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In-vitro Antioxidant, Antibacterial and Phytochemical Properties of Various Solvents Extracts of *Eclipta alba* Against Isolated ESBL Uropathogens

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Abstract: The main source of food, shelter, and many therapeutic techniques is provided by plants. Since ancient times, they have been used to treat a variety of human illnesses all throughout the world. Our study focused on assessing the antimicrobial activities and antioxidant activities of *Eclipta.alba (prostrata)* crude extracts (whole plant) using the in-vitro method. Different polarity-based solvent extracts of *Eclipta.alba* (prostrate) were screened against ESBL producing pathogens such as *Esherichia coli, klebsiella pneumoniae, Enterobacter aerogens, Proteus mirabilis, and Pseudomonas aeruginosa*. Out of all the tested extracts, aqueous extracts of *E. alba* exhibited the most effective activity against *K. pneumoniae* (18±0.577). Results obtained from all the extracts were compared with standard antibiotic amikacin. The antioxidant activity was determined using a DPPH assay and the data obtained showed that extracts of *E. alba (prostrate)* were highly antioxidant. GC-MS and HPLC analysis revealed the presence of significant bioactive compound. The reference compound used to compare the results of antioxidant values was Gallic acid. The study provides valuable evidence of the antibacterial activity of of *E.alba (prostrata)* crude extracts (Whole plant) in curing Urinary Tract Infections.

Keywords: *E. alba* • ESBL • Antioxidant • Gallic acid

Introduction

An infection that starts in the urinary system is known as a urinary tract infection (UTI). The kidneys, ureters, bladder, and urethra make up the urinary tract (Geetha et al., 2011). Due to their antibacterial characteristics, herbs have been utilised to treat UTIs since ancient times. India is one of the world's top producers of medicinal herbs and is rightfully referred to as the botanical garden of the globe since it has a wealth of historically well-documented and regularly used herbal medicine expertise. Due to their extensive safety profile, herbal medications have historically been utilised to treat a variety of disorders. Alkaloids, glycosides, resins, gums, mucilages, and other significant bioactive chemicals are important in plants that contribute to their therapeutic properties. *Eclipta alba* is a well-known species Bhringaraja is a small-branched annual herbaceous plant of the Asteraceae/Compositae family, which has a long history of usage in traditional remedies for its antimytotoxic, analgesic, antibacterial, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, and immune-modulatory effects (Manoj Kumar Pandey et al., 2011) The name *Eclipta alba* specifically relates to the colour of the blooms and implies white. In India, the plant is frequently used to make hair oils for long, healthy black hair. The root powder is used in Ayurveda to treat skin conditions, enlarged spleens, and hepatitis. The herb cures headaches when applied topically to the head while mixed with a little oil. To get rid of worms, newborns are given a mixture of the plant's leaf extract and honey. Children are sometimes given *Eclipta alba* when they have urinary tract infections To confirm the activity and determine the characteristics related to it, plants with potential antibacterial activity should be tested against an appropriate microbiological model. In the current investigation, the antibacterial activity of *Eclipta alba's* ethyl acetate,

methanol, and aqueous extracts was evaluated against uropathogen producers. to compare the antibacterial efficacy of *Eclipta alba (*L.) Hassk extracts to that of an antibiotic or other known antibacterial medication.

Material And Methods

Collection and source of plant material

The Eclipta alba(prostrata)(whole plant) (Family) Asteracea were collected during the month of June-August 2020 from in and around Haridwar(Uttarakhand) India.The plant materials were cleaned with distilled water and shade dried at room temperature. The plant materials were authenticated by the Botanical Survey of India, Dehradun.The plant material were shade dried for one to two weeks followed by the preparation of fine powder in a mixer grinder to obtain particle size less than 100µM. The powder was stored in a moisture-free environment for further use**.**

Preparation of Extracts

For the extraction, the powdered plant was extracted with solvents of increasing polarity. The Soxhlet extraction device was used to extract 25g of plant powder using a succession of increasing polarity solvents (organic ethyl acetate, methanol, and aqueous) (Vishnu et al., 2010). Extracts were concentrated and made solvent-free using a rotary evaporator.

Microorganisms:

ESBL uropathogens, including E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, and E. aerogens, were isolated from urine samples from outpatients with urinary tract infections (UTIs) who ranged in age from five to sixty-five (table 1) (Mishra et al., 2021). To verify their authenticity, Cowan & Steel's methodology used selective and differential culture media to study the cultural traits of isolates (Barrow et al., 2003).

Agar Well diffusion method for antibacterial activity

All E.alba extracts (ethyl acetate, methanol, and aqueous) were examined for their antibacterial activities using the agar well diffusion method (NCCLS, 2000; Chauhan et al., 2012). There were two different extract concentrations prepared: 1.0 mg/100 µl and 2.0 mg/100 µl. As a positive control, amikacin (1.0 mg/100 µl or 2.0 mg/100 µl) was used. The average zone of inhibition with standard error was used to represent the results.

Minimum Inhibitory Concentration (MIC) For determination of the minimum inhibitory concentration, tests were performed in microtiter plates using Muller-Hinton broth, and extracts were diluted in successive wells (**NCCLS 2000**). In each well 20µl of isolated uro-pathogen is added at the standard concentration of 5 X 10^5Cfu/ml. The experimental negative and positive controls were amikacin respectively. The plates were incubated for 24 hours at 37°C. The MIC was determined as the lowest concentration at which the extract or standard antibiotics exhibited no observable growth (turbidity).

Antioxidant activity:

DPPH free radical scavenging activity

The 2, 2-diphenyl-1-picryl-hydrazyl hydrate (DPPH) assay was performed to assess the ability of ethyl acetate, methanol, and aqueous extracts of the chosen plant to neutralize free radicals. 700 ml of the test sample solution was combined with 800 ml of the 0.1 M Tris HCl buffer (pH 7.4) in a test tube before 1 ml of the DPPH solution was added. The suspension was properly mixed before being incubated at room temperature in the dark. The DPPH solution was added, and 30 minutes later, the absorbance at 517 nm was measured. As a benchmark, gallic acid, a readily available antioxidant molecule, was used. For the blank preparation, 800 ml of Tris-HCl buffer and 1.7 ml of ethanol were combined (Farrukh et al. 2003). Percentage scavenging activity (%) was calculated by the formula.

DPPH Scavenging activity (%) = (AbsC AbsS/AbsC) ×100.

Where Abs S is the absorbance of the sample Abs C is the absorbance of the control.

The antioxidant activity of the extract was expressed as IC50. IC50 was calculated through linear regression analysis. IC50value is the concentration of extract that inhibits the formation of radicals by 50 %.

Phytochemical analysis of the extracts HPLC:

HPLC analysis was performed with the use of HPLC (make-Shimadzu) and LC Software 2010. 10 µl of the respective samples were injected, and the programme was run for 25 minutes (25min runtime). (Kumar, S., & Dhanani, T. (2013). The conventional marker compound was gallic acid. The UV detector was used to detect the light at 351nm.

Gas Chromatography and Mass Spectroscopy (GC-MS):

The identification of organic components in the provided sample, both qualitatively and quantitatively, was examined using gas chromatography and mass spectrometry. Using a GC-MS (Agilent: 7890-Jeol: Accu TOF GCV) system and an Elite 1 column, the possible bioactive chemicals of *E.alba* (whole plant) extracts were examined. Injector capacity was 2 l, temperature was 280 °C, and the carrier gas was helium with a flow rate of 1 ml/min. Using an isothermal for 5 minutes, the oven temperature was increased from 40 to 280 °C. Retention duration, MS fragment ions, and an assessment of their proportion to the total peak area were used to identify the bioactive chemicals. The components were identified by computer searches in a commercial library (Wiley 8 and NIST). The mass spectra of the peaks and those from the literature (Okwu, E. D. et.al.2010)

Results

A total of one hundred fifty-five (155) urine samples were collected. Results obtained showed the incidence of infection among female (70.32%) was considerably higher compared to male (29.68%). (Table-1) *Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, ,Proteus mirabilis,P.*aeruginosa Were found as ESBL pathogens in this present study. Sample extraction from the plants according to rising polarity index, extracts were made using the Soxhlet extraction method in a succession of different organic and aqueous solvents: ethanol (4.4), ethyl acetate (5.1), and water (9.0). The results obtained demonstrated the solvents' different levels of extractive strength. Water has the best ability for extraction compared to organic solvents like methanol and ethyl acetate. Water extract, which has the highest degree of polarity, was found to provide the highest yield, followed by ethyl acetate and methanol extract, which has the lowest yield. (Table -2) In the current study, antibacterial activity was assessed by measuring the inhibition zone in mm against several bacterial strains using the agar well diffusion method. The substantial inhibitory impact of (EA1, EA2, EA3) extracts on the chosen pathogens is confirmed by the findings of our antimicrobial study (Table: 3). The extract's bactericidal impact got more pronounced as concentration increased. EA3 extract (18±577) at a concentration of 2.0 mg/100 µl most efficiently inhibited *K.pneumoniae*, although EA2 and EA3 in that order shown less effective suppression. *P.miabilis* was resistant to EA1 and EA2 extract at the concentration of 1.0mg/100µl and 2.0mg/100µl and EA3 extract only at 1.0mg/100µl but EA3 extract of *Eclipta.alba(* 11.5±0.289 *)* could inhibit *P.miabilis* at a concentration of 2.0 mg/100µl respectively. *E.aerogens* was resistant to EA2 extract but EA1 and EA3 was active against the pathogens(17±0.577, 16±0.577) (13±0.577) (14±0.577) at both concentration.*K.pneumoniae* was resistant to EA1 and EA2at a concentration of

2.0mg/100µl , EA2 extract(14±0.577) and EA1 extract(15±0.577) show the activity against *K.pneumoniae* at the concentration of 1.0mg/100µl . *E. coli* was resistant to EA3 but could inhibit the growth of pathogen at EA1 and EA2 at both concentrations. *P.aeruginosa* was resistant to EA3 at both concentration and EA1 at the concentration of 1.0mg/µl but EA1 (10±0.577) at the concentration of 1.0mg/100µl and EA2 (11±0.577, 11±0.577)inhibit the pathogen at both concentration. Different *E. alba* extracts have antibacterial properties that were comparable to those of the common antibacterial medication Amikacin (positive control). Compared to the extracts, amikacin successfully suppressed infections. The range of 1.0-0.0312mg/ml was determined to be the minimal concentration needed to stop the development of microorganisms in the reference. (Table: (4,5,6). The two *E.alba* extracts, EA1 and EA3, were found to require the same concentration (0.0312 mg/ml) to inhibit the growth of *K.pneumoniae*, whereas EA3 extract inhibited *E.aerogens* at the same concentration (0.0312 mg/ml) as *K.pneumoniae*. *E. coli* had to be inhibited at a minimum inhibitory concentration of 0.125mg/ml and 0.0625 mg/ml (EA1, EA2 extract). EA2 extract needed a minimum inhibitory dose of 0.0312 mg/ml to inhibit the development of *P. aeruginosa*. All extracts, EA1, and EA3 had MIC, MBC, and MIC index values that were identical to those of amikacin for *K. pneumoniae* (0.0312 mg/ml, 0.0625 mg/ml, and 2), but differed for *E. aerogens* (0.0625mg/ml, 0.0312mg/ml, and 2 respectively). MBC values were higher than the MIC values . These results demonstrated the bactericidal abilities of the *E. alba* extracts. One of the free radicals frequently used to assess a compound's or a plant extract's potential to scavenge radicals is DPPH. DPPH scavenging activity and IC50 value of Gallic acid (5.65±0.401) compared with all the extract of*E.alba* showed a comparable good antioxidant activity (Fig. 2,3). The results obtained indicated that the free radical neutralizing activities of *E.alba* (whole plant)extract (EA2 and EA3)were the highest in comparison to the antioxidant activities(EA1). In the present study, the antioxidant power is measured using the DPPH assay. The DPPH(1,1-Diphenyl-2picrylhytrazyl) is stable purple colored free radical (absorbed at 517nm). if free radicals are scavenged, the color DPPH changes to yellow. This feature is used in this experiment to demonstrate free radical scavenging capacity in medicinal plants (Crude extract). The phytosignatures discovered in *E.alba*(whole plant) EA3 extract were determined to be the most effective free radical scavenger. When the results were compared to the reference compound gallic acid, a well-known antioxidant, a powerful polyphenol that exhibited high antioxidant activity, the antioxidant capacity was found to be concentration dependent in all cases. The graph is displayed in (Fig:-2). Polyphenols, flavonoids, and phenolic chemicals are active biosignatures in many plant-based therapeutics that protect cells from oxidated stress. **(Acharya et al., 2010).** The phytosignature profiling was not harmed throughout the extraction process, as the chromatogram of the extract displayed multiple peaks of different phytochemicals when using HPLC with a UV detector at a wavelength of 351nm to identify chemicals, however gallic acid was the compound of interest in the study(fig:4). The GC-MS analysis of the selected plant extracts have revealed the presence of several compounds. Traditionally water with highest polarity was the choice of solvent for extraction of phytochemicals but other research showed that organic solvents had more consistent antibacterial actions than those extracted with water. The polarity of solvent and the type of the bioactive chemicals recovered may be linked to the activities reported in organic solvents other than water. **(Dey et al., 2010; Rautela et al., 2018; Sharma et al., 2015).** Alkaloids, Tannins,

Cynoanogenic, Saponins, Glycosides, Flavonoids, Phenolic compounds, and Lignins are only a few examples of phtyoconstituents found in plants **(Upadhyay et al.,2014; Rautela et al., 2018; Sharma et al,2016).** Phytoconstituents responsible for wider range biological activities were identified in *Eclipta alba (prostrate*)extracts (EA1, EA2, EA3) (Table-7,8,9 and fig 5,6,7)). 2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester, Z- and Benzoic acid 3,4,5, trihydroxy (gallic acid) have antimicrobial,antioxidant properties. 9,12-Octadecadienoic acid (Z,z)-,Hexanoic acid, pent-2-en-4-ynyl ester have been reported to possess antimicrobial,R-propane-1,2-diol used to treat asthama, Glycine, N-methyl-n-propoxycarbonyl anti-inflammatory effects. This was also shown in our investigations where it was shown that the extracts were found to be highly antimicrobial in nature, possibly due to the chemicals present in diverse extract.

Table 1. Distribution rate of ESBL pathogens in infected patients infected with UTI and in relevance gender

Gender	Culture result (%)		Total (%)
	Positive	Negative	
Female	64 (73.56)	45 (66.16)	109 (70.32)
Male	23 (26.44)	23 (33.82)	46 (29.68)
Total	87 (56.12)	68 (43.9)	155 (100)

Table-2. Yield percentage and physical properties of *Eclipta alba (whole plant*) from different solvents.

S.No	Solvent	Yield%	Colour	State
1	Ethyl Acetate	3.04%	Blackish green	Viscous
2	Methanol	2.36%	Greenish brown	Powder
3	Aqueous	7.84%	Brown	Solid

Table 3: Antimicrobial effect of *E. alba (prostrate)whole plant extract against* ESBL producing Microbes

M/O	Amikacin		EA1		EA2		EA3	
Concentration (mg per 100µl)	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0
P.mirabilis	14±0.577	15±0.577	-	-	--	--	-	11.5±0.289
E.aerogens	18±0.577	17±0.577	17±0.577	16±0.577	--	--	13±0.577	14±0.577
K.pneumoniae	16±0.577	18±0.577	15±0.577	--	14±0.577	--	13±0.577	18±0.577
E. coli	13±0.577	14±0.577	9.5±0.289	11±0.577	10±0.577	11±0.577	--	--
P.aeruginosa	12±0.577	11±0.577	--	10±0.577	11±0.577	11±0.577	--	--

M/O= Microbes, P.m= *Proteus.mirabilis*, E.a= *E.aerogens*, K.p= *K.pneumonia*, E.c= *E.coli*, P.a= *P.aeruginosa* EA1=Ethyl Acetate, EA2= Methanol, EA3= Aqueous

Table-4 The MIC of Ethyl acetate (EA1) extract against isolated uropathogen

Microorganism	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)
E.a	1.0-0.0312	0.0312	0.0625	0.0625	0.125	2	2
E.c	1.0-0.0312	0.0312	0.0625	0.125	0.25	2	2
K.p	1.0-0.0312	0.0312	0.0625	0.0312	0.0625	2	2

Table-5. The MIC of Methanolic (EA2) extract against isolated uropathogens.

Microorganism	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)
P.a	1.0-0.0312	0.0312	0.0625	0.0312	0.0625	2	2
E.c	1.0-0.0312	0.0312	0.0625	0.0625	0.125	2	2

Table-6: The MIC of Aqueous (EA3) extract against isolated uropathogens.

Microorganism	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)
K.p	1.0-0.0312	0.0312	0.0625	0.0312	0.0625	2	2
E.a	1.0-0.0312	0.0312	0.0625	0.0312	0.0625	2	2

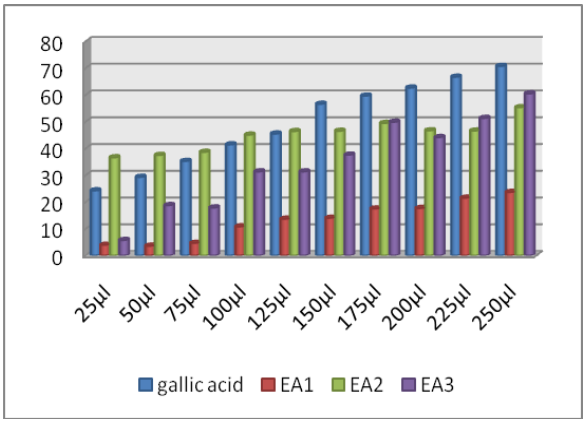

Fig 2: Graphical representation of antioxidant capacity of various extracts (EA1 (Ethyl actate), EA2 (Methanolic), EA3 (Aqueous)) of *E.alba (prostrata) whole plant* at increasing concentration with Gallic acid as reference compound.

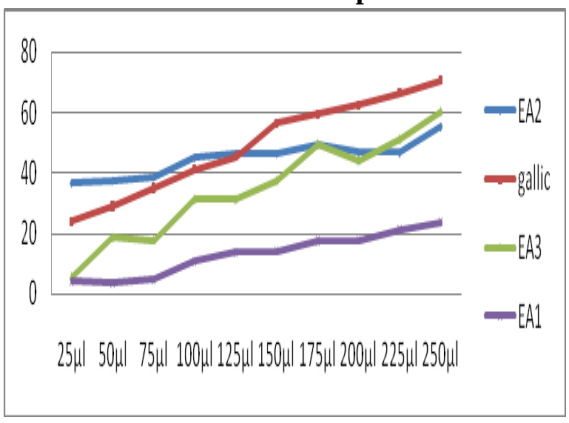

Fig:3 Graph showing DPPH scavenging activity of different solvent extract [Ethyl acetate (EA1) Methanolic (EA2), Aqueous (EA3)] of *Eclipta alba* (Whole plant)

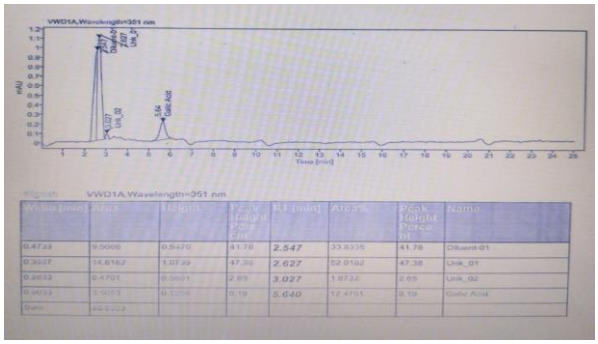

Fig: 4: HPLC analysis of *E.alba (prostrata)whole plant extract* detecting Gallic acid as the Principle component

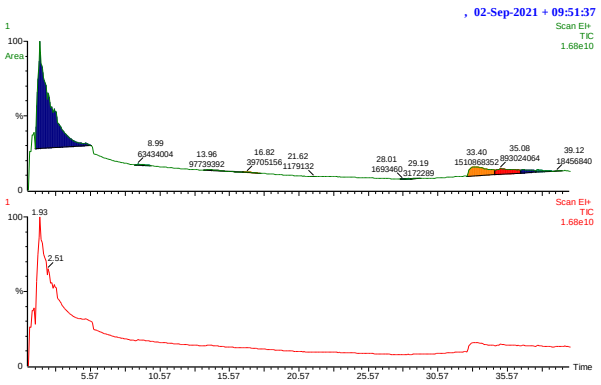

Fig:6 Chromatogram obtained from the GC-MS with methanolic extract of *Eclipta*

alba (prostrate)

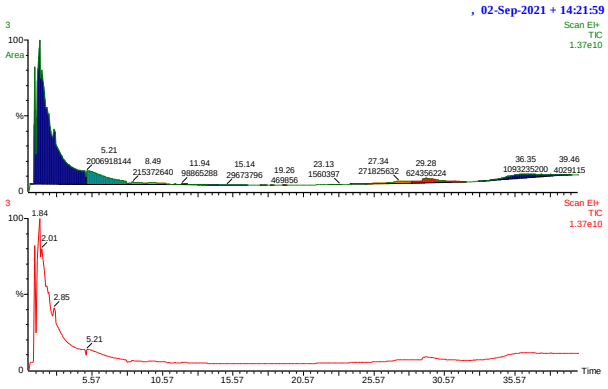

Fig 7: Chromatogram obtained from the GC-MS with Aqueous extract of *Eclipta alba (prostrate)*

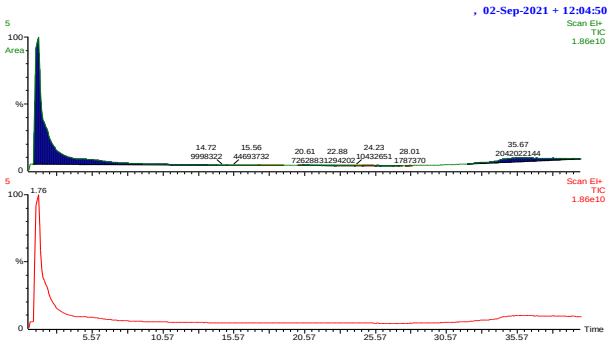

Fig:5 Chromatogram obtained from the GC-MS with the extract of Ethyl acetate extract of *Eclipta alba(prostrate)*

Table 7: **Phytochemicals referred in the Ethyl acetate extract of *Eclipta alba (prostrate) whole plant* by GC-MS**

S.no	Phytochemicals	Molecular Formula	Molecular weight	Ret time	Area
1	10-Undecenoic acid, 4,4-dimethyloxazoline derivative	$C_{15}H_{27}NO$	237.3810	20.61	7262883
2	4-Pyridinecarboxylic acid, methyl ester	$C_7H_7NO_2$	137.1360	14.72	9998322
3	2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester, (Z)	$C_{11}H_{18}O_2$	182.2594	22.88	1294202
4	N,n'-bis-(2-hydroxyethyl)-oxamide	$C_6H_{12}N_2O_4$	176.1705	20.61	7262883
5	p-acetoacetanisidide	$C_{11}H_{13}NO_3$	207.2258	14.72	9998322
6	Butanamide, N-(3-nitrophenyl)-	$C_{10}H_{12}N_2O_3$	208.2139	15.56	44693732
7	Cyclopropanecarboxamide, N,n-dihexyl	$C_{16}H_{31}NO$	253.4234	24.23	10432651
8	Glycine, N-methyl-n-propoxycarbonyl-, decyl ester	$C_{17}H_{33}NO_4$	315.4482	35.67	2042022144
9	1-Propylpiperidine	$C_8H_{17}N$	127.2273	14.72	9998322
10	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282.4614	35.67	2042022144
11	Pentanamide, N,n-dioctyl	$C_{21}H_{43}NO$	325.5722	35.67	2042022144
12	But-2-enamide, N,n-dihexyl-3-methyl	$C_{17}H_{33}NO$	267.4500	28.01	1787377
13	Benzoic acid, 3,4,5-trihydroxy-(Gallic acid)	$C_7H_6O_5$	170.1195	10.57	
14	Acetoxyacetic acid, 3, 5-dimethylphenyl ester	$C_{12}H_{14}O_4$	222.2372	28.01	1787377
15	Phenol, 3,5-dimethyl-, methylcarbamate	$C_{10}H_{13}NO_2$	179.2157	22.88	1294202
16	9,12-Octadecadienoic acid (Z,z)	$C_{18}H_{32}O_2$	280.4455	35.67	2042022144

Table 8: Phytochemicals referred in the Methanolic extract of *Eclipta alba (prostrate) whole plant* BY GC-MS

S. No	Phytochemicals	Molecular formula	Molecular weight	Ret. time	Area
1	Butane, 2-methyl	C_5H_{12}	72.1488	8.99	63434004
2	Cyclobutanecarboxylic acid, 4-nitrophenyl ester	$C_{11}H_{11}NO_4$	221.2093	28.01	1693460
3	Cyclohexanecarboxylic acid, 4-methoxyphenyl ester	$C_{14}H_{18}O_3$	234.2909	28.01	1693460
4	Glycine, N-methyl-n-allyloxycarbonyl-, dodecyl ester	$C_{19}H_{35}NO_4$	341.4855	35.08	893024064
5	Isonipecotic acid, n-butoxycarbonyl-, nonyl ester	$C_{20}H_{37}NO_4$	355.5121	39.12	18456840
6	2-Propanamine	C_3H_9N	59.1103	8.99	63434004
7	1,5-Dimethyl-1,4-cyclohexadiene	C_8H_{12}	108.1809	13.96	97739392
8	4-Pyridinecarboxylic acid, ethyl ester	$C_8H_9NO_2$	151.1626	16.82	38705156
9	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.4766	29.19	3172289
10	Methoxyacetamide, N-ethyl-n-(3-methylphenyl)	$C_{12}H_{17}NO_2$	207.2689	21.62	1179132
11	Hexanedioic acid, bis(phenylmethyl) ester	$C_{20}H_{22}O_4$	326.3863	33.40	1510868352
12	17-Octadecynoic acid, methyl ester	$C_{19}H_{34}O_2$	294.4721	33.40	1510868352
13	Methyl 2-hydroxystearate, Tms derivative	$C_{22}H_{46}O_3Si$	386.6843	39.12	18456840
14	9-Octadecenal	$C_{18}H_{34}O$	266.4620	28.01	1693460
15	1,11-Dodecadiene	$C_{12}H_{22}$	166.3031	16.82	38705156
16	Ethanedioic acid, dimethyl ester	$C_4H_6O_4$	118.0880	13.96	97739392
17	R-(-)-1,2-propanediol	$C_3H_8O_2$	76.0944	8.99	63434004

Table 9: Phytochemicals referred in the Aqueous extract of *Eclipta alba (prostrate)* by GC-MS

S. No	Phytochemicals	Molecular formula	Molecular weight	Ret time	Area
1	2,3,3-Trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone	$C_{14}H_{22}O$	206.3239	36.35	1093235200
2	Cyclopropanecarboxylic acid, 4-methoxyphenyl ester	$C_{11}H_{12}O_3$	192.2112	29.28	624356224
3	Octadecanoic acid, butyl ester	$C_{22}H_{44}O_2$	340.583	39.46	4029115
4	p-decyloxyaniline	$C_{16}H_{27}NO$	249.391	29.28	624356224
5	Cyclopropanecarboxamide, N,n-dihexyl-	$C_{16}H_{31}NO$	253.423	8.49	215372640
6	Octadecanoic acid, propyl ester	$C_{21}H_{42}O_2$	326.557	36.35	1093235200
7	Propanoic acid, 3-(4-benzyloxyphenyl)-, methyl ester	$C_{17}H_{18}O_3$	270.323	15.14	29673796
8	1,3-Benzenedicarboxylic acid, 5-nitro-, bis(1-methylethyl) ester	$C_{14}H_{17}NO_6$	295.287	23.13	1560397
9	Benzamide, N-(4-amino-9,10-dihydro-9,10-dioxo-1-anthracenyl)-	$C_{21}H_{14}N_2O_3$	342.3475	39.46	4029115
10	Benzoic acid, 3,4,5-trihydroxy-(gallic acid)	$C_7H_6O_5$	170.1195	5.21	2006918144
11	R-(-)-1,2-propanediol	$C_3H_8O_2$	76.0944	11.94	98865288

12	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	$C_{10}H_{16}O$	152.2334	11.94	98865288
13	Benzenamine, 4-(octyloxy)-	$C_{14}H_{23}NO$	221. 385	23.13	1560397
14	Hexanoic acid, pent-2-en-4-ynyl ester	$C_{11}H_{16}O_2$	180.2435	27.34	271825632

Discussion

In this investigation, we have shown that *Eclipta alba* whole plant extract has antibacterial action against ESBL uropathogens, with *K. pneumoniae* showing the strongest antibacterial activity. Because of the significant medical relevance of the antibacterial activity, tests have been conducted on it. Infections have significantly increased recently, and drug-resistant bacteria have emerged as a growing treatment challenge. It is well known that higher plants contain antimicrobial compounds since these compounds serve as a source of inspiration for new medication molecules and have a substantial positive impact on human health. Since they can accomplish the same goals as synthetic antimicrobial molecules without the negative side effects, plant-based antimicrobials offer tremendous medicinal potential. Pharmaceutical companies are now seeking for alternate options due to the growing resistance of microorganisms to the already prescribed antibiotics and the expensive expense of producing synthetic substances. Today, more investigation and study of plant-derived antimicrobials are required since they constitute a sizable untapped supply of medication. In the current investigation, it was discovered that the extract's inhibitory effect against all bacterial strains increased with an increase in concentration. Different workers in various systems came to similar conclusions (Elumalai et al., 2011). Some phytochemicals that were discovered in the plant extract could be the cause of the extract's inhibitory action on the growth of bacteria. The existence of numerous active principles in plants may be the cause of their antibacterial activity. Polyphenols and flavonoids, which may function as antibacterial components, are frequently found in plant extracts. Plant extracts' bioactivity is thought to be due to their phytochemical composition. A variety of pathogens, including *S. aureus, S. albus, S. pyogenes, P. aeruginosa, Salmonella gallinarum, B. subtilis,* and *E. coli,* have been shown to be susceptible to *Eclipta alba's* antibacterial properties (Sawhney,s s et.al 2011). Antioxidants are helpful in reducing the excess formation of free radicals, which happens in people as a result of persistent, severe, and long-lasting bacterial infections. A free radical called DPPH is frequently employed to test a compound's or a plant extract's potential to scavenge radicals. Antioxidant power has been found to depend on concentration. The following extracts lose their ability to scavenge free radicals in decreasing order: Ethyl acetate > methanol > water. Since the phytoconstituents in *E. alba* extracts were discovered to have antibacterial, antimicrobial, and antioxidant properties, the plant can be used to make herbal medicines to treat urinary tract infections. *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus* were among the bacteria against which *Eclipta prostrata* was evaluated for its antibacterial properties. (S. Karthikumar et al.2007). In the current investigation, it was discovered that the extract's inhibitory effect against all bacterial strains increased with an increase in concentration. In other systems, various workers came to similar conclusions (Khan et al., 2012; Elumalai et al., 2011). The presence of several phytochemicals that were discovered in the plant extract may be the cause of the extract's inhibitory action on the growth of bacteria. This plant's antibacterial

effectiveness against both gramme positive and gramme negative bacteria may be a sign that broad spectrum antibiotic chemicals are present (Doughari 2006 and Pandey et al., 2011). The current investigation supports the traditional medical system's reported usage of Eclipta alba to treat a variety of infectious disorders brought on by microorganisms. To fulfil the rising demand from the conventional medical system, this study promotes the growth of this extremely valuable medicinal plant.

Conclusion

Due to the presence of important bioactive principle compounds for the treating of various illnesses, the use of plant-based formulations has received a lot of attention. Antibiotics offer quick relief, but they also have a host of negative effects on the human body. The germs that cause urinary ailments in humans have evolved resistance, posing a severe challenge despite the fact that urinary tract infections can be treated with commercially available antibacterial medications. Among numerous traditional herbs used in the treatment of many ailments, *Eclipta alba* is a crucial plant., which are usually free from side effects, are economical and also easily accessible to humans. Our study's findings show that the *Eclipta alba* (whole plant) extracts EA2 and EA3 have potent bactericidal and antioxidant properties. The found phytochemicals are important in providing evidence for their usage in herbal medicines to treat urinary tract infections. The research can be expanded to include in vivo tests to identify the extract's precise mechanism of action. The generated information has served as the foundation for its usage as a phytotherapeutic agent to treat disease in the examples given.

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