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Research Article

SCREENING OF BIOPROTECTIVE PROPERTIES AND PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS OF *ECLIPTA ALBA* WHOLE PLANT

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ABSTRACT

The medicinal plants are laden with numerous effective antibacterial, antioxidants, anticancer agents which provide an alternative means of therapy to various infections caused by drug resistant bacteria, oxidative stress due to over-production of free radicals and leading to dreadful diseases like cancer and other physiological disorders. This study was conducted to evaluate the biological properties of different extracts of whole plant of *Eclipta alba* in terms of its antimicrobial, antioxidant, cytotoxic activities and phytochemical analysis to find out the active compounds responsible for these activities. Cell viability assay was performed using Human lung epithelial adenocarcinoma cell line (HCC-827). Our results demonstrated that extracts causes growth arrest and apoptosis in lung cancer cells and the growth inhibitory effects were found to be significant. Cell morphology was observed using phalloidin staining. GC-MS analysis showed the presence of compounds like Naphthoquinone, Hydrazine carboxamide of biological significance.

Keywords: *Eclipta alba*, Antioxidant, Cytotoxic, Human lung epithelial adenocarcinoma cell line (HCC-827), MTT, Phalloidin, GC-MS.

INTRODUCTION

Medicinal plants possess all the phytoconstituents which serves as a source of bioactive chemicals necessary for the significant pharmacological actions without any adverse effects. They can be used for the development of new classes of possibly safer drugs or medicines to cure various ailments. An increased resistance to present day available antibiotics has posed a problem worldwide due to the frequent use of antibiotics. The herbal drugs have always been used in healing various diseases because of the wide safety profile they provide. Important bioactive compounds contributing to the medicinal values in plants are alkaloids, glycosides, resins, gums, mucilages etc **(1).**The search of plant-based products has completely modified the drug discovery programme as herbal compounds have shown a promising effect in therapeutics. The diversity in the disease causing ability of bacteria has always presented a challenge in the treatment of their infections **(2).** Numerous plant-based substances show potential antitumour activity in several rodent and human cancer cell lines **(3)**. Phytosignatures such as vitamins (A, C, E, K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals have been found to exhibit antioxidant activities **(4; 5; 6).** *Eclipta alba* (L) is an annual herbaceous plant, commonly known as false daisy. It is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate belonging to family Asteraceae. Various extracts of *E. alba* has exhibited potent antimicrobial activity (**7, 8**), antioxidant power evaluated by DPPH and FRAP methods (**9, 10**) and immunomodulatory properties (**11**). *Eclipta alba* possess various biological properties and used for the treatment of various disorders like memory disorders, general tonic, edema, rheumatic pain treatments, digestion, hepatitis, enlarged spleen, antioxidant activity and Skin disorders **(12; 13; 14).** All the parts of *E. alba* contain various chemical constituents which have been used in different therapeutic cases. Major constituents are coumestans i.e. wedelolactone (I) and demethylwedelolactone (II), polypeptides, polyacetylenes, thiophene-derivatives, steroids, triterpenes and flavonoids. Coumestans are known to possess estrogenic activity **(15).** Wedelolactone exhibits various medicinal properties which is helpful in the treatment of hepatitis and cirrhosis (**16),** bacterial infection and hemorrhagic condition **(17).**

The study was performed to evaluate the biological properties of different extracts of whole plant of *Eclipta alba* as potential antimicrobial, antioxidant, cytotoxic values and also its phytochemical analysis was conducted to find out the active compounds responsible for these activities to search an alternative cure to respiratory infections.

MATERIALS AND METHODS

Plant collection

The dried whole plants of *Eclipta alba* were obtained from local market of Dehradun, India.

Chemicals and Reagents

2, 4, 6-tripyridyl-s-triazine (TPTZ), Mueller Hinton Agar were purchased from Hi-Media (Mumbai, India), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT, 98%) reagent, Fetal bovine serum (FBS) and streptomycin-penicillin antibiotic solutions were purchased from Sigma Aldrich, South Korea. The chemicals and reagents used for the study are of pure grade. The human lung epithelial adenocarcinoma cell line (HCC- 827) was procured from Korean cell bank, South Korea.

Preparation of plant extracts

25g of powder was taken as a thimble charge and organic {ethyl acetate (S1), methanol (S2)} and water (S3) extraction was done in succession using soxhlet extraction method **(18).** All the extracts were made solvent free and concentrated using rotary evaporator and preserved at 4°C in airtight bottle until further use.

Antimicrobial Susceptibility testing

Agar well diffusion method

The antimicrobial activity of all the extracts (ethyl acetate, methanol, aqueous) of *Eclipta alba* in reference was determined by agar well diffusion method **(19).** The extracts were dissolved in DMSO (dimethylsulphoxide) to obtain the concentration of 0.5mg/100µl and 1mg/100µl. The antibiotic gentamicin (0.5mg/100µl, 1mg/ 100µl) was used as positive control and DMSO as negative control. The tests were performed in triplicates and the final results were presented as the mean zone of inhibition and standard deviation were calculated.

Broth Dilution MIC test

The Minimal Inhibitory Concentration (MIC) of the plant extracts was determined by macro broth dilution assay **(20).** On the basis of the results obtained from Agar well diffusion method (ZOI) two-fold serial dilutions of all the extracts were prepared in well plates with Mueller-Hinton Broth (Hi-media, Mumbai, India) as diluents. 20µl of test microorganisms of the standard concentration (5×10^5 cfu/ml) was inoculated in the each dilution. Two-fold serial dilution of DMSO and gentamicin was used as experimental negative and positive control respectively. The plates were incubated at 37ºC for 24hours.

The lowest concentration at which the extract or standard drug showed no visible growth (turbidity) was taken as the MIC.

Determination of Minimum Bactericidal Concentration

20µl of the MIC test broth tube solutions were spread over MHA plates and incubated for 18-24h at 37ºC. The plates showing no single bacterial growth, the dilution was considered as MBC (Minimum Bactericidal Count) concentration of the extract that is bactericidal in nature. The MIC index (MIC/MBC) was performed to determine whether an extract is bactericidal (MIC/MBC <4) or bacteriostatic (MIC/MBC >4) in nature. MIC index values of greater than 4 and less than 32 are considered as bacteriostatic (**21**). The test was performed in triplicates and its mean MIC and MBC values were calculated. The results were expressed in terms of standard deviation.

Antioxidant power (Ferric Reducing Ability of Plant)

The FRAP assay was performed as described by **19.** The stock solution of various extracts of 2.5mg/ml concentration was prepared in DMSO. 10µl-100µl of extract was mixed with 1.5ml of FRAP reagent and the volume was adjusted to 5ml with distilled water. The tubes were incubated at 37ºC for 15minutes and absorbance was noted at 593nm.

Anticancer study

The human lung epithelial adenocarcinoma HCC-827 cell line was cultured and maintained in 90% DMEM media substituted with 10% Foetal Bovine serum (FBS) and 1% antibiotic for 24h. The media was then removed and the cell layer was washed with phosphate buffer saline (PBS) (0.1M pH7.0) to remove the traces of media. Later, 500 µl of trypsin-EDTA was added to the culture flask to remove the adherent cell layer from the flask. After 5min, 2ml of the media was added and single cells were collected. The cells were counted on the haemocytometer to get the exact viability and cell count for the experiments. 1×10^5 cells of the human lung epithelial adenocarcinoma-HCC-827 were used for the anticancer study of extracts in reference.

Cell Viability Assay

The viability of the cells was assessed by MTT (3, 4, 5-dimethylthiazol-2yl)-2-5-diphenyltetrazolium bromide) assay **(22).** 1 × 105 cells (The human lung epithelial adenocarcinoma-HCC-827) were incubated in DMEM (Dulbecco's modified Eagle medium) containing extracts of various concentrations (10µg/ml, 50µg/ml, and 100µg/ml) in 5% CO2 incubator at 37ºC. The metabolic activity of each concentration was assessed using MTT assay at 570nm after 24h and 48h.

Image Analysis

Phalloidin staining of the control and treated cells was performed to check the changes in the structure of cytoskeleton of the cancerous cells. Cells were fixed in 4% paraformaldheyde for 10 min, and were then permeabilized with 0.1% Triton-X 100 for 5min and after each step thorough rinsing with 0.01M PBS at room temperature (in LAF) was performed. The working solution of FITC labeled Phalloidin stain was made up in 1:200 dilutions with 1% BSA and cells were incubated for 15 min before imaging. The morphology of the cell cytoskeleton was observed under microscope (Nikon, TE-2000 U) **(23).**

Phytochemical analysis of the extracts

Gas Chromatography and Mass Spectroscopy (GC-MS)

The GC-MS analysis of ethyl acetate, methanol and water extracts were carried out using REX column. 2µl of samples were introduced via an all-glass injector working in the split mode, with helium as the carrier gas. Temperature programme: 70°C -300°C at 6º/min, with 10min hold at 300°C. The identification of components was accomplished using computer searches in commercial library (Wiley 8 and NIST).

RESULTS AND DISCUSSION

Extraction of the plant samples

Extracts were prepared in the series of different organic and water solvents based on increasing polarity index {Ethyl acetate **(4.4)**, Methanol **(5.1),** and Water **(9.0)**} using Soxhlet extraction method. Results obtained showed the variation in extracting power of the solvents. Water possessed highest extraction ability than the organic solvents like ethyl acetate and methanol. The maximum yield was obtained in the case of water extract which has maximum polarity followed by ethyl acetate and least by methanol (Table 1).

Antimicrobial Susceptibility testing

Traditional medicinal plants are yet to be systematically investigated against various pathogens, which have developed resistance to the present day antibiotics, drugs or any other means of treatment **(24).** Plants as antimicrobials should be tested against the specific pathogens to test their therapeutic values. The antimicrobial potential of plant extracts have been explored by a very large number of scientists world over **(25; 26).**

The screening results of our antimicrobial study confirms the strong inhibitory effect of (S1, S2 and S3) extracts on the chosen pathogens **(Table: 2, 3, 4, 5)**. Out of all the extracts tested and tried, S2 extract was found to exhibit strong inhibition on all the pathogens except *Streptococcus mutans*. The bactericidal effect of the extract became more prominent with the increasing concentration. *S. pyogenes* was most effectively inhibited by S2 extract (27±0.00) at a concentration of 0.5mg/100µl whereas comparatively less inhibition was shown by S3 and S1 in the order. *S. mutans* was resistant to S1 and S2 extracts but S3 extract of *E. alba* (9±1.63, 15±0.816) could inhibit the pathogen at a concentration of 0.5mg/100µl and 1mg/100µl respectively. S3 extract was active against *S. aureus* (25±0.816) at a concentration of 0.5mg/100µl followed by S2 (18±0.816) and S1 extract (17±0.816) at the same concentration. The activity of *K. pneumoniae* (15±0.816) and *S. pneumoniae* (17±0.816) were strongly suppressed by S3 extract than S2 and S1 extract. Antibacterial activities of various extracts of *E. alba* were comparable to those of standard antibacterial drug gentamicin (positive control). Gentamicin inhibited pathogens effectively when compared with the extracts. Evaluation of the minimum concentration required for the inhibition of growth of microorganisms in reference was found to be within the range of 0.5-0.0156mg/ml **(Table: 6, 7, 8)**. Concentration required to inhibit the growth of *S. aureus* was found to be same for all the extracts of *E. alba*, S1, S2 and S3 extracts (0.0156mg/ml) whereas S2 and S3 extract inhibited *S. pneumoniae* at the same concentration as *S. aureus* (0.0156mg/ml). The minimum inhibitory concentration required to inhibit *K. pneumoniae* was 0.0625mg/ml (S1 extract) and 0.125mg/ml (S2 and S3 extracts). The MIC, MBC and MIC index values of all the extracts (0.0156mg/ml, 0.0312mg/ml and 2) for *S. aureus* coincides with the values of Gentamicin whereas the values of S1 and S3 extracts (0.0156mg/ml, 0.0312mg/ml and 2) for *S.pyogenes* were same as that of the standard drug Gentamicin. The MIC, MBC and MIC index values of S3 extract (0.0156mg/ml, 0.0312mg/ml and 2) for *S. pneumoniae* were equal to the values of the reference drug showing the effectiveness of the bactericidal potential of the extracts as that of the chemically synthesized drug Gentamicin. MBC values were higher than the MIC values and MIC index values indicated the bactericidal properties of the extracts of *E.alba*.

Our data on antimicrobial activity of *E.alba* different extracts on the pathogens causing respiratory diseases in humans offers effective inhibition and found to be potent and is comparable with the studies done by **27, 28** and **29.** In the present day scenario, investigation on plants has increased all over the world and large evidences on the bioprotective properties of medicinal plants and their role in various traditional systems have been observed **(30).** In recent years there has been increase in the multiple drug resistance in human pathogens due to frequent use of chemically synthesized antibiotics which are commonly used in the treatment of their infections **(31; 32).** Bacteria have the capability to develop resistance to the drug and also transfer it, which is often used as antibacterial agent **(33).** The antibacterial action of the extracts may be of following types: Damage in microbial genome after treatment, damage in protein synthesis mechanism or structure, microbial membrane disintegration or specific enzyme inhibition.

Table 1: The yield, yield% and physical properties of *Eclipta alba*.

S. No.	Solvent Used	Yield (g/500ml)	Yield %	Colour	State
1.	Ethyl Acetate (S1)	0.59	2.36	Blackish green	Viscous
2.	Methanol (S2)	0.29	1.16	Greenish brown	Powder
3.	Water (S3)	1.54	6.16	Brownish	Solid

Table 2: The ZOI of control (Gentamicin) against different pathogenic pathogens

S. No.	Micro-Organisms	Zone of Inhibition (mm) Concentration of the drug in mg/100µl	
		0.5mg/100µl	1.0mg/100µl
1.	P1	28 ± 0.816	29 ± 0.816
2.	P2	31 ± 0.816	32 ± 0.816
3.	P3	27 ± 0.816	28 ± 0.816
4.	P4	31 ± 0.816	32 ± 0.816
5.	P5	29 ± 0.816	30 ± 0.816

Table 3: The antimicrobial activity of ethyl acetate extract against different pathogens

S. No.	Micro-Organisms	Zone of Inhibition (mm) Concentration of the drug in mg/100µl	
		0.5mg/100µl	1.0mg/100µl
1.	P1	10 ± 0.816	11 ± 0.816
2.	P2	17 ± 0.816	19 ± 0.816
3.	P3	Nil	Nil
4.	P4	21 ± 0.816	24 ± 0.816
5.	P5	14 ± 0.816	16 ± 0.816

Table 4: The antimicrobial activity of methanol extract against different pathogens

S. No.	Micro-Organisms	Zone of Inhibition (mm) Concentration of the drug in mg/100µl	
		0.5mg/100µl	1.0mg/100µl
1.	P1	13 ± 1.00	15 ± 0.816
2.	P2	18 ± 0.816	19 ± 0.816
3.	P3	Nil	3
4.	P4	27 ± 0.00	29 ± 1.73
5.	P5	11 ± 0.816	12 ± 0.00

Table 5: The antimicrobial activity of water extract against different pathogens

S. No.	Micro-Organisms	Zone of Inhibition (mm) Concentration of the drug in mg/100µl	
		0.5mg/100µl	1.0mg/100µl
1.	P1	15 ± 0.816	19 ± 0.816
2.	P2	25 ± 0.816	28 ± 1.63
3.	P3	9±1.63	15±0.816
4.	P4	23 ± 0.816	25 ± 0.816
5.	P5	17 ± 0.816	23 ± 0.816

Table 6: The MIC, MBC and MIC Index values of ethyl acetate extract against different pathogens.

Organism	Range (mg/ml)	MIC (control) (mg/ml)	MBC (control) (mg/ml)	MIC (extract) (mg/ml)	MBC (extract) (mg/ml)	MIC Index (control)	MIC Index (extract)
P1	0.5- 0.0156	0.0156	0.0312	0.0625	0.125	2	2
P2	0.5- 0.0156	0.0156	0.0312	0.0156	0.0312	2	2
P4	0.5- 0.0156	0.0156	0.0312	0.0156	0.0312	2	2
P5	0.5- 0.0156	0.0156	0.0312	0.0312	0.0625	2	2

Table 7: The MIC, MBC and MIC Index values of methanol extract against different pathogens

Organism	Range (mg/ml)	MIC (control) (mg/ml)	MBC (control) (mg/ml)	MIC (extract) (mg/ml)	MBC (extract) (mg/ml)	MIC Index (control)	MIC Index (extract)
P1	0.5- 0.0156	0.0156	0.0312	0.125	0.25	2	2
P2	0.5- 0.0156	0.0156	0.0312	0.0156	0.0312	2	2
P4	0.5- 0.0156	0.0156	0.0312	0.0312	0.0625	2	2
P5	0.5- 0.0156	0.0156	0.0312	0.0156	0.0312	2	2

Table 8: The MIC, MBC and MIC Index values of water extract against different pathogens.

Organism	Range (mg/ml)	MIC (control) (mg/ml)	MBC (control) (mg/ml)	MIC (extract) (mg/ml)	MBC (extract) (mg/ml)	MIC Index (control)	MIC Index (extract)
P1	0.5- 0.0156	0.0156	0.0312	0.125	0.25	2	2
P2	0.5- 0.0156	0.0156	0.0312	0.0156	0.0312	2	2
P4	0.5- 0.0156	0.0156	0.0312	0.0156	0.0312	2	2
P5	0.5- 0.0156	0.0156	0.0312	0.0156	0.0312	2	2

Antioxidant Power

Oxidative stress when increases causes damage in DNA leading to base damage, breaks in strands, alteration in gene expression, and ultimately mutagenesis **(34; 35).** It also causes various neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, Huntington's disease, Cardiovascular diseases, Respiratory diseases, Arthritis etc. The data obtained through antioxidant studies clearly depicted that the different extracts of *E. alba* possessed the capacity to neutralize the free radicals responsible for causing various diseases. The phytosignatures present in S2 extract of the *E.alba* were found to be the best free radical scavenger. The antioxidant power was concentration dependent in all the cases as the FRAP value exhibited rise with the increasing concentration of the extract. The S2 extract (52.5µM/l) at a concentration of 50×10^{-3} g/l showed the free radical scavenging power better than S1 (35.0µM/l) and S3 extract (18.0µM/l) at the same concentration. The results obtained were compared to the reference compound Gallic acid, a known antioxidant, a strong polyphenols showed high antioxidant power. The graphical representation is presented in **Fig 1.**

Many plant based medicines contain active biosignatures such as polyphenols, flavonoids and phenolic compounds that exert protective effect on cells from oxidative stress **(36).**

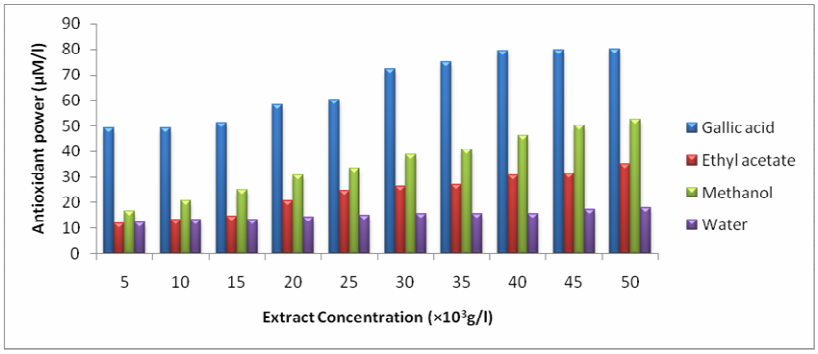

Fig 1: The antioxidant power (µM/l) of Gallic acid (reference) and various (S1, S2 and S3) extracts of *E.alba* at increasing concentration (g/l).

Anticancer activity

Plants have many bioactive compounds, which exhibits strong antioxidant activities. The antioxidants scavenge free radicals, protect the cell from highly reactive species and cure cancer. Many substances present in the human diet naturally act as potential chemopreventive agents so vegetables and fruits can provide prevention against cancer (37; 38). The human lung epithelial adenocarcinoma cell line (HCC-827) was used for anticancer study of different extracts (solvent-free) of *E.alba*. The different solvent-free extracts were tested for its antitumor activity on HCC-827 cell line using 3-(4, 5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT assay), which is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells to a purple formazon product. Results clearly showed that different solvent extracts of the plant in reference, exhibited effective to moderate inhibition on lung cancer cells. Inhibition of the growth of carcinoma cells was found to be concentration dependent. The potency of each plant extract to inhibit the cancerous growth was recorded in terms of decrease in viable cell count as compared to the control value. The inhibition of the growth of human lung epithelial adenocarcinoma cell line (HCC-827) has been found to be dose dependent and time dependent in S1 extract whereas S2 and S3 extracts showed slight variation in cell count. The S1 extract exhibited strong anti-tumor activity resulting into appreciable loss in viable cell count against the lung cancer cells followed by S2 and S3 extract in comparison to the control cells. The decrease in the viable cell count of cancerous cells was noticeable and increased with the increase in concentration of the S1 extract. The data is tabulated in **tables 8,9,10**. Bright Phase Microscopy shows reduction in the number of cancerous cells when treated with the extracts of *E. alba* that were observed to induce apoptosis as images shows changes in the cell morphology.

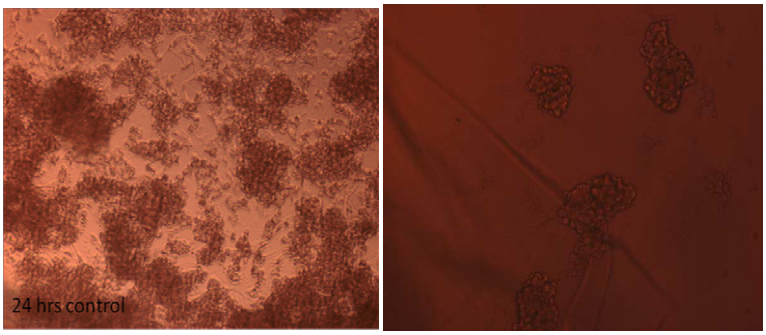

Fig. 2: Bright phase microscopy of Lung cancer cells after 24 h, Panel A shows the untreated cells (Control), Panel B represents cells treated with S3 extract.

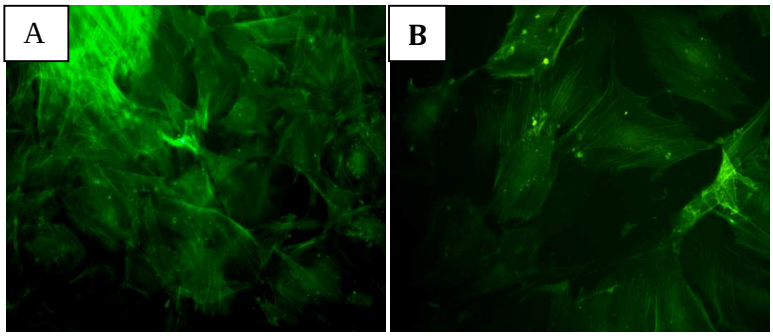

Fig. 3: Phalloidin stained control cells (Panel A), treated cells (Panel B) shows changes in cytoskeleton morphology and reduction in number of cells after 48h of treatment with extracts

Table 8: The effect of S1 extract on HCC-827 cells after 24h and 48h.

S. No.	Concentration (µg/ml)	Control (24h)	Treated (24h)	Control (48h)	Treated (48h)
1.	10	$1.57x10^5$	$0.02 x10^5$	$2.17x10^5$	$0.03x10^5$
2.	50	$1.57x10^5$	$0.03 x10^5$	$2.17x10^5$	$0.02 x10^5$
3.	100	$1.57x10^5$	$0.02 x10^5$	$2.17x10^5$	$0.03 x10^5$

Table 9: The effect of S2 extract on HCC-827 cells after 24h and 48h

S. No.	Concentration (µg/ml)	Control (24h)	Treated (24h)	Control (48h)	Treated (48h)
1.	10	$1.57x10^5$	$0.05 x10^5$	$2.17x10^5$	$0.02x10^5$
2.	50	$1.57x10^5$	$0.05 x10^5$	$2.17x10^5$	$0.05 x10^5$
3.	100	$1.57x10^5$	$0.06 x10^5$	$2.17x10^5$	$0.20 x10^5$

Table 10: The effect of S3 extract on HCC-827 cells after 24h and 48h.

S. No.	Concentration (µg/ml)	Control (24h)	Treated (24h)	Control (48h)	Treated (48h)
1.	10	$1.57x10^5$	$0.20 x10^5$	$2.17x10^5$	$0.17x10^5$
2.	50	$1.57x10^5$	$0.40 x10^5$	$2.17x10^5$	$0.42 x10^5$
3.	100	$1.57x10^5$	$0.50 x10^5$	$2.17x10^5$	$0.46 x10^5$

Phytochemical Analysis

Gas Chromatography and Mass Spectroscopy

Extraction of the specific phytochemical compounds from the plant's crude extract depends on the extraction procedure followed. Traditional medicinal plants use primarily water as the solvent but plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water in many studies reported. The activities reported in organic solvents other than water can be related to the polarity of the solvents and the nature of the bioactive compounds extracted **(39).** Plants possess a wide variety of phytoconstituents such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins and lignins **(40).** Various extracts of *E.alba* (S1, S2, and S3) were subjected to analysis and identification of phytoconstituents responsible for the wide array of biological activities **(Table 11, 12, 13)**. The extracts revealed the presence of various compounds like Hydrazine carboxamide, Naphthoquinones, Glycine, Carbamic acid etc., Hydrazine carboxamide is reported to possess antimicrobial activity and showed inhibition against some bacterial strains like *S. aureus, K. pneumoniae*, *E.coli, P.aeruginosa* and some fungal species, *A. niger, A. flavus, P.citrinum, C. albicans* and *Monascus purpereus* **(41),** which is also exhibited in our study where the extracts were found to be highly antimicrobial in nature which may be attributed to the compounds present in various extracts. Naphthoquinones are categorized under the category of phenolic compounds. They act by binding to DNA and proteins (enzymes) and inhibit the process of replication. They causes disturbance in cell and mitochondrial membranes leading to the interference with the respiratory chain electrons on the mitochondrial membranes which may be attributed to the anticancer activity of the plant. Derivatives of naphthoquinone show numerous biological activities such as antibacterial, antiviral, antitumour, cytotoxic, insect repellent, anti inflammatory, antipyretic properties. Plants with such constituent are used for the treatment of malignant and parasitic diseases in China and countries of South America **(42).** Glycine is an essential amino acid used as a metabolic product for the growth of bacteria. In excess inhibits the growth of bacteria and used as a non-specific antiseptic agent. Esters of Carbamic acid are used as muscle relaxants **(43).** The phytoconstituents identified in the extracts of *E.alba* were found to be antibacterial, antitumor and antioxidant in nature so the plant can be used in herbal drug formulation as a cure to respiratory infections which arises due to bacterial pathogenecity, oxidative stress caused by the overproduction of free radicals, that leads to the cancer when under uncontrolled conditions.

Table 11: The identified phytochemicals in the S1 extract were detected using GC-MS technique.

Peak no.	Phytochemicals	Molecular formula	Retention time (min)
1	Hydrazinecarboxyamide	CH_5N_3O	3.307
4	2-Propanone	C_3H_6O	3.613
10	Sclerosol	C_2H_6OS	7.110
22	Phoshine	CH_5P	7.903
23	Carbamic acid	CH_3NO_2	8.070
25	Methane-D3,Nitro-	CD_3NO_2	8.883
32	2H-Benzopyran-4-Carbonitrile,6-Fluoro-3,4-Dihydro-4-[(Methylthio)Methyl]	$C_{12}H_{12}FNOS$	16.767
34	2-Pyridinepropanoic acid	$C_{11}H_{13}NO_3$	46.097
38	Aminourea	CH_5N_3O	46.413

Table 12: The identified phytochemicals in the S2 extract were detected using GC-MS technique.

Peak no.	Phytochemicals	Molecular formula	Retention time (min)
3	N-(3,4,4-Trimethyl-1,2-Dioxethane-3-yl-MethoxyCarbonyl)Glycine	$C_9H_{15}NO_6$	3.620
6	Silane	$C_8H_{18}C_{12}OSi$	3.933
17	Acetamide	$C_2H_3C_{12}NO$	7.723
25	1H-Pyrimido[4,5,6-IJ][2,7]Naphthyridine-6-Carbonitrile,2-Ethyl-5,8-Dimethoxy-	$C_{14}H_{13}N_5O_2$	50.753
26	Acetonitrile-D3	C_2D_3N	50.860
28	3-Methoxy-5-(Methoxymethoxy)-7-Methyl-6-(3-(Trimethylsilyl)Propargyl)-1,4-Naphthoquinone	$C_{20}H_{24}O_5Si$	51.007
30	L-Alanine, Ethylester-	$C_5H_{11}NO_2$	52.513
32	Formamide,N-[(dibutylamino)methyl]-N-methyl-	$C_{11}H_{24}N_{20}$	53.240
34	Trans-2-((phenylthio)methyl)-1-(2-propenyl)-1,2,3,4-tetrahydronaphthalene	$C_{20}H_{22}S$	53.747
37	2-Acetonyl-3-cyano-2,3-dimethylcyclobutane-1-carboxylic acid	$C_{11}H_{15}NO_3$	54.043
45	5,5'-dicarboxy-3'-(2-chloroethyl)-4-(2-acetoxyethyl)-3,4'-dimethylpyrromethane	$C_{19}H_{23}ClN_2O_6$	56.893

Table 13: The identified phytochemicals in the S3 extract were detected using GC-MS technique.

Peak no.	Phytochemicals	Molecular formula	Retention time (min)
10	3,3'-[1,2-hydrazindiyl-bis(Carbonyloxymethylene)[Bis(3,4,4-trimethyl-1,2-dioxethane	$C_{14}H_{24}N_2O_8$	3.620
23	D5-Ethylnitrate	$C_2D_5NO_3$	7.740
25	Methane- D3	CD_3NO_2	8.850
20	Bis(Fluoromethyl)(Dimethyl)Silane	$C_4H_{10}F_2Si$	7.343
27	Erythro-1,2-Dimethyl-1-Methylthio-2-Hydroxyethane	$C_5H_{12}OS$	10.080

CONCLUSION

Much attention has been drawn towards the use of plant based formulations due to the presence of significant bioactive principle signatures for the treatment of many infections. Antibiotics provide immediate relief but with numerous side effects on the human body. Cure to the respiratory infections with the commercially available antibacterial drugs has been known but the pathogens causing respiratory afflictions in humans have developed resistance and posed a serious problem. The results of our study demonstrate the S2 extract of *Eclipta alba* (whole plant) to be a strong bactericidal and antioxidant agent and moderate cytotoxic agent. The phytochemicals identified plays a significant role in providing evidences for its use in herbal drug for the treatment of respiratory infections or Lung cancer when bacterial infections become chronic and uncontrolled oxidative stress occurs. The study is extendable to in vivo experiments for the determination of specific mode of action of the extract. The generated data has formed the basis for its use as a phytotherapeutic agent for the treatment of disease in reference.

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