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GC-MS ANALYSIS, ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF FRACTIONS OF *AGERATUM CONYZOIDES* LEAF AGAINST MDR *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM A HOSPITAL IN SOUTHERN NIGERIA

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ABSTRACT

The increasing number of multidrug-resistant bacterial pathogens causing diverse infections is a major public health concern worldwide, particularly in hospitals and other health care settings. So, the search for new alternative products to solve this problem is the question of the age. Plants are recognized in the pharmaceutical industry due to their broad spectrum of structural diversity and their wide range of pharmacological activities. This study is designed to assess the bioactive components, antibacterial and antibiofilm activity of the fractions of *Ageratum conyzoides* (Goat weed) leaf against multidrug-resistant (MDR) *Streptococcus pneumoniae* isolated from a hospital in Southern Nigeria. The test organism was collected from Microbiology Laboratory, University of Calabar Teaching Hospital (UCTH), Calabar, Nigeria. The isolate was authenticated by standard bacteriological methods. The test organism was subjected to antimicrobial susceptibility profiling using disc diffusion technique to determine multidrug resistance status. Based on previous preliminary *in vitro* screening, *A. conyzoides* leaf fractions were subjected to GC-MS analysis to identify and quantify various phytoconstituents. Antibacterial activity of the leaf fractions was determined by agar well diffusion method. MICs and MBCs were evaluated using microdilution assay. A modified crystal violet assay was used to determine antibiofilm activity of the fractions and Optical Densities (ODs) were recorded. Results showed that the isolate was multidrug-resistant with percentage resistance of 46.66%. Phytochemical screening revealed the presence of saponins, tannins, flavonoids alkaloids, triterpenes/steroids, cardiac glycosides and free anthraquinone in the plant. GC-MS analyses of fractions revealed various phytochemicals and their concentrations in percentage, which include phytol, benzenedicarboxylic acid, phenols, flavones, etc. Fractions from *A. conyzoides* (especially n-hexane and ethyl acetate fractions) demonstrated strong antibacterial activity with zones of inhibition ranging from 9mm to 23mm. MICs and MBCs of the various fractions varied, with the lowest MIC (6.25mg/mL) from n-hexane fraction. ODs indicated that the fractions had remarkable capacity to reduce biofilm formation. This study has demonstrated that the fractions of *A. conyzoides* leaf had varying degrees of antibacterial activity against bacterial planktonic and biofilm forms. Therefore, this evidence suggests that this plant can be used as alternative treatment measures to conventional antibiotics if properly harnessed.

KEYWORDS: *Multidrug-resistance, GC-MS, Ageratum conyzoides, fractions, biofilm.*

INTRODUCTION

Antibiotics are natural products synthesized by microorganisms that act against other microbes.[9] A few entirely synthetic molecules have been developed to tackle various diseases. Chemical modifications to the original antibiotic molecules have been made to increase potency, to improve solubility and pharmacokinetics and to evade resistance mechanisms.[1] This principle was first applied empirically to modify sulphanilamide and achieved great success with modifications to β-lactams.[10]

For years, many antimicrobial agents have been used to control or eliminate bacteria from hospitals and for the treatment of common bacterial infections of public health importance.[12,79] Antibiotics are one of our most powerful tools for fighting life-threatening infections.[13] Unfortunately, however, the irrational use of these antibacterial agents has produced strains of multiple antibiotic resistant bacteria in households, hospitals, etc.[14] Antibiotic resistance is the ability of microorganisms to defeat the drugs designed to kill them or inhibit their growth.[15]

Human pathogens such as *Staphylococcus aureus*, *Salmonella* t*yphi*., *Streptococcus pneumoniae*, *Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aeruginosa*, etc. have been isolated from different patients in several hospitals with some of the isolates recording high level of antimicrobial resistance to the commonly used antibiotics.[1,16]

Resistance to antibiotics has become a major public health problem worldwide as it reduces the effectiveness of treatments and increases morbidity, mortality, and health-care cost.[17,73] Another drawback of the indiscriminate use of conventional antimicrobials is their failure to treat infections caused by bacteria when they form biofilm.[10]

Microbial biofilms are communities of bacteria, embedded in a self-producing matrix, forming on living and non-living surfaces.[80] Biofilm-associated cells have the ability to adhere irreversibly on a wide variety of surfaces, including living tissues and indwelling medical devices as catheters, valves, prosthesis, and so forth.[18] Biofilm are considered an important virulence factor that causes persistent chronic and recurrent infections; they are highly resistant to antibiotics and host immune defense.[19] Bacteria protected within biofilm exopolysaccharides are up to 1,000 times more resistant to antibiotics than planktonic cells (free-living), which generates serious consequences for therapy and complicated treatment options. An estimated 75% of bacterial infections involve biofilms that are protected by an extracellular matrix, and most of these pathogens are implicated in nosocomial infections.[14,20] The increased biofilm resistance to conventional treatments enhances the need to develop new control strategies.[21]

Biofilm inhibition is considered as major drug target for the treatment of various bacterial infections, and pharmacological development of these drugs is now extensively studied.[22] In recent years, **s**everal green nonlethal strategies for biofilm control have been developed, because the mode of action of these novel antibiofilm agents is much less susceptible to the emergence of resistance. However, although they are promising strategies, they have disadvantages because none have been totally effective.[23]

One promising alternative is the search for naturally occurring compounds of plant origin capable of blocking biofilm formation and killing or inhibiting the growth of biofilm-forming bacterial pathogens.[24] Historically, plant extracts and their biologically active compounds have been a valuable source of natural products, which have played a central role in the prevention and treatment of diseases, helping to maintain human health.[76] Furthermore, they are widely accepted due to the perception that they are safe and have a long history of use in folk medicine to cure diseases since ancient times.[25] Today, more than 25% of prescribed drugs that are used in the treatment of diseases, in one way or the other contain natural substances that comes from plant.[26,27,28]

Ageratum is one of the genera which belongs to the family Asteraceae and consists of 30 species.[29] *Ageratum conyzoides* (Goat weed) is one of the mostly commonly known species of this genus. It is a tropical plant found commonly in western and eastern regions of Africa, in some regions of Asia and South America.[31] The leaves are consumed as vegetable[32] and has been traditionally used as a purgative, febrifuge, emetic, anti-spasmodic and anti-asthmatic.[33] The most common use of this plant is to cure wounds and burns.[6] In Nigeria, it is reportedly used in the treatment of typhoid fever and diarrhea.[3] It has also been found useful as styptic and anti-dysenteric, antimicrobial, anti-inflammatory, analgesic, antipyretic, antiparmedic, gastroprotective, anti-ulcer, insecticidal and herbicidal.[5,4,6]

However, there is need to scientifically study the phytochemical composition of *A. conyzoides* in order to unravel the basis for its medicinal value by using suitable qualitative and quantitative techniques. The combination of an ideal separation technique (Gas Chromatography) with the best identification technique (Mass Spectrometry) made GC-MS an ideal technique for qualitative and quantitative analysis of volatile and semi-volatile compounds.[2] GC separates the constituents; mass spectrometry determines the molecular weight of these compounds. Mass spectrometry is a powerful analytical technique for the identification of unknown compounds, quantification of known compounds and to elucidate the structure and chemical properties of molecules. Through MS spectrum the molecular weight of compounds can be determined. This method is mostly employed for the structural elucidation of organic compounds and for monitoring the existence of previously characterized compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously.[34]

Therefore, this study was designed to assess the bioactive components, evaluate antibacterial and antibiofilm activity of the fractions of *A. conyzoides* leaf against multidrug-resistant (MDR) *Streptococcus pneumoniae* isolated from a hospital in Southern Nigeria.

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh leaf of *A. conyzoides* were collected from Cross River National Park and jointly identified by the Departments of Botany, University of Calabar and Plant Science and Biotechnology, University of Cross River State, Nigeria.

Preparation of plant materials

The freshly collected leaf were washed thoroughly in tap water, followed by successive washing in distilled water, and air-dried under room temperature ($24^{o}C - 34^{o}C$) for

7 days. Upon drying, the leaf was pulverized using electrical blender. The powdered sample was stored in airtight containers and kept at room temperature until required.[36,38]

Extraction of plant material

Five hundred grams (500 g) of the powdered plant was weighed with electric weighing balance (Gerhardt, England) and transferred into 10 L glass extraction jar. The extraction was carried out by maceration for 72h at room temperature (25±2°C) using 99.9% methanol (5000 mL) as solvent with intermittent agitation for maximum extraction of phytochemicals. The liquid extract was filtered and concentrated in a rotary evaporator (LabTech Ltd., England) at 40^{o}C. The extract was weighed and stored at -4°C for further analysis.[37]

Partitioning of the leaf extract

The methanol leaf extract (50 g) of the plant was dissolved in distilled water (500 mL) and partitioned successively and exhaustively with n-hexane, dichloromethane and ethyl acetate using separating funnel (Pyrex, England). Their respective liquid fractions were concentrated at 40^{o}C to dryness using rotary evaporator. The fractions were weighed and stored in a refrigerator at -4^{o}C until when needed for further studies.

The percentage yield of methanol leaf extract of *A. conyzoides* was calculated using the formula:

$$\text{Percentage yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times \frac{100}{1}$$

Preliminary phytochemical screening of the extract

The qualitative phytochemical screening was conducted on the methanol extract of *A. conyzoides* leaf in accordance with standard methods to identify the various classes of bioactive compounds present.[72,78]

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the leaf fractions

Gas chromatography-mass spectroscopy (GC-MS), a hyphenated system that is a very compatible technique and the most commonly used technique for the identification and quantification of phytochemicals was employed in this study. The unknown organic compounds in the complex mixture can be determined by interpretation and also by matching the spectra with reference spectra.[39]

A solvent blank analysis of the fractions was first conducted using 1 µL of absolute methanol. Then 1 µL of the reconstituted solution was employed for GC-MS analysis as previously described with modifications.[40,41] GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Schimadzu GCMS-QP2010, employing the following conditions: Column Elite-1 fused silica capillary column (30×0.25 mm ID×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) as carrier gas at a constant flow of 1mL/ minute and a sample injection volume of 1 µL which was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Total run time was 30 min. The compounds were then identified from the GC-MS peaks, using library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectra of the components were compared with the database of spectrum of known components stored in the GC-MS library using National Industrial Securit Program (NISP) Search. The relative % amount of each component was calculated by comparing its average peak area to the total areas. The retention time, which is the time elapsed between injection and elution was also used in differentiating compounds. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.

Collection of test organism

A strain of pathogenic *Streptococcus pneumoniae* was obtained from the Microbiology Laboratory, University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State, Nigeria. The organism was authenticated by standard bacteriological protocol and molecular analysis using 16S rRNA gene sequencing by Sanger method.[42] The clinical bacterial isolate was maintained on nutrient medium at 37 °C for further study.

Determination of multidrug-resistant (MDR) status of test organism

The test isolate was investigated for multidrug-resistance status by Kirby Bauer disc diffusion method.[7,8] A total of fifteen (15) antibiotics belonging to six (6) classes of drugs were used in this study. The experiment was performed according to the guidelines given by.[43] Resistance of isolate to antibiotics was determined on Mueller-Hinton agar plates augmented with 5% sheep blood. The inoculum size of the organism was adjusted to the turbidity equivalent of 0.5 McFarland standard. The diameter of the zone of inhibition for each test antibiotic was measured and sensitivity or resistance estimated by comparing with zone-diameter interpretive standard.[43] Antibiotics discs (Oxoid Ltd., England) employed in this assay include ciprofloxacin (10 mcg), chloramphenicol (30 mcg), gentamicin (30 mcg), streptomycin (30 mcg), erythromycin (10 mcg), ampicillin (10 mcg), amikacin (10 mcg), levofloxacin (30 mcg), ceftazidime (20 mcg), cloxacillin (10 mcg), septrin (30 mcg), oxacillin (10 mcg), amoxillin (10 mcg), augmentin (30 mcg) and ciprofloxacin (20 mcg). Multidrug resistance status was taken as resistance to one drug in three or more groups of antibiotics.[43]

Evaluation of antibacterial activity of the leaf fractions

The antibacterial effects of the fractions against bacterial isolates were determined by Agar well diffusion technique.[44] Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were carried out on fractions that showed antibacterial activity. Broth micro-dilution method[45], with minor modifications was used to evaluate MIC.

To determine the antimicrobial activity of each fraction, 100 µL (0.1 mL) of fresh culture (approximately 10^6 CFU/mL and equivalent to 0.5 McFarland standard) was uniformly spread onto Muella-Hinton agar (MHA) plates using sterile glass spreader. Then, the plates were allowed to dry under room temperature for 10 minutes. After that, wells of 6mm in diameter were made in the agar using a sterilized cork borer and 100 µL of varying concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.50 mg/mL and 6.25 mg/mL) of each fraction was introduced into the wells. The concentrations were prepared using 10% dimethyl sulphuroxide (DMSO). Plates were incubated at 37^{o}C for 24 hrs. Antibacterial activity evidenced by the presence of clear inhibition zones around each well were measured in diameter and recorded. DMSO (10%) was used as negative control while chloramphenicol was used as positive control for comparing zones of inhibition as follows: Chloramphenicol (100 mg/mL): resistant (≤14), sensitive (≥15).[43,46]

Determination of minimum inhibitory concentration (MIC) of the fractions

One hundred microlitre (100 µL) of Muella-Hinton (MH) broth (Difco) plus different concentrations of the fractions were prepared and transferred to each microplate containing 96 wells to obtain dilutions of double strength, ranging from 6.25 mg/mL to 100 mg/mL. Then, 10 µL of fresh culture standardized according to McFarland 0.5% barium sulphate (approximately 1 x 10^6 CFU/mL) of test organisms was added. Microplates were incubated at 37^{o}C for 24 hrs. MIC value was estimated as the lowest concentration of the fraction that showed no turbidity after incubation. Bacterial suspension in broth was used as negative control, while broth containing standard drug (chloramphenicol) was used as positive control.[47]

Evaluation of minimum bactericidal concentration (MBC) of the fractions

To investigate MBC, 100 µL (0.1 mL) from each well that showed no visible growth was re-inoculated on Muella-Hinton agar plates and incubated at 37^{o}C for 24 hrs. MBC was evaluated as the lowest concentration of the fraction showing no bacterial growth.

Investigation of antibiofilm activity using leaf fractions of *A. conyzoides*

A modified crystal violet assay was employed to test the effect of fractions on biofilm formation as described by.[75] Two-fold serial dilutions of fractions were made in sterile 96 flat wells microliter plates containing 150 µL of Muella-Hinton broth per well. The test concentrations ranged from 6.25 mg/mL up to 100 mg/mL of each fraction. A 100 µL (0.1mL) of fresh bacterial suspension adjusted with (0.5 McFarland) was added to each well. Positive control (bacterial suspension in broth) and negative control (fraction in broth) were included. Following incubation at 37^{o}C for 24 hours, the content of each was gently removed by tapping the plates. The wells were washed with 200 µL of sterile distilled water to remove free floating bacteria. Biofilms formed by adherent cells in plate were stained with 0.1% crystal violet and incubated at room temperature for 30 minutes. Excess stain was rinsed off by thoroughly washing with distilled water and plates were fixed with 200 µL of 70% ethanol. Optical densities (OD_{600}) of stained adherent bacteria were measured using ELISA microplate reader (Sunrise $^{TM-}$TECAN, Switzerland).

RESULTS

Physical appearance and percentage yield of extract and fractions of *A. conyzoides* leaf

The nature and percentage yield of extract and fractions of *A. conyzoides* leaf is presented in Table 1. It revealed that n-hexane yielded more fractions (24.42%) than other solvents. The physical appearance of n-hexane fraction was dark brown in colour and powdery in form; while ethyl acetate was dark brown and sticky in nature. The crude methanol extract appeared light brown and solid hard with a percentage yield of 10%. The percentage yield of dichloromethane was 16.75% and was dark brown in colour and was a solid powder. The least percentage yield (12%) was obtained in the aqueous fraction and appeared light brown and powdery in colour and form, respectively.

Table 1: Nature and percentage yield of extract and fractions of the leaf of *A. conyzoides.*

Solvents	Colour/texture of extract/fraction	Weight of plant material/ extract used (g)	Extract/fraction yield (g)	Percentage yield (%)
Methanol	Light brown/solid hard	500.00	50.00	10.00
Dichloromethane	Dark brown/solid powder	12.00	2.01	16.75
n-hexane	Brownish/hard solid	12.00	2.93	24.42
Ethyl acetate	Dark brown/sticky powder	12.00	1.97	16.42
Aqueous	Light brown/powder	12.00	1.45	12.08

Preliminary screening of fractionated methanol leaf extract of *A. conyzoides*

The presence of some classes of phytochemicals such as saponins, tannins, flavonoids, alkaloids triterpenes/ steroids, cardiac glycosides and free anthraquinone was detected in methanol extract of *A. conyzoides* leaf (Table 2).

Table 2: Preliminary screening of methanol extract of *A. conyzoides* leaf.

S/N	Metabolite/Test	Methanol Extract
1.	Saponins (Frothing test)	+
2.	Tannins (5% $FeCl_3$ test)	+
3.	Flavonoids (Shinoda's reduction test)	+
4.	Alkaloids (Dragendorff's test)	+
5.	Triterpenes/Steroids (Lieberman's test)	+
6.	Cardiac glycoside (Salkowski test)	+
7.	Free anthraquinone (Borntrager's test)	+

- = Not present; + = presence

Gas chromatography-Mass spectrometric of the fractions of *A. conyzoides* leaf

Figure 1 reveals the total ion chromatogram (TIC) of n-hexane fraction of *A. conyzoides*. The active principles with their Retention Time (RT), Molecular Formula, Molecular Weight (MW), and Peak Area in percentage (quantity) and identified compound are presented in Table 3. The result revealed the existence of Benzenedicarboxylic acid (29.82%), 9-Octadecanamide (9.10%), 1-Octadecano (4.80%), Isopropyl palmitate (4.55%), and Benzofuran (4.15%), etc. A total of 50 phytochemical constituents were identified and quantified from the GC-MS analysis.

The total Ion Chromatogram (TIC) of dichloromethane fraction of *A. conyzoides* is shown in Figure 2. According to the result shown in Table 4, a total of 50 compounds were revealed in the fraction. The first five compounds in terms of quantity are 9-Octadecanamide (14.19%), Phytol (7.25%), Inden-1-one (6.68%), Acetic acid (6.60%) and Chromene (5.95%).

The spectral characteristics of ethyl acetate fraction of *A. conyzoides* are presented in Figure 3 and Table 5. The result indicates that a total of 54 phyto-compounds were quantitatively identified. A select few of the phyto-constituents include 9-Octadecanamide (7.03%), Benzenedicarboxylic acid (6.76%), Phenol (4.42%), n-Hexadecanoic acid (4.39%) and Verrucarol (3.36%).

Figure 4 presents the Total Ion Chromatogram (TIC) of aqueous fraction of *A. conyzoides*. The phyto-characteristics revealed the presence of 41 compounds including their quantities expressed as peak area percentage (Table 6). A few of the phytochemicals include 1,6-Dideoxyl-1-mannitol (27.68%), Butan-2-one isopropryl (8.05%), 1,4-Cyclohexane-diol (4.49%), 9-Octadecanamide (3.29%) and Vitamin E (3.92%).

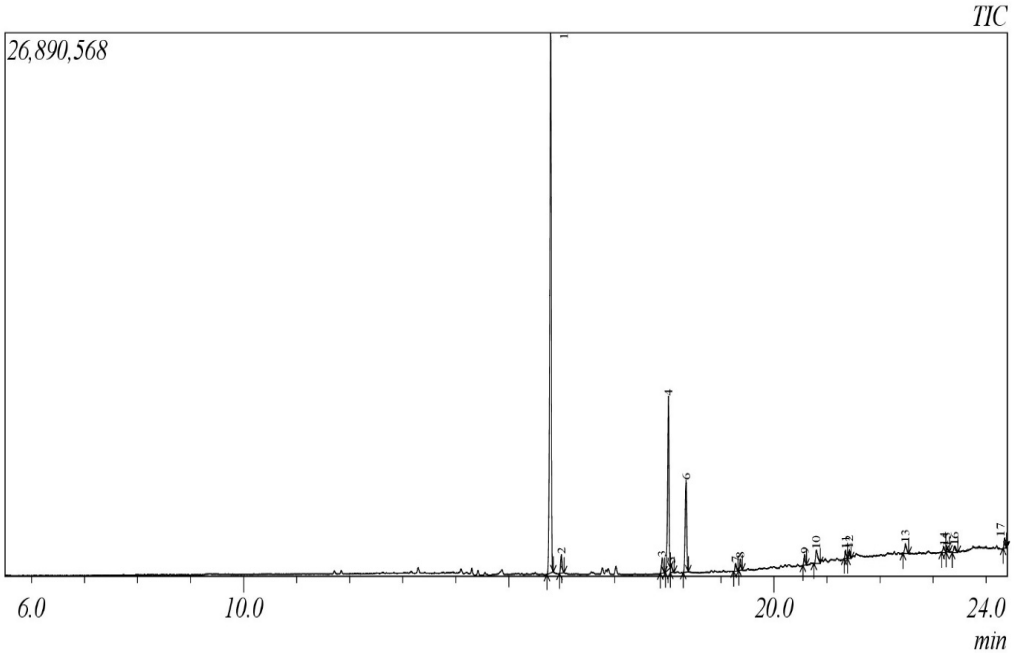

Figure 1: Total Ion Chromatogram (TIC) of n-hexane fraction of *A. conyzoides*.

Table 3: Phyto-components generated in the n-hexane fraction of *A. conyzoides* by GC-MS.

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	8.605	1.86	C_8H_8O	120	86	Benzofuran, 2,3-dihydro-
2	9.303	0.20	$C_{13}H_{20}O$	192	82	2(1H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-1,1,4a
3	9.524	1.07	$C_9H_{10}O2$	150	94	2-Methoxy-4-vinylphenol
4	10.924	0.23	$C_{10}H_{10}O_4$	194	90	Dimethyl phthalate
5	11.717	0.68	$C_{14}H_{22}O$	206	95	Phenol, 2,4-bis(1,1-dimethylethyl)-
6	12.323	0.66	$C_{12}H_{24}O_2$	200	94	Dodecanoic acid
7	12.655	0.36	$C_{16}H_{32}$	224	93	Cetene
8	12.927	0.69	$C_{12}H_{10}O_2$	186	83	1,4-Naphthalenedione, 2-ethyl-
9	13.367	0.64	$C_8H_{14}O_3$	158	72	1-(1-Hydroxy-1-methyl-ethyl)-cyclobutanecarboxylic
10	13.975	0.37	$C_{15}H_{20}O$	216	67	Octanal, 2-(phenylmethylene)-
11	14.075	0.45	$C_{13}H_{12}N_2O$	212	59	Benzoic acid, 2-phenylhydrazide
12	14.184	0.74	$C_{14}H_{28}O_2$	228	91	Tetradecanoic acid
13	14.360	1.74	$C_{12}H_{21}N$	179	78	2,3-Bis(1-methylallyl)pyrrolidine
14	14.526	2.09	$C_{19}H_{38}$	266	97	1-Nonadecene
15	14.606	0.75	$C_{16}H_{34}$	226	86	Hexadecane
16	14.715	2.00	$C_{17}H_{34}O_2$	270	88	Isopropyl myristate
17	14.939	5.36	$C_{22}H_{42}O_2$	338	89	Phytol, acetate
18	15.137	2.68	$C_{20}H_{40}O$	296	88	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
19	15.199	4.06	$C_{16}H_{34}O$	242	97	1-Hexadecanol
20	15.301	2.32	$C_{20}H_{40}O$	296	89	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
21	15.400	3.38	$C_{17}H_{24}O_3$	276	63	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-d
22	15.558	3.59	$C_{17}H_{34}O_2$	270	94	Hexadecanoic acid, methyl ester
23	15.717	9.82	$C_{20}H_{30}O_4$	334	94	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester
24	15.923	3.96	$C_{16}H_{32}O_2$	256	86	n-Hexadecanoic acid
25	15.983	4.15	$C_{15}H_{10}N_4O_2S$	310	69	1H-Benzofuro[3,2-e]indole, 1-[2-(aminocarbonothio
26	16.079	4.11	$C_{13}H_{18}O$	190	69	Phenol, 2-(1,1-dimethyl-2-propenyl)-3,6-dimethyl-
27	16.228	5.87	$C_{19}H_{38}$	266	97	1-Nonadecene
28	16.300	1.92	$C_{21}H_{44}$	296	93	Heneicosane
29	16.396	4.55	$C_{19}H_{38}O_2$	298	93	Isopropyl palmitate
30	16.918	4.83	$C_{18}H_{38}O$	270	96	1-Octadecanol
31	17.033	1.72	$C_{19}H_{36}O_2$	296	90	11-Octadecenoic acid, methyl ester
32	17.175	1.59	$C_{13}H_{28}$	184	68	Tridecane
33	17.292	0.36	$C_{19}H_{38}O_2$	298	88	Methyl stearate
34	17.432	0.56	$C_{18}H_{34}O_2$	282	87	cis-Vaccenic acid
35	17.715	1.08	$C_{16}H_{33}NO$	255	93	Hexadecanamide
36	18.098	1.50	$C_{19}H_{38}$	266	76	1-Nonadecene
37	19.436	0.72	$C_{10}H_{16}O$	152	75	cis-Verbenol
38	19.523	9.10	$C_{18}H_{35}NO$	281	94	9-Octadecenamide, (Z)-
39	19.757	1.38	$C_{18}H_{37}NO$	283	93	Octadecanamide
40	20.067	0.81	$C_{28}H_{58}O$	410	96	Octacosanol
41	20.135	0.70	$C_{18}H_{26}O$	258	71	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohex
42	20.218	0.53	$C_{15}H_{26}O$	222	80	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahyd
43	20.548	0.67	$C_{15}H_{24}O$	220	74	1H-3a,7-Methanoazulen-5-ol, octahydro-3,8,8-trime

44	20.817	0.77	$C_7H_{12}O_5$	176	65	Dimethyl 2-hydroxy-2-methylbutane-1,4-dioate
45	20.951	0.77	$C_{18}H_{36}O_2$	284	83	Decanoic acid, 2-ethylhexyl ester
46	21.136	0.77	$C_{24}H_{38}O_4$	390	94	Bis(2-ethylhexyl) phthalate
47	21.782	0.53	$C_{28}H_{58}O$	410	94	Octacosanol
48	22.551	0.47	$C_{18}H_{36}O_2$	284	82	Decanoic acid, 2-ethylhexyl ester
49	23.273	0.38	$C_{28}H_{58}O$	410	88	Octacosanol
50	23.946	0.48	$C_{30}H_{60}O_2$	452	73	Hexadecanoic acid, tetradecyl ester

SI = March Factor Based on Library

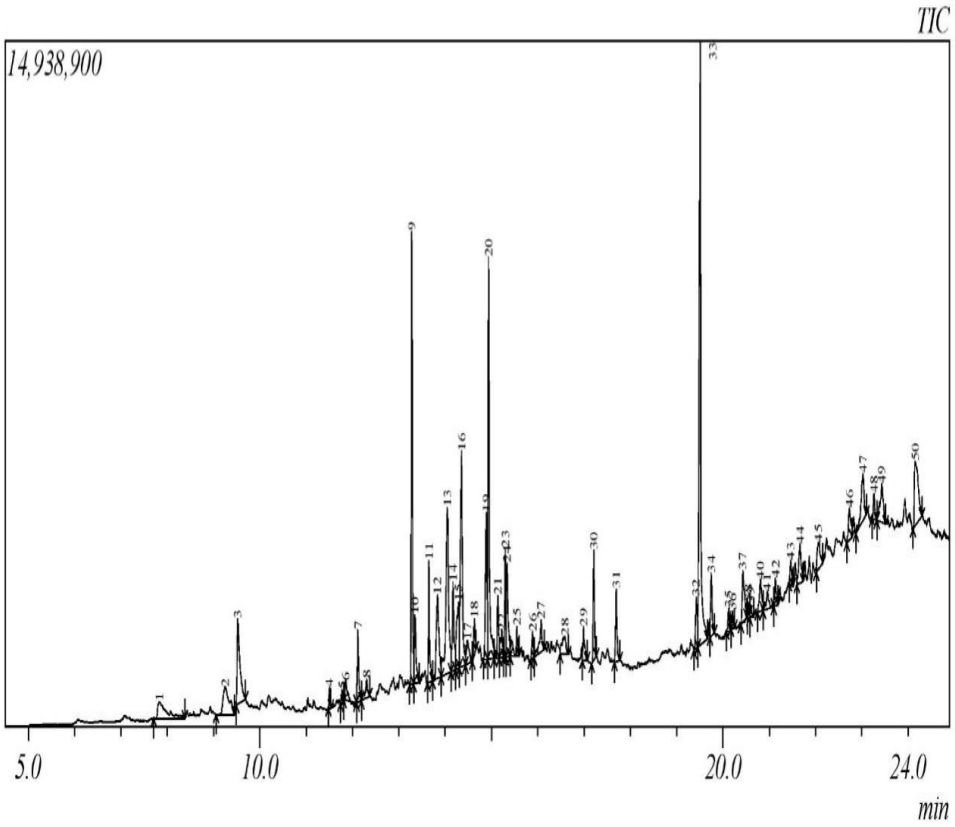

Figure 2: Total Ion Chromatogram (TIC) of dichloromethane fraction of *A. conyzoides.*

Table 4: Phytoconstituents of the dichloromethane fraction of *A. conyzoides* by GC-MS.

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	7.828	2.15	C_8H_8O	120	91	Benzofuran, 2,3-dihydro-
2	9.253	2.37	$C_6H_{14}O_4$	150	76	1,6-Dideoxy-l-mannitol
3	9.526	3.25	$C_9H_{10}O2$	150	94	2-Methoxy-4-vinylphenol
4	11.506	0.30	$C_{11}H_{14}O_3$	194	77	Bicyclo[4.4.0]dec-5-en-4-one-1-carboxylic acid
5	11.778	0.24	$C_{14}H_{25}ClO_4$	292	68	Diethylmalonic acid, monochloride, 5-methoxy-3-methy
6	11.85	0.51	$C_{11}H_{12}O_2$	176	84	2,2'-Isopropylidenedifuran
7	12.119	1.41	$C_9H_{10}N_4O_2$	206	74	1H-Tetrazole, 5-(3,4-dimethoxyphenyl)-
8	12.308	0.66	$C_{12}H_{18}O_2$	194	69	13-Oxadispiro[5.0.5.1]tridecan-1-one
9	13.286	6.68	$C_{15}H_{20}O$	216	88	1H-Inden-1-one, 7-(1,1-dimethylethyl)-2,3-dihydro-3,3-
10	13.353	1.62	$C_{13}H_{20}O_3$	224	79	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicy
11	13.653	1.95	$C_{14}H_{16}O_4$	248	77	Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-

12	13.838	3.06	$C_{13}H_{20}O_3$	224	72	Ppropiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcycl
13	14.052	6.60	$C_{14}H_{22}O_3$	238	73	Acetic acid, 2-(2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept
14	14.175	2.00	$C_{10}H_{14}OS_2$	214	76	Benzene, 1-[bis(methylthio)methyl]-4-methoxy-
15	14.291	2.26	$C_{13}H_{18}O_3$	222	69	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-ox
16	14.358	5.95	$C_{12}H_{20}O$	180	82	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene
17	14.483	0.83	$C_9H_{13}NO_3$	183	67	Normetadrenaline
18	14.634	0.66	$C_9H_{13}NO_3$	183	73	Normetadrenaline
19	14.891	3.15	$C_7H_{10}N_2O_3$	170	74	Propionic acid, 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyra
20	14.944	7.25	$C_{22}H_{42}O_2$	338	91	Phytol, acetate
21	15.142	1.27	$C_{20}H_{40}O$	296	91	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
22	15.233	0.83	$C_{15}H_{26}O_2Si$	266	55	Silane, dimethyl(2-isopropylphenoxy)butoxy-
23	15.302	1.62	$C_{20}H_{40}O$	296	91	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
24	15.339	2.27	$C_{17}H_{22}O_2$	258	61	10-Benzyloxytricyclo[4.4.0.0(3,8)]decan-4-ol
25	15.554	0.93	$C_{17}H_{34}O_2$	270	88	Hexadecanoic acid, methyl ester
26	15.89	0.54	$C_{16}H_{32}O_2$	256	88	n-Hexadecanoic acid
27	16.076	1.17	$C_{13}H_{18}O$	190	59	Benzene, 1-[1,1-dimethylethyl]-4-[2-propenyloxy]-
28	16.592	1.02	$C_{12}H_{14}O_2$	190	58	2,3-2H-Benzofuran-2-one, 3,3,4,6-tetramethyl-
29	16.992	0.71	$C_{19}H_{32}O_2$	292	85	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
30	17.215	1.86	$C_{20}H_{40}O$	296	96	Phytol
31	17.697	1.48	$C_{14}H_{29}NO$	227	92	Tetradecanamide
32	19.429	1.22	$C_{18}H_{31}ClO$	298	80	9,12-Octadecadienoyl chloride, (Z,Z)-
33	19.514	14.19	$C_{18}H_{35}NO$	281	94	9-Octadecenamide, (Z)-
34	19.751	1.23	$C_{18}H_{37}NO$	283	94	Octadecanamide
35	20.126	0.40	$C_{21}H_{30}N_4$	338	69	Diazoprogesterone
36	20.209	0.33	$C_{15}H_{26}O$	222	71	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-
37	20.434	1.60	$C_{15}H_{22}O_2$	234	69	Glaucic acid
38	20.545	0.19	$C_{15}H_{24}O$	220	64	1H-3a,7-Methanoazulen-5-ol, octahydro-3,8,8-trimethyl-6-methy
39	20.601	0.25	$C_{15}H_{26}O$	222	61	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-
40	20.813	1.00	$C_{25}H_{42}$	342	63	1H-Indene, 1-hexadecyl-2,3-dihydro-
41	20.958	0.74	$C_{15}H_{20}O_6$	296	50	Deoxynivalenol
42	21.135	0.58	$C_{24}H_{38}O_4$	390	82	Bis(2-ethylhexyl) phthalate
43	21.467	0.50	$C_{15}H_{22}O_4$	266	72	Verrucarol
44	21.665	1.33	$C_{12}H_{14}O_3$	206	77	Phenol, 2-methoxy-4-(1-propenyl)-, acetate
45	22.061	1.14	$C_{29}H_{48}O$	412	85	Stigmasterol
46	22.733	1.07	$C_{13}H_{20}O_2$	208	75	1-(2-Methoxymethyl-3,5,6-trimethylphenyl)ethanol
47	23.026	1.98	$C_{29}H_{50}O$	414	85	.beta.-Sitosterol
48	23.263	0.59	$C_{15}H_{26}O$	222	79	1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1
49	23.435	1.66	$C_{33}H_{54}O_6$	546	59	Cholestan-3,22,26-triol triacetate
50	24.154	3.41	$C_{17}H_{14}O_5$	298	79	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-(4-methoxyph

SI = March factor based on library

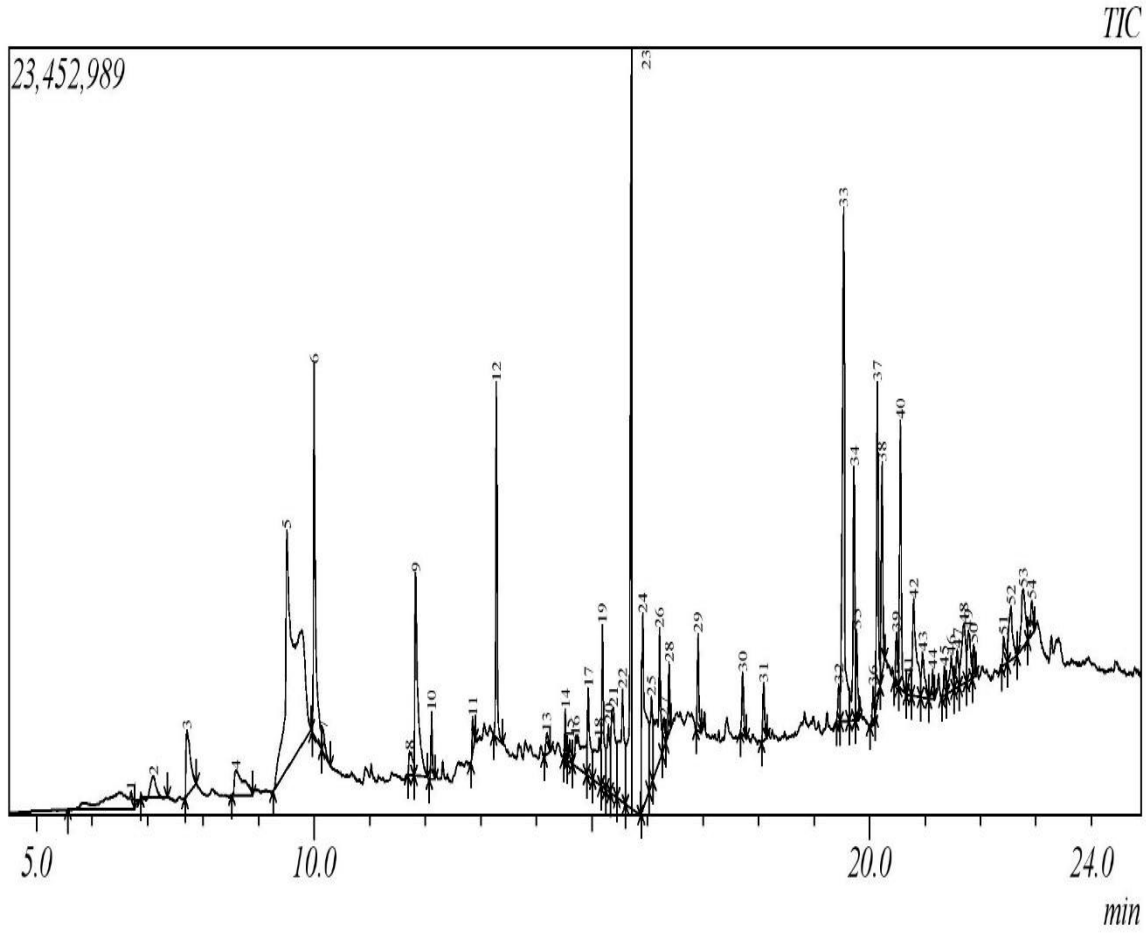

Figure 3: Total Ion Chromatogram (TIC) of ethyl acetate fraction of *A. conyzoides.*

Table 5: Phytoconstituents of the ethyl acetate fraction of *A. conyzoides* by GC-MS.

Peak	Retention Time/min	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	6.705	2.85	$C_{10}H_{18}O_2$	170	82	alpha-Methyl-alpha-[4-methyl-3-pentenyl]oxirane
2	7.103	0.59	$C_6H_{12}O_2$	116	93	1,4-Cyclohexanediol
3	7.71	1.71	C_8H_8O	120	94	Benzofuran, 2,3-dihydro-
4	8.593	1.41	C_8H_8O	120	90	Benzofuran, 2,3-dihydro-
5	9.511	15.52	$C_9H_{10}O_2$	150	80	2-Methoxy-4-vinylphenol
6	10.002	4.42	$C_{10}H_{12}O_2$	164	96	Phenol, 2-methoxy-3-(2-propenyl)-
7	10.167	0.39	$C_9H_8O_2$	148	88	Hydrocoumarin
8	11.717	0.53	$C_{14}H_{22}O$	206	90	Phenol, 2,4-bis(1,1-dimethylethyl)-
9	11.829	3.68	$C_{11}H_{12}O_2$	176	89	2,2'-Isopropylidenedifuran
10	12.116	0.57	$C_9H_{10}N_4O_2$	206	74	1H-Tetrazole, 5-(3,4-dimethoxyphenyl)-
11	12.858	0.34	$C_{13}H_{18}O_2$	206	73	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-oct
12	13.285	2.71	$C_{15}H_{20}O$	216	89	1H-Inden-1-one, 7-(1,1-dimethylethyl)-2,3-dihydro-
13	14.194	0.30	$C_{14}H_{28}O_2$	228	82	Tetradecanoic acid
14	14.524	0.34	$C_{22}H_{44}$	308	95	1-Docosene
15	14.603	0.31	$C_{15}H_{24}O$	220	74	Farnesene epoxide, E-
16	14.708	1.74	$C_{17}H_{34}O_2$	270	75	Isopropyl myristate
17	14.937	1.40	$C_{22}H_{42}O_2$	338	89	Phytol, acetate
18	15.133	1.47	$C_{20}H_{40}O$	296	81	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
19	15.196	1.90	$C_{16}H_{34}O$	242	96	1-Hexadecanol

20	15.3	1.23	$C_{20}H_{40}O$	296	89	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
21	15.388	2,32	$C_{13}H_{20}O$	192	64	Bicyclo[3.3.0]octan-2-one, 7-neopentylidene-
22	15.557	3.11	$C_{17}H_{34}O_2$	270	95	Hexadecanoic acid, methyl ester
23	15.717	6.76	$C_{20}H_{30}O_4$	334	93	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl est
24	15.921	4.39	$C_{16}H_{32}O_2$	256	88	n-Hexadecanoic acid
25	16.077	1.40	$C_{13}H_{18}O$	190	65	Phenol, 2-(1,1-dimethyl-2-propenyl)-3,6-dimethyl-
26	16.224	2.75	$C_{19}H_{38}$	266	97	1-Nonadecene
27	16.3	0.41	$C_{28}H_{58}$	394	87	Octacosane
28	16.394	0.58	$C_{19}H_{38}O_2$	298	92	Isopropyl palmitate
29	16.915	0.87	$C_{18}H_{38}O$	270	96	1-Octadecanol
30	17.719	0.66	$C_{16}H_{33}NO$	255	94	Hexadecanamide
31	18.097	0.62	$C_{21}H_{44}O$	312	95	1-Heneicosanol
32	19.45	0.43	$C_{17}H_{30}O_2$	266	80	7,10-Hexadecadienoic acid, methyl ester
33	19.539	7.03	$C_{18}H_{35}NO$	281	94	9-Octadecenamide, (Z)-
34	19.726	3.03	$C_{11}H_{17}BrO$	244	81	2-Adamantanol, 2-(bromomethyl)-
35	19.767	0.93	$C_{18}H_{37}NO$	283	92	Octadecanamide
36	20.071	0.39	$C_{28}H_{58}O$	410	92	Octacosanol
37	20.148	3.36	$C_{15}H_{22}O_4$	266	72	Verrucarol
38	20.23	2.07	$C15H_{26}O$	222	82	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro
39	20.49	0.58	$C_{23}H_{38}O_3$	362	72	17-Oxo-6.beta.-pentyl-4-nor-3,5-secoandrostan-3-oic a
40	20.56	2.75	$C_{15}H_{24}O$	220	74	Shyobunone
41	20.708	0.39	$C_{19}H_{28}O$	272	58	Androst-5,16-diene-3.beta.-ol
42	20.798	2.24	$C_{21}H_{32}O_4$	348	73	5.beta.-Pregnan-17.alpha.,21-diol-3,20-dione
43	20.96	0.87	$C_{15}H_{27}ClO_3$	290	71	Diethylmalonic acid, monochloride, 4-octyl ester
44	21.141	0.31	$C_{24}H_{38}O_4$	390	92	Bis(2-ethylhexyl) phthalate
45	21.354	0.33	$C_{15}H_{26}$	206	76	2,4a,8,8-Tetramethyldecahydrocyclopropa[d]naphthale
46	21.476	0.70	$C_{22}H_{32}O_2$	328	76	Retinol, acetate
47	21.583	0.61	$C_{20}H_{32}$	272	71	Naphthalene, 1,2,3,4-tetrahydro-2,6-dimethyl-7-octyl-
48	21.701	1.55	$C_{12}H_{14}O_3$	206	77	Phenol, 2-methoxy-4-(1-propenyl)-, acetate
49	21.785	0.96	$C_{18}H_{35}BrO_2$	362	77	Bromoacetic acid, hexadecyl ester
50	21.88	0.34	$C_{15}H_{26}O$	222	77	1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trim
51	22.417	0.45	$C_{15}H_{26}O$	222	75	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro
52	22.55	1.27	$C_{15}H_{24}O$	220	62	Tricyclo[6.3.0.0(5,7)]undecane, 1,8-epoxy-2,6,6,9-tetr
53	22.769	1.50	$C_{12}H_{16}O$	176	82	Benzeneethanal, 4-[1,1-dimethylethyl]-
54	22.925	0.64	$C_{14}H_{24}O$	208	72	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1

SI = March factor based on library

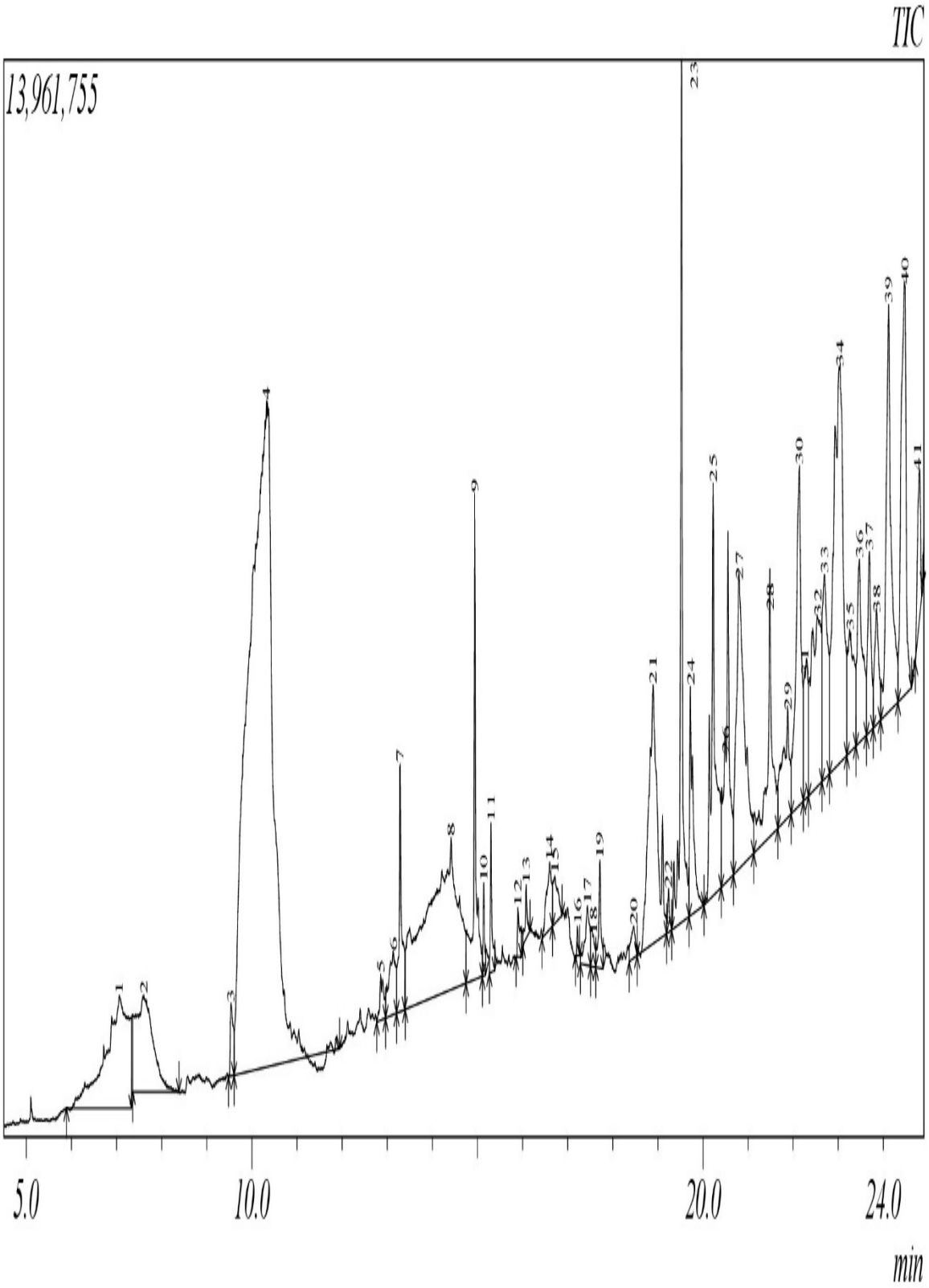

Figure 4: Total Ion Chromatogram (TIC) of aqueous fraction of *A. conyzoides.*

Table 6: Phytoconstituents of the aqueous fraction of *A. conyzoides* by GC-MS.

Peak	Retention Time/min	Peak Area %	Molecular Formula	Molecular Weight	SI	Name
1	7.063	4.49	$C_6H_{12}O_2$	116	80	1,4-Cyclohexanediol
2	7.608	2.84	$C_3H_8O_3$	92	81	Glycerin
3	9.540	0.37	$C_9H_{10}O_2$	150	93	2-Methoxy-4-vinylphenol
4	10.325	27.68	$C_6H_{14}O_4$	150	77	1,6-Dideoxy-l-mannitol
5	12.859	0.30	$C_{27}H_{48}O_3$	420	43	Cholestane-3,6,7-triol, (3.beta.,5.alpha.,6.beta.,7.beta.)-
6	13.135	0.73	$C_{23}H_{52}O_3Si_2$	432	36	Silane, dimethyl(dimethylpentyloxysilyloxy)tetradecylox
7	13.288	1.08	$C_{15}H_{20}O$	216	88	1H-Inden-1-one, 7-(1,1-dimethylethyl)-2,3-dihydro-3,3-
8	14.415	8.05	$C_{10}H_{17}NO$	167	64	Butan-2-one, 3-(2-ethynyl)(isopropyl)amino-
9	14.943	1.86	$C_{22}H_{42}O_2$	338	81	Phytol, acetate
10	15.142	0.22	$C_{20}H_{40}O$	296	92	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
11	15.302	0.40	$C_{20}H_{40}O$	296	93	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	15.902	0.21	$C_{16}H_{32}O_2$	256	89	n-Hexadecanoic acid
13	16.082	0.19	$C_{13}H_{18}O$	190	64	2,3,4,5,6-Pentamethyl acetophenone
14	16.604	0.58	$C_{18}H_{30}$	246	54	Spiro[2.7]dec-4-ene, 1,1,5,6,6,9,9-heptamethyl-10-meth
15	16.717	0.39	$C_{18}H_{36}N_2O_6Si_2$	432	39	Bis(trimethylsilyl) succinylacetoacetate diethoxime
16	17.218	0.06	$C_{20}H_{40}O$	296	88	Phytol
17	17.439	0.46	$C_{18}H_{32}O_2$	280	84	17-Octadecynoic acid
18	17.575	0.22	$C_7H_{15}NO_4Si$	205	53	2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undecane, 1-meth
19	17.714	0.45	$C_{14}H_{29}NO$	227	92	Tetradecanamide
20	18.462	0.25	$C_{25}H_{37}O_3P$	416	47	Butylphosphonic acid, hexyl 4-(2-phenylprop-2-yl)pheny
21	18.894	3.92	$C_{29}H_{50}O_2$	430	85	Vitamin E
22	19.242	0.15	$C_{23}H_{32}O_6$	404	69	Pregnan-17,21-diol-9,11-epoxy-3,20-dione, acetate
23	19.529	3.29	$C_{18}H_{35}NO$	281	94	9-Octadecenamide, (Z)-
24	19.727	1.33	$C_{10}H_{15}BrO$	230	80	Bicyclo[2.2.1]heptan-2-one, 1-(bromomethyl)-7,7-dimet
25	20.229	2.85	$C_{15}H_{26}O$	222	81	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1
26	20.508	2.23	$C_{19}H_{32}N_2O_3$	336	74	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,
27	20.807	4.11	$C_{21}H_{32}O_4$	348	69	5.beta.-Pregnan-17.alpha.,21-diol-3,20-dione
28	21.489	2.14	$C_{22}H_{32}O_2$	328	81	Retinol, acetate
29	21.881	1.31	$C_{25}H_{38}O_5$	418	69	3-Formoxy-12-ketocholanic acid
30	22.138	3.03	$C_{29}H_{48}O$	412	88	Stigmasterol
31	22.264	0.98	$C_{15}H_{22}O_2$	234	55	Methyl 4,6-tetradecadiynoate
32	22.545	2.97	$C_{15}H_{24}O_2$	236	66	Murolan-3,9(11)-diene-10-peroxy
33	22.692	1.80	$C_{19}H_{23}S_3O_3S$	388	56	2-(5,7-Di-tert-butyl-benzo[1,3]oxathiol-2-ylidene)-3
34	23.035	6.40	$C_{25}H_{34}O_7$	446	62	(22R)-6.alpha.,11.beta.,21-Trihydroxy-16.alpha.,17.
35	23.259	1.33	$C_{31}H_{46}O_2$	450	66	Phytonadione
36	23.468	1.65	$C_{30}H_{50}O$	426	86	.alpha.-Amyrin
37	23.692	1.04	$C_{17}H_{26}O_3$	278	80	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl

38	23.855	0.82	$C_{29}H_{50}O$	414	73	Cholestan-3-one, 4,4-dimethyl-, (5.alpha.)-
39	24.120	3.54	$C_{27}H_{48}O$	388	61	Cholestan-3-ol, (3.beta.,5.beta.)-
40	24.474	3.50	$C_{26}H_{20}O_8$	460	55	4-Acetoxy-6',7-dimethyl-5',8'-dimethoxy-1,2'-binaph
41	24.804	0.80	$C_{21}H_{22}O_8$	402	63	4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3

SI = March factor based on library

Susceptibility of clinical *Streptococcus pneumoniae* to antibiotics

Table 7 presents the susceptibility of *Streptococcus pneumoniae* isolate to antibiotics. Results indicate that the organism was resistant to seven (7) antibiotics out of the 15 drugs tested. The isolate was resistant to ampicillin (10 mm), amoxillin (11 mm), cloxacillin (13 mm), gentamycin (14 mm), erythromycin (9 mm) and amikacin (12 mm). However, varying susceptibilities were observed against the other antibiotics. More importantly, it was established that the isolate was multidrug-resistant.

Table 7: Antibiotic susceptibility of clinical *S. pneumoniae* isolate.

S/N	Antibiotic agents	Zones of clearance (mm)
1.	Ampicillin	10 (R)
2.	Amoxillin	11 (R)
3.	Cloxacillin	13 (R)
4.	Oxacillin	19 (S)
5.	Augmentin	25 (S)
6.	Gentamycin	14 (R)
7.	Streptomycin	15 (I)
8.	Amikacin	12 (R)
9.	Chloramphenicol	26 (S)
10.	Ciprofloxacin	19 (S)
11.	Levofloxacin	24 (S)
12.	Erythromycin	9 (S)
13.	Ceftazidine	19 (S)
14.	Norfloxacin	11 (R
15.	Septrin	14 (I)
Percentage resistance of *S. pneumoniae* to tested drugs is 46.66%		

R = resistance, S = sensitive, I = intermediate

Antibacterial activity of the fractions of leaf extract of *A. conyzoides*

Table 8 shows the antibacterial activities of the fractions of methanol leaf extract of *A. conyzoides*. The result indicates that the various fractions had antibacterial activities at various concentrations demonstrated by the zones of inhibition. The fraction obtained from n-hexane inhibited the growth of *S. pneumoniae* with 23.5 mm zone of inhibition (as the highest amongst the fractions) at 100 mg/mL concentration. Dichloromethane fraction demonstrated antibacterial activity against test isolate with a variety of zone sizes at different concentrations. The fraction produced a zone size of 17.5 mm against *S. pneumoniae* at 100 mg/mL concentration. Ethyl acetate fraction exhibited antibacterial activity with the zone of 19.5 mm against the organism at 100 mg/mL concentration. Aqueous fraction produced the least activity in relation to the performance of other fractions. The fraction inhibited the growth of *S. pneumoniae* with 15.5 mm zone size at the highest concentration (100 mg/mL). Generally, it was noted that zones of clearance reduced with decrease in concentration of all fractions.

Table 8: Antibacterial activity of the fractions of methanol leaf extract of *A. conyzoides.*

		Concentrations (mg/mL)/ zone of inhibition (mm)					Controls	
Plant fractions	**Isolate**	**100**	**50**	**25**	**12.5**	**6.25**	**CPC**	**DMSO**
n-hexane	*S. pneumoniae*	23.5±0.5	20.5±0.7	17.0±0.0	15.5±0.5	12±0.0	26 ±0.0	0
Dichloromethane	*S. pneumoniae*	17.5±0.5	15.5±0.7	12.0±0.0	9±0.5	0±0.0	25±0.0	0
Ethy lacetate	*S. pneumoniae*	19±0.5	17.5±0.7	15±0.0	13±0.5	10±0.0	24±0.0	0
Aqueous	*S. pneumoniae*	15.5±0.5	13.5±0.7	11.0±0.0	9±0.5	0±0.0	25±0.0	0

Values are mean of three replicates DMSO – dimethyl sulphuroxide; CPC – chloramphenicol; ± - mean standard deviation; mg/mL – milligram per millimetre; mm – millimetre.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of fractions of *A. conyzoides* against test organism

The obtained results showed that MICs and MBCs against tested MDR-bacterial isolate were concentration-dependent in all the fractions (Table 9). It was observed that cells of *S. pneumoniae* were inhibited and killed at 12.5mg/mL (MIC) and 25mg/mL (MBC), respectively by n-hexane fraction. DCM fraction had 12.5mg/mL as MIC and 50.0mg/mL as MBC. Ethyl acetate fraction demonstrated the same MIC and MBC (12.5mg/mL and 25.0mg/mL) as n-hexane fraction. Aqueous fraction of *A. conyzoides* could be said to be bacteriostatic rather than bactericidal as there was no MBCs recorded against the test organism. Also, there was high MIC (50mg/mL) recorded against *S. pneumoniae*.

Table 9: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of fractions of *A. conyzoides* on clinical bacterial isolate.

Plant fraction	Isolate	MIC (mg/mL)	MBC (mg/mL)
n-hexane	*S. pneumoniae*	12.5	25.0
Dichloromethane	*S. pneumoniae*	12.5	50.0
Ethyle acetate	*S. pneumoniae*	12.5	25.0
Aqueous	*S. pneumoniae*	50.0	NIL

Antibiofilm activity of the fractions of *A. conyzoides* against biofilm formation by *S. pneumoniae*

Table 10 presents the optical densities (ODs) recorded, which indicate antibiofilm formation by different fractions of the plants against test isolates at various concentrations. The results clearly show that effect was concentration-dependent. The best biofilm reduction is observed in higher concentrations of fractions (25 mg/mL, 50 mg/mL and 100 mg/mL) of the plant.

Table 10: Antibiofilm activity of the fractions of *A. conyzoides* against biofilm formation by clinical bacterial isolates.

		Optical Density (OD_{600}nm) / (Concentration in mg/mL)					Controls	
Plant fractions	**Isolate**	**100**	**50**	**25**	**12.5**	**6.25**	**+**	**-**
n-hexane	*S. pneumoniae*	0.012	0.039	0.297	0.417	0.532	1.623	0.000
Dichloromethane	*S. pneumoniae*	0.011	0.038	0.396	0.417	0.532	1.627	0.000
Ethyl acetate	*S. pneumoniae*	0.013	0.038	0.396	0.417	0.532	1.623	0.000
Aqueous	*S. pneumoniae*	0.010	0.035	0.096	0.317	0.532	1.623	0.000

+ = Positive control (bacterial suspension in broth); - = negative control (fraction in broth); nm = nanometre

DISCUSSION

This study was aimed at investigating new antibacterial compounds of *A. conyzoides* based on traditional medicinal use for growth and biofilm inhibitory activity targeting identified multidrug-resistant bacteria isolated from clinical specimens. Results obtained in this study through colonial characteristics, microscopy, biochemical characterization and molecular analysis using 16S rRNA gene sequencing, identified *S. pneumoniae*. This pathogen has been isolated from diverse hospitals worldwide and reported to have also been etiologic agents of community and hospital acquired infections.[48,49,50]

The negative effect by which antibiotics are prescribed by unprofessional personnel in the health-care system occasioned by under dosing, over prescription and outright fake drug racketeering have led to the development of resistance by a variety of pathogens.[51,77] This unpalatable phenomenon is even aggravated when these bacterial pathogens are existing in a biofilm state. Consequently, this has led to high morbidity, mortality and health-care cost.[30,52] This study revealed the presence of multidrug-resistant clinical *S. pneumoniae* after subjecting it to fifteen (15) commonly used antibiotics by disc diffusion method. The test organism had 46.66% percentage drug resistance (i.e., resistance to seven antibiotics in more than two classes of drugs). This finding is consistent with other studies, which have reported the resistance of *S. pneumoniae* to penicillins, aminoglycosides and macrolides.[30,53]

It is interesting to find bioactive compounds of plant extracts, where modern chemotherapy has failed, with activity against MDR strains. In addition, novel anti-infectives that operate through different mechanisms of action, including disruption of membrane function and structure, interruption of DNA and RNA synthesis and function, interference with intermediate metabolism, induction of coagulation constituents and interruption of normal cell communication (quorum sensing) are required.[54] Plants are a good source of natural products for the recovery of bioactive compounds.[55] However, a small number of plants have been investigated for their antimicrobial activity.[67]

In this study, methanol was used as primary extraction solvent. It was selected as an extraction solvent because it is one of the best solvents used for the extraction of

antimicrobial substances.[56,57] Moreover, methanol polarity ensured the extraction of polar and moderately polar and non-polar active compounds from plants against microorganisms like terpenoids, tannins, flavonoids and polyphenols.[58]

The detection of different classes of phytochemicals such as saponins, tannins, flavonoids, alkaloids, triterpenes/steroids, cardiac glycosides and free anthraquinones in the leaf of *A. coznyzoides* collaborates other research reports.[56,59] These secondary metabolites have been reported to have considerable antibacterial activities.[57]

The quantity of active components in crude extracts from medicinal plants may be small or diluted but when fractionated, these components become concentrated and therefore exhibit greater antibacterial activity. Thus, fractions from crude medicinal extracts have great potential as antimicrobial agents and can be used as potential sources for antibacterial formulations in the treatment of infectious diseases caused by microbial pathogens.[60]

The susceptibility of MDR-resistant clinical bacterial isolate to the fractions of *A. conyzoides* using agar well diffusion technique is demonstrated in this investigation. However, the agar well diffusion assay is considered a qualitative technique and is mainly used for selecting extracts with antimicrobial activity, mostly when diameter zones of inhibition are ≥10 mm.[61,62] According to the result obtained in this study, the zones of inhibition ranged from 9 – 23 mm. An inhibition zone of ≤10 mm was chosen as a cut-off point for bacteria resistance to plant fractions. The aqueous fraction was bacteriostatic but not bactericidal while others were both bacteriostatic and bactericidal. N-Hexane is a non-polar solvent which must have easily extracted the lipid (fatty acids) soluble phytochemicals such as essential oils and coumarins and diffusion rates of these phytochemicals within the agar matrix may explain the wider zone of inhibition observed.[60] Also, the higher activity of n-hexane fraction at a concentration as low as 6.25mg/mL is attributed to the presence of compounds such as phytol and benzenedicarboxylic acid.[57]

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results are comparable to those obtained in the agar-well diffusion technique, because the lowest MIC and MBC obtained corresponded with the fractions that elaborated the best antibacterial activity. The non bactericidal activities are represented as zero (table 8). Generally, n-hexane fraction demonstrated better antibacterial activity followed by ethyl acetate fraction. Aqueous fraction showed moderate activity to the test isolate. This was also reported in other empirical studies.[60,63]
Gas chromatography-mass spectrometry (GC-MS) analyses of sub-fractions of methanolic leaf extract of the plant revealed at molecular level enormous presence of diverse phyto-components including phenols (known disinfectants). Similarly, other fractions including ethyl acetate, DCM and aqueous were found active against test organism at various concentrations indicating that both polar and nonpolar fractions were active against test organisms though nonpolar had higher activity.[60,63] The presence of flavonoids, which contain subclass compounds such as taxifolin (flovanol) is abundant in several plants including *A. conyzoides*; it is an important anti-oxidant while isoflavone, known by its anti-inflammatory and anti-oxidant properties, has been shown to interact with animal and human estrogenic receptors.[65] This compound is often mentioned as responsible for wound healing properties.[64] This explains why this plant is used as folklore medicine. Also, the high antibacterial activities observed in the fractions could be explained by the synergism amongst phytochemicals, e.g., between phenolics and flavonoids, and the assertion that saponins weaken the membranes of bacteria thereby enhancing the penetration of other bioactive components. Synergistic effects enhance activity against microorganisms. This suggestion agrees with the findings of.[64] This combinatorial positive interaction is of vital importance in phytomedicine; it helps to overcome difficulties associated with always isolating a single active ingredient, or to enhance the efficacy of apparently low doses of active constituents in herbal products.[66]

Bacterial biofilm remains a global threat to health due to high refractoriness to treatment and the ability to aggravate nosocomial infections. Hence, search for novel efficacious molecules to tackle this problem is a priority.[74] In this study, the activities of the plant's fractions were tested against the biofilms of the test organism. The ability of antibacterial agents to inhibit formation of or destruction of biofilms hold promise for reducing colonization of surfaces and epithelial mucosa by microbes.[6] In this study, all the fractions prevented the formation of biofilm.[67] Inhibition of biofilm formation can be explained by the presence of flavonoids, previously reported as quercetin, kaemferol, naringenin, and apigenin, which are capable of reducing biofilm synthesis because they can suppress the activity of the autoinducer-2 responsible for cell-to-cell communication.[69]

The excellent ability of the plant's fractions to interfere with the initial stage of biofilm formation of the clinical bacterial isolate may be attributed to interference with forces such as Brownian, sedimentation, Lifshift-Van der Waals and electrostatic interactions forces that favour the deposition and adherence of bacteria to surfaces.[70] Also, since certain organic and inorganic molecules and other nutrients are important for cell growth and hence cell adhesion[71], it is possible that the plant's fractions may inhibit the availability of nutrients. The active plants fractions may hold promise for reduction of colonization of surfaces and various epithelial tissues of the body, thereby preventing infections.

CONCLUSION

The capacity of bacterial pathogens to resist antibacterial compounds especially when they are embedded in biofilm increased the interest in the search for new agents that are effective against bacteria in this mode of growth. In this context, many species of plants provide an enormous diversity of phytochemicals with a range of biological effect, namely antibacterial properties against clinically relevant bacteria. Moreover, it is known that phytochemicals act in synergy through different mechanisms from those of synthetic drugs, which make these compounds ideal candidates to reduce infections. Some phytochemicals have also the ability to control biofilms, affecting essential processes for bacterial growth. Our results have shown that the fractions of *A. conyzoides* had remarkable activity on the planktonic and sessile forms of the clinical bacterial isolate investigated. Therefore, this evidence suggest that this plant can be used as alternative treatment measures to conventional antibiotics if properly harnessed.

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