GC-MS Analysis and Anti-bacterial activity of *Juniperus communis* L.

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Abstract

The present investigation focused on the qualitative analysis of components of essential oil using GC-MS and antibacterial efficacy of essential oil of *Juniperus communis* L. The oils were extracted from the leaves using hydro-distillation method. The antibacterial activity of the extracted essential oils was evaluated against *Escherichia coli* (MTCC No. 723), *Klebsiella pneumoniae* (MTCC No. 4032) and *Salmonella typhimurium* (MTCC No. 3231) using Kirby-Bauer disc diffusion method recommended by Clinical Laboratory Standard Institute (CLSI) formerly (NCCLS). Nineteen different compounds have been identified. Essential oil activity was found most effective against *S. typhimurium* whereas found least effective against *K. pneumoniae*. Hence, essential oil from leaves of *J. communis* exhibit great potential for the development of eco-friendly, non-toxic, cost-effective anti-bacterial formulations.

Key words: Essential oil, Antibacterial activity, Kirby-Bauer Disc diffusion method, GC-MS.

1. Introduction:

J. communis L. is distributed throughout the cool temperate Northern Hemisphere including America, Europe and Asia. The essential oil, infusions, decoctions, and alcoholic extracts are used in different fields (pharmaceuticals, alcoholics, etc.) [1-3]. Composition of essential oils of the plants can be changes due to environmental factors, such as the soil or climate in which the plants are grown and by different harvesting methods or distillation techniques. In the last years a number of publications have reported the composition of the berries and leaves essential oil of the *Juniperus* species [4-9].

2. Material and method:

2.1. Collection of plant materials:

The plant materials of *J. communis* L. were collected from Roxburgh Garden, Department of Botany, University of Allahabad, in the month of July [23]. Leaves were crushed and hydrolyzed using a Clevenger type Apparatus for 4-6 hours. Essential oil of *J. communis* (juniper) was nearly transparent. Oil content was stored at 4°C until analysis [23].

2.2. GC-MS: The extracted essential oil samples were analyzed by GCMS.

GCMS conditions on which your samples were analysed are as below:

- Instrument : Perkin Elmer SQ8 C MS with Clarus 680 GC
- Capillary Column : Elite 5 MS (30m x0.25mmx 0.25um)
- Oven Programming : 60-250 @ 3°C/min, then 320 @ 5°C/min final hold 10 min.
- Helium Carrier flow : 1 ml/minute
- Split ratio : 1:80
- Injector Temp. : 280° C
- EI ionization : 70 eV
- m/z : 50 to 500 amu
- Scan time : 0.5 sec
- Inter Scan Delay : 0.15 sec
- Source Temp. : 250° C
- Inlet Line Temp. : 250° C.

2.3. Identification of components:

Identification as well as the proportionate percentage of each component was done by comparing its average peak area of to the total areas the identification of compounds was performed by comparing their mass spectra with data from and IST 08 National institute of standards and technology us and will it libraries spectrum of unknown compounds was compared with spectrum of known compounds stored in the data libraries and their molecular formula molecular weight and number of heads used in the used to identify the name of components from data library.

2.4. Antibacterial Screening - Essential oils were screened for antibacterial activity against *E. coli*, *K. pneumoniae* and *S. typhimurium*. Zone of Inhibition (ZOI) were determined using Kirby Bauer disc diffusion method recommended by Clinical Laboratory Standard Institute (CLSI). Dilutions of the oil were prepared in DMSO. Different concentration of oil were tested using standard disc, which were incubated at 37 °C for 24 hours [22, 23]. The final zone of inhibition were recorded after 24 hours. Streptomycin was used as standard drug for this experiment. DMSO was used as a positive control whereas formaldehyde was used as negative control.

3. Results and Discussion:

3.1. Percent yield: % yield = weight of oil / weight of sample x 100.

Juniperus commmunis = 0.30%

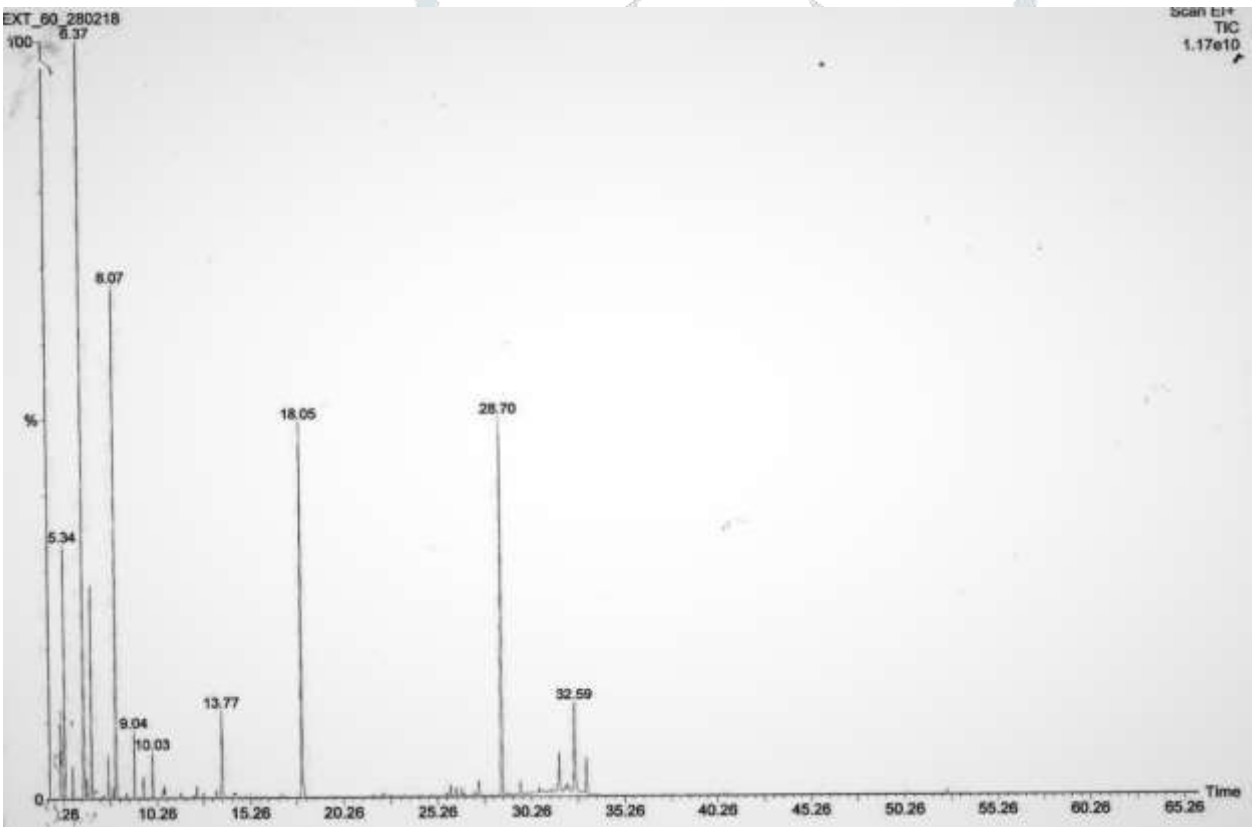
3.2. GC-MS:

In the present study, GC-MS analysis revealed the presence of various phytochemical constituents in EO of *J. communis*. The identification of the phytoconstituents was confirmed on the basis of CAS, retention time and molecular formula [10-13]. Nineteen compounds have been identified on twenty different retention times. Results are depicted in table 1. Gas chromatogram in graph 1.

Table 1. Compounds identified in the EO of *J. communis* L. by GC-MS.

S.No	R.T.	CAS	Name of Compound	M.F.	M.W
1	5.079	13466789	Bicyclo[4.1.0]hept-3-ene, 3,7,7-trimethyl {Delta.3-Carene}	$C_{10}H_{16}$	136
2	5.147	99832	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl) {Alpha-Phellandrene}	$C_{10}H_{16}$	136
3	5.35	80568	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl {Alpha-Pinene}	$C_{10}H_{16}$	136
4	5.77	79925	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene {Camphene}	$C_{10}H_{16}$	136
5	6.353	3387415	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl) {Sabinene}	$C_{10}H_{16}$	136
6	6.502	127913	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene {2-Beta-Pinene} {Nopinene}	$C_{10}H_{16}$	136
7	6.787	123353	1,6-Octadiene, 7-methyl-3-methylene {Beta-Myrcene}	$C_{10}H_{16}$	136
8	7.912	99876	P-Cymene	$C_{10}H_{14}$	134
9	7.654	586629	Cyclohexene, 1-methyl-4-(1-methylethylidene) {Alpha-Terpinolene}	$C_{10}H_{16}$	136
10	8.061	138863	Cyclohexene, 1-methyl-4-(1-methylethenyl) {D-Limonene}	$C_{10}H_{16}$	136
11	9.037	99854	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl) {Gamma-Terpinene}	$C_{10}H_{16}$	136
12	9.525	17699160	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol {Trans-Sabinene hydrate}	$C_{10}H_{18}O$	154
13	10.026	586629	Cyclohexene, 1-methyl-4-(1-methylethylidene) {Alpha-Terpinolene}	$C_{10}H_{16}$	136
14	13.78	562743	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) {Terpinen-4-ol}	$C_{10}H_{18}O$	154

15	18.036	125122	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, {Exobornyl acetate}	$C_{12}H_{20}O_2$	196
16	28.689	639996	o-Menth-8-ene-4-methanol {Elemol}	$C_{15}H_{26}O$	222
17	31.739	1209718	2-Naphthalenemethanol	$C_{15}H_{26}O$	222
18	32.498	473154	Eudesm-4(14)-en-11-ol {Beta-Eudesmol}	$C_{15}H_{26}O$	222
19	32.62	473165	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)-2-propanol {Alpha-Eudesmol}	$C_{15}H_{26}O$	222
20	33.23	489861	Guaiol	$C_{15}H_{26}O$	222



Graph 1. Gas chromatogram of *Juniperus communis* L.

3.3. Antibacterial activity of essential oil of *J. communis*.

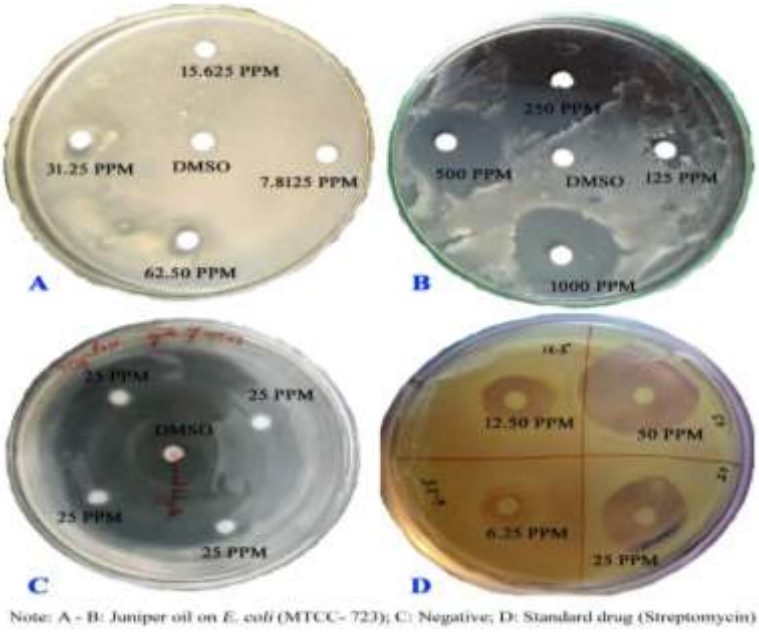
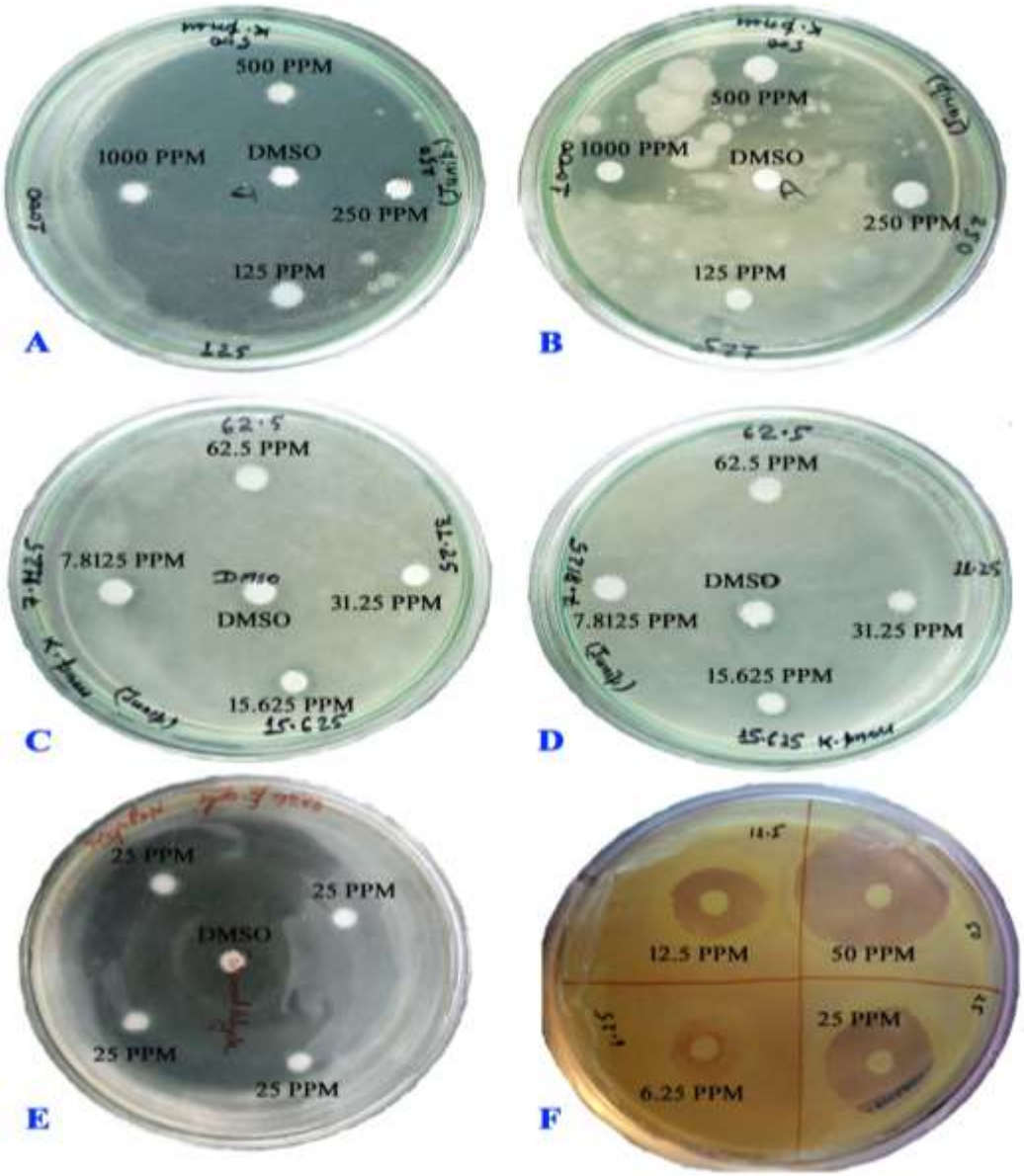


Figure 1. Antibacterial activity of *J. communis* essential oil against *E. coli* (MTCC-723).

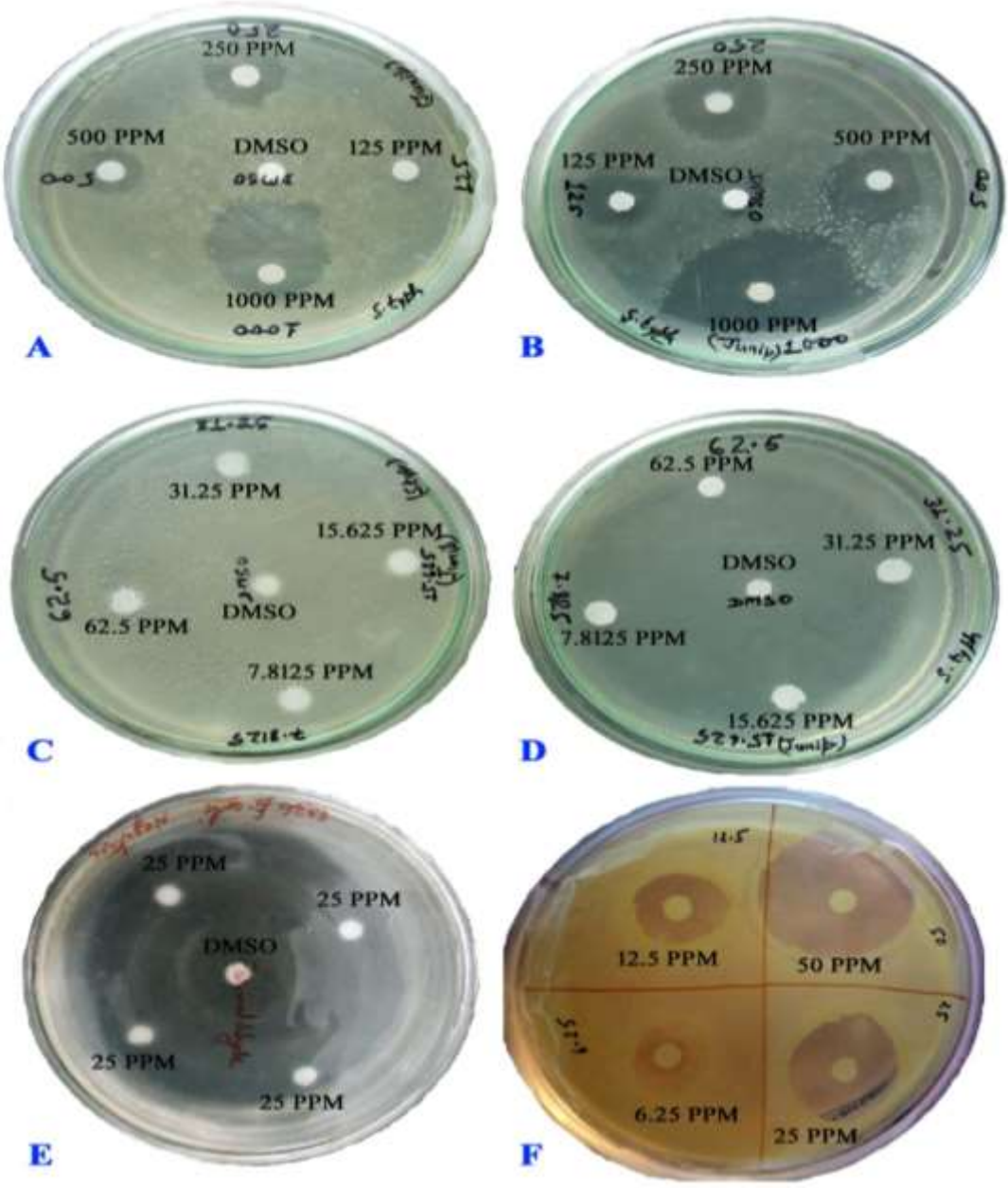
At 1000 ppm, the zone of inhibition (ZOI) of essential oil of *J. communis* was 19.5 mm against *E. coli* (723). For 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm and 7.8125 ppm; the ZOI was 13.5 mm, 16.5 mm, 11 mm, 6.5 mm, 6 mm, 6.5 mm and 6.5 mm respectively. No growth was recorded for negative control.



Note: A - D: *Juniperus* oil on *K. pneumoniae*; E: Negative; F: Standard drug (Streptomycin)

Figure 2. Antibacterial activity of *J. communis* essential oil against *K. pneumoniae.*

At 1000 ppm, the zone of inhibition (ZOI) of essential oil of *J. communis* was 15 mm against *K. pneumoniae*. For 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm and 7.8125 ppm; the ZOI was 14.5 mm, 10.5 mm, 8 mm, 8 mm, 8 mm, 8 mm and 7 mm respectively. No growth was recorded for negative control.



Note: A-D: Juniperus oil on *S. typhimurium*; E: Negative; F: Standard drug (Streptomycin)

Figure 3. Antibacterial activity of *J. communis* essential oil against *S. typhimurium*.

At 1000 ppm, the zone of inhibition (ZOI) of essential oil of *J. communis* was 23 mm against *S. typhimurium*. For 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm and 7.8125 ppm; the ZOI was 16.5 mm, 15.5 mm, 9.5 mm, 8 mm, 7 mm, 7 mm and 6.5 mm respectively. No growth was recorded for negative control.

Table 2. Mean ZOI values of EO of *J. communis* against bacterial pathogens

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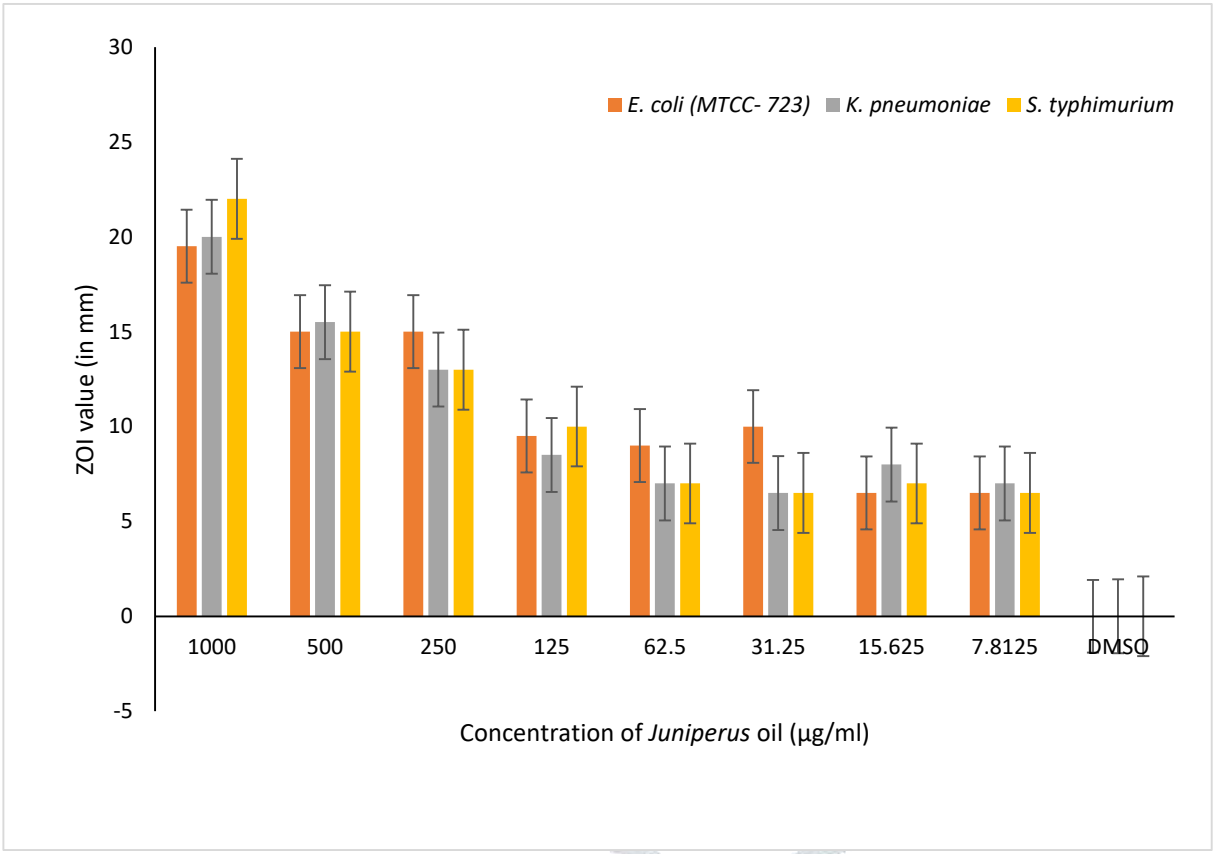
***Juniperus* oil conc. (in ug/ml)**	**1000**	**500**	**250**	**125**	**62.5**	**31.25**	**15.625**	**7.8125**	**DMSO**
E. coli	19.5	13.5	16.5	11	6.5	6	6.5	6.5	0
K. pneumoniae	15	14.5	10.5	8	8	8	8	7	0
S. typhimurium	23	16.5	15.5	9.5	8	7	7	6.5	0



Graph 2. Graphical representation of antibacterial activities of EO of *J. communis*.

It was found that EO of *J. communis* was found to be most active against *S. typhimurium* at higher ppm. A significant activity was also found against *E. coli* (MTCC-723) and *K. pneumoniae* also [23-26].

Conclusion:

Essential oil of *J. communis* exhibit great potential for the development of eco-friendly, non-toxic, cost-efficient and antibacterial herbal formulations [20-26].

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