WJPMR, 2026, 12(7), 960-469

Research Article

Impact Factor: 7.533

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

ISSN (O): 2455-3301
ISSN (P): 3051-2557
Coden USA: WJPMBB

ASSESSMENT OF ANTIBACTERIAL ACTIVITIES OF *ALOE VERA* L. (*IN VIVO* & *IN VITRO* REGENERATED) WHOLE LEAF AND INNER GEL EXTRACTS

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DOI: https://doi.org/10.5281/zenodo.21155532

How to cite this Article: Neelofar Khanam*[1,2,3], G. K. Sharma[3] (2026). Assessment Of Antibacterial Activities Of Aloe Vera L. (In Vivo & In Vitro Regenerated) Whole Leaf And Inner Gel Extracts. World Journal of Pharmaceutical and Medical Research, 12(7), 960–469.

Article Received on 05/06/2026 **Article Revised on 25/06/2026** **Article Published on 03/07/2026**

ABSTRACT

The present study evaluates the antibacterial potential of *Aloe vera* L. whole leaf and inner gel extracts derived from both *in vivo grown* and *in vitro* regenerated plants. A range of clinically significant bacterial strains, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Bacillus cereus*, were selected to assess antimicrobial efficacy. Various solvent extracts (n-hexane, chloroform, ethyl acetate, methanol, and aqueous) were prepared and tested using the disc diffusion method, followed by determination of minimum inhibitory concentrations (MIC) through agar dilution. The results demonstrated that most solvent extracts exhibited significant antibacterial activity against the tested pathogens, whereas aqueous extracts showed no inhibitory effect. Among all extracts, ethyl acetate fractions displayed the highest antibacterial efficacy, producing larger zones of inhibition and lower MIC values, followed by methanol, chloroform, and n-hexane extracts. Notably, *in vitro* regenerated plant extracts, particularly inner gel, showed greater antibacterial activity compared to *in vivo* samples. *Pseudomonas aeruginosa* exhibited resistance to most extracts, while other pathogens showed varying degrees of sensitivity. The enhanced antibacterial activity may be attributed to the presence of bioactive phytochemicals such as flavonoids, alkaloids, phenolics, and glycosides. The findings indicate that *Aloe vera* L. possesses broad-spectrum antibacterial properties effective against both Gram-positive and Gram-negative bacteria. This study highlights the potential of *Aloe vera* L. as a natural source for developing alternative antimicrobial agents, particularly in the context of increasing antibiotic resistance.

KEYWORDS: *Aloe vera* L., Antibacterial activity, Phytochemical extracts, Minimum inhibitory concentration (MIC) and *In vitro* regeneration.

INTRODUCTION

In present investigation, number of bacterial strains of human health significance including *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Bacillus cereus* were tested against different solvent extracts of *Aloe vera* L. (both *in vivo* grown and *in vitro* regenerated) whole leaf and inner gel, at the same time positive and negative controls were also taken to cross check the obtained results.

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 50ºC 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^{o}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 (95ºC) distilled water. The infusion was left overnight under refrigeration (4ºC) 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 – 47.5ºC. The frozen extract was then freeze dried to a powder, weighed, transferred into separate vial and preserved at 4ºC 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 – 47.5ºC. The dried extracts thus obtained was weighed, transferred into separate vials and preserved at 4ºC for future analysis.

The antibacterial activity of different extracts of *Aloe vera* L. (both *in vivo* grown and *in vitro* regenerated) whole leaf and inner gel were tested against number of bacterial strains of human health significance including *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Bacillus cereus.* The bacterial stock cultures were maintained on Nutrient Agar (NA) and stored at 4^{o}C.

Determination of antibacterial potential of *Aloe vera* L.

Paper discs impregnated with specific antibiotics or the test substances were placed on the surface of the Muller Hinton Agar medium inoculated with the target organisms. The plates were incubated and the zones of inhibition around each disc were measured.

The medium was prepared by dissolving 33.9 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121ºC for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured onto 100 mm petriplates (25 ml/plate).

5% Dimethyl Sulphoxide (DMSO) was used as negative control, while Gentamycin and Tetracycline taken as standard positive controls and 0.5 McFarland opacity standard was also taken.

Assay

Antibacterial 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] All the bacterial strains were maintained on Nutrient Agar (NA). Pure culture of bacterial strains from the plate were inoculated into MHA plate and sub cultured at 37ºC for 24 hours.

The inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/l saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a microbial suspension of 1×10^6 CFU/ml. Standardized inoculum was transferred and spread evenly on MHA plates to yield a lawn culture. Sterile Whatmann No. 1 filter paper discs (5mm diameter) impregnated plant extracts (100 µg/disc) were placed on the inoculated MHA plates, and allowed to diffuse for half an hour at room temperature and then all the bacterial strains containing plates were incubated at 37ºC. Disc containing 5% DMSO served as negative control while Gentamycin and Tetracycline (100 µg/disc) served as positive control.

The plates were observed after 48h for the presence of inhibition of microbial growth that was indicated by the clear zone around the disc. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity.[4, 5] All experiments were carried out in triplicates under strict aseptic conditions. The activities were expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm.[6]

Determination of Minimum Inhibitory Concentrations (MIC)

MIC test was performed by Agar Dilution Method.[7] Different concentrations of chloroform, methanol, ethyl acetate, *n*-hexane and aqueous extracts of *Aloe vera* L. (both *in vivo* grown and *in vitro* regenerated) whole leaf and inner gel from 0.125 to 16 mg/ml (0.125, 0.250, 0.5, 1.0, 2.0, 4.0, 8.0 and 16 mg/ml) were prepared in DMSO which was then filtered through membrane filter. The extracts were mixed thoroughly with 14 ml of autoclaved MHA medium and poured into sterile petriplates and were allowed to solidify. Inoculum suspensions (10 µl) of various microbial isolates were seeded on individual agar plates of MHA medium. Growth control was prepared by inoculating 10 µl of each culture suspension on 15 ml MHA medium without any extract or solvent (drug-free medium). Solvent control was prepared by pouring 1ml of DMSO to 14 ml of MHA medium accordingly, followed by seeding of cultures. The plates were allowed to diffuse for 1h at room temperature and incubated at 37ºC for 48h. Minimum inhibitory concentration (MIC), was determined as the lowest concentration of antimicrobial agent that inhibited the visible growth of a microorganism after proper incubation.

Statistical analysis

All the analysis were carried out in triplicates and expressed as mean ± SD. Analysis of variance (ANOVA) were performed using the one-way analysis of variance. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

RESULTS

The antibacterial activity of different extracts including *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts of *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and inner gel were determined by disc diffusion assay. Subsequently the detection of minimum inhibitory concentrations (MIC) was also done in current study. The results found are explained as further.

Assessment of antibacterial activities

Antibacterial potentials were determined to study the efficacy of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel extracts. Total 9 clinically significant bacterial strains including *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Bacillus cereus* were used for this task. All the strains tested were showing measurable zone of inhibition.

As shown in the Table 1 – 4, the standard positive control depicted inhibition diameter ranging from 17.57 ± 1.42 mm to 25.05 ± 0.86 mm in case of Gentamycine 100 µg/disc while 14.33 ± 0.07 mm to 31.84 ± 0.19 mm in case of Tetracyclin 100 µg/disc, against the tested organisms.

All the extracts except aqueous extracts of whole leaf and inner gel samples were showing significant antibacterial activities in disc diffusion assay against most of the microbial genre tested. Among all the samples ethyl acetate extracts were showing the larger zone of inhibition compared to other extract, as shown in the Table 1 – 4. Methanol, Chloroform and then *n*-hexane extracts were also showing the significant inhibition zone against the pathogens tested while aqueous extracts were totally unable to inhibit any of the pathogens tested (Table 1 – 4).

Among all the bacterial strains tested with various extracts of different samples including whole leaf and inner gel extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. *Pseudomonas aeruginosa* was showing the resistance, while only one standard i.e. tetracycline was able to inhibit *Pseudomonas aeruginosa* showing inhibition zone of 14.33 ± 0.07 mm, whereas gentamycin was not showing any measurable inhibition zone against the *Pseudomonas aeruginosa* and *Enterococcus faecalis.* While *Enterococcus faecalis* was showing sensitivity against tetracycline (inhibition zone 19.23 ± 0.18) and all the extracts of *Aloe vera* L. whole leaf and inner gel as well. So that instead of *Pseudomonas aeruginosa* all pathogens tested including *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Bacillus cereus* were showing the significant sensitivity against standard antibiotics as well as all the extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel (Table 1 – 4).

In the present investigation all the pathogens tested were showing varying degree of inhibition with different extracts including *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel. Among all the extracts, ethyl acetate extracts were showing the strong inhibitory activity towards all the pathogens tested, in other words the ethyl acetate extracts were depicted the higher degree of antibacterial activities and when we compare among the different samples of *Aloe vera* L. including *in vitro* grown whole leaf and only gel as well as *in vivo* grown whole leaf and only gel, the *in vitro* regenerated only gel extracts were showing the highest degree of inhibition (Table 1 – 4).

Overall, from all the results (Table 1 – 4) it can be concluded that *Aloe vera* L. has a significant antimicrobial activity against the bacterial strains of human health significance and can be used as the antibacterial remedies in the preparation of antimicrobial drugs/medicines.

Minimum inhibitory concentrations (MIC)

MIC was determined for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts of leaves of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel samples, by Agar Dilution method (Table 5 – 8). Ethyl acetate extracts had lowest MIC, followed by methanol, chloroform and *n*-hexane extracts, whereas aqueous extracts were not tested for their MIC because they were unable to inhibit any of the pathogens tested in disc diffusion method done in initial part of this study.

For the samples of *in vitro* regenerated *Aloe vera* L. whole leaf extracts (Table 5), the *n*-hexane extract was showing MIC of 4.0 mg/ml. against *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cereus*. Whereas MIC of 8.0 mg/ml. against *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia* and

Bacillus subtilis. The chloroform extract showed MIC of 1.0 mg/ml. against *Proteus vulgaris* and *Bacillus cereus* was inhibited at MIC of 2.0 mg/ml. followed by *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* that showed MIC of 4.0 mg/ml. and *Enterococcus faecalis, Klebsiella pneumonia* and *Bacillus subtilis* were inhibited at 8.0 mg/ml. in chloroform extract of *in vitro* regenerated *Aloe vera* L. whole leaf samples. The ethyl acetate extract was showing MIC of 0.5 mg/ml. for *Proteus vulgaris*. *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cerius* were inhibited at a MIC of 2.0 mg/ml. MIC of 4.0 mg/ml. were detected against *Klebsiella pneumonia* and *Bacillus subtilis* in ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. whole leaf samples. Methanol extract of *in vitro* regenerated *Aloe vera* L. whole leaf samples were showing the MIC of 0.5 mg/ml. against *Proteus vulgaris*, while *Staphylococcus aureus*, *Enterococcus fecalis* and *Bacillus cereus* were inhibited at MIC of 2.0 mg/ml. Growth of *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis* were inhibited at MIC of 4.0 mg/ml., followed by *Klebsiella pneumonia* which was inhibited at the MIC of 8.0mg/ml (Table 5).

As shown in Table 6, among the samples of *in vitro* regenerated *Aloe vera* L. only gel extracts examined, the MIC of *n*-hexane extract was found 2.0 mg/ml. against the growth of *Proteus vulgaris*. The growth of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Bacillus cereus* were inhibited at the MIC of 4.0 mg/ml. *Proteus vulgaris* were found to inhibit at MIC of 0.5 mg/ml. with chloroform extract. Chloroform extract of *in vitro* regenerated *Aloe vera* L. only gel samples were showing the MIC of 1.0 mg/ml. against the growth of *Escherichia coli* followed by the MIC of 2.0 mg/ml. against *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cereus*. The growth of *Enterococcus faecalis*, *Klebsiella pneumonia* and *Bacillus subtilis* were found to inhibit at the MIC of 4.0 mg/ml. Growth of *Proteus vulgaris* was inhibited at MIC of 0.250 mg/ml., followed by *Escherichia coli* that showed MIC of 0.5 mg/ml. *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cerius* were inhibited at 1.0 mg/ml in ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. only gel samples. MIC of 2.0 mg/ml. was found against the *Enterococcus faecalis*, *Klebsiella pneumonia* and *Bacillus subtilis*. Methanol extracts were found to inhibit the growth of *Escherichia coli* and *Proteus vulgaris* those were inhibited at the MIC of 0.5mg/ml. At the same time MIC of 1.0 mg/ml. was found to inhibit the growth of *Bacillus cereus* followed by MIC of 2.0 mg/ml. which was found to prevent the growth of *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis*. The growth of *Klebsiella pneumonia* was found to inhibit at the MIC of 4.0 mg/ml. with methanol extract of *in vitro* regenerated *Aloe vera* L. only gel extract (Table 6).

As shown in the Table 7, *n*-hexane extract of *in vivo* grown *Aloe vera* L. whole leaf extract was depicted the MIC of 4.0 mg/ml. against *Proteus vulgaris*, followed by MIC of 8.0mg/ml. against the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cerius*. The growth of *Salmonella typhi*, *Enterococcus faecalis* and *Klebsiella pneumonia* were found to inhibit at the MIC of 16.0 mg/ml. The chloroform extract of *in vivo* grown *Aloe vera* L. whole leaf samples were detected to inhibit the growth of *Proteus vulgaris* at the MIC of 2.0 mg/ml., followed by *Bacillus cereus* which was inhibited at the MIC of 4.0 mg/ml. The growth of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis* were found to inhibit at the MIC of 8.0 mg/ml., whereas *Klebsiella pneumonia* was found to inhibit at the MIC of 16.0 mg/ml. Ethyl acetate extract of *in vivo* grown *Aloe vera* L. whole leaf samples were showing the MIC of 1.0 mg/ml. against *Proteus vulgaris* & 2.0 mg/ml against *Salmonella typhi*. *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtillis* and *Bacillus cerius* were inhibited at MIC of 4.0 mg/ml in ethyl acetate extract of *in vivo* grown *Aloe vera* L. whole leaf samples. Methanol extract of *in vivo* grown *Aloe vera* L. whole leaf samples were found to show MIC of 1.0 mg/ml. against the growth of *Proteus vulgaris*, followed by MIC of 4.0 mg/ml. against the growth of *Escherechia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cerius*. The growth of *Enterococcus faecalis*, *Klebsiella pneumonia* and *Bacillus subtilis* were found to inhibit at MIC of 8.0 mg/ml (Table 7).

Result in Table 8 has shown that among the samples of *in vivo* grown *Aloe vera* L. only gel, *n*-hexane extract extract were depicted the MIC of 4.0 mg/ml. against the growth of *Proteus vulgaris*, *Staphylococcus aureus* and *Bacillus cereus* followed by the MIC of 8.0 mg/ml. against the growth of *Escherichia coli*, *Salmonella typhi*, *Enterococcus faecalis*, *Klebsiella pneumonia* and *Bacillus subtilis*. The chloroform extracts of *in vivo* grown *Aloe vera* L. only gel samples were showing the MIC of 2.0 mg/ml. against *Proteus vulgaris*, followed by the MIC of 4.0 mg/ml. against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cereus*. At the same time MIC of 8.0 mg/ml. was found to prevent the growth of *Enterococcus faecalis*, *Klebsiella pneumonia* and *Bacillus subtilis*. Ethyl acetate extract of *in vivo* grown *Aloe vera* L. only gel inhibited *Protius vulgaris* at MIC of 1.0 mg/ml., while *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cerius* at MIC of 2.0 mg/ml. *Enterococcus faecalis*, *Klebsiella pneumonia* and *Bacillus subtilis* were inhibited at MIC of 4.0 mg/ml. Simultaneously methanol extract of *in vivo* grown *Aloe vera* L. only gel samples were found to show MIC of 1.0 mg/ml. to prevent the growth of *Proteus vulagaris* followed by the MIC of 2.0 mg/ml. against the growth of *Bacillus cereus*. The growth of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Enterococcus faecalis* were found to inhibit at the MIC of 4.0 mg/ml. Subsequently the growth of *Klebsiella pneumonia* and *Bacillus subtilis* were found to inhibit at the MIC of 8.0 mg/ml (Table 8).

Among all the bacterial pathogens of human health significance tested the *Pseudomonas aeruginosa* was not examined for its minimum inhibitory concentration because it was not showing any inhibition zone when tested for its antibacterial activity against any of the extracts studied including *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel samples. Instead of *Pseudomonas aerugenosa* all the pathogens of clinical significance tested including *Escherechia coli*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Bacillus cereus* were showing their minimum inhibitory concentrations (MIC) against serial 2-fold dilution prepared from the *in vivo* grown and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel samples. These results depicted that *Aloe vera* L. has a good quality of antibacterial activities and can be used in the preparation of antimicrobial agents.

Table 1: Antibacterial activity of *in vitro* regenerated *Aloe vera* L. whole leaf extracts by disc diffusion method (zone of inhibition in mm at 100 µg/disc)

S.No.	Microorganisms (Pathogens)	*n*-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous	Gentamycin	Tetracycline
1	*Escherichia coli*	15.28 ± 0.47*	17.81 ± 0.90	21.49 ± 1.87	18.06 ± 0.34	NI	25.05 ± 0.86	31.84 ± 0.19
2	*Pseudomonas aeruginosa*	NI	NI	NI	NI	NI	NI	14.33 ± 0.07
3	*Proteus vulgaris*	10.82 ± 1.24	12.63 ± 0.18	16.20 ± 0.06	13.28 ± 1.63	NI	17.57 ± 1.42	21.42 ± 0.26
4	*Salmonella typhi*	11.06 ± 0.91	14.50 ± 1.02	17.54 ± 1.46	15.02 ± 0.10	NI	20.35 ± 0.60	22.10 ± 0.74
5	*Staphylococcus aureus*	10.14 ± 0.57	12.74 ± 0.36	15.62 ± 0.08	14.73 ± 0.24	NI	23.08 ± 1.12	25.10 ± 0.43
6	*Enterococcus faecalis*	10.70 ± 0.25	13.57 ± 0.49	16.02 ± 0.86	14.34 ± 0.21	NI	NI	19.23 ± 0.18
7	*Klebsiella pneumonia*	09.33 ± 1.42	11.38 ± 0.50	14.87 ± 0.16	12.29 ± 0.83	NI	24.72 ± 0.36	26.04 ± 0.25
8	*Bacillus subtilis*	13.58 ± 0.20	17.23 ± 0.57	21.34 ± 1.49	18.76 ± 0.36	NI	23.28 ± 0.03	28.05 ± 1.52
9	*Bacillus cereus*	10.19 ± 0.24	11.36 ± 0.08	17.54 ± 0.90	14.28 ± 0.33	NI	20.42 ± 0.37	24.60 ± 0.91

The values are given as Mean ± SD of triplicates.
The concentration of extracts used was 100 µg/disc.
* Inhibitory zone in mm, including diameter of disc (5.0 mm)
NI - No Inhibition.

Table 2: Antibacterial activity of *in vitro* regenerated *Aloe vera* L. only gel extracts by disc diffusion method (zone of inhibition in mm at 100 µg/disc).

S.No.	Microorganisms (Pathogens)	*n*-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous	Gentamycin	Tetracycline
1	*Escherichia coli*	18.73 ± 0.30*	20.24 ± 0.52	25.40 ± 1.54	20.80 ± 0.02	NI	25.05 ± 0.86	31.84 ± 0.19
2	*Pseudomonas aeruginosa*	NI	NI	NI	NI	NI	NI	14.33 ± 0.07
3	*Proteus vulgaris*	12.34 ± 0.14	15.58 ± 1.36	19.36 ± 0.93	16.57 ± 0.42	NI	17.57 ± 1.42	21.42 ± 0.26
4	*Salmonella typhi*	14.59 ± 0.24	17.20 ± 0.12	21.43 ± 0.82	18.36 ± 0.33	NI	20.35 ± 0.60	22.10 ± 0.74
5	*Staphylococcus aureus*	13.62 ± 0.19	16.04 ± 0.52	19.33 ± 0.36	18.24 ± 0.04	NI	23.08 ± 1.12	25.10 ± 0.43
6	*Enterococcus faecalis*	13.83 ± 1.56	16.36 ± 0.02	20.25 ± 0.83	17.47 ± 0.19	NI	NI	19.23 ± 0.18
7	*Klebsiella pneumonia*	11.18 ± 0.32	13.52 ± 0.26	18.72 ± 1.20	16.87 ± 0.18	NI	24.72 ± 0.36	26.04 ± 0.25
8	*Bacillus subtilis*	15.26 ± 0.28	20.43 ± 0.63	25.32 ± 0.52	21.56 ± 0.12	NI	23.28 ± 0.03	28.05 ± 1.52
9	*Bacillus cereus*	13.82 ± 0.39	14.07 ± 0.27	22.25 ± 0.63	18.63 ± 1.04	NI	20.42 ± 0.37	24.60 ± 0.91

The values are given as Mean ± SD of triplicates
The concentration of extracts used was 100 µg/disc
* Inhibitory zone in mm, including diameter of disc (5.0 mm)
NI - No Inhibition

Table 3: Antibacterial activity of *in vivo* grown *Aloe vera* L. whole leaf extracts by disc diffusion method (zone of inhibition in mm at 100 µg/disc).

S.No.	Microorganisms (Pathogens)	*n*-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous	Gentamycin	Tetracycline
1	*Escherichia coli*	11.49 ± 1.82*	13.57 ± 0.42	18.15 ± 0.05	14.03 ± 0.56	NI	25.05 ± 0.86	31.84 ± 0.19
2	*Pseudomonas aeruginosa*	NI	NI	NI	NI	NI	NI	14.33 ± 0.07
3	*Proteus vulgaris*	08.65 ± 0.28	10.18 ± 0.57	13.24 ± 1.35	11.48 ± 0.24	NI	17.57 ± 1.42	21.42 ± 0.26
4	*Salmonella typhi*	09.50 ± 0.17	10.34 ± 1.12	15.62 ± 0.30	12.55 ± 0.38	NI	20.35 ± 0.60	22.10 ± 0.74
5	*Staphylococcus aureus*	09.04 ± 0.24	11.47 ± 0.26	12.31 ± 0.52	11.25 ± 0.63	NI	23.08 ± 1.12	25.10 ± 0.43
6	*Enterococcus faecalis*	07.43 ± 0.61	10.38 ± 0.19	12.57 ± 0.60	10.33 ± 0.42	NI	NI	19.23 ± 0.18
7	*Klebsiella pneumonia*	07.28 ± 0.39	07.62 ± 0.46	10.39 ± 0.56	09.16 ± 1.29	NI	24.72 ± 0.36	26.04 ± 0.25
8	*Bacillus subtilis*	11.06 ± 0.91	15.53 ± 0.24	18.72 ± 0.36	16.29 ± 0.20	NI	23.28 ± 0.03	28.05 ± 1.52
9	*Bacillus cereus*	07.35 ± 0.33	08.20 ± 0.53	14.54 ± 1.06	11.55 ± 0.82	NI	20.42 ± 0.37	24.60 ± 0.91

The values are given as Mean ± SD of triplicates
The concentration of extracts used was 100 µg/disc
* Inhibitory zone in mm, including diameter of disc (5.0 mm)
NI - No Inhibition

Table 4: Antibacterial activity of *in vivo* grown *Aloe vera* L. only gel extracts by disc diffusion method (zone of inhibition in mm at 100 µg/disc).

S.No.	Microorganisms (Pathogens)	*n*-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous	Gentamycin	Tetracycline
1	*Escherichia coli*	13.54 ± 0.24*	15.39 ± 0.86	20.65 ± 0.28	16.56 ± 0.04	NI	25.05 ± 0.86	31.84 ± 0.19
2	*Pseudomonas aeruginosa*	NI	NI	NI	NI	NI	NI	14.33 ± 0.07
3	*Proteus vulgaris*	09.08 ± 0.18	11.42 ± 0.07	14.36 ± 0.33	12.24 ± 0.29	NI	17.57 ± 1.42	21.42 ± 0.26
4	*Salmonella typhi*	10.20 ± 0.12	12.74 ± 0.36	16.55 ± 0.82	14.91 ± 1.48	NI	20.35 ± 0.60	22.10 ± 0.74
5	*Staphylococcus aureus*	10.03 ± 1.57	12.26 ± 0.74	14.62 ± 0.12	13.47 ± 0.06	NI	23.08 ± 1.12	25.10 ± 0.43
6	*Enterococcus faecalis*	09.27 ± 0.75	12.86 ± 0.24	13.43 ± 0.39	12.33 ± 0.54	NI	NI	19.23 ± 0.18
7	*Klebsiella pneumonia*	07.48 ± 0.02	09.20 ± 0.29	12.57 ± 0.52	10.04 ± 0.51	NI	24.72 ± 0.36	26.04 ± 0.25
8	*Bacillus subtilis*	12.57 ± 0.20	16.26 ± 0.38	20.33 ± 0.72	17.29 ± 0.83	NI	23.28 ± 0.03	28.05 ± 1.52
9	*Bacillus cereus*	08.02 ± 0.30	09.50 ± 0.18	15.85 ± 0.22	12.06 ± 0.35	NI	20.42 ± 0.37	24.60 ± 0.91

The values are given as Mean ± SD of triplicates
The concentration of extracts used was 100 µg/disc
* Inhibitory zone in mm, including diameter of disc (5.0 mm)
NI - No Inhibition

Table 5: Minimum inhibitory concentration (MIC) of *in vitro* regenerated *Aloe vera* L. whole leaf extracts.

S.No.	Microorganisms (Pathogens)	MIC (mg/ml.)				
		n-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous
1	*Escherichia coli*	8.0	4.0	2.0	4.0	NT
2	*Pseudomonas aeruginosa*	NT	NT	NT	NT	NT
3	*Proteus vulgaris*	4.0	1.0	0.5	0.5	NT
4	*Salmonella typhi*	4.0	4.0	2.0	4.0	NT
5	*Staphylococcus aureus*	4.0	4.0	2.0	2.0	NT
6	*Enterococcus faecalis*	8.0	8.0	2.0	2.0	NT
7	*Klebsiella pneumonia*	8.0	8.0	4.0	8.0	NT
8	*Bacillus subtilis*	8.0	8.0	4.0	4.0	NT
9	*Bacillus cereus*	4.0	2.0	2.0	2.0	NT

NT - Not Tested

Table 6: Minimum inhibitory concentration (MIC) of *in vitro* regenerated *Aloe vera* L. only gel extracts.

S.No.	Microorganisms (Pathogens)	MIC (mg/ml.)				
		***n*-Hexane**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Aqueous**
1	*Escherichia coli*	4.0	1.0	0.5	0.5	NT
2	*Pseudomonas aeruginosa*	NT	NT	NT	NT	NT
3	*Proteus vulgaris*	2.0	0.5	0.250	0.5	NT
4	*Salmonella typhi*	4.0	2.0	1.0	2.0	NT
5	*Staphylococcus aureus*	4.0	2.0	1.0	2.0	NT
6	*Enterococcus faecalis*	4.0	4.0	2.0	2.0	NT
7	*Klebsiella pneumonia*	4.0	4.0	2.0	4.0	NT
8	*Bacillus subtilis*	4.0	4.0	2.0	2.0	NT
9	*Bacillus cereus*	4.0	2.0	1.0	1.0	NT

NT - Not Tested

Table 7: Minimum inhibitory concentration (MIC) of *in vivo* grown *Aloe vera* L. whole leaf extracts.

S.No.	Microorganisms (Pathogens)	MIC (mg/ml.)				
		***n*-Hexane**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Aqueous**
1	*Escherichia coli*	8.0	8.0	4.0	4.0	NT
2	*Pseudomonas aeruginosa*	NT	NT	NT	NT	NT
3	*Proteus vulgaris*	4.0	2.0	1.0	1.0	NT
4	*Salmonella typhi*	16.0	8.0	2.0	4.0	NT
5	*Staphylococcus aureus*	8.0	8.0	4.0	4.0	NT
6	*Enterococcus faecalis*	16.0	8.0	4.0	8.0	NT
7	*Klebsiella pneumonia*	16.0	16.0	4.0	8.0	NT
8	*Bacillus subtilis*	8.0	8.0	4.0	8.0	NT
9	*Bacillus cereus*	8.0	4.0	4.0	4.0	NT

NT - Not Tested

Table 8: Minimum inhibitory concentration (MIC) of *in vivo* grown *Aloe vera* L. only gel extracts.

S.No.	Microorganisms (Pathogens)	MIC (mg/ml.)				
		***n*-Hexane**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Aqueous**
1	*Escherichia coli*	8.0	4.0	2.0	4.0	NT
2	*Pseudomonas aeruginosa*	NT	NT	NT	NT	NT
3	*Proteus vulgaris*	4.0	2.0	1.0	1.0	NT
4	*Salmonella typhi*	8.0	4.0	2.0	4.0	NT
5	*Staphylococcus aureus*	4.0	4.0	2.0	4.0	NT
6	*Enterococcus faecalis*	8.0	8.0	4.0	4.0	NT
7	*Klebsiella pneumonia*	8.0	8.0	4.0	8.0	NT
8	*Bacillus subtilis*	8.0	8.0	4.0	8.0	NT
9	*Bacillus cereus*	4.0	4.0	2.0	2.0	NT

NT - Not Tested

DISCUSSION

Interestingly, this study recorded a notable susceptibility of the resistant strains, to all the extracts including whole leaf and inner gel extracts from *in vitro* and *in vivo* grown *Aloe vera* L., suggesting that components contained in that particular extracts may provide an alternate strategy for combating these organisms and could improve treatment of infections caused by these organisms. Many previous studies reported the inability of natural antimicrobial agents to inhibit growth of Gram-negative bacteria[8,9], perhaps, because of the presence of complex cell wall structure that usually reduces penetration of bacterial cells by extracts. The remarkable findings of this study are that *Aloe vera* L. extracts are equally effective against both Gram-positive and Gram-negative bacteria.

Gel extracts seemed to contain higher amounts antibacterial components as compared to whole leaf extracts. When compared between *in vitro* regenerated *Aloe vera* L. and *in vivo* grown *Aloe vera* L., it was found that *in vitro* regenerated plants were showing greater antibacterial activities. Shin, *et al*., recently demonstrated that phenolic compounds, in addition to isothiocyanates, could be responsible for antibacterial activity of wasabi.[10]

The antibacterial effect of the plant extracts of *Aloe vera* L. may be due the presence of different phytochemicals such as alkaloids, phytosterols, steroids, glycosides, flavonoids and phenolics.[11,12] Karou *et al*., have reported that the alkaloids present in *Sida acuta* showed antimicrobial activity against the tested microorganisms.[13]

Quinlan, *et al.*, worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates.[14] Flavonoids, another constituent of *Aloe vera* L. leaf extracts shown to exhibit a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties.[15]

All the solvent extracts were showing the different degree of inhibition against all the bacterial strains tested. This study also included *E. faecalis* which is resistant to penicillin and streptomycin and *P.aeruginosa* resistant to penicillin, because these opportunistic bacteria can cause life threatening infections in humans, especially, in a nosocomial environment.[16, 17] While *E. faecalis* showed the significant sensitivity against *Aloe vera* L. extracts in the current study. Some workers used norfloxacin and tetracycline as a positive control because it inhibits the growth of both Gram-positive and Gram-negative bacteria.[18,19] The negative control, dimethylsulphoxide (DMSO), is a colourless liquid and an important polar solvent which dissolves both polar and non-polar compounds from a plant. It is miscible in a wide range of organic solvents including water. It has the distinctive property of penetrating the human skin very readily. In the present study 5% DMSO were used as negative control, which was found unable to inhibit any pathogenic strain tested.

Shahzad, *et al*., used Streptomycin and Gentamycin, as reference standard antibiotics along with the different preparations of *Aloe vera* gel to test the antimicrobial activities. They found that all the four preparations of *Aloe vera* gel exhibited reasonably good inhibitory activities compared with the standard reference antibiotics.[20]

Subramanian, *et al.*, also observed remarkable antibacterial activities with ethanolic extracts of *Aloe vera* gel even at low concentrations compared with the standard antibiotics and support the view that *Aloe vera* is a potent antimicrobial agent compared with the conventional antibiotics.[21] The results of the study by Coopoosamy and Magwa also revealed that lowest concentrations of ethyl acetate and ethanol crude extracts of *Aloe excelsa* resulted in complete inhibition of visible growth of pathogenic bacteria compared with the control antibiotics, chloramphenicol and streptomycin sulphate.[22] The poor activity of the aqueous extract against most microbial strains investigated in this study is in accordance with the earlier studies of.[23] Since water can dissolve polar compounds, due to the insolubility of the active compounds in water or hot water and denaturation of the active compounds during extraction process could be the reasons for the lower activity of the aqueous extracts.[24, 25, 26]

It is also observed from the results that the ethyl acetate and methanol extracts had wide antibacterial activity against both Gram positive and Gram-negative bacteria. This may be due to the capability of ethyl acetate and methanol to dissolve both polar and non-polar compounds. The activity of the extracts against the Gram-negative bacteria is noteworthy as these bacteria are known to exhibit high degree of resistance to conventional antibiotics.[27, 28, 25]

MIC results obtained in the present study showed that ethyl acetate extracts of *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and inner gel had greater antibacterial activities, 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.*[28]

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.[29, 30, 31, 32] Therefore, in view of these results, the ability of the extracts to inhibit the growth of several bacterial species is an indication of the broad-spectrum antibacterial potential of *Aloe vera* L. which makes the plant a candidate for bio-prospecting for antibacterial drugs.

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 antibacterial potentials.

CONCLUSION

Assessment of antibacterial activities of *in vivo* and *in vitro* regenerated *Aloe vera* L. leaf gel and whole leaf extracts was attempted in current investigation. The antibacterial activity of different extracts of *Aloe vera* L. (both *in vivo* grown and *in vitro* regenerated) whole leaf and inner gel were tested against number of clinically important bacterial strains related to human health including *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis, Bacillus cereus* by disc diffusion assay. Ethyle acetate extracts were found to be more potent in inhibiting all the bacterial strains tested, other extracts including methanol, chloroform and *n*-Hexane were also showing the significant zone of inhibition except the aqueous extract, which was unable to inhibit any bacterial strain. Detection of minimum inhibitory concentrations (MIC) was also done. MIC results obtained in the present study showed that ethyl acetate extracts of *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and inner gel had greater antibacterial activities, as ethyl acetate extracts required the lesser concentrations to inhibit the bacterial strains followed by methanol, chloroform and *n*-hexane extracts, while aqueous extracts were found unable to inhibit any of the bacterial strains tested. Thats why it can be concluded that *Aloe* *vera* L. has a significant potential against bacterial strains of human health significance.

ACKNOWLEDGEMENT
Authors are highly thankful to Dr. B. R. Singh, Principal Scientist, IVRI, Izzatnagar Bareilly (U.P.) India, for his kind co-operation and skilful guidance and also grateful to IFTM University, Moradabad (U.P.) India and Hindu College, Moradabad (U.P.) India.

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