

**ORIGINAL ARTICLE**DOI: <https://doi.org/10.3329/mediscope.v7i2.49444>**Antibacterial effect of Dimethyl sulfoxide extract of *Aloe vera* (*Aloe barbadensis*) leaf gel against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae******SD Haque¹, AMM Al-Amin², B Islam³, N Nazneen⁴, SN Karim⁵, MA Rahman⁶****Abstract**

An exploratory study based on laboratory experiment was carried out to determine the antibacterial effect of Dimethyl sulfoxide (DMSO) extract of *Aloe vera* leaf gel (DAE) against standard strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* in the Department of Pharmacology & Therapeutics in collaboration with the Department of Microbiology, Mymensingh Medical College, Mymensingh, Bangladesh. DMSO extract was used in five different concentrations (100, 200, 300, 400 and 500 µg/ml). Dose dependent inhibitory effect was seen against the test organisms using disc diffusion method. Zone of inhibition (ZOI) were 8 mm, 13 mm, 15 mm, 16 mm and 21 mm against *S. aureus*; 0 mm, 8 mm, 13 mm, 15 mm and 18 mm against *P. aeruginosa*; 8 mm, 11 mm, 13 mm, 16 mm and 20 mm against *E. coli*; 0 mm, 9 mm, 12 mm, 14 mm and 18 mm against *K. pneumoniae* at 100, 200, 300, 400 and 500 µg/ml respectively. The minimum inhibitory concentration (MIC) was assessed by broth dilution technique. The MICs of DAE for *S. aureus*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* were 300 µg/ml, 400 µg/ml, 400 µg/ml and 450 µg/ml respectively. From the study it was observed that DMSO extract of *Aloe vera* leaf gel possesses antibacterial effect against the test pathogens. The findings highlight the need for further extensive study to detect and isolate the biologically active ingredients present in the *Aloe vera* leaves which are responsible for antibacterial effect. Hopefully, that would lead to the discovery of new and more potent antimicrobial agents originated from *Aloe vera*.

Keywords: *Aloe vera*, Antibacterial effect, DMSO extract.

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Introduction

Microorganisms such as bacteria, fungi and virus have the ability to cause life-threatening infections in human beings all over the world. Diseases due to the pathogenic bacteria represent a critical problem to human health and safety. Antimicrobial resistance is a serious threat to mankind because many of the infection causing bacteria have become multidrug resistant. The resistance developed by the bacteria to various drugs restricts the choice of antibiotics for therapy. It is of utmost importance to search for new infection fighting strategies.¹ For centuries, medicinal plants have been used in the traditional system of medicine in the curing of various types of diseases. Different species of medicinal plants have been analyzed for biologically active ingredients known to have pharmacological properties and many of the studied plants have shown antimicrobial property.² The use of plant extracts, with known antimicrobial properties, can be of great significance in the treatment of various microbial infections.³

Aloe vera (*Aloe barbadensis miller*) is a plant, which belongs to the Liliaceae family and is mostly succulent having a whorl of elongated, pointed leaves. The name is derived from the Arabic word 'Alloeh' which means 'bitter'. It refers to the bitter taste of the liquid contained in the leaves. Modern clinical use of Aloe vera began in the 1920s. Since then, numerous researches have been conducted regarding its significant therapeutic potentials when used both topically and parenterally.⁴ Aloe vera is a stem less or sometimes very short-stemmed succulent plant growing up to 60-100 cm tall. The green leaves are thick and fleshy with some varieties showing white flecks on the upper and lower stem surfaces.⁵ When the green skin of a leaf is removed, a clear mucilaginous substance is seen that contains fibers, water and also the ingredient to retain the water in the leaf. The gel of Aloe vera is contained in the leaves. The gel contains 99.3% of water, the remaining 0.7%

is made up of solids with carbohydrates constituting for a large part of it.³ Aloe vera has been described as an antibacterial agent. Aloe vera gel has anthraquinones as an active compound, which is structural analogue of tetracycline. The anthraquinones act like tetracycline which inhibits bacterial protein synthesis by blocking the ribosomal A site (where the aminoacylated tRNA enters). Therefore, the bacteria cannot grow in the media containing Aloe vera extract. Polysaccharides of Aloe vera gel have been attributed direct antibacterial activity through the stimulation of phagocytic leucocytes to destroy bacteria.⁶

Several studies have indicated the effectiveness of Aloe vera gel against both gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus subtilis* etc.^{3,4,5} Therefore, the aim of the present study is to evaluate the antibacterial effects of Ethanolic extract of Aloe vera leaf gel. This study may be helpful to emphasize the importance of natural products to control antibiotic resistant bacteria, which have been a major threat to human health.

Materials and methods

This laboratory based exploratory study was carried out in the Department of Pharmacology & Therapeutics in collaboration with the Department of Microbiology, Mymensingh Medical College, Mymensingh, during the period from July 2017 to June 2018.

Materials required

- Aloe vera leaves were bought from local market of Mymensingh.
- Test organisms: Reference strains viz., *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 13883) were collected from Department of Microbiology,

Mymensingh Medical College, Mymensingh.

Extraction of aloe gel:

Mature, healthy and fresh leaves of Aloe vera were bought from local market of Mymensingh city. The leaves were washed out in running tap water for 5 minutes and rinsed with distilled water (D/W). After that they were dissected longitudinally and the colourless parenchymatous tissue (aloe gel) was scraped out without the fibres using a sterile knife.

Inoculation of bacterial cultures on nutrient medium:

- H Pure cultures were maintained on nutrient agar plates regularly.
- H Inoculum from the primary culture plate was prepared by touching the top of the colony of the test organism with a sterile wire loop.
- H The loop was streaked all over the surface of the medium three times, rotating the plate through an angle of 60°C after each application. Finally, the loop was passed round the edge of the agar surface.
- H The cultures were kept in incubator for 24 hours at 37°C and stored at 4°C.

Preparation of Bacterial Suspension:

For each of the test organisms the separate inoculums were prepared in the following ways. 3-5 similar colonies from 18-24 hours old nutrient agar plates were picked up with sterile wire loop and mixed with sterile normal saline. The turbidity was adjusted with 0.5 McFarland turbidity standards by adding saline or colony to the suspension as required. The turbidity of 0.5 McFarland standards corresponds to 1.5×10^8 organisms/ml.

Preparation of DMSO stock solution:

1 gm of DMSO extract powder was dissolved in 10 ml of distilled water (D/W) to get a concentration of 0.1 gm i.e. 100 mg/ml which was labeled as stock solution-1. Again from the above solution 1 ml was taken and dissolved in 99 ml distilled water to get a

concentration of 1mg/ml which was labeled as stock solution-2. From the above stock solution-2, different concentrations such as 100, 200, 300,400 and 500 µg/ml were prepared.

Calculation:

1 ml of stock solution-2 was mixed with 9 ml of D/W. So, the total amount is 10 ml.

This 10 ml solution contains 1000 µg extract (as described before).

So, 1 ml of this solution contains $(1000 \div 10)$ µg = 100 µg.

So, the final concentration is 100 µg/ml.

Similar procedure was applied to prepare working solutions having concentrations of 200, 300, 400 and 500 µg/ml. This calculation is given below in tabulated form:

Table 01: Preparation of working solutions containing DMSO Aloe vera extract (DAE)

Sl. No.	Amount of stock solution-2 (ml)	Amount of D/W (ml)	Final concentration (µg/ml)
1.	1	9	100
2.	2	8	200
3.	3	7	300
4.	4	6	400
5.	5	5	500

Antibacterial sensitivity test by disc diffusion method:

Antibacterial sensitivity test was performed by Modified Kirby-Bauer disc diffusion technique as following. After matching with 0.5 McFarland standards for each isolates, a sterile cotton swab was dipped into bacterial suspension and streaked in three directions on the surface of Mueller-Hinton agar plates and then left for 5-10 minutes in room temperature. By using sterile forceps the blank paper discs (6 mm in diameter) were placed on the surface of the plates. Then with the help of micropipette 5 µl amount of different concentrations of DAE were put over the

blank discs and left for five minutes. Then the plates were incubated at 37°C for 24 hours. After that zone of inhibition for respective organisms were measured in mm by using ruler.

Determination of the Minimum Inhibitory Concentration (MIC):

The minimum inhibitory concentration (MIC) is the concentration giving the least inhibitory activity and below which there is no further inhibition. The MIC of the DMSO extract was obtained by dissolving various volume of DAE stock solution-2 (1 mg/ml or 1000 µg/ml) in various volume of nutrient broth.

Preparation of different concentrations of working EAE solution:

Set-I: Working solution was made by mixing 9 ml of DAE stock solution-2 with 1 ml of Nutrient broth (N/B). So the total amount became 10 ml. As stated previously, 1 ml of DAE stock solution-2 contains 1 mg extract.

So, 9 ml of DAE stock solution-2 contains (1×9) mg = 9 mg or (9×1000) µg = 9000 µg

stock solution-2 + 1 ml N/B) of the preparation contain = 9000 µg of DAE.

So, 1 ml of the preparation contains (9000÷10) µg = 900 µg DAE.

The final concentration is 900 µg/ml.

Then Sets-II, III, IV, V, VI, VII, VIII and IX respectively were made in different test tubes by mixing measured amount of DAE stock solution-2 with measured amount of nutrient broth medium. The concentrations of these sets were 800 µg/ml, 700 µg/ml, 600 µg/ml, 500 µg/ml, 400 µg/ml, 300 µg/ml, 200 µg/ml and 100 µg/ml DAE respectively (Table 02).

Three types of controls were also used.

Control-I was made with 10 ml of DAE stock solution-2 (inoculated with bacterial suspension) in test tubes.

Control-2 was made with 10 ml of nutrient broth medium (inoculated with bacterial suspension) in test tubes.

Control-3 was made with 10 ml of nutrient broth medium (not inoculated with bacterial suspension) in test tubes (Table 02).

Table 02: Composition and different concentrations of working DAE solutions and the controls.

No. of Sets	DAE stock solution-2 (ml)	Nutrient broth Medium (ml)	Total (ml)	Concentration of DAE (µg/ml)	Test Organism (µl)
Set-I	9	1	10	900	20
Set-II	8	2	10	800	20
Set-III	7	3	10	700	20
Set-IV	6	4	10	600	20
Set-V	5	5	10	500	20
Set-VI	4	6	10	400	20
Set-VII	3	7	10	300	20
Set-VIII	2	8	10	200	20
Set-IX	1	9	10	100	20
Set-X (C-1)	10	0	10	1000	20
Set-XI (C-2)	-	10	10	-	20
Set-XII (C-3)	-	10	10	-	-

extract. Which means, total 10 ml (9 ml DAE

Inoculation of bacterial suspension in test

tubes:

After matching the turbidity of bacterial suspension with 0.5 McFarland standards, 20 µl or one drop (0.02 ml) of bacterial suspension of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* were separately added with different concentrations of working DAE in separate test tubes. These inoculums were also added to the Control-1 & 2, but not to Control-3.

Examination of growth of test organisms in different concentrations of DAE:

After 18 to 24 hours of incubation at 37 °C, the growth of test organisms in different preparations of DAE were examined and compared against that of controls by matching their turbidity. The clear preparations were considered as “No growth” of bacteria and turbid ones, as “Growth of bacteria”. The MIC was reported as lowest concentration of DAE required to prevent the visible growth of test organisms.

A. Results of Antibacterial sensitivity test by disc diffusion method:

Figure 01 shows the sensitivity of test organisms to DAE. Respective ZOI against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* are stated below:

Against *Staphylococcus aureus*, ZOI were 8 mm, 13 mm, 15 mm, 16 mm and 21 mm at 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml concentrations respectively.

Against *Pseudomonas aeruginosa*, ZOI were 0 mm, 8 mm, 13 mm, 15 mm and 18 mm at 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml concentrations respectively.

Against *Escherichia coli*, ZOI were 8 mm, 11 mm, 13 mm, 16 mm and 20 mm at 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml concentrations respectively.

Against *Klebsiella pneumoniae*, ZOI were 0 mm, 9 mm, 12 mm, 14 mm and 18 mm at 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml and 500 µg/ml concentrations respectively.

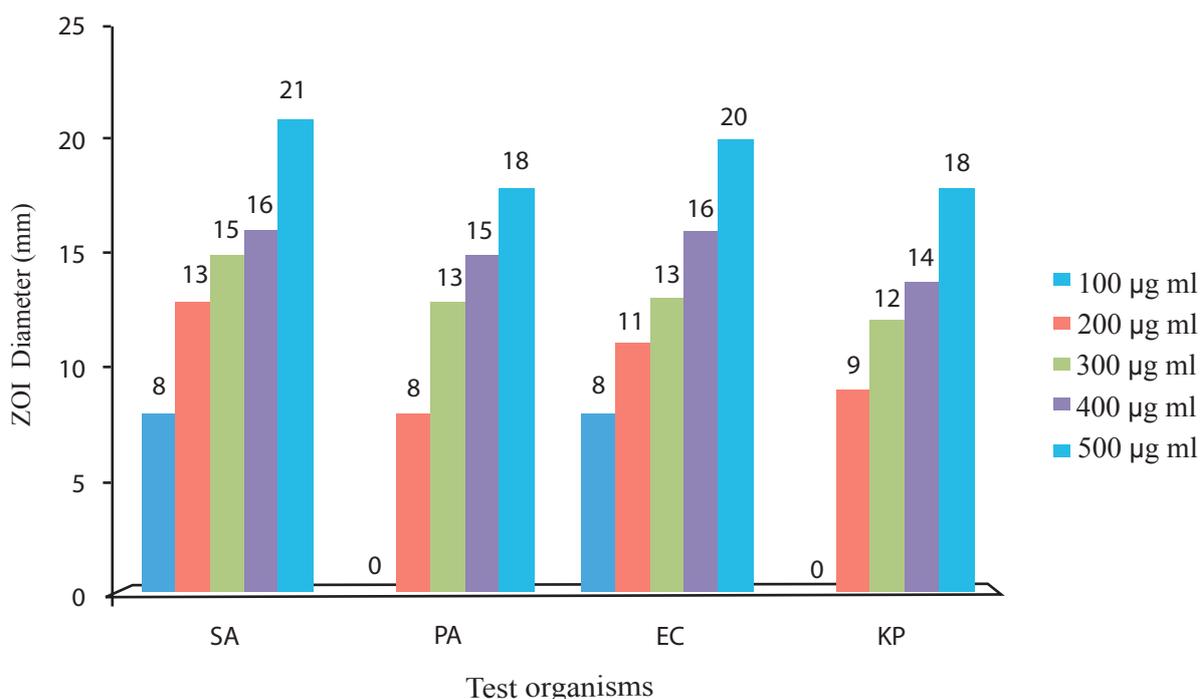


Figure 01: Multiple bar diagram showing Zone of inhibition at different concentrations of DAE Results

B. Results of Minimum Inhibitory Concentration (MIC) by broth dilution technique:

As stated before, the minimum inhibitory concentration (MIC) is the concentration giving the least inhibitory activity and below

which there is no further inhibition. Table 03 shows that the initial MICs for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* are 300, 400, 400 and 500 µg/ml respectively.

Table 03: MIC of DAE against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*

No. of Sets	Concentration of DAE (µg/ml)	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
Set-I	900	No Growth	No Growth	No Growth	No Growth
Set-II	800	No Growth	No Growth	No Growth	No Growth
Set-III	700	No Growth	No Growth	No Growth	No Growth
Set-IV	600	No Growth	No Growth	No Growth	No Growth
Set-V	500	No Growth	No Growth	No Growth	No Growth
Set-VI	400	No Growth	No Growth	No Growth	Growth
Set-VII	300	No Growth	Growth	Growth	Growth
Set-VIII	200	Growth	Growth	Growth	Growth
Set-IX	100	Growth	Growth	Growth	Growth
Set-X (C-1)	Pure stock DAE (with bacteria)	No Growth	No Growth	No Growth	No Growth
Set-XI (C-2)	N/B media (with bacteria)	Huge Growth	Huge Growth	Huge Growth	Huge Growth
Set-XII (C-3)	N/B media (no bacteria)	No Growth	No Growth	No Growth	No Growth

After performing the initial experiment, a second experiment was done to find out more precise MICs for the test organisms. Now, measured amounts of DMSO extract, Nutrient

broth and organisms are taken in different test tubes to make required concentration of DAE. The calculation is given below in tabulated form.

Table 04: Composition and different concentrations of working DAE solutions for repeat experiment (can be correlated with Table 02)

Organism	DAE stock solution-2 (ml)	Nutrient broth Medium (ml)	Total (ml)	Concentration of DAE (µg/ml)	Test Organism (µl)	Observation	MIC (µg/ml)
<i>Staphylococcus aureus</i>	2.5	7.5	10	250	20	Growth of bacteria	300 *
<i>Pseudomonas aeruginosa</i>	3.5	6.5	10	350	20	Growth of bacteria	400 *
<i>Escherichia coli</i>	3.5	6.5	10	350	20	Growth of bacteria	400 *
<i>Klebsiella pneumoniae</i>	4.5	5.5	10	450	20	No Growth of bacteria	450

*From initial experiment.

From the aforementioned experiments it can be stated that, the MICs of DAE against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* are 300 µg/ml, 400 µg/ml, 400 µg/ml and 450 µg/ml respectively.

Discussion

In the present study, in vitro antibacterial activity of the DMSO extract of Aloe vera leaf gel was quantitatively evaluated on the basis of zone of inhibition by disc diffusion method and the minimum inhibitory concentration was assessed by broth dilution technique. Different concentrations of the extract exhibited varying degrees of inhibitory effect. Several studies have been conducted to evaluate the antibacterial properties of Aloe vera. In a study⁷ the antimicrobial activity of DMSO extracts of Aloe barbadensis Miller (Aloe vera) gel was examined against pathogenic organisms including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. Three different concentrations (100, 200 and 400 µg/ml) of DMSO gel extracts of Aloe vera were used to determine the inhibitory effect on the pathogens. Highest ZOI were observed against *Staphylococcus aureus*, which were 12 mm, 14 mm and 16 mm respectively at 100, 200 and 400 µg/ml of DMSO extract. This result is almost similar to the findings of present study (8 mm, 13 mm and 16 mm at 100, 200 and 400 µg/ml). Lowest ZOI were observed against *Pseudomonas aeruginosa*, which are 6 mm, 8 mm and 10 mm respectively at 100, 200 and 400 µg/ml. This finding is also a bit consistent with present study results because, in this study, ZOI were 0 mm, 8 mm and 15 mm respectively at the aforementioned concentrations. ZOI against *Escherichia coli* were 8 mm, 12 mm and 15 mm at 100, 200 and 400 µg/ml of Aloe vera extract (In present study, 8 mm, 11 mm and 16 mm respectively). Finally, against *Klebsiella pneumoniae*, ZOI were 8 mm, 10 mm and 13 mm at 100, 200 and 400 µg/ml which are somewhat compat

ible with present study findings (0 mm, 9 mm and 14 mm respectively).

Antibacterial effect of DMSO extract of Aloe vera gel against various oral pathogens including *Staphylococcus aureus* was tested by Jain et al.⁸ Antibacterial property was detected using disc diffusion method. At 100% concentration average ZOI was 6.6 mm in case of *Staphylococcus aureus*. At 50% concentration ZOI was 6.1 mm. At lower concentrations (25%, 12.5%) there was no effect against the bacteria. This result does not coincide with present study because ZOI against *Staphylococcus aureus* ranged from 8-21 mm across different concentrations (100-500 µg/ml). This significant difference evident in the results may be due to the use of clinical strains of organisms by the aforementioned researchers.

Antimicrobial activity of Aloe vera gel investigated against multi-drug resistant (MDR) *Pseudomonas aeruginosa* isolated from patients with burn wound infections.⁹ Aloe vera gel was extracted using DMSO. MIC of Aloe vera was measured by broth dilution technique. The results obtained from antibacterial activities of Aloe vera gel showed that 89.4% isolates were inhibited by Aloe vera gel extract at the MIC □ 400 µg/ml. The MIC values of Aloe vera gel against the remaining 10.6% isolates were 800 µg/ml. In present study MIC against *Pseudomonas aeruginosa* was 400 µg/ml which is compatible for majority of the organisms used in the aforementioned research.

Conclusion

From the study it is clearly evident that DMSO extract of Aloe vera leaf gel has a dose dependent inhibitory effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. Relatively higher concentrations of the extracts showed higher degrees of inhibition against the test organisms. Further studies are required to detect and isolate the biologically active ingredients present in the Aloe vera

leaves which are responsible for antibacterial effect. The practice of using medicinal plants like Aloe vera as supplementary or alternative medicine in developing countries will reduce not only the clinical burden of drug resistance development but also the side effects and cost of the treatment as compared to synthetic compounds.

References

1. Jothi Karumari R, Vijayalakshmi K, Tamilarasi L, Balasubramanian E. Antibacterial Activity of Leaf Extracts of Aloe Vera, Ocimum Sanctum and Sesbania Grandiflora against the Gram Positive Bacteria. *Asian Journal of Biomedical and Pharmaceutical Sciences*. 2014;4(35):60-63.
2. Yebpella GG, Adeyemi Hassan MM, Hammuel C, Magomya AM, Agbaji AS, Okonkwo EM. Phytochemical screening and comparative study of antimicrobial activity of Aloe vera various extracts. *African Journal of Microbiology Research*. 2011;5(10):1182-1187.
3. Kedarnath, Kaveri MK, Chirkod VB, Patil CS. Antimicrobial Activity of Aloe vera leaf extract. *International Journal of Applied Biology and Pharmaceutical Technology*. 2013;4(4):286-290.
4. Bashir A, Saeed B, Mujahid TY, Jehan N. Comparative study of antimicrobial activities of Aloe vera extracts and antibiotics against isolates from skin infections. *African Journal of Biotechnology*. 2011;10(19):3835-3840.
5. Irshad S, Butt M, Younus H. In-Vitro antibacterial activity of Aloe Barbadensis Miller (Aloe Vera). *International Research Journal of Pharmaceuticals*. 2011; 1(2):59-64.
6. Radha MH, Laxmipriya NP. Evaluation of biological properties and clinical effectiveness of Aloe vera: A systematic review. *Journal of Traditional and Complementary Medicine*. 2015;5:21-26.
7. Devi DL, Srinivas B, Rao NB. An evaluation Antimicrobial Activity of Aloe barbadensis Miller (Aloe vera) Gel Extract. *Journal of Pharmaceutical and Biomedical sciences*. 2012;21(21):1-4.
8. Jain S, Rathod N, Nagi R, Sur J, Laheji A, Gupta N, Agrawal P, Prasad S. Antibacterial Effect of Aloe Vera Gel against Oral Pathogens: An In-vitro Study. *Journal of Clinical and Diagnostic Research*. 2016;10(11):41-44.
9. Goudarzi M, Fazeli M, Azad M, Seyedjavadi SS, Mousavi R. Aloe vera Gel: Effective Therapeutic Agent against Multidrug-Resistant *Pseudomonas aeruginosa* Isolates Recovered from Burn Wound Infections. *Chemotherapy Research and Practice*. 2015. Article ID 639806, 5 pages <http://dx.doi.org/10.1155/2015/639806>