

A COMPARATIVE STUDY BETWEEN DISC DIFFUSION METHOD AND MIC (MINIMUM INHIBITORY CONCENTRATION) TO TEST THE EFFICIENCY OF ANTIMICROBIAL ACTIVITIES OF *ALOE VERA L.* EXTRACTSNeelofar Khanam^{*1,2,3}, G. K. Sharma³¹Department of Medical Laboratory Sciences, College of Allied and Healthcare, SCPM Medical College, Lucknow Road, Haripur, Gonda - 271003, Uttar Pradesh, India.²School of Biotechnology, IFTM University, Moradabad-244001, Uttar Pradesh, India.³Division of Biotechnology, Department of Botany, Hindu College, Moradabad-244001, Uttar Pradesh, India.***Corresponding Author: Neelofar Khanam**Department of Medical Laboratory Sciences, College of Allied and Healthcare, SCPM Medical College, Lucknow Road, Haripur, Gonda - 271003, Uttar Pradesh, India. DOI: <https://doi.org/10.5281/zenodo.21155574>**How to cite this Article:** Neelofar Khanam^{*1,2,3}, G. K. Sharma³ (2026). A Comparative Study Between Disc Diffusion Method And Mic (Minimum Inhibitory Concentration) To Test The Efficiency Of Antimicrobial Activities Of *Aloe Vera L.* Extracts. World Journal of Pharmaceutical and Medical Research, 12(7), 470–473.This work is licensed under [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by-nc/4.0/).

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ABSTRACT

The present study evaluates and compares the efficiency of two widely used antimicrobial susceptibility testing methods, namely the disc diffusion method and the minimum inhibitory concentration (MIC), in determining the antimicrobial potential of *Aloe vera L.* extracts. Various clinically significant microorganisms, including bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, etc.), fungi (*Aspergillus spp.*), and yeast (*Candida spp.*), were selected for the investigation. Crude extracts were prepared from both whole leaf and inner gel of *Aloe vera L.*, derived from *in vivo* grown and *in vitro* regenerated plants. Different solvents such as ethyl acetate, methanol, chloroform, n-hexane, and water were used for extraction. Antimicrobial activity was assessed using the Kirby-Bauer disc diffusion technique, while MIC values were determined using the agar dilution method. Comparative analysis revealed that ethyl acetate extracts exhibited the highest antimicrobial activity against most tested pathogens, followed by methanol, chloroform, and n-hexane extracts. Aqueous extracts showed negligible or no inhibitory effect. The results obtained from both disc diffusion and MIC methods were largely consistent, although minor variations were observed due to differences in microbial susceptibility, extract diffusion, and solvent properties. The study confirms that *Aloe vera L.* possesses significant broad-spectrum antimicrobial properties, attributed to its bioactive phytochemicals. Furthermore, the findings highlight that MIC provides a more precise quantitative evaluation, whereas disc diffusion offers a rapid qualitative assessment. Overall, this comparative study supports the potential application of *Aloe vera L.* extracts in developing alternative antimicrobial agents and emphasizes the importance of method selection in antimicrobial testing.

KEYWORDS: *Aloe vera L.*, Antimicrobial activity, Minimum inhibitory concentration (MIC), Disc diffusion method and Phytochemical extracts.**INTRODUCTION**

In present investigation, number of pathogens of clinical significance including bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Bacillus cereus*), fungi (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium digitatum*) and yeast (*Candida albicans*, *Candida utilis*) were used for the comparative study between disc diffusion method and minimum

inhibitory concentration (MIC) to test the efficiency of antimicrobial activities of *Aloe vera l.* extracts.

MATERIALS AND METHODS**Preparation of crude extract**

Leaves of the *Aloe vera L.* were collected from the already *in vitro* propagated and properly acclimatized 9-12 months old plants. *In vitro* propagation was the previous phase of our study to produce quality plant material to meet industrial requirement. Simultaneously leaves from 9-12 months old *in vivo* grown *Aloe vera L.*

plants were also collected. Freshly collected *Aloe vera* L. leaves were washed with distilled water, followed by disinfecting with ethanol 70%. Later, in case of whole leaf crude extract preparation, leaves were chopped into the small pieces and were exposed to 50°C for 3 days to get dried. After complete drying, leaf parts were powdered using electric grinder, simultaneously in case of only gel crude extract preparation, upper green skin/rind of leaves was removed and latex was cut into small pieces and both types of leaf materials were homogenized separately. The homogenized materials were extracted with ethanol (95%). The ethanol from the extracted leaf materials was evaporated at 65°C temperature in water bath. The solvent was completely removed and dried to get powder. All the powdered plant materials including whole leaf and only gel were used for the preparation of aqueous and solvent extracts.

Aqueous extract

Extracts were prepared using the modified method of Case.^[1] 1:3 (w/v) ratios were used for the powdered leaf material and distilled water for extract preparation. The pulverized leaf material was used to prepare an infusion in hot (95°C) distilled water. The infusion was left overnight under refrigeration (4°C) to prevent any possible contamination. After 24 h the extracts were kept in rotary shaker at 100 rpm for 1 h and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at - 47.5°C. The frozen extract was then freeze dried to a powder, weighed, transferred into separate vial and preserved at 4°C for future analysis.

Solvent extracts

As in case of aqueous extract here also 1:3 (w/v) ratios were used for the powdered leaf material and different solvents for extract preparation. The pulverized leaves material was mixed with sufficient quantity of solvents

viz., hexane, ethyl acetate, methanol and chloroform. It was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at - 47.5°C. The dried extracts thus obtained was weighed, transferred into separate vials and preserved at 4°C for future analysis.

Antimicrobial activities of *Aloe vera* L. (both *in vivo* grown and *in vitro* regenerated) whole leaf and inner gel extracts were carried out by disc diffusion method using the Kirby-Bauer technique^[2,3] and also the minimum inhibitory concentration (MIC) test was performed by Agar Dilution Method^[4] and finally a comparative study was done between disc diffusion method and minimum inhibitory concentration (MIC) to test the efficacy of antimicrobial activities of different extracts of *Aloe vera* L. (both *in vivo* grown and *in vitro* regenerated) whole leaf and inner gel against the number of pathogens of human health significance including bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Bacillus cereus*), fungi (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium digitatum*) and yeast (*Candida albicans*, *Candida utilis*).

RESULTS

Ethyl acetate extract of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel showed better antimicrobial activities followed by methanol, chloroform and then *n*-hexane extract, when the results of disc diffusion assay and MIC were compared. All the results of comparison between disc diffusion assay and MIC of *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and only gel samples are shown in the Table 1 – 4.

Table 1: Comparison of antimicrobial activity and MIC of *in vitro* regenerated *Aloe vera* L. whole leaf extracts.

S.No.	Microorganisms (Pathogens)	Results of Disc Diffusion					Results of MIC			
		He	Ch	Ea	Me	Aq	He	Ch	Ea	Me
1	<i>Escherichia coli</i>	++	++	++	++	-	-	+	+	+
2	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-
3	<i>Proteus vulgaris</i>	+	+	++	+	-	+	+	+	+
4	<i>Salmonella typhi</i>	+	+	++	++	-	+	+	+	+
5	<i>Staphylococcus aureus</i>	+	+	++	+	-	+	+	+	+
6	<i>Enterococcus faecalis</i>	+	+	++	+	-	-	-	+	+
7	<i>Klebsiella pneumonia</i>	+	+	+	+	-	-	-	+	-
8	<i>Bacillus subtilis</i>	+	++	++	++	-	-	-	+	+
9	<i>Bacillus cereus</i>	+	+	++	+	-	+	+	+	+
10	<i>Aspergillus niger</i>	+	+	+	+	-	-	-	+	+
11	<i>Aspergillus flavus</i>	+	+	++	++	-	-	+	+	+
12	<i>Aspergillus fumigatus</i>	+	+	+	+	-	-	-	+	+
13	<i>Penicillium digitatum</i>	+	+	++	+	-	-	+	+	+
14	<i>Candida albicans</i>	+	++	++	++	-	+	+	+	+
15	<i>Candida utilis</i>	+	+	++	+	-	+	+	+	+

He - *n*-Hexane, Ch - Chloroform, Ea - Ethyl acetate, Me - Methanol, Aq - Aqueous.

Disc diffusion results : ++ = ≥ 15 mm, + = ≥ 10 mm, - = ≤ 9 mm,

MIC results : + = ≤ 4.0 mg/ml, - = ≥ 5.0 mg/ml

Table 2: Comparison of antimicrobial activity and MIC of *in vitro* regenerated *Aloe vera* L. only gel extracts.

S.No.	Microorganisms (Pathogens)	Results of Disc Diffusion					Results of MIC			
		He	Ch	Ea	Me	Aq	He	Ch	Ea	Me
1	<i>Escherichia coli</i>	++	++	++	++	-	+	+	+	+
2	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-
3	<i>Proteus vulgaris</i>	+	++	++	++	-	+	+	+	+
4	<i>Salmonella typhi</i>	+	++	++	++	-	+	+	+	+
5	<i>Staphylococcus aureus</i>	+	++	++	++	-	+	+	+	+
6	<i>Enterococcus faecalis</i>	+	++	++	++	-	+	+	+	+
7	<i>Klebsiella pneumonia</i>	+	+	++	++	-	+	+	+	+
8	<i>Bacillus subtilis</i>	++	++	++	++	-	+	+	+	+
9	<i>Bacillus cereus</i>	+	+	++	++	-	+	+	+	+
10	<i>Aspergillus niger</i>	+	++	++	++	-	-	+	+	+
11	<i>Aspergillus flavus</i>	++	++	++	++	-	+	+	+	+
12	<i>Aspergillus fumigatus</i>	+	+	++	++	-	+	+	+	+
13	<i>Penicillium digitatum</i>	+	++	++	++	-	-	+	+	+
14	<i>Candida albicans</i>	++	++	++	++	-	+	+	+	+
15	<i>Candida utilis</i>	++	++	++	++	-	+	+	+	+

He - n-Hexane, Ch - Chloroform, Ea - Ethyl acetate, Me - Methanol, Aq - Aqueous.

Disc diffusion results : ++ = ≥ 15 mm, + = ≥ 10 mm, - = ≤ 9 mm,

MIC results : + = ≤ 4.0 mg/ml, - = ≥ 5.0 mg/ml

Table 3: Comparison of antimicrobial activity and MIC of *in vivo* grown *Aloe vera* L. whole leaf extracts.

S.No.	Microorganisms (Pathogens)	Results of Disc Diffusion					Results of MIC			
		He	Ch	Ea	Me	Aq	He	Ch	Ea	Me
1	<i>Escherichia coli</i>	+	+	++	+	-	-	-	+	+
2	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-
3	<i>Proteus vulgaris</i>	-	+	+	+	-	+	+	+	+
4	<i>Salmonella typhi</i>	+	+	++	+	-	-	-	+	+
5	<i>Staphylococcus aureus</i>	+	+	+	+	-	-	-	+	+
6	<i>Enterococcus faecalis</i>	-	+	+	+	-	-	-	+	-
7	<i>Klebsiella pneumonia</i>	-	-	+	+	-	-	-	+	-
8	<i>Bacillus subtilis</i>	+	++	++	++	-	-	-	+	-
9	<i>Bacillus cereus</i>	-	-	+	+	-	-	+	+	+
10	<i>Aspergillus niger</i>	-	+	+	+	-	-	-	-	-
11	<i>Aspergillus flavus</i>	-	+	+	+	-	-	-	-	-
12	<i>Aspergillus fumigatus</i>	-	-	+	+	-	-	-	-	-
13	<i>Penicillium digitatum</i>	-	+	+	+	-	-	-	+	-
14	<i>Candida albicans</i>	+	+	++	+	-	-	-	+	+
15	<i>Candida utilis</i>	+	+	++	+	-	-	-	+	+

He - n-Hexane, Ch - Chloroform, Ea - Ethyl acetate, Me - Methanol, Aq - Aqueous.

Disc diffusion results : ++ = ≥ 15 mm, + = ≥ 10 mm, - = ≤ 9 mm,

MIC results : + = ≤ 4.0 mg/ml, - = ≥ 5.0 mg/ml

Table 4: Comparison of antimicrobial activity and MIC of *in vivo* grown *Aloe vera* L. only gel extracts.

S.No.	Microorganisms (Pathogens)	Results of Disc Diffusion					Results of MIC			
		He	Ch	Ea	Me	Aq	He	Ch	Ea	Me
1	<i>Escherichia coli</i>	+	++	++	++	-	-	+	+	+
2	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-
3	<i>Proteus vulgaris</i>	+	+	+	+	-	+	+	+	+
4	<i>Salmonella typhi</i>	+	+	++	+	-	-	+	+	+
5	<i>Staphylococcus aureus</i>	+	+	+	+	-	+	+	+	+
6	<i>Enterococcus faecalis</i>	+	+	+	+	-	-	-	+	+
7	<i>Klebsiella pneumonia</i>	+	+	+	+	-	-	-	+	-
8	<i>Bacillus subtilis</i>	+	++	++	++	-	-	-	+	-
9	<i>Bacillus cereus</i>	+	+	++	+	-	+	+	+	+
10	<i>Aspergillus niger</i>	+	+	+	+	-	-	-	+	-
11	<i>Aspergillus flavus</i>	-	+	++	+	-	-	-	+	+

12	<i>Aspergillus fumigatus</i>	-	+	+	+	-	-	-	-	-
13	<i>Penicillium digitatum</i>	-	+	++	+	-	-	-	+	-
14	<i>Candida albicans</i>	+	+	++	++	-	-	+	+	+
15	<i>Candida utilis</i>	+	+	++	+	-	-	-	+	+

He - *n*-Hexane, Ch - Chloroform, Ea - Ethyl acetate, Me - Methanol, Aq - Aqueous.

Disc diffusion results : ++ = ≥ 15 mm, + = ≥ 10 mm, - = ≤ 9 mm,

MIC results : + = ≤ 4.0 mg/ml, - = ≥ 5.0 mg/ml

DISCUSSION

After comparing the results of disc diffusion method and MIC, ethyl acetate extract was found as strongest amongst all the extracts tested. Overall, it was concluded that *Aloe vera* L. leaf and inner gel is having potential antimicrobial components and specifically ethyl acetate extract of *Aloe vera* L. whole leaf and inner gel were having more effective antimicrobial properties as ethyl acetate extracts required the lesser concentrations to inhibit the pathogens followed by methanol, chloroform and *n*-hexane extracts, while aqueous extracts were found unable to inhibit any of the pathogens tested. The results of this study are in accordance with the findings of Aliero *et al.*, on *S. hyacinthoides*.^[5]

The outcomes of the present study also reflected the presence of potent phytochemicals in solvent extracts of whole leaf and inner gel of *Aloe vera* L. which could be responsible for its antimicrobial activity.^[6,7,8,9] Therefore, in view of these results, the ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad-spectrum antimicrobial potential of *Aloe vera* L. which makes the plant a candidate for bio-prospecting for antimicrobial drugs. Comparisons of the results between disc diffusion and MIC were also strongly supported that the different extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel had valuable antimicrobial properties. Only few variations in results between the disc diffusion and MIC results could be due to the different susceptibility of the bacterium to the plant extract, the rate of growth of bacteria, solvents used to extract the plant compounds and the rate of plant extract diffusion.^[10]

Therefore, several studies by different workers including results and observations of the current investigation strongly supports that the active components present in *Aloe vera* L. possesses strong antimicrobial potentials.

CONCLUSION

Comparisons of the results between disc diffusion and MIC were also strongly supported that the different extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel have valuable antimicrobial properties. Furthermore, the findings highlight that MIC provides a more precise quantitative evaluation, whereas disc diffusion offers a rapid qualitative assessment.

Overall, this comparative study supports the potential application of *Aloe vera* L. extracts in developing

alternative antimicrobial agents and emphasizes the importance of method selection in antimicrobial testing.

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