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Preliminary screening of active compounds with the evaluation of the anti-Pseudomonas aeruginosa bacteria activity of ashwagandha (Withania Somnifera) leaves extracted by different types of solvents

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ABSTRACT

Ashwagandha (Withania Somnifera L.) plant is a medicinal plant used to treat various ailments in Iraq by herbalists, the pharmacological evaluation of its leaves despite the rich pharmacological potential of its roots was not done, in this re- search, the phytochemicals from the Ashwagandha (Withania Somnifera L.) leaves were extracted by different types solvents (carbon tetrachloride, chloroform, ethanol and water respectively), presence flavonoids, tannins, saponin, steroids in water and ethanol extracts while the saponin and steroids only were present in chloroform and carbon tetrachloride extracts, the water and ethanolic extracts were found to be good inhibitor or good active against Pseudomonas aeruginosa bacteria, and this activity may be belong to polyphenolic compounds such as flavonoids and tunnies com- pounds, and by comparing the GC/MS analysis chromatogram of extracted sample with NIST database entries, identification compounds by peak value of the unknown compound in chromatogram. In chloroform extract among the 16 phytochemicals identified, 1,2,3,5- Cyclohexanetetrol is the most abundant compound present followed by n-Hexadecanoic acid (Palmitic acid), then Acetic acid, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, 5-Hydrxoy methyl furfural, Caryophyllene oxide, α-Tocopherol-β-D-mannosidic respectively, other compounds revealed are n-Decanoic acid, Formic acid, cis-3-Hexenylpyruvate, 4-Octyl tetradecyl Phthalate ester, etc. In ethanol extract among the 21 phytochemicals identified, O- Butyl hydroxylamine is the most abundant compound present followed by Hexanoic acid ethyl ester, then Oleyl alcohol, trifluoroacetate, Oleic acid, etc.

Keywords: ***Withania somnifera L., Leaves, Pseudomonas aeruginosa, GC/MS, natural***

INTRODUCTION

The herbal medicine had pass used in the past decades is to thrust heed to wild cultivated natural plants and to return to what is known as traditional medicine or herbal medicine (Arab medicine), which was used for previous centuries in Iraq mainly for medicine treatment and health care [1]. Ashwagandha (Withania somnifera L.) plant belongs to the Solanaceae family, it is a wild plant grown in semi-arid mild regions in the south and mid-Iraq [2].

The different parts of this plant contain different active compounds with a high pharmaceutical value [3,4]. Ashwagandha is an anoint medicinal herb with multiple health benefits. The roots crud and extract can reduce anxiety and stress, help fight depression, boost fertility and testosterone in men, and boost brain function [5]. In the Ayurvedic and Greek systems, the leaves of the plant are used for tumor and tubercular glands. Leaves are bitter in taste and used as an anthelmintic [1,2] [6,7], and anticholinesterase activities [8]. The root decoction boiled with milk and ghee is recommended for the treatment of sterility in women. while roots are only used in the treatment of constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, excess spermatorrhoea in men, and loss of muscular energy [4]. Previous research has also shown that it is anti-stress, neuroprotective, anti-arthritic, anti-tumor, analgesic, and anti-inflammatory (maintaining health).[9], and indicate that W. somnifera possesses important bioactive properties which could be attributed to the high amounts of phenolic compounds.[10].

Pseudomonas aeruginosa is a type of germ that can cause infections in humans, mostly in hospital patients, it causes pneumonia [11]. Many Pseudomonades aeruginosa isolates are resistant to a large range of antibiotics and many demonstrate additional resistance to term unsuccessful treatment [12]. Withania somnifera L. has been widely studied for therapeutic purposes and possesses several pharmacological properties, including antioxidative, analgesic, anti-ulcerative, antiepileptic, and antibacterial properties [13,14].

The leaves of Ashwagandha alcoholic extract have the potential to induce senescence-like growth arrest and differentiation in glioma cells, these led to the formulation of a unique combination formula of alcoholic extract components that caused enhanced differentiation of glial cells [15], in other way of Ashwagandha leaf extract, cholinergic properties by provided biochemical and molecular evidence during brain disorders associated with cholinergic dysfunction [16], and quickly reduces Ag+ to Ag0 and enhances synthesis of silver nanoparticles with highly an-ti-microbial activity.[17], and trimethylene glycol is an active sleep-inducing component of Ashwagandha leaves and could potentially be useful for insomnia therapy[18], so, W. somnifera is a rich source of withanolides and other bioactive constituents, which can be used as a safe drug for various chronic diseases due to the minimal side effects in various researches pre-clinical. These researches are interesting and signify that more clinical trials should be conducted to prove the efficacy and other potential therapeutic effects in human settings [19,20.21].

The goal of this research included, Withania somnifera L. leaves extraction, and preliminary screening of the main active compound using different solvents such as carbon tetrachloride, chloroform, ethanol 80%, and water (deionized W.) respectively. Also, the aim included evaluation the of all extracts against Pseudomonas aeruginosa bacteria, the present

work aimed to identify the chemical compounds present in ethanol and chloroform extracts using GC- MS analyses, and this research contributes to the chemotaxonomic characterization of ethanol and chloroform extracts and validates these compounds as a potential source of natural antimicrobial and bacteria.

Materials and Methods

1.1. Plant material

The leaves of the plant Withania somnifera L. were collected from Al-Saidiya District, Baghdad, Iraq. The plant material was collected during April and washed with tap water to remove the debris, dried for two weeks at room temperature in the shade. The dried powder was packed in tightly closed containers and stored for phytochemical and antibacterial studies.

1.2. Leaves Extraction

Leaves powder of Withania somnifera L. 20 g was defatting with carbon tetrachloride (200mL) by Soxhlet apparatus till exhaustion (for 8hs) then the mare was extracted by different solvents including chloroform, ethanol 80%, and water (deionized W.). The carbon tetrachloride, chloroform, and ethanolic extracts were dried by rotary evaporator while water (deionized W.) extract was dried by lyophilized apparatus.[12]

1.3. Chemical Tests

Detection of the main active compounds using chemical substances in general.

1.3.1. Flavonoids Test

Ethanolic potassium hydroxide (2mL) was added to (1mL) of extract of leaves. If flavonoid compounds are present in the extract a result yellow color is detected.

1.3.2. Terpenoids Test.

In a test tube mix (5mL) aqueous leaves extract with (2mL) chloroform, then add carefully (3mL) of concentrated sulphuric acid to the mixture to form a layer. If the terpenoid constituents are present, an interface with a reddish-brown color is found.

1.3.3. Saponin Test.

Powdered (1g) leaves sample was boiled together with (10mL) D.W. and then filtered, in a test tube mixed (5mL) of the filtered sample with (5mL) of D.W. and shacked vigorously to get a steadily permanent fourth. The formation of an emulsion by mixing the persistent forth with olive oil (3 drops) indicates the presence of saponins.

1.3.4. Tannins Test.

In a test tube (0.5g) of powdered plant, the sample is boiled with (20mL) of D.W. and then filtered, added to the filtered sample (0.1%) ferric chloride, if the presence of tannins that show observed brownish-green or a blue-black coloration.

1.3.5. Steroids Test.

In the test tube ethanolic leaves extract add (1mL) chloro- form, (2-3mL) of acetic anhydride, and drops of concentration H2SO4. If the presence of steroids the upper layer in the test tube turns into a red sulphuric acid layer showing yellow with green fluorescence.

After the detection of the main active compounds using chemical substances in general, all extracts were dried under laboratory temperature, and different solvents to investigate of residual of the main active compounds, the solvents were used CCl4. CHCl3, EtOH 80%, and deionized water respectively.

1.4. IR Infrared (IR)

FTIR spectrophotometer is a valuable tool for functional groups identified present in the plant extract. I.R. spectral studies were carried out in the solid state from leaves extracts for solvents (water, ethanol 80%, chloroform, and carbon tetrachloride), as pressed KBr pellets, and using Shimadzu transform (FT-IR) spectrophotometer in the range of 400-4000/cm.

1.5. Mass Spectroscopy

To tell us to identify and separate the individual plant extract components by GC-MS instrument; What are plant extract components composed of? How much of each of the components are present in plant extract of chloroform and ethanolic? The identification of a compound in GC-MS is not only by comparing its retention time to a standard with (GC) only but also by using its mass spectrum, making this a tremendously powerful analytical tool.

1.6. Antibacterial study

The Pseudomonas aeruginosa bacteria used in this study were obtained from Al-Yarmouk Hospital. The antibacterial culture was collected from a solid medium and inserted-ed in the petri dishes (that contain the nutrient agar) by using incubation loops, then incubated these petri dishes at 37 0C for 24 hours. After incubation, the cultures were ready for use in the experiment. The leaf plant extract's antibacterial activity was tested by the agar-well-diffusion method. The gel puncture method was done by using the sterile corn borer to make wells of 6mm size in the agar of each plate. The bacteria with DMSO (Dimethyl sulfoxide) were considered as negative control while the amoxicillin was considered as position control, then (1mL) of bacteria suspension was added in the wells in each plate. The water, ethanol 80%, chloroform, and carbon tetrachloride leaves extracts were tested for antibacterial activity against Pseudomonas aeruginosa.

Results and Discussion

Preliminary phytochemical examination of ashwagandha leaves extracts based on the polarity of different solvents, namely, water, ethanol 80%, chloroform, and carbon tetrachloride, respectively, detected the existence of flavonoids, tannins, saponins, and

steroids in abundance in water and ethanol 80% extracts, while the saponins and steroids are few in chloroform and carbon tetrachloride extracts. The summary of results is obtained in Table 1. These results referred to the water and ethanol 80% sol- -vents had more polarity percentage compared with chloroform and carbon tetrachloride.

Table1. Qualitative profile of the phytochemical found in leaves extracts of Ashwagandha.

No.	Solvents	Flavor-Noid	Tan-nine	Saponin	Steroids
1	Water	+++	+++	+++	++
2	EtOH 80%	+++	+++	+++	++
3	$CHCl_3$	-	-	+	+
4	CCl_4	-	-	+	+

3.2 Identification IR Method of function groups

Spectra were measured in terms of T%, and the peaks at specific Û cm^{-1} (Wavenumber) were assigned by functional group bonding as per the reference given in the instrument manual of Shimadzu FTIR. The explanation of IR spectrum values of plant leaves extract with CCl_4, $CHCl_3$, EtOH, and Water are shown in Figure1, Figure2, Figure3, and Figure4, respectively, and the explanation of IIR spectrum values of plant Leaves extract with CCl_4, $CHCl_3$, EtOH, and Water as in the Table2, Table3, Table4. Table5, respectively.

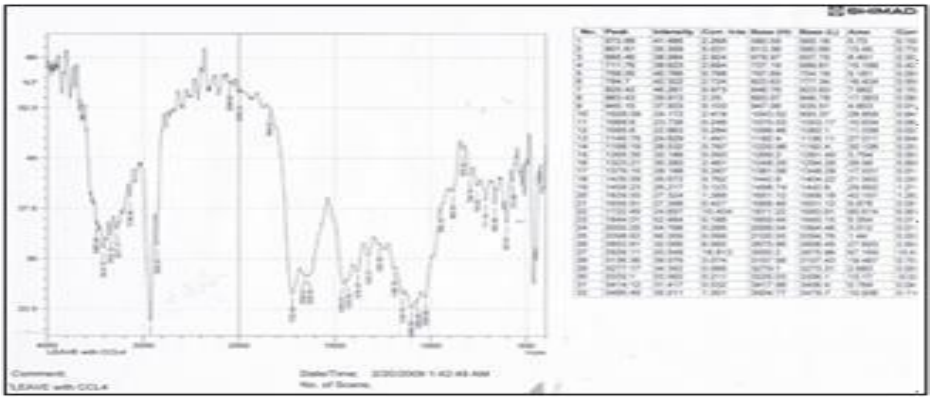


Figure 1. IR Spectrum of Leaves extract with CCl_4.

Table 2. Explanation of IR spectrum values of plant leaf extracts with CCl_4.

No.	Û cm-1	Transfer measure	
1.	3485.5,3414	N-H	
2.	3333, 3277	N-H amide	
3.	3136, 2926	O-H	
4.	2852	C-H hydrogen	Aldehyde

5. 2098 C≡C
6. 1722 C=O ester
7. 1656 C=O Amide
8. 1639, 1458 N-H Amide or Primary or Secondary amine
9. 1435, 1379, 1323 C-O-H
10. 1265, 1188,1145 C-O ether

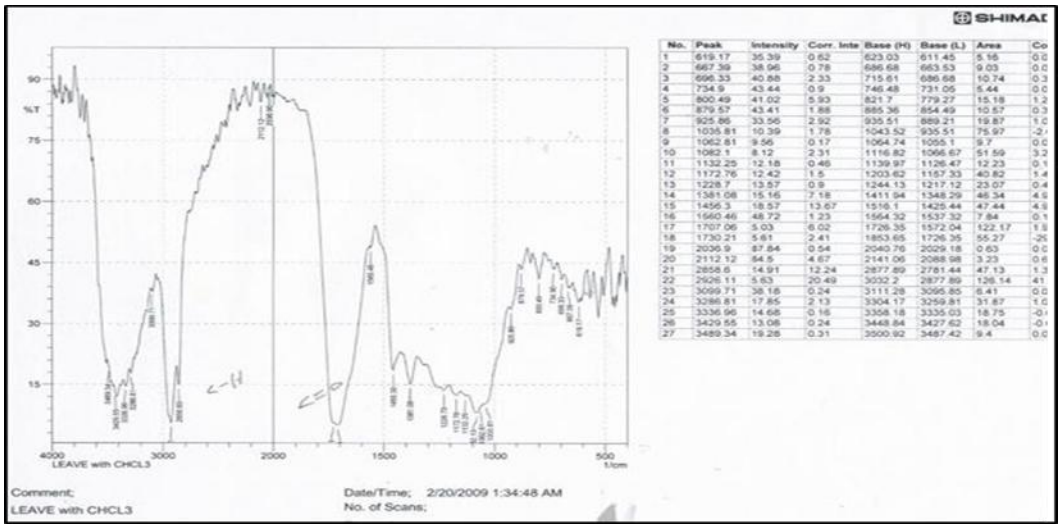
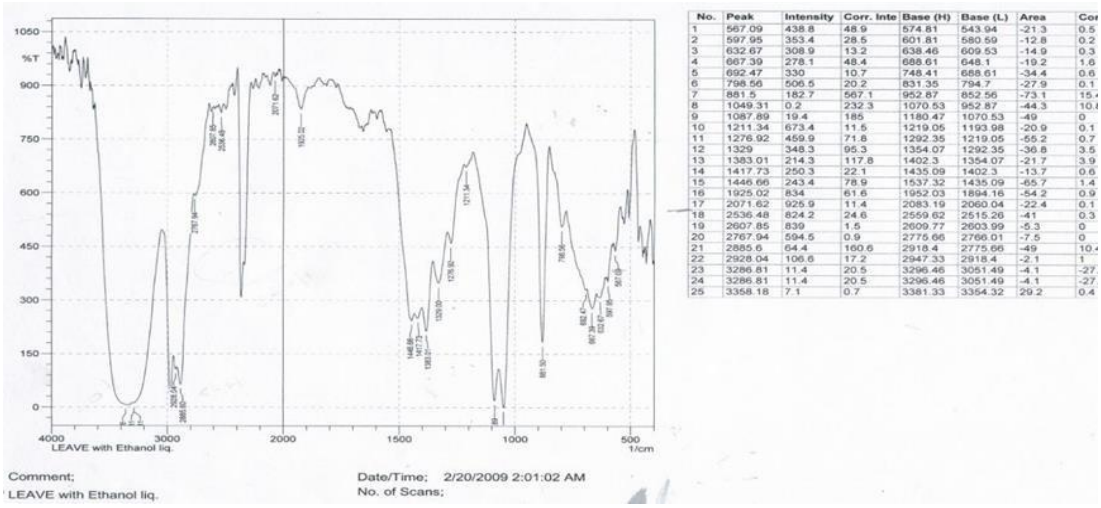


Figure 2. IR Spectrum of Leaves extract with $CHCl_3$.

Table 3. Explanation of IR spectrum values of plant leaves extract with $CHCl_3$.

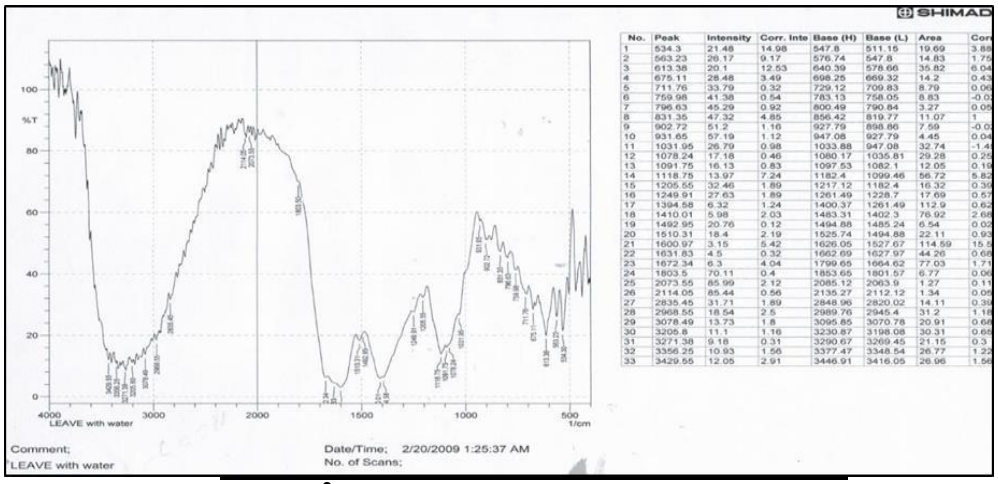
No.	Û cm-1	Transfer measure
1.	3489, 3429	N-H
2.	3336, 3286	N-H amide
3.	3099, 2926	O-H
4.	2852	C-H Aldehyde hydrogen
5.	2098	C≡C
6.	1722	C=O ester
7.	1656	C=O Amide
8.	1639	N-H Amide or Primary amine
9.	1458	N-H Secondary amine
10.	1435,1379, 1323	C-O-H
11.	1265,1188, 1145	C-O ether



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Cor
1	567.09	438.8	48.9	574.81	543.94	-21.3	0.5
2	597.95	353.4	28.5	601.81	580.59	-12.8	0.2
3	632.67	308.9	13.2	638.46	609.53	-14.9	0.3
4	667.39	278.1	48.4	688.61	648.1	-19.2	1.6
5	692.47	330	10.7	748.41	688.61	-34.4	0.6
6	798.56	506.5	20.2	831.35	794.7	-27.9	0.1
7	881.5	182.7	567.1	952.87	852.56	-73.1	15.
8	1049.31	0.2	232.3	1070.53	952.87	-44.3	10.
9	1087.89	19.4	185	1180.47	1070.53	-49	0
10	1211.34	673.4	11.5	1219.05	1193.98	-20.9	0.1
11	1276.92	459.9	71.8	1292.35	1219.05	-55.2	0.7
12	1329	348.3	95.3	1354.07	1292.35	-36.8	3.5
13	1383.01	214.3	117.8	1402.3	1354.07	-21.7	3.9
14	1417.73	250.3	22.1	1435.09	1402.3	-13.7	0.6
15	1446.66	243.4	78.9	1537.32	1435.09	-65.7	1.4
16	1925.02	834	61.6	1952.03	1894.16	-54.2	0.9
17	2071.62	925.9	11.4	2083.19	2060.04	-22.4	0.1
18	2536.48	824.2	24.6	2559.62	2515.26	-41	0.3
19	2607.85	839	1.5	2609.77	2603.99	-5.3	0
20	2767.94	594.5	0.9	2775.66	2766.01	-7.5	0
21	2885.6	64.4	160.6	2918.4	2775.66	-49	10.
22	2928.04	106.6	17.2	2947.33	2918.4	-2.1	1
23	3286.81	11.4	20.5	3296.46	3051.49	-4.1	-27
24	3286.81	11.4	20.5	3296.46	3051.49	-4.1	-27
25	3358.18	7.1	0.7	3381.33	3354.32	29.2	0.4

Figure 3. IR Spectrum of Leaves extract with EtOH.

Table 4. Explanation of IR spectrum values of plant leaves extract with EtOH Units.



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Cor
1	534.3	21.48	14.98	547.8	511.15	19.69	3.88
2	563.23	26.17	9.17	576.74	547.8	14.83	1.75
3	613.38	20.1	12.53	640.39	578.66	35.82	6.04
4	675.11	28.48	3.49	698.25	669.32	14.2	0.43
5	711.76	33.79	0.32	729.12	709.83	8.79	0.06
6	759.98	41.38	0.54	783.13	758.05	8.83	-0.0
7	796.63	45.29	0.92	800.49	790.84	3.27	0.05
8	831.35	47.32	4.85	856.42	819.77	11.07	1
9	902.72	51.2	1.16	927.79	898.86	7.59	-0.0
10	931.65	57.19	1.12	947.08	927.79	4.45	0.04
11	1031.95	26.79	0.98	1033.88	947.08	32.74	-1.4
12	1078.24	17.18	0.46	1080.17	1035.81	29.28	0.25
13	1091.75	16.13	0.83	1097.53	1082.1	12.05	0.19
14	1118.75	13.97	7.24	1182.4	1099.46	56.72	5.82
15	1205.55	32.46	1.89	1217.12	1182.4	16.32	0.39
16	1249.91	27.63	1.89	1261.49	1228.7	17.69	0.57
17	1394.58	6.32	1.24	1400.37	1261.49	112.9	0.62
18	1410.01	5.98	2.03	1483.31	1402.3	76.92	2.68
19	1492.95	20.76	0.12	1494.88	1485.24	6.54	0.02
20	1510.31	18.4	2.19	1525.74	1494.88	22.11	0.93
21	1600.97	3.15	5.42	1626.05	1527.67	114.59	15.5
22	1631.83	4.5	0.32	1662.69	1627.97	44.26	0.68
23	1672.34	6.3	4.04	1799.65	1664.62	77.03	1.71
24	1803.5	70.11	0.4	1853.65	1801.57	6.77	0.06
25	2073.55	85.99	2.12	2085.12	2063.9	1.27	0.11
26	2114.05	85.44	0.56	2135.27	2112.12	1.34	0.05
27	2835.45	31.71	1.89	2848.96	2820.02	14.11	0.38
28	2968.55	18.54	2.5	2989.76	2945.4	31.2	1.18
29	3078.49	13.73	1.8	3095.85	3070.78	20.91	0.68
30	3205.8	11.1	1.16	3230.87	3198.08	30.31	0.65
31	3271.38	9.18	0.31	3290.67	3269.45	21.15	0.3
32	3356.25	10.93	1.56	3377.47	3348.54	26.77	1.22
33	3429.55	12.05	2.91	3446.91	3416.05	26.96	1.56

No.	Û cm-1	Transfer measure
1.	3358	≡C -H
2.	3286	N-H amide
3.	2928, 2885, 2727, 2607, 2536	O-H Carboxylic acid
4.	2071	C≡C
5.	1417, 1383, 1329, 1276	C-O-H
6.	1211, 1087, 1049	C-O alcohol
7.	881, 796, 692, 667	=C-H

Figure 4. IR Spectrum of Leaves extract with H2O.

Table 5. Explanation of IR spectrum values of plant leaves extract with H2O.

No.	Û cm-1	Transfer measure
1.	3429	≡C-H
2.	3356	N-H
3.	3271	N-H amide
4.	3205, 3078,2968	O-H Carboxylic acid
5.	2835	C-H Aldehyde hydrogen
6.	2114, 2073	C≡C
7.	1672, 1631,	C=O Amide
8.	1600, 1510, 1492	C=C
9.	1410, 1394, 1249	C-O-H
10.	1205, 1118, 1091, 1078, 1031	C-O Alcohol
11.	931, 902, 831, 796	=C-H

1.1. GC-Mass Spectroscopy Assay

The GC- Mass Spectroscopy Assay of Ashwagandha leaves from chloroform and ethanol extracts; In the chloroform extract, sixteen compounds were found through the results of GC- Mass Spectroscopy chromatogram analysis of the extract leaves, as shown in Figure 5 and Table 6, Learn about the compounds formula (MF), identification, and percentage in extract. Compound 1 as formic acid with MF [CH_2O_2] (m/z 46), constitutes 1.35% of chloroform extract; Compound 2 as Acetic acid with MF [$C_2H_4O_2$] (m/z 60), it constitutes 5.15% of chloroform extract; Compound 3 as 1-hydroxy 2-propanone with MF [$C_3H_6O_2$] (m/z 74), it constitutes 0.64% of chloroform extract; Compound 4 as ethenyl 2-Propenoate ester with MF [$C_5H_6O_2$] (m/z 98), it constitutes 0.26% of chloroform extract; Compound 5 as N, N, O-Tri- acetyl hydroxylamine with MF [$C_6H_9NO_4$] (m/z 159). it constitutes 0.35% of chloroform extract; Compound 6 as 3,5-dihydroxy-6-methyl- 2,3- dihydro - 4H-pyran-4-one with MF [$C_6H_8O_4$] (m/z 144), it constitutes 4.79% of chloroform extract; Compound 7 as 5-Hydroxy methyl furfural with MF [$C_6H_6O_3$] (m/z 126), it constitutes 3.68% of chloroform extract; Compound 8 as 1,1,3-trimethyl Cyclopentane with MF [C_8H_{16}] (m/z 112), it constitutes 0.35% of chloroform extract; Compound 9 as Caryophyllene oxide with MF [$C_{15}H_{24}O$] (m/z 220), it constitutes 2.43% of chloroform extract; Compound 10 as 1,2,3,5-Cyclohexanetetrol with MF [$C_{15}H_{24}O$] (m/z 148), it constitutes 64.16% of chloroform extract; Compound 11 as n-Decanoic acid with MF [$C_{10}H_{20}O_2$] (m/z 172), it constitutes 1.67% of chloroform extract; Compound 12 as n-Hexadecanoic acid with MF [$C_{16}H_{32}O_2$] (m/z 256), it constitutes 10.15% of chloroform extract; Compound 13 as cis-3-Hexenylpyruvate with MF [$C_9H_{14}O_3$] (m/z 170), it constitutes 1.30% of chloroform extract; Compound 14 as 1,2-Pentanediol with MF [$C_{30}H_{50}O_4$] (m/z 104). it constitutes 0.66% of chloroform extract; Compound 15 is 4-octyl tetradecyl Phthalate ester with MF [$C_{30}H_{50}O_4$] (m/z 474), constitutes 1.02% of chloroform extract; Compound 16 as α- Tocopherol-β-D-mannoside with MF [$C_{35}H_{60}O_7$] (m/z 592), it constitutes 1.02% of chloroform extract

Table 6. Compounds in Chloroform extract of Ashwagandha leaves from GC-MS

-	Compound Name	Formula	M. mass	Area %	R.Time
1.	Formic acid	CH2O2	46	1.35	2.098
2.	Acetic acid	C2H4O2	60	5.15	2.226
3.	1-Hydroxy 2-propanone	C3H6O2	74	0.64	2.434
4.	Ethenyl 2-Propenoate ester	C5H6O2	98	0.26	2.965
5.	N, N, O-Triacetyl hydroxylamine	C6H9NO4	159	0.35	3.242
6.	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	C6H8O4	144	4.79	8.901
7.	5-Hydroxy methyl furfural	C6H6O3	126	3.68	10.294
8.	1,1,3-Trimethyl Cyclopentane	C8H16	112	0.70	15.050
9.	Caryophyllene oxide	C15H24O	220	2.43	15.245
10.	1,2,3,5-Cyclohexanetetrol	C15H24O	148	64.16	16.143
11.	n-Decanoic acid	C10H20O2	172	1.67	17.191
12.	n-Hexadecanoic acid	C16H32O2	256	10.15	19.370
13.	cis-3-Hexenylpyruvate	C9H14O3	170	1.30	21.110
14.	1,2-Pentanediol	C30H50O4	104	0.66	21.303
15.	4-Octyl tetradecyl Phthalate ester	C30H50O4	474	1.02	24.690
16.	α-Tocopherol-β-D-mannoside	C35H60O7	592	2.04	24.823

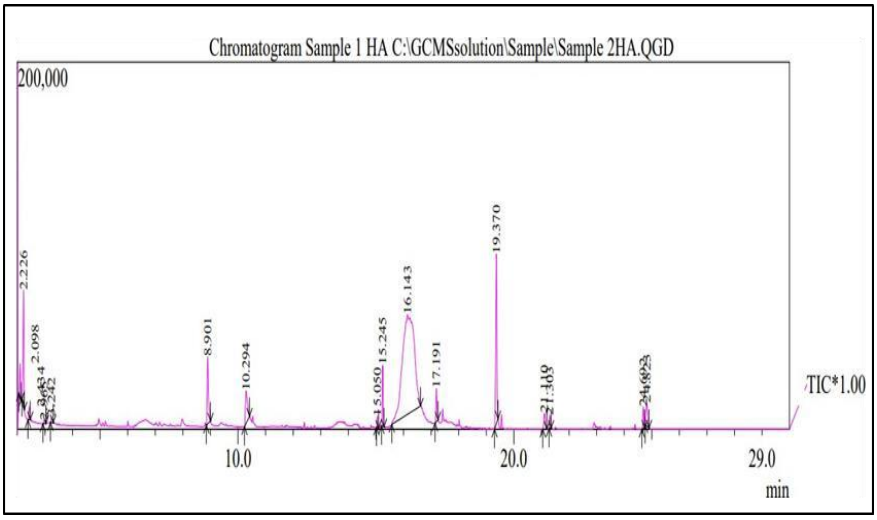


Figure 5. GC-MS Spectrum of Leaves extract with Chloroform.

In the ethanol extract of Ashwagandha leaves, twenty one compounds were found through the results of GC-Mass Spectroscopy chromatogram analysis, as show in Figure 6, and fourteen compounds are the largest percentage in the alcoholic leaves extract as show in Table 7, Compound 1 as Hexanoic acid, ethyl ester with MF [$C_8H_{16}O_2$] (m/z 144.2114), it constitutes 20.03% of ethanol extract; Compound 2 as O-Butyl hydroxylamine with MF [$C_4H_{11}NO$] (m/z 89.14), it constitutes 52.70% of ethanol extract; Compound 3 as N- ethyl propanamide with MF [$C_5H_{11}NO$] (m/z 101.15), it constitutes 0.17% of ethanol extract; Compound 4 as 1,3,6 Trioxocane with MF [$C_5H_{10}O_3$] (m/z 118.13), it constitutes 0.64% of ethanol extract; Compound 5 as Butanoic acid, ethyl ester with MF [$C_6H_{12}O_2$] (m/z 116.1583), it constitutes 0.20% of ethanol extract; Compound 6 as Oleyl alcohol, trifluoroacetate with MF [$C_{20}H_{35}F_3O_2$] (m/z 364.4859), it constitutes 7.36% of ethanol extract; Compound 7 as Cis- Vaccenic acid with MF [$C_{18}H_{34}O_2$] (m/z 282.4614), it constitutes 1.71% of ethanol extract; Compound 8 as Oleic acid with MF [$C_{18}H_{34}O_2$] (m/z 282.4614), it constitutes 5.65% of ethanol extract; Compound 9 as Cis 9-hexadecenal with MF [$C_{16}H_{30}O$] (m/z 238.4088), it constitutes 0.87% of ethanol extract; Compound 10 as 13-octadecenal, Z- with MF [$C_{18}H_{34}O$] (m/z 266.5), it constitutes 1.12% of ethanol extract; Compound 11 as (S)(+)Z-13-Methyl 11-pentadecena- 1-ol acetate with MF [$C_{18}H_{34}O_2$] (m/z 282.4614), it constitutes 0.58% of ethanol extract; Compound 12 as (S)(+)Z-13-Methyl 11-pentadecena-1-ol acetate with MF [$C_{18}H_{34}O_2$] (m/z 282.4614). it constitutes 1.49% of ethanol extract; Compound 13 as 7-Tetradecenal, Z- with MF [$C_{14}H_{26}O$] (m/z 210.3556). it constitutes 0.84% of ethanol extract; Compound 14 as Oxirane, tetradecyl- with MF [$C_{16}H_{32}O$] (m/z 240.4247), it constitutes 0.47% of ethanol extract; Compound 15 as Cis-13-Octadecenoic acid with MF [$C_{18}H_{34}O_2$] (m/z 282.4614), it constitutes 0.84% of ethanol extract; Compound 16 as 9-methyl-z, z-10,12-hexadecadien-1-ol acetate with MF [$C_{19}H_{34}O_2$] (m/z 294.4879), it constitutes 0.57% of ethanol extract; Compound 17 as 13-Otadecenal, Z- with MF [$C_{18}H_{34}O$] (m/z 266.5), it constitutes 1.02% of ethanol extract; Compound 18 as 9- Octadecenoic acid, Z-, methyl ester with MF [$C_{19}H_{36}O_2$] (m/z 296.4879), it constitutes 0.43% of ethanol extract; Compound 19 as Erucic acid with MF [$C_{22}H_{42}O_2$] (m/z 338.5677), it constitutes 0.10% of ethanol extract; Compound 20 as Oleic acid with MF [$C_{18}H_{34}O_2$] (m/z 282.4614), it constitutes 1.84% of ethanol extract; Compound 21 as Cis 9-hexadecenal with MF [$C_{16}H_{30}O$] (m/z 238.4088), it constitutes 1.36% of ethanol extract.

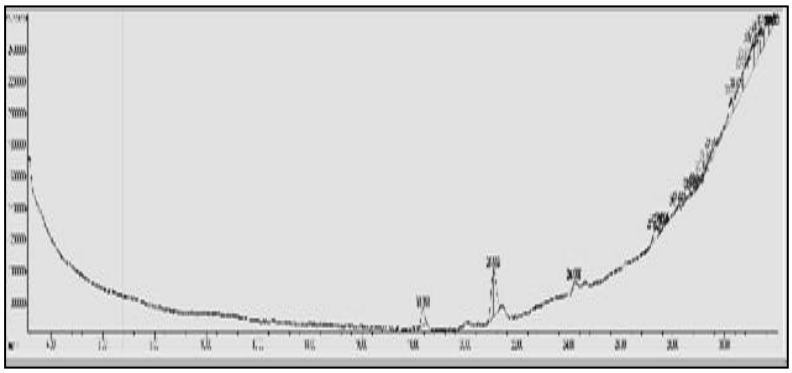


Figure 6. GC-MS Spectrum of Leaves Extract with Ethanol.

Table 7. Compounds in Ethanol extract of Ashwagandha leaves from GC-MS.

No.	Compound Name	Formula	M. mass	Area %	RTime
1.	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	144.2114	20.03	18.351
2.	O-Butyl hydroxylamine	$C_4H_{11}NO$	89.14	52.70	21.084
3.	N- ethyl- Propanamide	$C_5H_{11}NO$	101.15	0.17	21.346
4.	1,3,6 Trioxocane	$C_5H_{10}O_3$	118.13	0.64	21.411
5.	Butanoic acid, ethyl ester	$C_6H_{12}O_2$	116.1583	0.20	21.433
6.	Oleyl alcohol, trifluoroacetate	$C_{20}H_{35}F_3O_2$	364.4859	7.36	27.317
7.	Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.4614	1.71	27.357
8.	Oleic acid	$C_{18}H_{34}O_2$	282.4614	5.65	27.401
9.	Cis 9-hexadecenal	$C_{16}H_{30}O$	238.4088	0.87	29.109
10.	13-octadecenal, Z-	$C_{18}H_{34}O$	266.5	1.12	29.147
11.	(S)(+)Z-13-Methyl 11-pentadecena-1-ol acetate	$C_{18}H_{34}O_2$	282.4614	0.58	29.190
12.	(S)(+)Z-13-Methyl 11-pentadecena-1-ol acetate	$C_{18}H_{34}O_2$	282.4614	1.49	29.245
13.	7-Tetradecenal, Z-	$C_{14}H_{26}O$	210.3556	0.84	29.289
14.	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240.4247	0.47	29.370
15.	Cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	0.84	29.507
16.	9-methyl-z,z-10,12-hexadecadien-1-ol acetate	$C_{19}H_{34}O_2$	294.4879	0.57	29.567
17.	13-Otadecenal, Z-	$C_{18}H_{34}O$	266.5	1.02	29.692
18.	9- Octadecenoic acid, Z-, methyl ester	$C_{19}H_{36}O_2$	296.4879	0.43	29.741
19.	Erucic acid	$C_{22}H_{42}O_2$	338.5677	0.10	29.769
20.	Oleic acid	$C_{18}H_{34}O_2$	282.4614	1.84	29.878
21.	Cis 9-hexadecenal	$C_{16}H_{30}O$	238.41	1.36	30.702

1.2. Antibacterial assay

The water and ethanolic extracts at 500 concerts showed more activity as anti-P. aeruginosa bacteria compared with chloroform and carbon tetrachloride, as shown in Figure 7. The inhibiter zones were 22,20 mm of water and ethanolic extracts while 15 and 12 mm of chloroform and carbon tetrachloride respectively. These results referred to more phenolic compounds in water and ethanol extracts such as flavonoids, tannin, and steroids which are considered antiseptic compounds. The inhibitory zones of water, ethanol, chloroform, and carbon tetrachloride extracts are presented in Table 8.

Table 8. The inhibition zone of Ashwagandha leaves extracts against Pseudomonas aeruginosa bacteria.

Extract with	Water	Ethanol 80%	Chloroform	CCl4	DMSO	Amoxicillin
Inhibition Zone (mm)	22	20	13	12	Zero	18

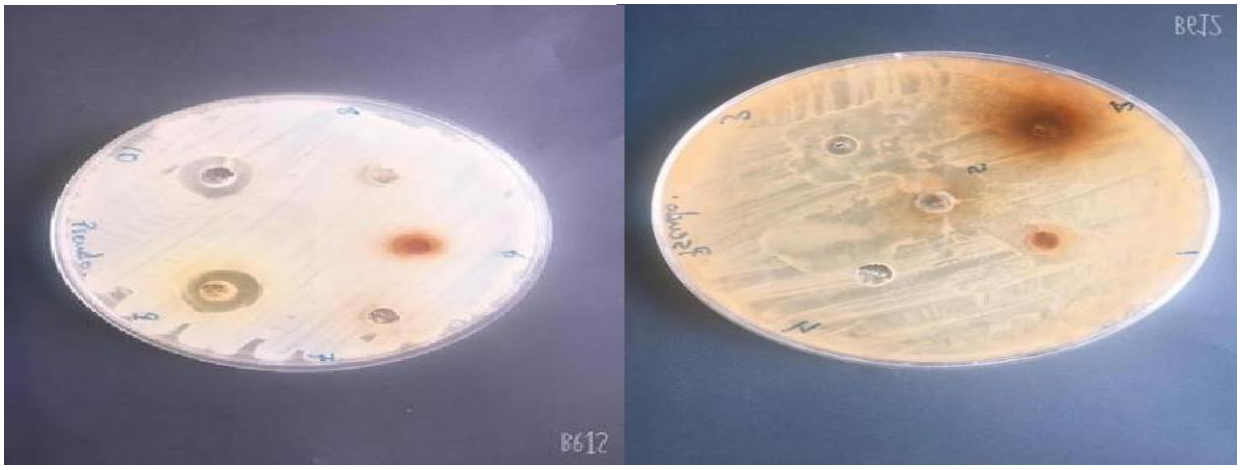


Figure7. Showed anti-P. aeruginosa activity. 2-Leaves Extract with CCl4, 5- Leaves Extract with water, 9- Leaves Extract with Ethanol, 10- Leaves Extract with Chloroform.

Flavonoids are synthesized by plants as a defense reaction against plant microbial infection, thus extracts from plants rich in flavonoids are used as bactericidal aid. They act by deactivating bacteria toxins or inhibiting the synthesis of bacterial DNA or they act by altering the outer bacterial cell membrane. The water and ethanolic extracts were found to be good inhibitors or good active against *Pseudomonas aeruginosa bacteria, and this* activity may belong to polyphenolic compounds such as flavonoids and tunnies' compounds. As part of scientific research to extract bioactive natural products with novel structures from plants, we measured a phytochemical examination of the leaves of W. somnifera extract using- Mass Spectroscopy Assay, by comparing the chromatogram of GC-Mass Spectroscopy Assay peak value of the unknown compound with entries in NIST database, the identification of compounds was done. Among the 16 phytochemicals identified in chloroform extract, 1,2,3,5-Cyclohexanetetrol is the most abundant compound present (64.16%) in the extract, followed by n- Hexadecanoic acid (10.15%), then Acetic acid (5.15%), 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran4one(4.79%), 5-Hydrxoy methyl furfural (3.68%), Caryophyllene oxide (2.43%), α-Tocopherol-β-D-mannoside (2.04%), n-Decanoic acid (1.67%), Formic acid (1.35%), cis-3-Hexenylpyruvate (1.30%), 4-Octyl tetradecyl Phthalate ester (1.02%), 1,2-Pentanediol (0.66%), 1- Hydroxy 2-propanone (0.64%), N, N, O-Triacetyl hydroxylamine (0.35%), 1,1,3-Trimethyl Cyclopentane (0.35%), Ethenyl 2-Propenoate ester (0.26%). And

the 21 phytochemicals identified in ethanol extract, O-Butyl hydroxylamine is the most abundant compound present (52.70%) from ethanol extract, followed by Hexanoic acid, ethyl ester (20.03%), then Oleyl alcohol, trifluoroacetate (7.36%), Oleic acid, Cis 9-hexadecenal .est. An analysis of the leaves of this medicinal plant shows some compounds with rich phar-pharmaceutical potential, which requires more scientific research experiments to identify and discover some new drugs that may be found in the leaves of this plant.

Conclusions

Four kinds of solvents were used to extract Withania Somifera L. leaves, the phytochemical investigation of leaves extract revealed the presence of important bioactive compounds such as flavonoids, tannins, saponin, and steroids.

The present study indicated that the plant leaves contain an antibacterial compound that can be further developed as phytomedicine for the therapy of infection.

2. Acknowledgments

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