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ANTIMICROBIAL EFFICACY OF WEDELIA GLAUCA (ORTEGA) O. HOFFM. EX HICKEN AND PHYTOCHEMICAL ANALYSIS USING GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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ABSTRACT

Plants as a treasure of bioactive compounds continue to play a major role in sustaining human health. The aim of this study is to evaluate the antimicrobial efficacy of *Wedelia glauca* stem extracts against the pathogens causing urinary tract infections and to screen them to identify the phytochemical constituents present in the extracts. Individual extraction was done by soxhlation using five solvents. Agar well diffusion method was used to determine the antimicrobial activity. Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, carbohydrates, proteins & amino acids, steroids, triterpenoids, tannins, reducing sugar, and saponins in different plant stem extracts. Most of the extracts were shown substantial inhibition activity against Gram-positive compared to gram-negative bacteria. Acetone extract showed better activity against all the bacterial and fungal cultures studied. The GC- MS analysis of acetone extract revealed the presence of various potential compounds which attributes to the results obtained.

KEYWORDS: ***Wedelia glauca,*** **phytochemical analysis, antimicrobial activity, plant extraction, solvents, antimicrobial resistance, GC-MS.**

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections in the general population, with an estimated overall incidence rate of 18 per 1000 person per year. It is the most frequent bacterial infection recorded in older people. In addition, UTIs are a major cause of hospital admissions and are associated with significant morbidity and mortality as well as a high economic burden.[1] Antibiotics are one of our most important weapons in fighting bacterial and fungal infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria[2-3] The resistance among various microbial species (infectious agents) to different antimicrobial drugs has emerged as a cause of public health threat all over the world at a terrifying rate. Due to the pacing advent of new resistance mechanisms and decrease in efficiency of treating common infectious diseases, it results in failure of microbial response to standard treatment, leading to prolonged illness, higher expenditures for health care, and an immense risk of death.[4] With the trend in emerging diseases, there is an increased interest to revert back to the knowledge of traditional medicine, with hopes to discover novel biological activities within these plants.[5] Against microorganisms, many reports have demonstrated the validness of old herbs so the plants provide a rich source for modern medicine to attain new principles.[6] Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called as phytochemicals.[7, 8] In an effort to expand the spectrum of antibacterial agents from natural resources, *Wedelia glauca* belonging to Asteraceae family has been selected. The genus *Wedelia* (Family Asteraceae, tribe Heliantheae, subtribe Ecliptinae) consists of 60 species distributed in tropical and warm temperature regions, including India, Burma, Ceylon, China, and Japan. A number of plants from the genus *Wedelia* are used as traditional herbal medicines throughout the world and they have been reported to possess hepatoprotective, antipyretic-analgesic, bactericidal, molluscicidal, hypoglycemic and antitumour activities.[9] *Wedelia glauca* is a perennial and herbaceous plant, 30 to 80 centimetres high, with stiff stems and simple or compound lanceolate basal leaves with 2-3 teeth. The yellow inflorescences are arranged in terminal capitates heads.[10] The aim of the present study was to examine the antibacterial and antifungal potential of *Wedelia glauca* and to screen the presence of various phytochemical constituents present in the plant. This study gives first time description of phytochemical screening and antimicrobial activity of stem extracts of *Wedelia glauca.*

MATERIALS AND METHODS

Plant Materials

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

Preparation of the Crude Extracts

The healthy stems were collected from *Wedelia glauca* plant were shade dried at room temperature for two weeks then homogenized to a fine powder and stored in an airtight glass container at 4°C until the further process. In this study, petroleum ether (P.E), ethyl acetate (E.A), acetone (A), ethanol (E) and aqueous (W) were used as solvents in the ratio 1:10 (w/v). Solvents were been chosen based on the polarity, Petroleum ether (non-polar), Ethyl acetate and Acetone (polar aprotic), Ethanol and Water(aqueous) were belongs to polar protic solvents. Their dielectric contstants were 2.0, 6.02, 21, 24.55, and 80 respectively. These solvents were been used for the individual extraction of the plant material by soxhlation. The extracts were subjected to rotary evaporation in order to eliminate the solvent and the obtained semisolid extracts were stored in an airtight container at 4º C in the freezer for further use. The extract was exposed to 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 50mg of the extract was dissolved in 1ml of 5 percent dimethyl sulphoxide (DMSO). It was sterilized by filtration using 0.22 µm millipore filter.[11]

Phytochemical Analysis

The preliminary phytochemical analysis was carried out on the different extracts using standard procedures[11-14] to identify the phytochemical constituents. They are

Detection of Alkaloids

To 0.5 ml extract were treated with few drops of 1ml 2N HCl to this few drops of Mayer's reagent / Dragandorf reagent and Hager's reagent were added. Orange precipitate, Orange color, White or Yellow precipitate shows the presence of alkaloids.

Test for Flavonoids

Test tubes containing 0.5ml of test extracts, 5-10 drops of dilute Hcl and small piece of zinc or magnesium were added and the solution was boiled for few min. In the presence of flavanoids, reddish pink or dirty brown color is produced.

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 2ml of chloroform and concentrated sulphuric acid was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Test for Terpenoids
Salkwoski reaction
0.5ml of the test extracts was mixed in 0.2ml of chloroform and conc. H2SO4 (0.3ml) was carefully added to form a layer. A reddish brown coloration in the inter phase formed indicate the presence of terpenoids.

Test for Proteins
Millon's test: Small portion of the extract when mixed with 2ml of Millon's reagent, a white precipitate appeared which turned red upon 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 min. The solution appears green, yellow or red depending upon the reducing sugar present.

Test for Tannins
To 1-2ml of test extracts, few drops of 5% aqueous Fecl3 solution were added. A bluish black color, which disappears on addition of a few ml of dilute H2SO4 followed by the formation of yellowish brown precipitate indicate the presence of tannins.

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

Culture Media
Muller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) was prepared according to the manufacturer's instruction, autoclaved and dispensed at 20ml per plate in petri dishes. Set plates were incubated overnight to ensure sterility before use.[11]

Antibacterial assay
Using the well diffusion method, Twenty-four-hour broth culture of the respective bacteria was adjusted to a turbidity of 0.5 McFarland standards. Each of this bacterial culture was swabbed over sterile MHA plates separately by using sterile cotton swabs. A well with diameter 6mm 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, 100µl was added to the each well, final concentration was made up to 1, 2, 3, 4 and 5mg respectively. The plates were kept at 4˚C for 1hr for diffusion of extract, thereafter the plates were incubated at 37˚C for 24hrs. Ampicillin (1 mg respectively) was served as the positive reference standard to determine the sensitivity of the tested microbial strains. The Antibacterial activity was determined by measuring the Zones of inhibition in diameter produced after incubation and results were expressed in millimetres (mm).[11]

Antifungal study
Agar well diffusion method was used for the in vitro screening of antifungal activity against 2 pathogenic fungal strains. The culture of organisms was maintained on Sabouraud dextrose agar. Activated cultures of fungal strains in Sabouraud's broth were adjusted as per McFarland standard. Each of the diluted cultures was swabbed on sterile SDA plates separately by using sterile cotton swabs. The plate was dried for 30 minutes at room temperature. A well with diameter 6mm 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, 100µl was added to each well, final concentration was made up to 1, 2, 3, 4 and 5mg respectively. Fluconazole (1 mg) was used as the positive reference, and the plates were kept at 4˚C for 1hr for the prediffusion of the extract. Then the plates were incubated for 48 - 72 hours at 37°C. The zone of inhibition on fungal growth was measured in diameter (mm).[11]

STATISTICAL ANALYSIS

The data obtained after determining the zone of inhibition was 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 significant)

Gas chromatography-mass spectrometry (GC-MS) analysis
Gas chromatography-mass spectrometry (GC-MS) analysis GC-MS analysis of the acetone extract of the stem 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), 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

As the current antimicrobial therapies using the antibiotics of chemical origin for treating various infections causes serious side effect and leads to the emergence of antimicrobial resistance among the

pathogens, the need for identifying new leads from alternative therapies especially from plant source is essential. In ayurvedic and ethnomedicine, plants and their derived products play an important role as chemotherapeutic agents.[15] Plants derived compounds exhibited significant activity not only against cancer, it also proved to be effective in combatting bacterial, fungal, viral infections, inflammatory, arthritis, diabetic, wound healing and much more.According to the World Health Organization (WHO), medicinal plants would be the greatest resource to find a range of drugs and bioactive compound. As a result, such plants should be investigated to better understand their properties, protection and effectiveness.[16] Still there are many unexplored plants are there, if those plants are identified and examined it may result in several effective drugs of plant based origin with minimal or no side effects. This will aide the human race to combat antimicrobial resistance and the side effects caused by the synthetic drugs. In this investigation, an effort was taken to explore the antimicrobial potential of the all the five stem extracts of *W. glauca* and to analyze its phytochemical richness. To check the presence of secondary metabolites preliminary phytochemical analysis has been performed and the results were tabled (Table 1). The overall results revealed the presence of various phytochemical compounds like alkaloids, flavonoids, carbohydrates, proteins & amino acids, steroids, triterpenoids, tannins, reducing sugar and saponins. Among the solvents used acetone and aqueous extract showed the presence for eight out of nine phytochemicals studied. Followed by petroleum ether and ethyl acetate extracts shown the presence for five phyto compounds while ethanol extract shown the presence for four phytoconstituents. The difference among the solvent system over the presence of different phytocompounds shows their potential in extraction. These classes of phytochemicals are well known for medicinal properties against various types of pathogenic attacks and consequently, could recommend for the treatment of various ailments.[16] Antimicrobial analysis has been performed against the pathogenic microorganisms isolated from the patients infected with urinary tract infections. Extracts of *W. glauca* has been tested against pathogenic bacterial cultures and fungal cultures. The results were observed and recorded (Table 2 – 11). As in correlation with results obtained in preliminary phytochemicals acetone stem extract of the plant exhibited a better consistent activity when compared to all other solvents. Higher zone of inhibition of 1.4±0.99 mm was observed against *Proteus* sp and *Streptococcus* sp. respectively. This is one evidence that we can say that the phytochemical constituents present in the extract can be a prominent reason for this activity. It has shown a promising activity against gram positive and gram negative bacteria as well as it shown inhibition of fungal culture at the concentration of 2mg. At the concentration of 5 mg 1.4±0.99 mm and 1.3±1.00 mm of zone of inhibition was observed against *Aspergillus* sp and *Candida* sp. respectively. The inhibition was in dose dependent manner. On the other hand the aqueous extract shown minimal activity in comparison with the acetone extract. Earlier a study reported that there was two possibilities that may be responsible 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.[17] From this, it is obvious that acetone extract extracted the higher quantity of vital phytochemical compounds which may be the difference of activity between the acetone and aqueous extracts. The extracts of Petroleum ether, ethyl acetate and ethanol extracts shown a moderate level of activity. Among the bacterial cultures examined *Citrobacter* sp., *Enterobacter* sp., *E. coli* and *Pseudomonas* sp. displayed higher level of resistance. All the extracts were been effective against *Bacillus* sp. and shown good range of inhibition against *Streptococcus* sp., *Staphylococcus* sp., and *Proteus* sp. Results indicates that all these extracts were having slightly higher activity against gram positive bacteria than the gram negative bacteria. Among the fungal cultures, the plant extracts of concentration 3 mg and above had inhibited the growth of both the fungal cultures studied. While acetone and ethanol extracts has inhibited the growth of *Aspergillus* sp. at the concentration of 2 mg. Against *Candida* sp. acetone petroleum ether extract and acetone extract shown the inhibition at the concentration of 2mg. Ampicillin, Flucoconazole were used as positive control for bacteria and fungi respectively. 5 percent DMSO was used to dissolve the extracts, hence it was examined as negative control, which showed no activity. Among all the five extracts, acetone extract was found to retain higher amount of phytoconstituents which possessed most potent antibacterial and antifungal activity. Accordingly, this most potent extract was subjected GC-MS analysis. Normally, phytocompounds are highly complexes, so for their analysis by GC-MS method well appropriate due to its sensitivity and selectivity.[16] In order screen phytochemical compounds present in the acetone extract the GC-MS analysis was been performed. The analysis spectrum of acetone extract showed seven outstanding peaks occupying higher percent area (Figure. 1). Based on the retention time and peaks obtained it has been compared with the available library and literatures to find the compounds. The compounds identified through the library search were tabled (Table 13). It revealed the presence for vital compounds like á-Terpinyl acetate, dl-Limonene, á-Phellandrene, Kaur-16-en-19-ol, Terpinen-4-ol, which were already been reported for their antitumour, anti HIV activity, antiaflatoxigenic activity, angiogenesis, antiseptic activity, antiulcer activity[18-24]. The activity of the other compounds had to be explored. With the results obtained it is evident that this plant *W. glauca* will play a crucial in finding potent drugs in the future.

Table 1
Phytochemical screening of Wedelia glauca stem extracts

S No.	Phytochemicals	Plant stem extracts				
		P.E	E.A	A	E	W
1	Alkaloids	-	+	+	+	+
2	Flavonoids	+	-	+	-	+
3	Carbohydrates	+	+	+	-	+
4	Proteins & amino acids	+	+	+	+	+
5	Steroids	-	-	+	-	+
6	Triterpenoids	-	-	+	-	-
7	Tannins	-	+	-	-	-
8	Reducing sugar	+	-	+	+	+
9	Saponins	+	+	+	+	+

Keys
- ***+ Presence of the compound; - Absence of the compound.***
- ***petroleum ether (P.E), ethyl acetate (E.A), acetone (A), ethanol (E) and aqueous (W) extracts respectively.***

Table 2
Antimicrobial activity of different extracts of Wedelia glauca on Bacillus sp.

	1mg	2mg	3mg	4mg	5mg
PE	0.9±0.57	0.9±0.57	1.0±0.63	1.1±0.77	1.1±0.77
E. A	0.9±0.57	1.0±0.63	1.0±0.63	1.1±0.77	1.1±0.77
A	1.0±0.63	1.0±0.63	1.0±0.63	1.2±0.52	1.2±0.52
E	0.8±0.34	0.9±0.57	1.0±0.63	1.1±0.77	1.0±0.63
W	-	0.9±0.57	0.8±0.34	0.8±0.34	1.0±0.63
Ampicillin	1.9±0.52				

- indicates no zone of inhibition

Table 3
Antimicrobial activity of different extracts of Wedelia glauca on Klebsiella Sp.

	1mg	2mg	3mg	4mg	5mg
PE	-	-	0.9±0.57	1.1±0.77	1.1±0.77
E. A	-	-	0.9±0.57	1.0±0.63	1.1±0.77
A	0.8±0.34	0.9±0.57	1.0±0.63	1.2±0.52	1.3±1.00
E	-	-	0.9±0.57	1.0±0.63	1.3±1.00
W	-	0.9±0.57	1.1±0.77	1.2±0.52	1.2±0.52
Ampicillin	0.9±0.57				

Table 4
Antimicrobial activity of different extracts of Wedelia glauca on Proteus sp.

	1mg	2mg	3mg	4mg	5mg
PE	-	1.1±0.77	1.1±0.77	1.1±0.77	1.2±0.52
E. A	-	-	1.1±0.77	1.3±1.00	1.3±1.00
A	1.1±0.77	1.1±0.77	1.2±0.52	1.3±1.00	1.4±0.99
E	-	-	1.1±0.77	1.2±0.52	1.2±0.52
W	-	-	1.0±0.63	1.1±0.77	1.2±0.52
Ampicillin	1.0±0.63				

Table 5
Antimicrobial activity of different extracts of Wedelia glauca on E. coli

	1mg	2mg	3mg	4mg	5mg
PE	-	-	0.9±0.57	1.0±0.63	1.1±0.77
E. A	-	-	-	1.0±0.63	1.0±0.63
A	-	-	1.0±0.63	1.1±0.77	1.2±0.52
E	-	-	-	0.9±0.57	1.2±0.52
W	-	-	-	1.0±0.63	1.0±0.63
Ampicillin	-				

Table 6
Antimicrobial activity of different extracts of Wedelia glauca on Staphylococcus sp.

	1mg	2mg	3mg	4mg	5mg
PE	-	-	0.8±0.34	1.0±0.63	1.1±0.77
E. A	-	-	0.8±0.34	0.9±0.57	1.0±0.63
A	0.9±0.57	1.0±0.63	1.0±0.63	1.1±0.77	1.3±1.00
E	-	1.0±0.63	1.0±0.63	1.1±0.77	1.2±0.52
W	-	-	0.9±0.57	1.1±0.77	1.2±0.52
Ampicillin	1±0.63				

Table 7
Antimicrobial activity of different extracts of Wedelia glauca on Streptococcus sp.

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

Table 8
Antimicrobial activity of different extracts of Wedelia glauca on Pseudomonas sp.

	1mg	2mg	3mg	4mg	5mg
PE	-	-	-	0.9±0.57	1.1±0.77
E. A	-	-	-	1.1±0.77	1.1±0.77
A	-	-	0.9±0.57	1.2±0.52	1.3±1.00
E	-	-	-	-	1.0±0.63
W	-	-	0.9±0.57	1.0±0.63	1.2±0.52
Ampicillin	-				

Table 9
Antimicrobial activity of different extracts of Wedelia glauca on Enterobacter sp.

	1mg	2mg	3mg	4mg	5mg
PE	-	-	-	0.9±0.57	0.9±0.57
E. A	-	-	-	-	1.0±0.63
A	-	-	1.0±0.63	1.3±1.00	1.3±1.00
E	-	-	-	0.9±0.57	0.9±0.57
W	-	-	-	0.9±0.57	1.0±0.63
Ampicillin	-				

Table 10
Antimicrobial activity of different extracts of Wedelia glauca on Citrobacter sp.

	1mg	2mg	3mg	4mg	5mg
PE	-	-	-	0.9±0.57	1.1±0.77
E. A	-	-	-	-	0.9±0.57
A	-	-	-	1.1±0.77	1.3±1.00
E	-	-	-	-	-
W	-	-	-	0.9±0.57	0.9±0.57
Ampicillin	-				

Table 11
Antimicrobial activity of different extracts of Wedelia glauca on Aspergillus sp.

	1mg	2mg	3mg	4mg	5mg
PE	-	-	1.0±0.63	1.0±0.63	1.2±0.52
E. A	-	-	0.9±0.57	1.0±0.63	1.0±0.63
A	-	1.0±0.63	1.3±1.00	1.4±0.99	1.4±0.99
E	-	0.9±0.57	0.9±0.57	1.1±0.77	1.1±0.77
W	-	-	0.9±0.57	1.0±0.63	1.0±0.63
Fluconazole	1.6±0.77				

Table 12
Antimicrobial activity of different extracts of Wedelia glauca on Candida albicans

	1mg	2mg	3mg	4mg	5mg
PE	-	0.9±0.57	0.9±0.57	1.2±0.52	1.2±0.52
E. A	-	-	1.1±0.77	1.1±0.77	1.2±0.52
A	-	1.0±0.63	1.2±0.52	1.3±1.00	1.3±1.00
E	-	-	0.9±0.57	1.0±0.63	1.0±0.63
W	-	-	1.0±0.63	1.0±0.63	1.2±0.52
Fluconazole	1.5±0.22				

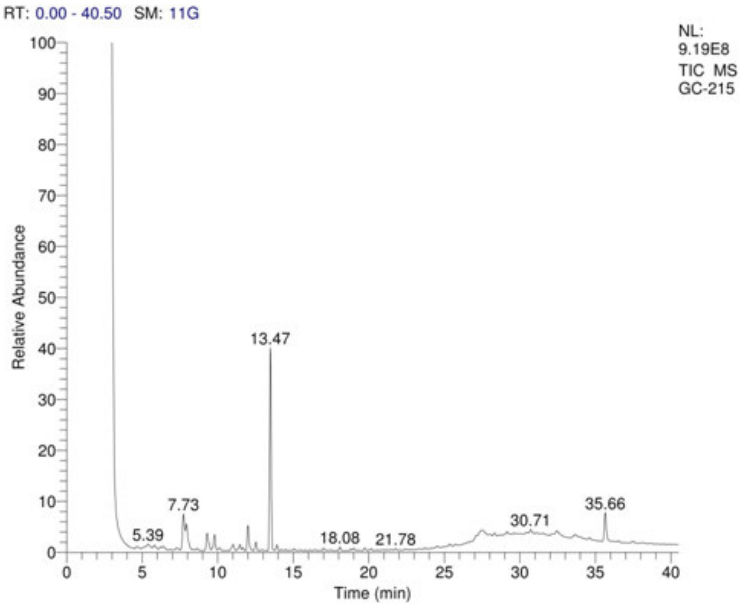

Figure 1
The gas chromatography-mass spectrometry spectral peaks of acetone stem extract of W. glauca

Table 13
GC-MS identified compounds of acetone extract plant extract

S. No	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
	GC chromatogram with library match of peaks with R.T 5.39 minutes					
1	897	á-Terpinyl acetate	18.84	$C_{12}H_{20}O_2$	196	2.37
2	870	dl-Limonene	9.71	$C_{10}H_{16}$	136	2.37
3	849	á-Phellandrene	6.47	$C_{10}H_{16}$	136	2.37
4	843	R(+)-LIMONEN	5.96	$C_{10}H_{16}$	136	2.37
	GC chromatogram with library match of peaks with R.T 7.73 minutes					
5	897	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (CAS)	50.93	$C_{10}H_{18}O$	154	13.65
6	902	l-4-Terpineol	36.98	$C_{10}H_{18}O$	154	13.65
7	860	Terpinen-4-ol	50.93	$C_{10}H_{18}O$	154	13.65
8	863	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	36.98	$C_{10}H_{18}O$	154	13.65
	GC chromatogram with library match of peaks with R.T 13.47 minutes					
9	970	MYRISTCIN	54.6	$C_{11}H_{12}O_3$	192	35.72
10	941	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- (CAS)	54.6	$C_{11}H_{12}O_3$	192	35.72
11	950	CROWEACIN	26.51	$C_{11}H_{12}O_3$	192	35.72
12	955	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	54.6	$C_{11}H_{12}O_3$	192	35.72
13	930	CIS-ISOMYRISTICIN	12.07	$C_{11}H_{12}O_3$	192	35.72
	GC chromatogram with library match of peaks with R.T 18.08 minutes					
14	797	1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)	29.76	$C_{12}H_{20}O_2$	196	0.74
15	986	7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nona ne	16.24	$C_{15}H_{26}O_2$	238	0.74
16	730	Bicyclo[3.2.0]heptan-2-one, 5-formylmethyl-6-hydroxy-3,3-dimethyl-6-vinyl-	4.6	$C_{13}H_{18}O_3$	222	0.74
17	715	1-Cyclohexene-1-methanol, à,2,6,6-tetramethyl-	2.88	$C_{11}H_{20}O$	168	0.74
18	678	(1,5,5,8-Tetramethyl-bicyclo[4.2.1]non-9-yl)-acetic acid	2.65	$C_{15}H_{26}O_2$	238	0.74
	GC chromatogram with library match of peaks with R.T 19.73 minutes					
19	841	4,5-dihydroxy-4-(3-methyl-2-butenyl)benzoic acid methyl ester	46.71	$C_{13}H_{16}O_4$	236	0.54
20	876	Dimethyl 2,5-furandipropionate	39.46	$C_{12}H_{12}O_5$	236	0.54
21	875	(+) spathulenol	2.93	$C_{15}H_{24}O$	220	0.54
	GC chromatogram with library match of peaks with R.T 25.34 minutes					
22	923	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	53.67	$C_{20}H_{40}O$	296	0.52
23	892	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R*,R*-(E)]]- (T-PHYTOL)	21.21	$C_{20}H_{40}O$	296	0.52
	GC chromatogram with library match of peaks with R.T 29.64 minutes					
24	902	Kaur-16-en-19-ol	28.96	$C_{20}H_{32}O$	288	0.5
25	864	[17-(14-C]-18-hydroxyaphidicol-16-ene	18.13	$C_{20}H_{32}O$	288	0.5
26	770	METHYL ENT-16-KAUREN-19-OATE	5.04	$C_{21}H_{32}O_2$	316	0.5
27	740	Kaur-16-en-18-oic acid, methyl ester, (4á)-	5.04	$C_{21}H_{32}O_2$	316	0.5
	GC chromatogram with library match of peaks with R.T 30.71 minutes					
28	687	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-HEXADECAMETHYLOCTASILOXANE #	12.04	$C_{16}H_{50}O_7Si_8$	578	1.42
	GC chromatogram with library match of peaks with R.T 32.44 minutes					
29	722	1H-Purin-6-amine, [(2-fluorophenyl)methyl]- (CAS)	35.68	$C_{12}H_{10}FN_5$	243	2.63
30	841	Heptasiloxane, hexadecamethyl-	6.65	$C_{16}H_{48}O_6Si_7$	532	2.63
	GC chromatogram with library match of peaks with R.T 33.66 minutes					
31	840	9,19-Cyclolanost-24-en-3-ol, acetate, (3á)-	46.6	$C_{32}H_{52}O_2$	468	1.83
32	879	9,19-Cyclolanost-24-en-3-ol, (3á)-	26.83	$C_{30}H_{50}O$	426	1.83
	GC chromatogram with library match of peaks with R.T 35.66 minutes					
33	814	13-Docosenamide, (Z)-	40.94	$C_{22}H_{43}NO$	337	6.68
34	817	9-OCTADECENAMIDE	22.34	$C_{18}H_{35}NO$	281	6.68
35	813	8-Methyl-6-nonenamide	3.04	$C_{10}H_{19}NO$	169	6.68

CONCLUSION

It is a high time to contain the emergence of multidrug resistance amongst the uropathogens as well as to find better efficient antibiotics with zero side effects. The overall study concluded that the stem extracts of *W.glauca* contains several diversified phytocompounds which possess the efficacy to inhibit the growth of pathogens causing urinary tract infections. The present investigation concluded that the *W. glauca* is enriched with various compounds of diverse activity. On further analysis this plant will be a good healthcare and alternative formulation of synthetic drugs.

CONFLICT OF INTEREST

Conflict of interest declared none.

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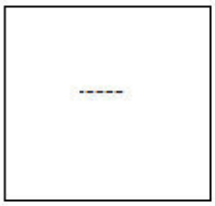

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