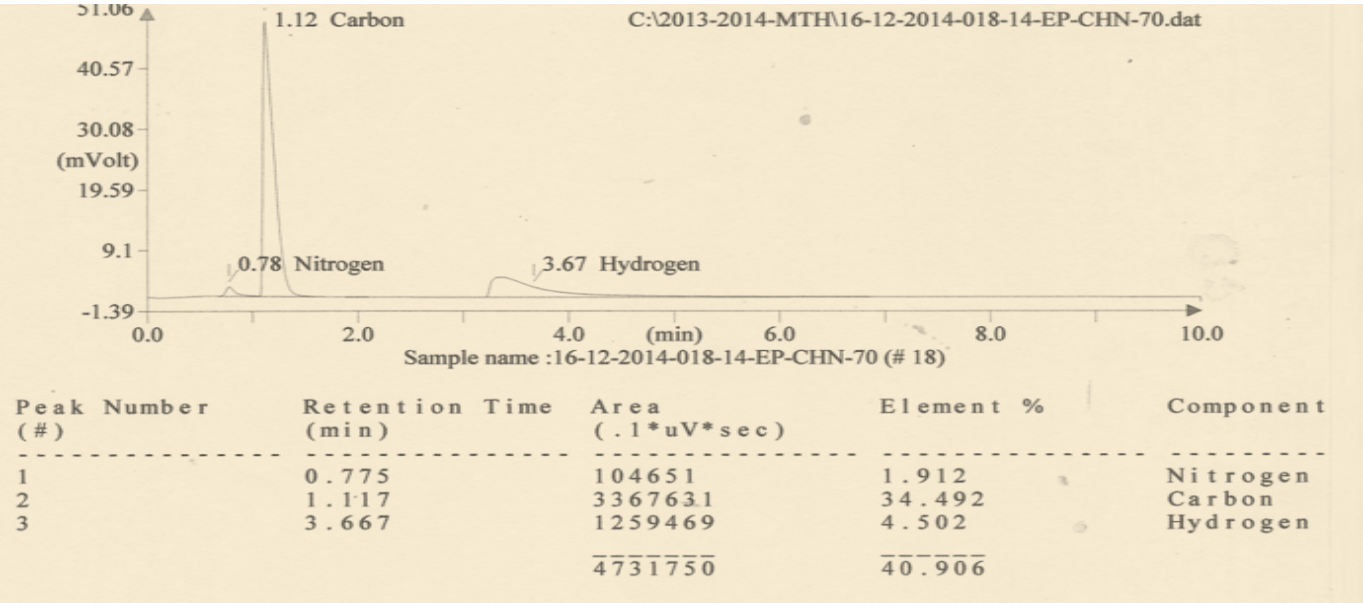
FTIR Spectroscopy Analysis: Preparation of plant extract: Powdered leaf samples of *Eclipta prostrata* was extracted with acetone, methanol and water by maceration process. Concentrated extracts have been analysed by using FTIR spectroscopy (Model-Nicoleti S 5) for determination of herbal functional groups.

Results and discussion

CHNS analysis: Analysis was carried out to know the organic composition of carbon, hydrogen, nitrogen and sulphur, and results are presented in Figure-1. Reddy *et. al*[11] analysed CHNS of *Eclipta prostrata* by CHNS analyser, which showed that 37.27% carbon, 5.19 % hydrogen but nitrogen and sulphur were not detected. Similar results were found in present investigation hydrogen (4.5%), carbon (34.49%) and also nitrogen has also been reported and quantified about 1.91%. Carbon, hydrogen and nitrogen are basic elements and these all are found actively participating in most of the metabolic reactions occurred in living beings.

ICP-MS analysis: Elemental analysis of *Eclipta prostrata* leaves by ICP-MS confirmed presence of pharmaceutically essential elements such as B, Na, Mg, P, K, Ca, Cr, Mn, Fe, Se, Mo, Co, Ni, Cu and Zn. The results of ICP-MS analysis have been recorded in Table-1. In *Eclipta prostrata* leaves Boron has been determined to be 35.10 ppm. Some researchers proved that B can successfully treat vaginal yeast infections and some suggested that B might be useful to decrease symptoms of osteoarthritis but it also shows some side effects, it is unsafe when taken by mouth in high doses which causes skin inflammation, irritation, vomiting, diarrhoea and also increase estrogen level[12]. Mg, Na, P, K and Ca are essential elements and were quantified maximum in amount such as 4525.8 ppm, 210.3 ppm, 6683.7 ppm, 5050.3 ppm and 1379.5 ppm respectively. Mg maintains equilibrium of osmotic pressure and catalyses many reactions enhanced by enzymes. It also helps in formation and functioning of bones and muscles[13]. K/Na ratio in food prevents hypertension and atherosclerosis[14]. Ca and P are main components for bone growth[15,16]. Mn, Fe and Se are pharmacologically active elements and also they have some medicinal properties e.g. Se have an antioxidant property[17], Mn derivatives have antipyretic, antibiotic, antiemesis and hypocholesterolemic properties[18]. Fe is essential element in formation of blood haemoglobin in human body[19]. Mn (175.8 ppm), Fe (144.8 ppm) and Se (154.1 ppm) were found in sufficient amount in *Eclipta prostrata* leaves. Cr is possibly essential element and helpful in regulating carbohydrate, lipoprotein and nucleic acid metabolism[20]. It was quantified as 4.3 ppm in present study. Zn and Cu are important. Zn is an essential growth supplement and for insulin production[21]. Cu plays active role in metabolic processes, which was found in adequate concentration in the leaves of *Eclipta prostrata*, Zn was 39.9 ppm and Cu was 21.86 ppm. According to WHO, concentration of Mo (0.8 ppm), Co (0.98 ppm) and Ni (3.96 ppm) has been found below the permissible level[22].

Toxic elements like Li, Be, Hg, and Bi are found totally absent but some traces of heavy metals also have been observed like As (0.16 ppm), Cd (0.08 ppm), Sn (8.57 ppm) and Pb (0.29 ppm). Heavy metals and nonessential Al (575.63) and Ba (12.09 ppm) were found in maximum concentration in the leaves of *Eclipta prostrata*.



Peak Number (#)	Retention Time (min)	Area (.1*uV*sec)	Element %	Component
1	0.775	104651	1.912	Nitrogen
2	1.117	3367631	34.492	Carbon
3	3.667	1259469	4.502	Hydrogen
		4731750	40.906	

Figure-1: Elemental Analysis of Eclipta prostrata (L.) L. Leaves by CHNS Analyser.

Table-1: Elemental Analysis of *Eclipta prostrata* (L.) L. Leaves by ICP-MS.

Eclipta prostrata (L.) L. Leaves			
Elements	Conc (ppm)	Elements	Conc(ppm)
7 Li [1]	0.00	60 Ni [1]	3.96
9 Be [1]	0.00	63 Cu [1]	21.86
11 B [1]	35.10	66 Zn [1]	39.98
23 Na [1]	210.361	75 As [1]	0.16
24 Mg [1]	4525.84	82 Se [1]	154.144
27 Al [1]	575.63	95 Mo [1]	0.785
31 P [1]	6683.73	107 Ag [1]	0.13
39 K [1]	5050.3	111 Cd [2]	0.08
43 Ca [1]	1379.521	118 Sn [1]	8.57
52 Cr [1]	4.279	137 Ba [1]	12.90
55 Mn [1]	175.837	208 Pb [2]	0.29
56 Fe [1]	144.870	202 Hg [1]	0.00
59 Co [1]	0.989	209 Bi [1]	0.00

FTIR analysis: The FTIR spectroscopic analysis showed the presence of essential phytoconstituents and results were recorded in Table-2, Figure-2, 3 and 4. FTIR spectra analysis was used to identify the functional groups of active phytoconstituents. The acetone, methanolic and aqueous extract of *Eclipta prostrata* leaves showed characteristic absorption bands at 3320.61 cm^{-1}, 3287.42 cm^{-1} and 3270.67 cm^{-1} for O-H (stretch) hydroxyl groups. These absorption peaks confirm the presence of alcoholic and phenolic compounds and also flavonoids[23]. The peak obtained at 2852.37 cm^{-1}, 2922.38 cm^{-1} in acetone extract, 2979.91 cm^{-1} in methanolic extract and 2980.03 cm^{-1} in aqueous extract indicated the presence of C-H stretching while the peak obtained at 1376.47 cm^{-1} in acetone extract and 1348.47 cm^{-1} in methanolic extract indicated the presence of C-H bending for alkanes. Characteristic peak for alkenes C=C (stretch) was only obtained in acetone extract at 1651.15 cm^{-1} but absent in other extracts of *Eclipta prostrata.* These peaks indicated the presence of terpens[24] in *Eclipta prostrata.* The peaks found in acetone extract at 1026.02 cm^{-1}, 1045.77 cm^{-1}, 1073.44 cm^{-1} and in methanolic extract at 1031.7 cm^{-1}, where as in aqueous extract at 1020.04 cm^{-1} is assigned to ether group C-O (stretch). Ketone group C=O (stretch) has been only recorded in acetone extract at 1709.54 cm^{-1}. O-H (stretch) and C=O (stretch) exhibited presence of flavonoid contents where as C=O (stretch) and C-O (stretch) revealed presence of glycosides[25]. The peaks obtained at 1247.86 cm^{-1}, 1158.88 cm^{-1}, 1261.50 cm^{-1}, 1266.7 $cm^{-1}$for acetone, methanol and aqueous extracts indicate the presence of amine group C-N (stretch). Abundant peaks of 1º and 2º amines, N-H and C-N have been found in acetone extract at 719.2 cm^{-1}, 788.5 cm^{-1}, 837.73 cm^{-1} and methanolic extracts at 667.83 cm^{-1} , 769.07 cm^{-1}, 858.02 cm^{-1} of *Eclipta prostrata.* Aromatic compounds, C=C (stretch) have been detected in all extracts of *Eclipta prostrata* leaves at 1454.92 cm^{-1}, 1605.88 cm^{-1} in acetone extract, 1606.22 cm^{-1} in methanol extract and 1403.42 cm^{-1}in aqueous extract. The presence of alcohol, alkanes, alkenes, amines, primary & secondary amines and aromatic compounds in *Eclipta prostrata* leaves extracts, exhibited the occurrence of alkaloids in maximum quantity[26]. The nitrile compounds with multiple bonding have been obtained at 2025.19 cm^{-1}, 2158.96 cm^{-1} in acetone extract and at 2158.57 cm^{-1} in methanolic extract of *Eclipta prostrata* leaves. Nitro compounds with N-O (stretch) were found only in aqueous extract at 1556.86 cm^{-1}. Phosphorus containing functional groups, P-H also have been detected in methanolic as well as aqueous extracts of *Eclipta prostrata* leaves at 2361.37 cm^{-1} and 2360.10 cm^{-1} respectively, revealed presence of phosphorus and nitrogen containing secondary metabolites.

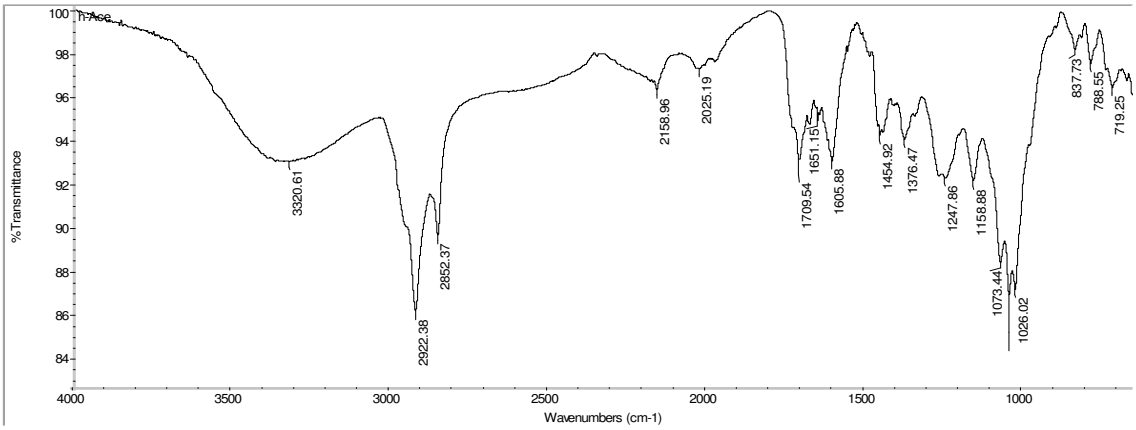


Figure-2: FTIR spectrum Acetone extract of *Eclipta prostrata* leaves

Table-2: FTIR Spectra Analysis of *Eclipta prostrata* (L.) L. Leaves extracts.

Sr.No.	*Eclipta prostrata* Functional groups	Component (Peaks)	Absorption spectrum, Frequency (cm^{-1})			
			Wave number cm^{-1} (Reference Article)[27,28]	Acetone Extract	Methanolic Extract	Aqueous Extract
1.	Alcohols and Phenols	O-H Stretch (Strong, Broad)	3200-3600	3320.61	3387.42	3270.67
2.	Alkanes	C-H Stretch (Strong) C-H bending (Variable)	2850-3000 1350-1480	2852.37 2922.38 1376.47	2979.91 1348.47	2980.03
3.	Alkenes	C=C Stretch	1630-1680	1651.15	--	--
4.	Ether	C-O Stretch (Strong)	1000-1300	1026.02 1045.77 1073.44	1031.7	1020.04
5.	Ketone	C=O Stretch	1705-1725	1709.54	---	---
6.	Amine	C-N Stretch (Medium-Weak)	1080-1360	1247.86 1158.88	1261.50	1266.7
7.	1º and 2º Amines	N-H C-N (Medium-Weak)	660-900	719.2 788.5 837.73	667.83 769.07 858.02	---
8.	Aromatic	C=C Stretch (Medium-Weak)	1400-1600	1454.92 1605.88	1606.22	1403.42
9.	Nitrile Compound	Multiple Bonding	2000-2300	2025.19 2158.96	2158.57	---
10.	Nitro	N-O Stretch (Strong)	1515-1560	---	---	1556.86
11.	Phosphorus Functions	P-H Phosphine (Medium)	2280-2440	---	2361.37	2360.10

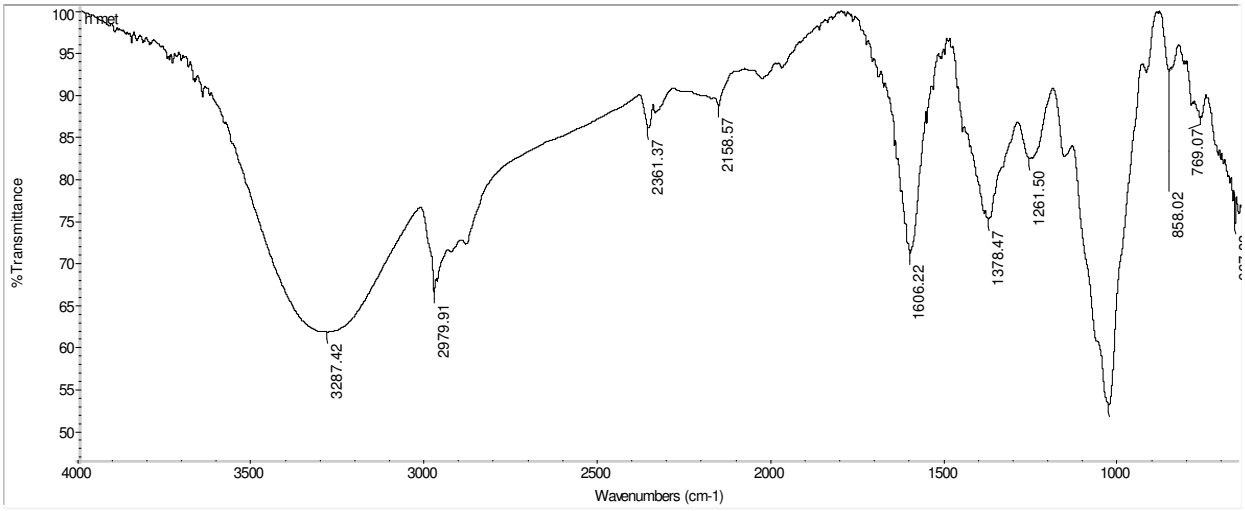


Figure-3: FTIR spectrum Methanolic extract of *Eclipta prostrata* leaves

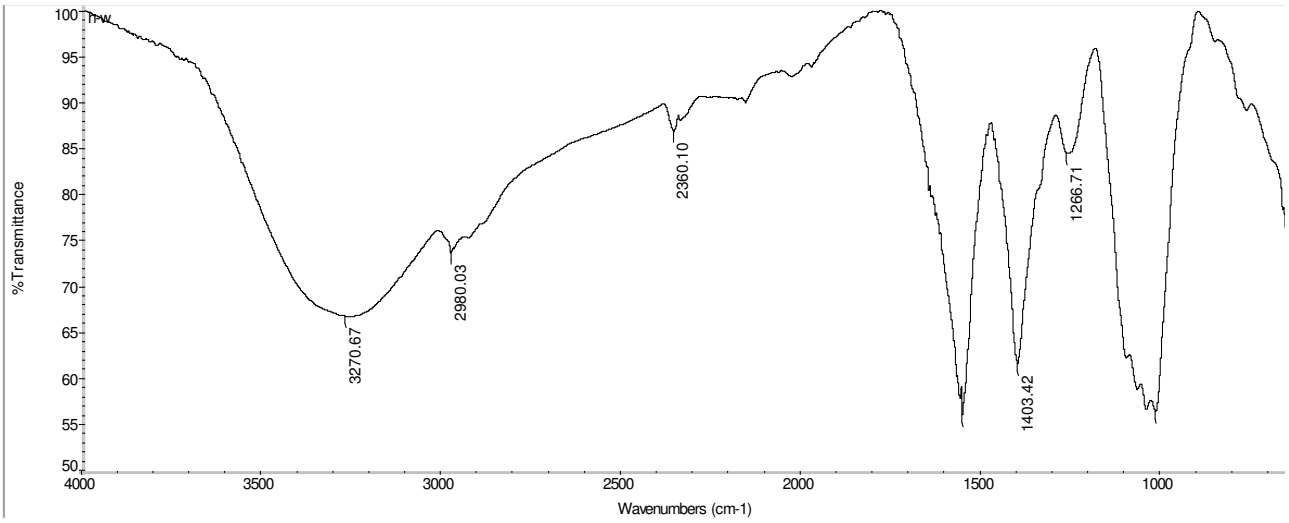


Figure-4: FTIR spectrum Aqueous extract of *Eclipta prostrata* leaves

Conclusion

Results of elemental analysis of *Eclipta prostrata* leaves have been found important to correlate pharmacological properties of plant. The obtained data of elemental concentration has been found rich source of biologically essential elements which may play most important role in therapeutic activity and curative ability of plant. The plant contains active trace elements whose activity is responsible for curing certain dangerous diseases. The FTIR spectroscopy study confirmed the presence of many functional groups like O-H, C-H, C=C, C-O, C=O, C-N, N-H, N-O, P-H stretching. The presence of characteristic functional groups has been detected in *Eclipta prostrata* leaves extracts such as hydroxyl, alcohols, phenols, alkanes, alkenes, ether, ketone, amine, aromatic compounds, nitrile compounds, nitro compounds and phosphorus functional groups. The pharmacological essential compounds have been detected in various extracts of *Eclipta prostrata* leaves which would be responsible for the various medicinal characteristics and properties.

Further studies are needed to identify unknown bioactive components, their isolation, characterization and structure elucidation which may be responsible to investigate unknown pharmacological properties.

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