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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS AND *IN VITRO* ANTIMICROBIAL SCREENING OF *WEDELIA GLAUCA* (ORTEGA) O. HOFFM. EX HICKEN

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ABSTRACT

Objective: Continued resistance toward the antibiotics urges us to explore newer antibiotics. Plants are being the safer source of antibiotics with lesser or no side effects. This study was designed to study the presence of phytochemical constituents and antibacterial activity of leaf and flower extracts of *Wedelia glauca* against urinary tract infection causing pathogens.

Methods: The plant leaves were extracted with five different solvents based on the polarity. The extraction was done using soxhalation. Antimicrobial activity was determined by agar well diffusion method for both the sample and standard. The acetone plant extract was subjected to gas chromatography-mass spectrometry (GC-MS) analysis for screening phytoconstituents.

Results: Preliminary phytochemical screening revealed the presence of diverse phytoconstituents in the plant. The different extracts exhibited a considerable antimicrobial potential. Among the solvents used acetone extract showed comparably better antimicrobial activity with 100% of inhibition rate with the maximum zone of inhibition of 1.6±0.77 mm against *Staphylococcus* sp. and *Aspergillus* sp. at the concentration of 5 mg. GC-MS analysis provided 8 major peaks which revealed the existence of a variety of bioactive compounds which may attribute to the efficacy of the plant.

Conclusion: *W. glauca* leaf and flower extracts displayed a broad spectrum of antibacterial and antifungal activity and can be considered as a potential source of newer antibiotic compounds.

Keywords: *Wedelia glauca,* Urinary tract infections, Antimicrobial resistance, Antibacterial, Antifungal, Soxhalation, Phytochemical analysis, Chromatography-mass spectrometry.

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INTRODUCTION

Among the most common infectious diseases, urinary tract infections (UTIs) are commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million [1,2]. Antimicrobial resistance (AMR) to various classes of antimicrobials to uropathogens continues to be a major health problem in different parts of the world [3,4]. The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives [5]. Infectious diseases caused by AMR microbes and the treatment are the serious problems in the field of medical science today world over. The development of an alternative drug line to treat such infectious diseases is urgently required [6]. Developing world, especially the countries of Southeast Asia, Western and Central Africa, India, and Pakistan are the most vulnerable to various infectious pandemic diseases [7]. Increasing AMR among microbes caused the emergence of new resistant phenotypes and further caused the development of new antimicrobial compounds [8]. It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health-care systems [9,10].

Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs, and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [11]. The therapeutic potential of medicinal plants as a source of noble natural antioxidants and antimicrobial agents has been well recognized all across the globe [12]. Plants have unlimited ability to produce a wide variety of secondary metabolites most of which are aromatic compounds, including alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins [13] forming the basis of plant-derived antimicrobial compounds. Plant-derived antimicrobial substances are plant originated secondary metabolites and have great concern because of their antibiotic activity without conferring resistance [14,15]. Screening of various bioactive compounds from plants has to lead to the discovery of a new medicinal drug which has efficient protection and treatment roles against various diseases [16]. The medicinal plants are a rich source of the secondary metabolites such as alkaloids, glycosides, steroids, and flavonoids, which are a potential source of drugs. Nearly one-third of the pharmaceuticals are plant origin. As all the plants are able to synthesize a multitude of organic molecules/phytochemicals, they are referred to as "secondary metabolites" [17].

Wedelia glauca (Asteraceae) is an herbaceous, invasive, perennial plant native to South America, and widely distributed in Argentina, Chile, Uruguay, Paraguay, Bolivia, and Brazil. It was introduced in the Southeastern United States (Louisiana and Florida), where it has been described with the former name *Pascalia glauca* and also in Spain (provinces of Madrid and Valencia) [18]. *Wedelia* genus it is an interesting source of potential bioactive molecules, as iridoids compounds, flavonoids, diterpenoids derivatives, phytosteroids, with antioxidant, anti-inflammatory, antimicrobial, hepatoprotective activity, analgesic and antihistamine, anti-implantation, antiasthmatic activities, and anticancer activity [19]. In this study, an effort has been made to determine the significance of *W. glauca* on its richness in phytochemical constituents and its antibacterial and antifungal properties.

METHODS

Plant materials

The plant material was collected from the regions of Coimbatore, Tamil Nadu, India, from September to November 2012. The sample was identified and authenticated by the Botanical Survey of India, Coimbatore, India. The authentication voucher no. - BSI/SRC/5/23/2013-14/Tech/1461.

Preparation of the crude extracts

Well-grown healthy leaves and flowers of *W. glauca* plant were collected and shade-dried at room temperature for a week. It was homogenized to a fine powder and stored in an airtight glass container at 4°C until the further process. The extraction of the plant materials was carried out using a Soxhlet extractor. Based on the polarity the solvents were selected for the study. The solvent used were petroleum ether (PE), ethyl acetate (EA), acetone (A), ethanol (E), and aqueous (W) in the ratio 1:10 (w/v). Individual extraction was been performed. The extract liquid was subjected to rotary evaporation to remove the solvent. The semi-solid extract obtained was stored in an airtight container at 4°C in the freezer for further use. The dried extract was exposed to ultraviolet (UV) light (200-400 nm) for 24 hrs and checked frequently for sterility by streaking on nutrient agar plates. For antimicrobial activity, a volume of 50 mg of the extract was dissolved in 1 ml of 5% dimethyl sulfoxide (DMSO). It was sterilized by filtration using 0.22 µm millipore filter [20].

Phytochemical analysis

The preliminary phytochemical analysis was carried out on the different extracts using standard procedures [20,21] to identify the phytochemical constituents. They are:

- Detection of alkaloids: The extracts were treated with few drops of dilute HCl and filtered. The filtrate was tested with the following reagents:
- Dragendroff's reagent: The extract was dissolved in methanol and few drops of Dragendorff's reagent is added. Orange red precipitate shows the presence of alkaloids.
- Test for flavonoids: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.
- Test for carbohydrates: Benedict's test: To 1 ml of the filtrate, 5 ml of Benedict's reagent were added. The mixture was heated; appearance of red precipitate indicated the presence of reducing sugars.
- Test for steroids: The extract was mixed with 2 ml of chloroform and concentrated sulfuric acid was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.
- Test for terpenoids: About 5 ml of extract was mixed in 2 ml of chloroform, and concentrated sulfuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.
- Test for proteins: Millon's test: Small portion of the extract when mixed with 2 ml of Millon's reagent, a white precipitate appeared which turned red on gentle heating that confirmed the presence of protein.
- Test for reducing sugar: Benedict's test: Mixed equal volume of Benedict's reagent and test solution in a test tube. Heated in a boiling water bath for 5-10 minutes. The solution appears green, yellow, or red depending on the reducing sugar present.
- Test for tannins: Small quantities of various extracts were taken separately in water and tested for the presence of tannins. The extract was treated with dilute FeCl3 solution. A blue dark green or violet color was obtained.
- Test for saponins: The extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Collection and maintenance of pathogens

Nine pathogenic bacteria used for the study were *Bacillus* sp., *Staphylococcus* sp., *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., *Escherichia coli*, *Streptococcus* sp., *Enterobacter* sp., and *Citrobacter* sp., and the two fungal cultures were *Candida albicans* and *Aspergillus* sp. isolated from patients had been collected from "Sri Ramakrishna Hospital," Coimbatore, and maintained on nutrient agar slants, blood agar slants, and sabouraud dextrose agar (SDA) slants in cold room at 4°C.

Culture media

Mueller-Hinton agar (MHA) and SDA were prepared according to the manufacturer's instruction, autoclaved and dispensed at 20 ml per plate in Petri dishes. Set plates were incubated overnight to ensure sterility before use [20].

Antibacterial assay

Using the well diffusion method, 24-hrs broth culture of the respective bacteria was adjusted to a turbidity of 0.5 McFarland standards. In brief, 0.2 ml broth culture of the respective bacterial strain was dispensed into a 20 ml sterile nutrient broth and incubated for 24 hrs at 37°C and standardized at 1.5×106 CFU/ml by adjusting the optical density to 0.1 at 600 nm and performed on UV/visible - spectrophotometer. Each of this bacterial culture was swabbed over sterile MHA plates separately using sterile cotton swabs. A well with diameter 6 mm was made using sterile cork borer. The bottoms of the wells were sealed by pouring 20-50 µl of molten MHA into the scooped out wells. Subsequently, from the prepared extract in DMSO, 100 µl was added to the each well, final concentration was made up to 1, 2, 3, 4, and 5 mg, respectively. The plates were kept at 4°C for 1 hr for the diffusion of extract, thereafter, the plates were incubated at 37°C for 24 hrs. Ampicillin (1 mg) was served as the positive reference standard to determine the sensitivity of the tested microbial strains. The antibacterial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation, and the results were expressed in millimeters (mm) [20].

Antifungal study

In vitro screening of antifungal activity was carried out against 2 pathogenic fungal strains using the agar well diffusion method. The culture of organisms was maintained on SDA. Activated cultures of fungal strains in Sabouraud's broth were adjusted to 1×108 CFU/ml as per the McFarland standard. Each of the diluted cultures was swabbed on sterile SDA plates separately using sterile cotton swabs. The plate was dried for 30 minutes at room temperature. A well with diameter 6 mm was made using sterile cork borer. The bottoms of the wells were sealed by pouring 20-50 µl of molten SDA into the scooped out wells. From the prepared extracts in DMSO, 100 µl was added to each well, the final concentration was made up to 1, 2, 3, 4, and 5 mg, respectively. Fluconazole (1 mg) was used as the positive reference, and the plates were kept at 4°C for 1 hr for the pre-diffusion of the extract. Then, the plates were incubated for 48-72 hrs at 37°C. The zone of inhibition of fungal growth was measured in diameter (mm) [20].

Statistical analysis

The data obtained after determining the zone of inhibition were recorded and mean and standard deviation was calculated. Data have been expressed as mean ± standard deviation. Statistical analysis was performed with GraphPad Prism version 5 using ANOVA ($p<0.0001$ is considered to be statistically significant).

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the acetone extract of the leaves and flower of *W. glauca* was carried out using thermo GC - trace ultraversion: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non-polar column (30 m × 0.25 mm), and film thickness 0.25 µm. Helium was used as the carrier gas at a constant flow of 1 ml/minute and an injection volume of 1.0 µl, and the temperature programming was set with initial oven temperature at 70°C and held for 2 minutes and the temperature of the oven was raised to 260°C for 10 minutes and raised 6°C/minute and final temperature was 280°C for 10 minutes. Mass spectra were recorded over 50-650 (m/z) range. The components were identified by comparison of their mass spectra

with those of mass spectral library, as well as by comparison of their retention time either with those of authentic compounds or with literature values.

RESULTS AND DISCUSSION

In the present study, the healthy leaves and flowers of *W. glauca* were collected, dried, and extracted with five different solvents, namely, PE, EA, acetone, ethanol, and aqueous. Based on the polarity the solvents had been selected. Preliminary phytochemical analysis of the all five extracts revealed the presence of diverse phytochemical constituents which were observed and tabled (Table 1).

The discovery of antibiotics has revolutionized the management of infectious diseases more efficiently and timely. As by the famous saying, "Every invention and discovery has its own downside," the bacteria are developing resistance to those antibiotics, becoming recalcitrant, and troublemaker to treatment [22]. The emergence of multidrug resistance among the uropathogens and other microorganisms urge the researchers to identify newer antibiotics with zero side effects. This study examines the antimicrobial potential of *W. glauca*. All the assays had been performed in triplicates. The solvent DMSO (5%) was used to dissolve the plant extracts. The activity of DMSO has also been studied as the negative control for all assays and showed no inhibition of microbial growth. The activity was examined based on the zone of inhibition produced around the wells and tabled (Tables 2-12), respectively.

Among the different extracts, acetone extract shown comparatively better activity than all other extracts. With this result, the acetone extract has been taken for further analysis in GC-MS. The results of the GC-MS analysis provided 8 major peaks (Fig. 1) which clearly indicate the presence of different phytochemical compounds. Based on the corresponding peaks, the top five compounds with higher probability have been noted (Table 13).

The objective of the study is to find the antimicrobial potential of leaf and flower extract of *W. glauca* against uropathogens. Further, to find out the bioactive principle behind the activity. The preliminary phytochemical analysis revealed the presence of important secondary metabolites from all five extracts of the plant. Collectively, it showed the presence of alkaloids, flavonoids, carbohydrates, proteins, amino acids, sterols, triterpenoids, tannins, and free sugar. There is a variation in the extraction of these phytochemical constituents by different solvents. This may be based on the polarity of the solvent and its extraction capacity. All of these secondary metabolites, making the plants a potential source of drugs. Medicinal plants represent rich sources of antimicrobial agents used medicinally in different countries and are a source of many potent drugs used in traditional medicine [23]. In this study, five different concentrations, they are 1, 2, 3, 4, and 5 mg, of the five extracts of *W. glauca* were analyzed. Each extract of five concentrations had been tested against nine bacteria, which included both Gram-positive and Gram-negative bacteria, and two fungal cultures. The results clearly revealed maximum zone inhibition in a dose-dependent manner. The tested bacterial strains showed a different pattern of inhibition. Among the two studied fungal cultures, ethanol and acetone showed better activity. Acetone extract shown higher activity against *Aspergillus* sp. with 1.6±0.77 mm and against *Candida albicans* with 1.5±0.22 mm of zone of inhibition. Although the zone of inhibition of the extracts is equal to the standard antibiotic, this study has shown that these extracts possess antifungal potential. Out of the nine bacterial cultures, *Citrobacter* sp., (Table 10) *Enterobacter* sp., (Table 9) and *E. coli* (Table 5) being more resistant toward the plant extracts as well as the standard antibiotic. Against these three organisms acetone extract exhibited higher activity with 1.3±1.00 mm, 1.4±0.99 mm, and 1.5±0.22 mm of zone of inhibition, respectively. While *Bacillus* sp. is susceptible all the extracts studied (Table 2). Against *Bacillus* sp. acetone extract has shown highest zone of inhibition with

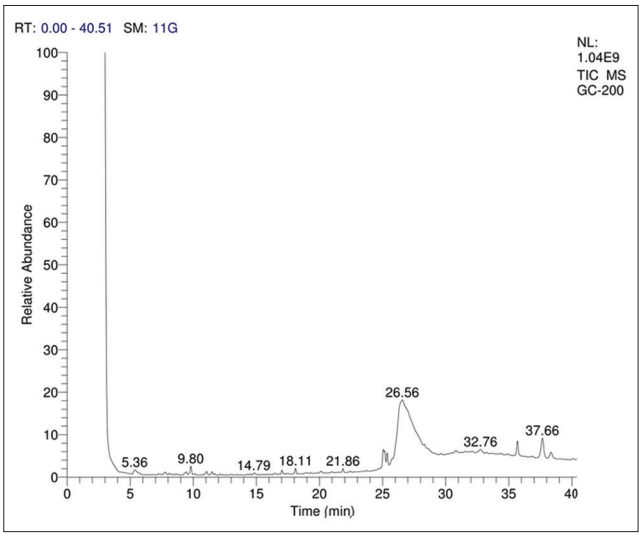

Fig. 1: The gas chromatography-mass spectrometry spectral peaks of acetone extract of *Wedelia glauca*

Table 1: Preliminary phytochemical screening of different extracts of *W. glauca*

Phytochemicals	Plant extracts				
	PE	EA	A	E	W
Alkaloids	-	+	+	+	-
Flavonoids	-	-	+	-	+
Carbohydrates	+	-	-	+	+
Proteins and amino acids	-	-	+	-	-
Steroids	+	-	+	-	+
Triterpenoids	+	-	+	-	+
Tannins	-	+	-	-	-
Reducing sugar	+	-	-	-	+
Saponins	+	+	+	+	+

+: Presence of the compound, -: Absence of the compound. PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous, *W. glauca: Wedelia glauca*

Table 2: Antimicrobial activity of different extracts of *W. glauca* on *Bacillius* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	0.9±0.57	1.0±0.63	1.1±0.77	1.2±0.52	1.3±1.00
EA	1.1±0.77	1.1±0.77	1.2±0.52	1.2±0.52	1.3±1.00
A	0.9±0.57	1.0±0.63	0.9±0.57	1.0±0.63	1.4±0.99
E	1.0±0.63	1.2±0.52	1.0±0.63	1.0±0.63	1.0±0.63
W	1.1±0.77	1.1±0.77	1.0±0.63	1.1±0.77	1.0±0.63
Ampicillin	1.9±0.52				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

Table 3: Antimicrobial activity of different extracts of *W. glauca* on *Klebsiella* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	1.1±0.77	1.3±1.00	1.0±0.63	1.1±0.77
EA	-	1.2±0.52	1.3±1.00	1.3±1.00	1.1±0.77
A	1.0±0.63	1.2±0.52	1.3±1.00	1.1±0.77	1.3±1.00
E	1.3±1.00	1.2±0.52	1.3±1.00	1.0±0.63	1.1±0.77
W	1.0±0.63	1.0±0.63	1.3±1.00	1.1±0.77	0.9±0.57
Ampicillin	0.9±0.57				

-: Indicates no zone of inhibition, *W. glauca: Wedelia glauca*, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

1.4±0.99 mm. All the extracts with the minimum concentration of 1 mg have exhibited inhibition effect on the organism. All the extracts shown good inhibition activity against *Klebsiella* sp., (Table 3) followed *Proteus* sp., (Table 4) and *Staphylococcus* sp. (Table 6). Against the above pathogens, maximum zone of inhibition was attained by acetone extract with 1.3±1.00 mm, and aqueous extract with 1.3±1.00 mm and 1.6±0.77, respectively. The plant extracts have inhibited the growth of *Pseudomonas* sp. (Table 8) considerably. Acetone extract shown higher zone of inhibition with 1.4±0.99 mm. From the results obtained, it is evident that this plant extracts showed a broad spectrum of activity against both Gram-positive and Gram-negative organisms. Among the five extracts investigated, acetone extract of the plant exhibited greater activity constantly against all the pathogens studied, it includes both bacterial cultures and fungal cultures. This activity may be due to the presence of secondary metabolites. Although all the extracts revealed the presence of different phytochemical constituents, the activity of acetone extract may be due to its ability in the extraction of

Table 4: Antimicrobial activity of different extracts of *W. glauca* on *Proteus* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	1.0±0.63	0.9±0.57	0.9±0.57	1.1±0.77
EA	-	0.9±0.57	1.2±0.52	1.3±1.00	1.1±0.77
A	1.1±0.77	1.1±0.77	1.2±0.52	1.3±1.00	1.2±0.52
E	0.9±0.57	1.0±0.63	0.9±0.57	1.3±1.00	0.9±0.57
W	-	-	1.0±0.63	1.3±1.00	1.3±1.00
Ampicillin	1.2±0.52				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

Table 5: Antimicrobial activity of different extracts of *W. glauca* on *E. coli*

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	-	-	-	1.0±0.63
EA	-	-	-	1.1±0.77	1.2±0.52
A	-	1.1±0.77	1.3±1.00	1.3±1.00	1.5±0.22
E	-	-	1.0±0.63	1.2±0.52	1.3±1.00
W	-	-	-	1.1±0.77	1.2±0.52
Ampicillin	-				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous, *E. coli: Escherichia coli*

Table 6: Antimicrobial activity of different extracts of *W. glauca* on *Staphylococcus* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	-	1.0±0.63	1.1±0.77	1.2±0.52
EA	-	1.1±0.77	1.1±0.77	1.3±1.00	1.2±0.52
A	1.3±1.00	1.1±0.77	1.3±1.00	1.3±1.00	1.6±0.77
E	1.0±0.63	1.2±0.52	1.2±0.52	1.2±0.52	1.2±0.52
W	-	-	1.0±0.63	1.0±0.63	1.6±0.77
Ampicillin	1±0.63				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

Table 7: Antimicrobial activity of different extracts of *W. glauca* on *Streptococcus* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	-	0.9±0.57	1.0±0.63	1.1±0.77
EA	-	1.1±0.77	1.1±0.77	1.1±0.77	1.2±0.52
A	1.1±0.77	1.2±0.52	1.3±1.00	1.2±0.52	1.2±0.52
E	1.0±0.63	1.1±0.77	1.1±0.77	1.2±0.52	1.2±0.52
W	-	-	-	1.2±0.52	1.3±1.00
Ampicillin	0.9±0.57				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

Table 8: Antimicrobial activity of different extracts of *W. glauca* on *Pseudomonas* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	1.3±1.00	0.9±0.57	1.1±0.77	1.3±1.00
EA	-	1.3±1.00	1.1±0.77	1.1±0.77	1.2±0.52
A	-	0.9±0.57	0.9±0.57	1.2±0.52	1.4±0.99
E	-	1.0±0.63	1.1±0.77	1.3±1.00	1.3±1.00
W	-	-	0.9±0.57	0.9±0.57	1.0±0.63
Ampicillin	-				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

Table 9: Antimicrobial activity of different extracts of *W. glauca* on *Enterobacter* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	-	-	-	-
EA	-	-	-	-	1.2±0.52
A	-	-	0.9±0.57	1.0±0.63	1.4±0.99
E	-	-	-	1.0±0.63	1.0±0.63
W	-	-	-	-	-
Ampicillin	-				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

Table 10: Antimicrobial activity of different extracts of *W. glauca* on *Citrobacter* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	-	-	-	1.0±0.63
EA	-	-	-	-	1.1±0.77
A	-	-	-	1.1±0.77	1.3±1.00
E	-	-	-	-	1.1±0.77
W	-	-	-	-	-
Ampicillin	-				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

Table 11: Antimicrobial activity of different extracts of *W. glauca* on *Aspergillus* sp.

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	-	0.9±0.57	1.0±0.63	1.3±1.00
EA	-	-	1.3±1.00	1.0±0.63	1.1±0.77
A	-	0.9±0.57	1.3±1.00	1.3±1.00	1.6±0.77
E	-	0.9±0.57	1.0±0.63	1.1±0.77	1.3±1.00
W	-	-	1.0±0.63	1.0±0.63	1.1±0.77
Ampicillin	2.2±0.77				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

large quantities of phytochemicals. A previous study has shown two possibilities that may account for the higher antibacterial activity of the extract are the nature of biologically active compounds (alkaloids, flavonoids, tannins, triterpenoids which may be enhanced in the presence of the extract) and stronger extraction capacity of solvent that may yield a greater number of active constituents responsible for the antibacterial activity [24]. This may also be the reason for the higher bacterial and fungal activity shown by acetone extract. The acetone extract shown the presence of flavonoids. Flavonoids also play a major role in inhibiting microorganisms. A study claims that the flavonoids reported in the extract may be one of the major constituent responsible for registering the antibacterial activity. Flavonoids have been reported as a major class of natural products with anti-infective activity. Many research showed that the structure of flavonoids and antibacterial activity have a close relationship [25].

Table 12: Antimicrobial activity of different extracts of *W. glauca* on *Candida albicans*

Extracts/ standard drug	1 mg	2 mg	3 mg	4 mg	5 mg
PE	-	-	0.9±0.57	1.0±0.63	1.2±0.52
EA	-	1.0±0.63	1.1±0.77	1.3±1.00	1.3±1.00
A	0.9±0.57	1.0±0.63	1.0±0.63	1.3±1.00	1.5±0.22
E	-	1.0±0.63	0.9±0.57	1.1±0.77	1.3±1.00
W	-	-	1.0±0.63	1.1±0.77	1.1±0.77
Fluconazole	2.4±0.57				

W. glauca: Wedelia glauca, PE: Petroleum ether, EA: Ethyl acetate, A: Acetone, E: Ethanol, W: Aqueous

GC-MS analysis the presence of diverse phytoconstituents in the acetone extract of leaves and flowers of *W. glauca.* The library search list of the GC-MS shown the presence of compounds like ç-Terpinene, 1-P-menthen-8-yl acetate, beta-phellandrene, á-Terpinyl acetate, 4,7,7-trimethylbicyclo[4.1.0]hept-4-en-3-ol, 4,4-dimethyl-cyclohex-

Table 13: GC-MS analysis of acetone extract of *W. glauca*

RSI	Compound name	p	Molecular formula	Molecular weight	Area %
GC chromatogram with library match of peaks with R.T 5.36 minutes					
766	ç-Terpinene	7.39	$C_{10}H_{16}$	136	2.22
748	1-P-menthen-8-YL acetate	4.92	$C_{12}H_{20}O_2$	196	2.22
760	Beta-phellandrene	4.54	$C_{10}H_{16}$	136	2.22
798	á-Terpinyl acetate	4.19	$C_{12}H_{20}O_2$	196	2.22
765	Limonen-10-yl acetate	3.86	$C_{12}H_{18}O_2$	194	2.22
GC chromatogram with library match of peaks with R.T 9.80 minutes					
772	4,4-Dimethyl-cyclohex-2-en-1-ol	8.92	$C_8H_{14}O$	126	2.61
743	(1R,2R,3S,5R)-(-)-2,3-pinanediol	8.92	$C_{10}H_{18}O_2$	170	2.61
749	6-Ethyl-7-hydroxy-4-octen-3-one	6.65	$C_{10}H_{18}O_2$	170	2.61
737	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl- (CAS)	6.65	$C_{10}H_{18}O_2$	170	2.61
746	4-Octen-3-one, 6-ethyl-7-hydroxy-.	6.65	$C_{10}H_{18}O_2$	170	2.61
GC chromatogram with library match of peaks with R.T 14.79 minutes					
937	(+) Spathulenol	69.34	$C_{15}H_{24}O$	220	0.64
918	(-)-Spathulenol (CAS)	69.34	$C_{15}H_{24}O$	220	0.64
901	1H-cycloprop[e] azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]-	20.02	$C_{15}H_{24}O$	220	0.64
896	1H-cycloprop[E] azulen-7-OL, decahydro-1,1,7-trimethyl-4-methylene-	20.02	$C_{15}H_{24}O$	220	0.64
861	Isospathulenol	4.11	$C_{15}H_{24}O$	220	0.64
GC chromatogram with library match of peaks with R.T 18.11 minutes					
984	7-acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane	24.12	$C_{15}H_{26}O_2$	238	1.1
789	1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	21.31	$C_{12}H_{20}O_2$	196	1.1
746	Bicyclo[3.2.0]heptan-2-one, 5-formylmethyl-6-hydroxy-3,3-dimethyl-6-vinyl-	9.06	$C_{13}H_{18}O_3$	222	1.1
721	1-Cyclohexene-1-methanol, à,2,6,6-tetramethyl- (CAS)	3.03	$C_{11}H_{20}O$	168	1.1
717	1-Cyclohexene-1-ethanol, 2,6,6-trimethyl-	2.2	$C_{11}H_{20}O$	168	1.1
GC chromatogram with library match of peaks with R.T 21.86 minutes					
918	Hexadecanoic acid, methyl ester	44.14	$C_{17}H_{34}O_2$	270	0.71
912	Hexadecanoic acid, methyl ester (CAS)	44.14	$C_{17}H_{34}O_2$	270	0.71
917	Pentadecanoic acid, 14-methyl-, methyl ester (CAS)	28.51	$C_{17}H_{34}O_2$	270	0.71
GC chromatogram with library match of peaks with R.T 26.56 minutes					
864	9,12-Octadecadienoic acid (Z, Z)- (CAS)	24.92	$C_{18}H_{32}O_2$	280	56.77
847	9-Octadecenoic acid (Z)- (CAS)	22.02	$C_{18}H_{34}O_2$	282	56.77
831	9,12-Octadecadienoyl chloride, (Z, Z)-	5.27	$C_{18}H_{31}ClO$	298	56.77
821	17-Octadecynoic acid	4.66	$C_{18}H_{32}O_2$	280	56.77
807	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	3.1	$C_{16}H_{28}O_3$	268	56.77
GC chromatogram with library match of peaks with R.T 32.76 minutes					
793	Campesterol	35.47	$C_{28}H_{48}O$	400	0.48
791	Ergost-5-en-3-ol, (3á,24R)- (CAS)	35.47	$C_{28}H_{48}O$	400	0.48
791	Ergost-5-EN-3á-OL	34.09	$C_{28}H_{48}O$	400	0.48
840	Ergost-5-en-3-ol, (3á,24R)- (CAS)	35.47	$C_{28}H_{48}O$	400	0.48
778	5-Cholestene-3-ol, 24-methyl-	10.03	$C_{28}H_{48}O$	400	0.48
GC chromatogram with library match of peaks with R.T 37.66 minutes					
923	5-Methoxy-8,8-dimethyl-6-[(E)-2-methylbut-2-enoyl]-4-phenyl-2H,8H-benzo[1,2-b: 3,4-b']dipyran-2-one	35.92	$C_{26}H_{24}O_5$	416	7.82
912	Trans-2,8-diphenyl-2-methyl-1,1-bis (methylthio)-1,2-dihydroazeto[2,1-b] quinazoline	23.92	$C_{25}H_{24}N_2S_2$	416	7.82
907	1,2-O-Isopropylidene-4-ethenyl-3-O-tosyl-(6S)-à, D-glucofuramose	19.28	$C_{17}H_{20}O_8S_2$	416	7.82
951	1-(p-Methoxyphenyl)-3-phenyloxy-4-oxoazetidine-2-N-benzylN-methylenenitrone	7.04	$C_{24}H_{22}N_2O_4$	402	7.82
939	Calophyllolide	4.05	$C_{26}H_{24}O_5$	416	7.82

GC-MS: Gas chromatography-mass spectrometry, *W. glauca: Wedelia glauca*

2-en-1-ol, (+) spathulenol, isospathulenol, 1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl), hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid (Z,Z)- (CAS), 17-octadecynoic acid, campesterol, Ergost-5-EN-3á-OL, calophyllolide, and many more compounds shown (Table 13) is known to have higher potential in combating bacterial, fungal, and viral pathogens. Some of the compounds were reported for its anti-cancer property [26-28]. A previous review on genus *Wedelia* concluded that the thorough screening of literature available on genus *Wedelia* depicted the fact that it is a popular remedy among the various ethnic groups, Ayurvedic, and traditional practitioners for the treatment of ailments. Researchers are exploring the therapeutic potential of this plant as it has more therapeutic properties which are not known [19]. Through this present study, we explored the antibacterial and antifungal potential of *W. glauca*, by this, we can understand that this plant is also possessing higher potential in treating ailments, infections, and diseases like all the other species in the genus *Wedelia*. Further studies on the plant will definitely lead to the discovery of newer medications in the future.

CONCLUSION

The antimicrobial activity of *W. glauca* and nature of active principles present in the extracts of this plant is demonstrated for the first time against the pathogenic bacterial and fungal cultures. Among the different extracts studied acetone extract exhibited a better activity and revealed the presence of diverse secondary metabolites. GC-MS results of acetone extract clearly revealed the presence of various vital compounds which is responsible for the better activity of the plant. These results prove that this plant will be a promising source in the future research for the development of new antibiotic drugs for both bacterial and fungal infections.

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