ORIGINAL ARTICLE

Cytomorphological and Preliminary Phytochemical Screening of *Eclipta alba* (L.) Hassk

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Abstract

Aim: Present study deals with the morphological, cytological and phytochemical characterization of *Eclipta alba*. **Materials and Methods:** The plant materials (*Eclipta alba* whole plant) were collected from "Banga" region of Punjab, India. They were then studied for various morphological features, meiosis and phytochemicals. Fourier Transform Infrared Spectroscopy (FTIR) and Wavelength Dispersive X-Ray Fluorescence (WD-XRF) have been done to understand the different functional groups and elemental detail. **Results and Discussion:** Three morphotypes *i.e.,* prostrate, semi-erect and erect has been described for *Eclipta alba* but with a constant chromosome number 2n=22 in all forms. Alkaloids, amino acid, carbohydrates, flavonoids, glycosides, gums and mucilage, phenolics, reducing sugars, tannins *etc*. were reported in aqueous and ethanol extracts of these forms. Further, Fourier Transform Infrared Spectroscopy has strengthened the phytochemical observations. Elements like Potassium, Calcium, Chlorine, Magnesium, Iron *etc*. were analyzed through Wavelength Dispersive X-Ray Fluorescence. **Conclusion:** Three morphotypes with same chromosome number have been described for *Eclipta alba*. The prostrate form seems to be better than other forms in terms of phytoconstituents and elements.

Key words: *Eclipta alba*, Morphology, Meiosis, Phytochemicals, Elements

INTRODUCTION

Eclipta alba (L.) Hassk (Asteraceae) is one of the important traditional medicinal plants. It is an annual herb, commonly known as false daisy in English and "Bhringoraj" or "Bhringraj" in Bangladesh and India.[1] The plant is useful in the treatment of memory disorders, edema, fevers, rheumatic joint pains, digestion, hepatitis, enlarged spleen, and skin problems.[2-4] In India, the plant is commonly used in hair oil for healthy black and long hair.[5] *E. alba* is monospecific and exists in a variety of climatic conditions that has resulted in phenotypic diversity. Three morphotypes of *E. alba,* i.e., erect, intermediate and prostrate have already been described.[6] Phytochemical analysis of plant yields a number of compounds of various pharmacological activities. These biological active compounds are useful in the synthesis of medicines.[7] Some important phytochemicals of plants include alkaloids, tannins, flavonoids, and phenolics. Different plant parts may possess a variety of these compounds. Thus, knowledge of such biologically active constituents is desirable and may be useful in the synthesis and designing of new and alternate medicines.

During present study, morphological, cytological, and phytochemical parameters of *E. alba* have been taken into consideration. Besides this, Fourier transform infrared (FTIR) spectroscopy and wavelength dispersive X-ray fluorescence (WD-XRF) analysis have also been undertaken to study the functional groups and to detect macro and micro elements, respectively. An effort has also been made to correlate the cytomorphological variations with the phytochemicals and elemental details of three forms of *E. alba* from the state of Punjab.

MATERIALS AND METHODS

Collection and Identification of Material

The plant material (*E. alba* whole plant) was collected in 2014 from "Banga" region of Punjab. The plant specimens were

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identified using different floras, manuals and by consulting the herbarium of the Botany Department, Panjab University, Chandigarh.

Morphological Study

The plant material was studied for various morphological features such as leaf color, shape, venation, arrangement, type of inflorescence, flower color *etc*. These characteristics will be helpful in establishing the morphotypes.

Meiotic Study

The young flower buds of three forms of *E. alba* were collected and fixed in freshly prepared fixative 3:1 (ethanol: glacial acetic acid) for 24-48 h. They were then shifted to 70% ethanol and preserved in a refrigerator until further use. Anthers were excised from the buds and squashed in a drop of 1% acetocarmine stain on a glass slide and covered with the cover slip. The slide was then heated gently and pressed by thumb pressure between the two folds of the filter paper to spread the cells**.** The pollen mother cells having well separated and well-stained chromosomes were studied and photomicrographed.

Preparation of Extracts

The collected plant material was washed thoroughly for 3-4 times in running tap water and then with distilled water for proper cleaning. They were then allowed to dry at room temperature. The dried plant material was powdered using an electric grinder and kept separately in air tight containers**.** The extracts were prepared in different solvents.

Ethanol Extract

Ten (10 g) whole plant powder of each of the three morphotypes of *E. alba* was extracted in 130 ml of ethanol. The extraction was carried out using Soxhlet apparatus at a temperature ranging between 50°C to 60°C. After the extraction process is over, the extract was allowed to evaporate at room temperature until it reaches the 1/3rd volume of the original extract. Then, it was stored at 4°C in screw cap bottles.

Aqueous Extract

Twenty (20 g) whole plant powder of each of the three morphotypes was added separately to three conical flasks containing 100 ml of distilled water. These flasks were kept on an orbital shaker at room temperature for 24 h. The mixtures were then filtered through muslin cloth, and the resultant filtrates were again filtered through Whatmann's Filter No. 1. The final extracts were collected in vials and stored in the refrigerator until further use.

Preliminary Phytochemical Screening

The aqueous and ethanol extracts of three morphotypes of *E. alba* were screened for the presence of various phytoconstituents using the standard procedure. Various tests performed for phytochemicals are as follows.

Alkaloids

Mayer's test: 3 ml of extract was treated with 1 ml of 1% HCl. The mixture was heated for 20 min, cooled and then filtered. 1 ml of extract was treated with Mayer's reagent.

Observations: Creamy (yellow) precipitates indicate the presence of alkaloids.[8]

Amino acids

Ninhydrin test: To 1 ml of extract, 1 ml of ninhydrin reagent was added and boiled for few minutes.

Observations: Formation of blue color indicates the presence of amino acids.[9]

Anthocyanin

1 ml of extract is added to 1 ml each of 2N HCl and NH_3.

Observations: Appearance of pinkish red color which turns into blue violet indicates the presence of anthocyanin.[9]

Betaxanthin

To 1 ml of extract, 0.5 ml of 2M NaOH was added and heated for 5 min at 100°C.

Observations: Formation of yellow color indicates the presence of betaxanthin.[10]

Carbohydrates

Molisch's test: 1 ml of extract was treated with 5 drops of Molisch reagent. The test tube was hold at an angle and 2 ml of conc. H_2SO_4 was added along the sides of the test tube.

Observations: Violet ring at the junction was formed. It means carbohydrates are present.[11,12]

Coumarin

1.5 ml of 10%, aqueous NaOH was added to 1 ml of extract.

Observations: Formation of yellow color indicates the presence of coumarin.[9]

Flavonoids

NaOH test: 1 ml of extract was treated with 1 ml of 10% aqueous NaOH solution.

Observations: Intense yellow color formation indicates the presence of flavonoids.

H_2SO_4 test: 1 ml extract was treated with 10 drops of conc. H_2SO_4.

Observation: Appearance of yellow color indicates the presence of flavonoids.[8,11]

Glycosides

$FeCl_3$ test: 2 ml of extract was treated with 5 ml of conc. H_2SO_4 and boiled for 20 min in water bath. The mixture was cooled and neutralized with 20% KOH and divided it into two parts. 3 drops of $FeCl_3$ were added to one part.

Observations: Green to black precipitates shows the presence of glycosides.[11,13]

Gums and mucilage

1 ml of extract was slowly added to 2.5 ml of ethanol under constant stirring.

Observations: Creamy precipitates indicate the presence of gums and mucilage.[13,14]

Oxalate

To 2 ml of extract, 25 drops of acetic acid glacial were added.

Observations: Formation of greenish black color or precipitates indicates the presence of oxalate.[15]

Phenolics

2 ml of extract was treated with the 6-7 drops of 10% aqueous $FeCl_3$.

Observations: Formation of blue or green color indicates the presence of phenolics.[13,14]

Phlobatannins

1 ml of the extract was boiled with 8 drops of 2% aqueous HCl.

Observations: Formation of red precipitates indicates the presence of phlobatannins.[16]

Proteins

Xanthoproteic test: 1 ml of extract was treated with 15 drops of conc. HNO_3.

Observations: Yellow coloration confirms the presence of proteins.[9]

Quinones

1 ml of extract was treated with 10 drops of Conc. HCl.

Observations: Formation of yellow precipitates or color indicates the presence of quinones.[15]

Reducing sugars

Fehling's test: 1 ml of each of Fehling's solution "A" and "B" was heated on spirit lamp. The mixture was allowed to cool, and 1 ml of extract was added. The mixture was then heated gently.

Observations: Formation of brown red colored precipitates confirms the presence of reducing sugars.[17]

Resin

1 ml of extract was treated with caustic soda.

Observations: A red coloration showed the presence of resin.[18]

Saponins

Froth test: To 2 ml of plant extract was shaken vigorously in a test tube.

Observations: Formation of froth indicates the presence of saponins.[8]

Starch

Iodine test: To 1.5 ml of extract, 10 drops of dil. iodine solution were added.

Observations: A blue coloration indicates the presence of starch.[19]

Steroids

H_2SO_4 test: 12-15 drops of conc. H_2SO_4 were added to 1 ml of extract in a test tube.

Observations: Formation of slightly red color indicates the presence of steroids.[8]

Tannins

$FeCl_3$ test: 1 ml of extract was treated with 2 drops of $FeCl_3$.

Observations: Appearance of dark green color indicates the presence of tannins.

KOH test: Add 1 ml of plant extract to 1 ml of 10% potassium hydroxide (freshly prepared).

Observations: Dirty white precipitates indicate that tannins are present.[13,17]

Terpenoids

Salwoski's test: About 1 ml of extract was mixed well with 2 ml of chloroform. Then, 2ml of conc. H_2SO_4 was added carefully from the sides of the test tube.

Observations: Reddish brown color at the interface indicates the presence of terpenoids.[11,13]

FTIR Spectroscopy

The whole plant powder each of the three forms of *E. alba* was studied for the presence of different functional groups using Perkin Elmer Spectrum 400 FTIR/FT-FIR spectrometer. The test was conducted at Sophisticated Analytical Instrumentation Facility (SAIF), Central Instrumentation Laboratory (CIL) and the University Centre for Instrumentation and Microelectronics (UCIM), Panjab University, Chandigarh.

WD-XRF Spectroscopy

The WD-XRF spectroscopy has been done to detect various macro and micro elements using WD-XRF Tiger 8 spectrometer. The whole plant powder each of the three forms of *E. alba* was used for this purpose. The test was performed at SAIF, CIL and UCIM, Panjab University, Chandigarh, India.

RESULTS AND DISCUSSION

Botanical Description

E. alba is a small annual herb, commonly known as false daisy or Bhringraj. It is a member of family Asteraceae. As it grows widely at moist places throughout the world as a wild weed, thus called as moisture- loving herb. Three morphotypes, i.e., prostrate, semi-erect, and erect of this species have been described during this investigation.

Prostrate Form [Figure 1a]

It is a creeping herb. The stem is slender, soft, much branched, and light green to reddish-purple in color. Stems and branches bear small, stiff white hairs that are appressed upward and have swollen nodes, often rooting at nodes. Leaves are simple, opposite, sessile or subsessile and light green in color. Leaf blades are lanceolate or elliptic-lanceolate with serrate margin and reticulate venation. Leaf base is attenuate with acute or sub-acute tip. Short and randomly dispersed hairs are found on both the surfaces of the leaves. Inflorescence is in flower heads, solitary or two heads together on axillary or terminal on long and slender peduncle with involucral bracts which are 8-10 in number. The involucral bracts are green in color form the base of flower head and are in two rows. Outer ones

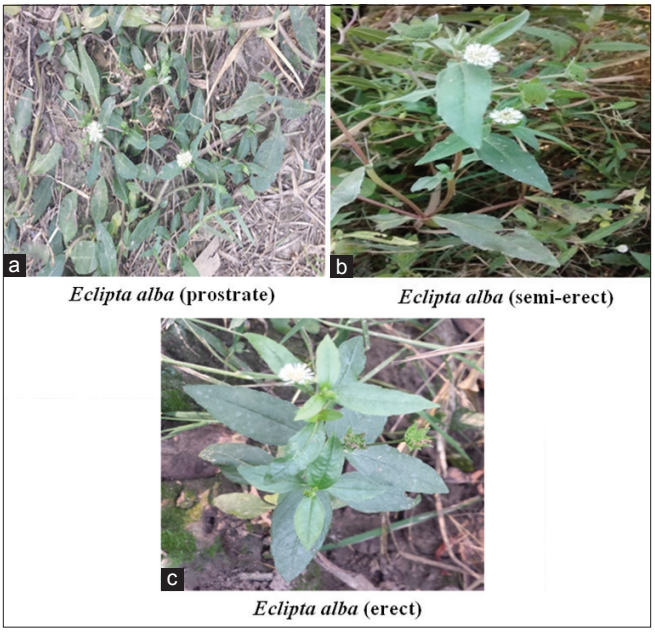

Figure 1: (a-c) Morphotypes of *Eclipta alba*

are ovate with acute apex and possess stiff, appressed hairs whereas inner ones are short and glabrous. The receptacle is flat. A cluster of small, sessile, white flowers is present on heads. Each flower head consists of numerous ray florets surrounding disc florets. The ray florets are white, ligulate whereas, the disc florets are tubular and dull white in color. Pale yellow colored anthers protrude from the disc florets. The ray florets are pistillate whereas disc florets are bisexual. The tap roots are cylindrical, greyish brown and well developed.

Semi-erect Form [Figure 1b]

This is similar to the prostrate form except that leaves are dark green in color and lower branches of the stem are creeping to the substratum while main stem grow toward the upper side.

Erect Form [Figure 1c]

It is also similar to the previous forms in morphological features, but the plant is tall, upright and leaves are light green in color.

Earlier, Ramakrishnan studied the morphological detail of *E. alba* Hassk along with ecological factors and examined various characters such as root, stem, leaves, and inflorescence.[20] These characters have also been studied during this investigation. A new species of *Eclipta* and its allies were previously reported from Eastern Asia and described for morphological characters such as leaf shape, leaf margin, leaf tip, inflorescence, and fruit.[21] North Indian populations of *E. alba* were divided into three morphotypes, i.e., erect, intermediate and prostrate on the basis of habit, size and shape of leaves, hairiness, and size of flower head.[22] Similarly, on account of leaf color and growth habit of the

plants, three morphotypes have been described from the single site (Banga) in the state of Punjab.

Meiotic Study

The meiotic study has revealed the presence of 2n=22 chromosomes in all the three morphotypes of *E. alba*. The course of meiosis is normal with 11 bivalents at diakinesis stage [Figure 2]. In the previous study, Gupta examined some Indian compositae cytologically and reported regular meiosis in *E. alba*.[23] Our findings are in conformity with this study. Similarly, in another study, 11 bivalents were reported both at diakinesis and metaphase stages in this species.[24] This has further corroborated the presently studied chromosome number of this species. Recently, Saggoo *et al*.[6] studied seasonal variation in chiasma frequency among three morphotypes of *E. alba* and reported $n = 11$ in all the three forms which tallies with this chromosome count in three morphotypes of *E. alba.*

Phytochemical Screening

Phytochemical study in three forms of *E. alba* has witnessed the presence of alkaloids, amino acid, anthocyanin, betaxanthin, carbohydrates, coumarin, flavonoids, glycosides, gums and mucilage, oxalate, phenolics, phlobatannins, proteins, quinones, reducing sugars, resin, saponins, starch, steroids, tannins, and terpenoids [Table 1]. Flavonoids and glycosides were found only in ethanol extracts. Alkaloids, betaxanthin, carbohydrates, coumarin, oxalate, proteins, resin, starch, tannins, and terpenoids have been reported both in aqueous and ethanol extracts but in variable amount. Anthocyanin has been reported invariably in prostrate and semi-erect but absent in erect form. Gums and mucilage, phenolics, phlobatannins, and saponins have been observed in all aqueous extracts. Steroids have reported only in erect form whereas quinones are absent in prostrate form but present in other two. Different phytoconstituents are known to be useful in the treatment of various human diseases. Thus, the presence of phytochemicals authenticates the medicinal potential of this species.

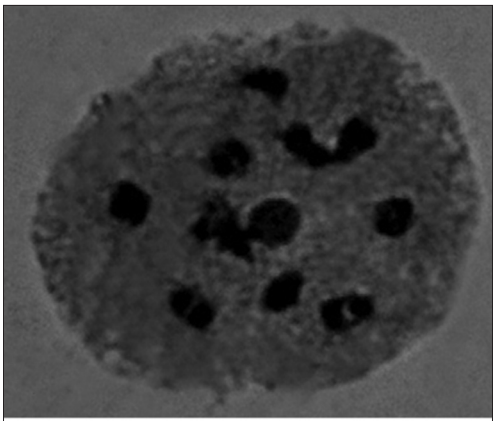

Figure 2: 11 bivalents at diakinesis stage in *Eclipta alba*

Earlier, Saggoo *et al*.[22] conducted the quantitative analysis of leaves of three forms of *E. alba* and suggested that carbohydrates, proteins, phenols, and saponins were comparatively higher in erect and intermediate form as compared to prostrate form. However, the qualitative analysis of whole plant powder of three forms of *E. alba* showed that prostrate form contain more number of phytoconstituents than other two forms. Phenols, flavonoids, tannins, alkaloids, terpenoids, and steroids were previously studied

Table 1: Phytochemical screening in three morphotypes of *Eclipta alba*

Phytochemical (s)	*Eclipta alba*					
	Prostrate		Semi-erect		Erect	
	Aq.	Eth.	Aq.	Eth.	Aq.	Eth.
Alkaloids						
Mayer's reagent test	±	+	±	+	±	+
Amino acid						
Ninhydrin test	±	−	−	±	±	−
Anthocyanin	−	±	−	±	−	−
Betaxanthin	±	±	±	+	+	±
Carbohydrates						
Molisch test	+	+	±	+	±	+
Coumarin	+	+	+	+	+	+
Flavonoids						
NaOH test	−	+	−	+	−	±
H_2SO_4 test	−	+	−	+	−	±
Glycosides	−	+	−	±	−	+
Gums and mucilage	+	−	+	−	+	−
Oxalate	+	+	+	±	±	+
Phenolics	+	−	+	−	+	−
Phlobatannins	+	−	+	−	+	−
Proteins						
Xanthoproteic test	±	+	±	+	±	+
Quinones	−	−	−	±	±	±
Reducing sugars	+	−	+	+	+	−
Resin	+	+	+	+	+	±
Saponins						
Froth test	+	−	±	−	±	−
Starch	+	+	+	+	±	+
Steroids						
H_2SO_4 test	−	−	−	−	±	±
Tannins						
$FeCl_3$ test	+	+	+	+	+	+
KOH test	+	+	+	+	+	±
Terpenoids						
$CHCl_3$ test	±	+	+	+	+	±

Aq.: Aqueous, Eth.: Ethanol, +: Present, −: Absent, ±: Traces

in methanol and aqueous extracts of *E. alba*.[25] But during present investigation, amino acid, anthocyanin, betaxanthin, carbohydrates, coumarin, glycosides, gums and mucilage, oxalate, phlobatannins, proteins, quinones, reducing sugars, resin, saponins, and starch have also been found. Similarly, carbohydrates, protein, glycosides, saponin, steroids, flavonoids, alkaloids, tannins, and terpenoids were screened in various extracts prepared from aerial parts of *E. alba*.[26] The presently studied phytochemicals are in agreement with their observations irrespective of the use of whole plant powder extract. In another study, alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, reducing sugars, anthraquinones, and cardiac glycoside were reported from the ethanol, chloroform, benzene, petroleum ether, and aqueous extracts.[27] Their results have corroborated our findings related to the phytoconstituents in three forms of *E. alba*.

Phytochemical screening of leaves, stem, and root or even whole plant of *E. alba* using different solvents such as petroleum ether, ethyl acetate, ethanol, methanol, fractions of hexane, carbon tetrachloride, chloroform, dichloromethane, and water were conducted in the previous studies.[7,28-35] 15 phytoconstituents such as alkaloids, amino acids, carbohydrates, coumarin, flavonoids, glycosides, mucilage, phenolics, proteins, reducing sugars, saponin, starch, steroids, tannins, and terpenoids were reported in these studies. They have also been reported in presently studied whole plant powder of ethanol and aqueous extracts of *E. alba*. To the best of our knowledge, quinones, resin, phlobatannins, oxalate, anthocyanin, and betaxanthin have not been reported in earlier studies in *E. alba*. Hence, likely be the first report for this species.

FTIR Spectroscopy

FTIR spectroscopy has depicted various functional groups present in the sample. The IR spectra of the whole plant powder of three forms of *E. alba* are given in Figures 3-5. The peak values and probable functional groups are presented in Table 2.

WD-XRF Spectroscopy

This technique is widely used for the quantitative analysis of major, minor, and trace elements. The comparative account of elements in three forms of *E. alba* is presented in Table 3. The elements such as sodium (Na), calcium (Ca), chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), selenium (Se), bromine (Br), rubidium (Rb), and ruthenium (Ru) are present in all the three forms but in quite variable amounts. Molybdenum (Mo) is absent in semi-erect form whereas, Mn is present at same concentration in erect and semi-erect forms. Phosphorus (P), zinc (Zn), and rhenium (Re) have been reported in all three forms and in the same concentration. The results have shown that erect form is a good source of chlorine (Cl). The elements such as magnesium (Mg), silicon (Si), potassium (K), calcium (Ca), iron (Fe) and many other are present abundantly in prostrate form.

According to the previous report, plant parts like leaf, stem, and root of *E. alba* contains higher concentration of elements such as Na, Mg, K, Ca, Cu, Zn, and Fe as compared to *Eclipta prostrata*.[36] However during this study, prostrate form of *E. alba* contains these elements

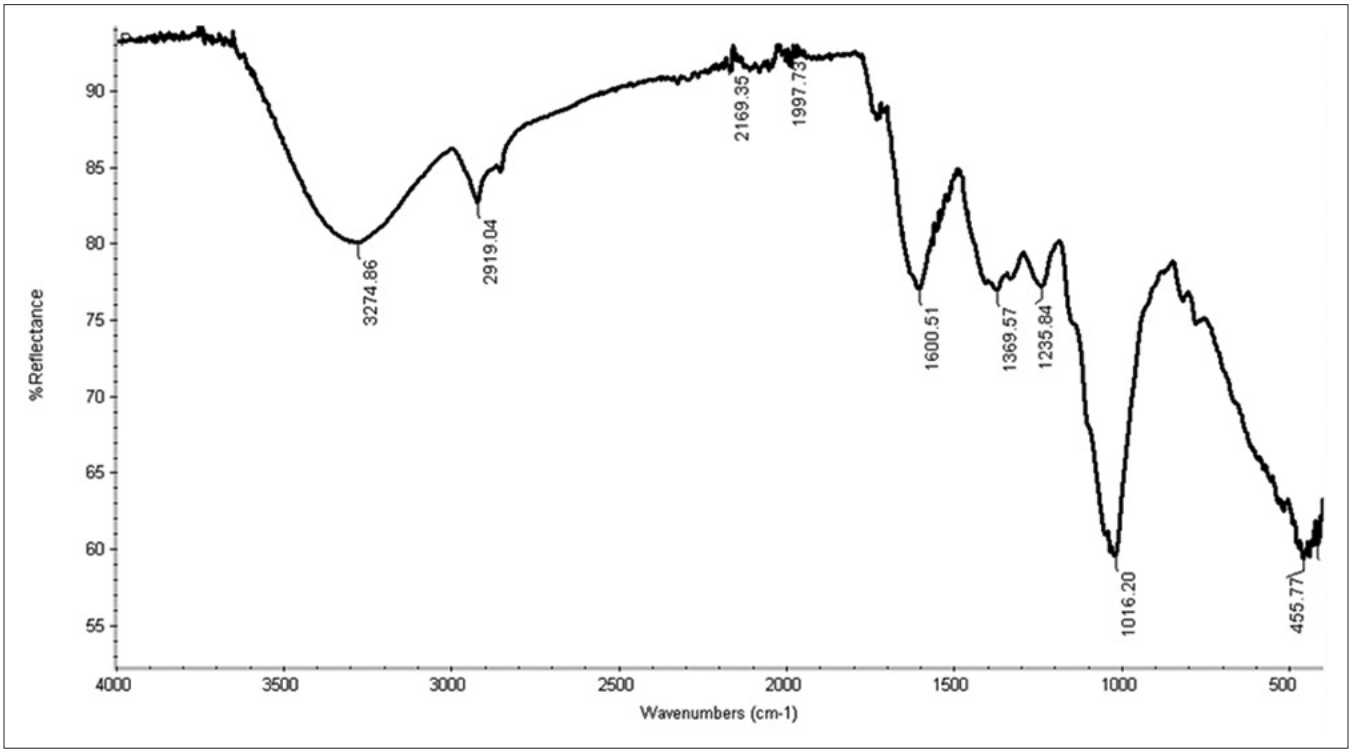

Figure 3: Fourier transform infrared of *Eclipta alba* (prostrate)

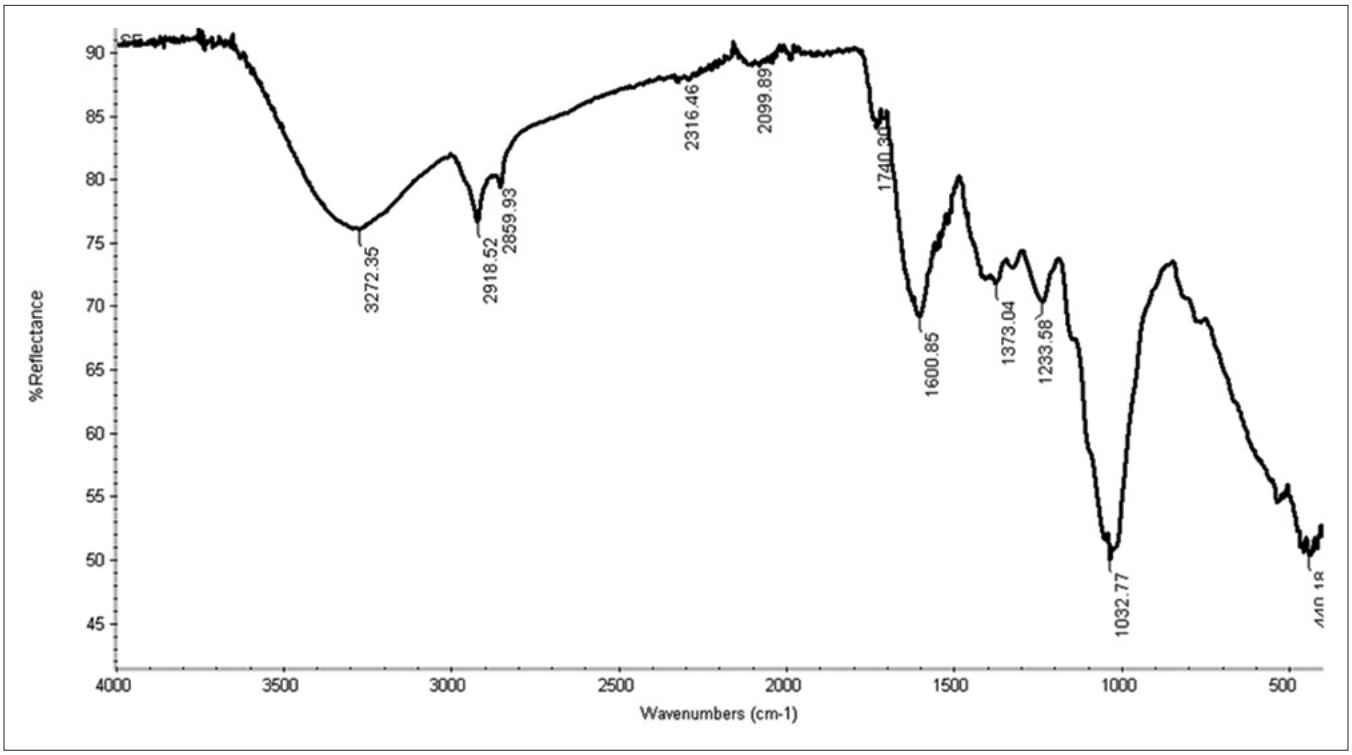

Figure 4: Fourier transform infrared of *Eclipta alba* (semi-erect)

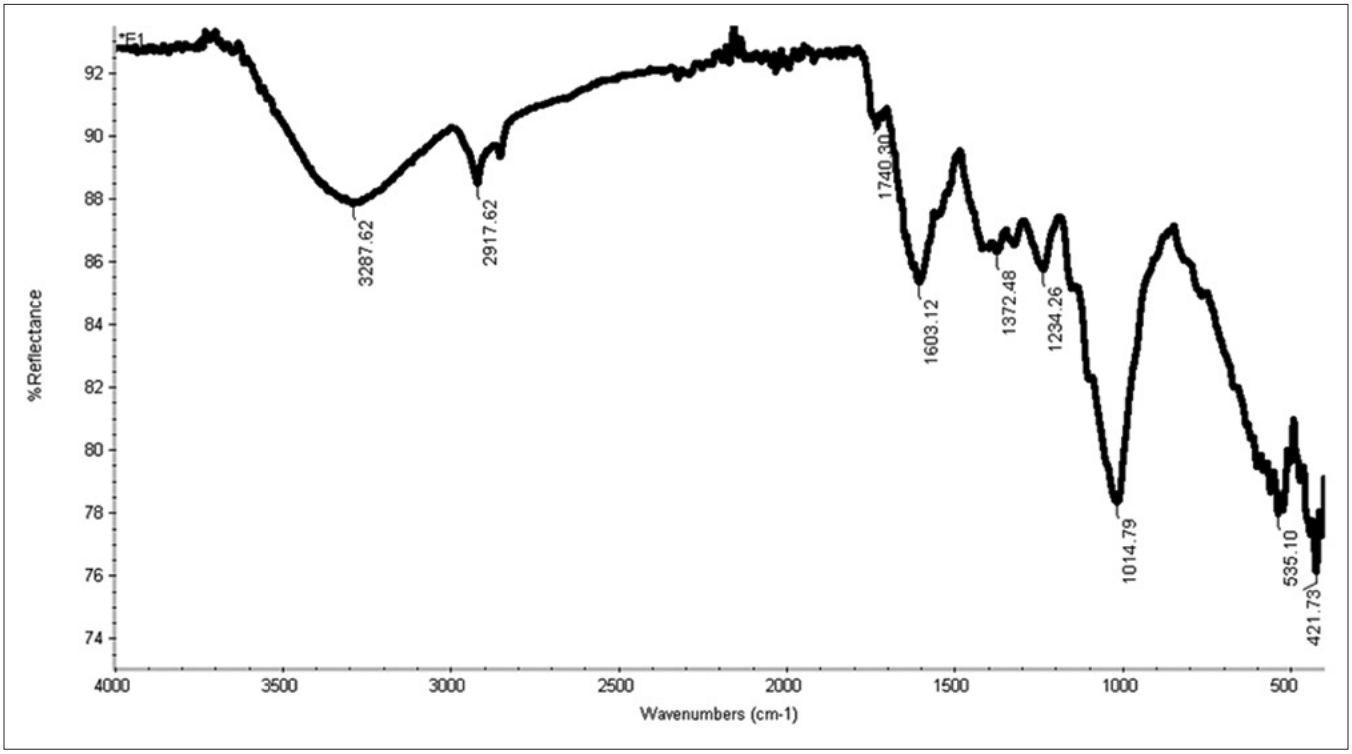

Figure 5: Fourier transform infrared of *Eclipta alba* (erect)

at slightly higher concentration than other two forms. It may likely be because of the use of whole plant powder. Similarly, 11 elements were studied in whole plant sample by Anuradha *et al.*[37] Some of these elements such as Na, Mg, Al, Si, P, S, Cl, K, and Ca have also been reported in three forms of *E. alba* during this study. Comparative WD-XRF analysis of three morphotypes seems to be the first report. These elements are known to affect various biochemical processes of human body. Thus, their determination is of utmost importance to quantify the medicinal potential of

Table 2: Comparative IR frequencies of *Eclipta alba* (morphotypes)

Peak values			Functional groups	Literature IR frequencies range for the respective functionality[38] (cm^{-1})
Prostrate (cm^{-1})	Semi-erect (cm^{-1})	Erect (cm^{-1})		
3274.86	3272.35	3287.62	O-H stretching in alcohols, phenols, and carboxylic acids (hydrogen bonded; broad absorption)	3400-3200
2919.04	2918.52	2917.62	Alkanes sp^3 C-H stretch	3000-2840
-	2859.93	-	Stretch, aldehyde hydrogen (-CHO) consists of a pair of weak bonds	2860-2800
-	2316.46	-	Carbonate stretching (for CO_2 formation)	2360-2000
2169.35	2099.89	-	C≡C stretching in alkynes	2250-2100
			X=C = Y type of vibration in allene, ketene, isocyanates, isothiocyanates	2270-1940
1997.73	-	-	X=C = Y type of vibration in allene, ketene, isocyanates, isothiocyanates	2270-1940
-	1740.30	1740.30	C=O stretch in carbonyl compounds (aldehydes, ketones)	1740-1705 (aldehyde, ketone)
1600.51	1600.85	1603.12	C≡C stretch	1660-1600
			N-H bond in 1° amines	1640-1560
1369.57	1373.04	1372.48	C-F stretch	1400-1000
			Nitro group has a strong absorption	1390-1300
1235.84	1233.58	1234.26	C=O bending in ketones appears as a medium intensity peak	1300-1100
			Phenyl alkyl ethers give two strong bonds C-O stretch in alcohols, ethers, esters, carboxylic acids, anhydrides	1250-1040
			C-N stretch in amines	1350-1000
			Aryl fluorides absorb	1250 and 1100
			Asymmetric S=O stretch in Sulfones, Sulfonyl chlorides, Sulfates, Sulfonamides	1350-1140
1016.20	1032.77	1014.79	C-N stretch in amines C-O stretch in alcohols, ethers, esters, etc.	1350-1000
-	-	535.10	C-X for bromides/iodide	<667
455.77	440.18	421.73	C-X for bromides/iodide	<667

IR: Infrared

plant species. This information is likely be useful for the synthesis and designing of medicines.

CONCLUSION

Morphological characterization of populations of *E. alba* has described three morphotypes, i.e., prostrate, semi-erect, and erect for this species but with a constant chromosome number 2n=22 in all forms. Phytochemical analysis of these forms has revealed the presence of alkaloids, amino acid, anthocyanin, betaxanthin, carbohydrates, coumarin, flavonoids, glycosides, gums and mucilage, oxalate, phenolic compounds, etc. The number of phytoconstituents in prostrate form is slightly more whereas erect and semi-erect forms have similar phytochemicals. Further, the FTIR analysis has suggested various functional groups associated with the reported phytochemicals. WD-XRF spectroscopy detected various elements such as Na, Mg, Al, Si, Cl, K, P, S, Ca, Mn, Fe, Ni, Cu, Zn, Br, *etc*. Hence, this study has justified the use of this plant in traditional health-care systems. Based on the findings, prostrate form seems to be better as it contains

Table 3: WD-XRF data for morphotypes of *Eclipta alba* (mg/g)*

Elements	*Eclipta alba*		
	Prostrate	Semi-erect	Erect
$_{11}$Na	1.6	1.7	2.2
$_{12}$Mg	6	5.5	5.8
$_{13}$Al	1.1	1.3	0.7
$_{14}$Si	15.7	14.1	14.3
$_{15}$P	3.6	3.6	3.6
$_{16}$S	4.3	3.3	3.7
$_{17}$Cl	4.4	4.2	5.6
$_{19}$K	40.3	34.9	35.3
$_{20}$Ca	13.2	10.9	12
$_{22}$Ti	0.087	0.083	0.051
$_{24}$Cr	0.019	0.016	0.013
$_{25}$Mn	0.3	0.2	0.2
$_{26}$Fe	0.8	0.7	0.5
$_{28}$Ni	0.006	0.007	0.008
$_{29}$Cu	0.046	0.04	0.038
$_{30}$Zn	0.001	0.001	0.001
$_{34}$Se	0.042	0.037	0.033
$_{35}$Br	0.054	0.061	0.063
$_{37}$Rb	0.011	0.01	0.012
$_{38}$Sr	0.074	0.063	0.065
$_{40}$Zr	0.005	0.007	0.004
$_{42}$Mo	0.019	-	0.019
$_{44}$Ru	0.021	0.018	0.022
$_{75}$Re	0.1	0.1	0.1
$C_6H_{10}O_5$	908.2	919.1	915.6

*The error in concentration values of the elements ranges from~5% up to 20%. WD-XRF: Wavelength dispersive X-ray fluorescence

useful elements in the higher concentration than other two forms. Thus, it can be explored in future for its utility in the medicinal preparations.

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