

Evaluating the Antimicrobial Efficacy of Different Extracts of *Aloe species* on Human Pathogens

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ABSTRACT: Plant extracts possess unique antimicrobial agents is the topic attaining great popularity among the researchers to abolish current health issues. So a study was conducted to examine the antimicrobial activity of *Aloe species* viz. *Aloe barbadensis*, *Aloe rupestris*, *Aloe juvenna* and *Gasteria pulchra* in different solvents like ethanol, methanol, petroleum ether and aqueous solution against human pathogens namely *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Aspergillus niger* and *Candida albicans* by disc diffusion method. The result observed was ethanolic as well as methanolic extracts of *Aloe species* exhibited more inhibitory effect than petroleum ether and aqueous solution. *Aloe barbadensis*, *Aloe rupestris*, *Aloe juvenna* and *Gasteria pulchra* were effective against tested pathogens. *Aspergillus niger* as well as *Candida albicans* were also susceptible to *Aloe* leaf extracts given inhibitory zones ranging from 6mm to 11mm. Results of these findings will be a gain for the therapeutic field.

KEYWORDS: Antimicrobial drugs, Antifungal, *Aloe species*, Pathogens, Disc diffusion, Zone of Inhibition.

I. INTRODUCTION

Sprouting of infectious diseases is a threat to the coming world. Researchers have to flourish the answers for the present health challenges from hidden nature. Many plant species are medically powerful to combat infectious pathogens due to pharmacological properties of having secondary metabolites [1]. Researchers are focusing on the biologically compounds from plant species used in herbal medicine for the development of drugs [2].

Aloe species are shrubby, succulent, perennial, pea green color plants belonging to Asphodeloideae family. There are more than 500 species widely distributed in China, Japan, Russia, South Africa, the United States, Jamaica, Latin America and India [3]. *Aloe vera* (Linn), also known as *Aloe barbadensis* of family Liliaceae is widely distributed in Asia, Africa and other tropical areas [4]. *Aloe Vera* plant has a long history of therapeutic ability and therefore has been promoted to combat large variety of clinical conditions. *Aloe vera* is a succulent stemless plant growing upto 60-100 cm tall. The leaves are thick and fleshy green with white flecks on the upper and lower stem surfaces. Leaves of *Aloe rupestris* are thick, shaggy, deep green, unspotted and bordered by red teeth and form a spiraled rosette at the stem apex. *Aloe juvenna* is a dwarf aloe with multibranching stems. It forms smallish spikey rosettes from base. *G. pulchra* is very decorative, tall, narrow shrubby succulent with long finger-like marbled foliage. Leaves are almost vertical, glabrous, glossy, smooth to touch, grey-green to dark green in colour. The present study was aimed to examine the antimicrobial activity of *Aloe species* in different solvents like ethanol, methanol, petroleum ether and aqueous solution on human pathogens like *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium* and in fungal strains like *Aspergillus niger* and *Candida albicans*.

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II. MATERIALS AND METHODS

Four Aloe species such as Aloe barbadensis, Aloe rupestris, Aloe juvenna and Gasteria pulchra were collected from different plant nurseries of Ghaziabad (Uttar Pradesh) and Alwar (Rajasthan) in India. Fresh leaves were dissected and lacerated into small pieces using sterilized knife and shade dried for 5 days. The dried leaves were then blended in an electrical blender. The dried powder so obtained is stored in separate vials at 4°C.

i. PREPARATION OF PLANT EXTRACTS

The protocol for preparing the plant extracts was slightly modified from [5] et al. 2011. The dried powders of all Aloe species were extracted with different solvents like ethanol, methanol, petroleum ether and aqueous solution. 10g of powdered leaves were soaked in 100ml of each solvents viz. ethanol, methanol, and petroleum ether for 24hrs. For aqueous solution preparation 10g of powdered leaves of all Aloe species were soaked in 100 ml of distilled water, for 24hrs. The contents were then filtered through Whatman's filter paper no.1. The filtrate was collected and centrifuged at 10,000rpm for 15 minutes in a refrigerated centrifuge. The supernatant was collected to check the antibacterial activity.

ii. MICROBIAL STRAINS

The bacterial cultures viz. Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium and fungal cultures like Aspergillus niger and Candida albicans used for present investigation were obtained from School of Sciences Department, Noida International University, U.P. These organisms were cultured in slants for 24 hrs. Slant cultures of organisms were subcultured to inoculums using 0.85 NaCl₂.

iii. ANTIMICROBIAL ASSAY

The antimicrobial activities were carried out by disc diffusion method in triplicates. Sterile nutrient agar was prepared and poured in petriplates. 100µl of inoculum containing test organisms like Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus and Salmonella typhimurium were spreaded over the nutrient agar. Discs were impregnated with 50µl of Aloe extracts in different solvents like ethanol, methanol, petroleum ether and aqueous solution. After the impregnation, discs were placed on the surface of Nutrient agar evenly and antimicrobial susceptibility was read after 24 hours incubation in BOD at 37°C. For fungal assay, Sterile Potato Dextrose Agar (PDA) agar was prepared and poured in petriplates. 100µl of inoculum containing test organisms like Aspergillus niger and Candida albicans were spreaded over the PDA. Discs were impregnated with 50µl of Aloe extracts in different solvents like ethanol, methanol, petroleum ether, aqueous solution and crude extracts. After the impregnation, discs were placed on the surface of PDA evenly and antimicrobial susceptibility was read after 48 hours incubation in BOD at 25°C. Antimicrobial activity was determined by measuring the zone of inhibition around the discs and statistically evaluated in terms of Mean ± Standard deviation.

III. RESULTS AND DISCUSSION

Antimicrobial activity of different preparations of Aloe extracts was investigated in-vitro by disc diffusion method against human pathogens. Aloe species were found effective against human pathogens like Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus and Salmonella typhimurium. The measurements of zone of inhibition varied in each species according to the solvents added. The microbial growth inhibition of ethanol, methanol, petroleum ether and aqueous solution of Aloes involved in antibacterial activity

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Table I: Measurements of zone of inhibition of *Aloe barbadensis* in different extracts against human pathogens in terms of Mean and Standard Deviation (mm).

| EXTRACTS | BACTERIAL STRAINS | | | | |
|------------------|-------------------|--------------------|-----------------------|-----------------|-------------------|
| | <i>E coli</i> | <i>Klebshiella</i> | <i>Staphylococcus</i> | <i>Bacillus</i> | <i>Salmonella</i> |
| Ethanol | 14±2 | 21±1 | 14.6±1.15 | 17.3±1.52 | 22.6±2.08 |
| Methanol | 12.66±1.5 | 16.3±2.51 | 10.3±2.08 | 10±1 | 13.3±1.52 |
| Petroleum ether | 9.6±1.52 | 11.6±0.57 | 12±1 | 15.3±1.52 | 12.6±1.52 |
| Aqueous solution | 8±1 | 8.6±1.5 | - | 6.33±0.57 | - |

Among the four species tested, methanolic extracts of *Aloe rupestris* showed the maximum zone of inhibition in *Klebsiella pneumoniae* and *Salmonella typhimurium*. Also, *Salmonella typhimurium* was highly susceptible to all the *Aloe species*.

Table II: Measurements of zone of inhibition of *Aloe rupestris* in different extracts against human pathogens in terms of Mean and Standard Deviation (mm).

| EXTRACTS | BACTERIAL STRAINS | | | | |
|------------------|-------------------|--------------------|-----------------------|-----------------|-------------------|
| | <i>E coli</i> | <i>Klebshiella</i> | <i>Staphylococcus</i> | <i>Bacillus</i> | <i>Salmonella</i> |
| Ethanol | 16±1 | 12.6±1.52 | 13.6±1.52 | 14±2.64 | 23.3±3.05 |
| Methanol | 15.3±1.52 | 29.3±1.52 | 15.3±1.52 | 15.3±2.3 | 28.6±1 |
| Petroleum ether | 14.6±0.57 | 17.3±1.52 | 13±2 | 11.3±2.3 | 13.6±1 |
| Aqueous solution | 8±2 | - | - | - | - |

Table III: Measurements of zone of inhibition of *Aloe juvenna* in different extracts against human pathogens in terms of Mean and Standard Deviation (mm).

| EXTRACTS | BACTERIAL STRAINS | | | | |
|------------------|-------------------|--------------------|-----------------------|-----------------|-------------------|
| | <i>E coli</i> | <i>Klebshiella</i> | <i>Staphylococcus</i> | <i>Bacillus</i> | <i>Salmonella</i> |
| Ethanol | 18.33±0.57 | 16.33±0.57 | 13±1 | 10±1 | 14±2 |
| Methanol | 13±1 | 17±2 | 14.66±1.52 | 14.66±2.08 | 12±2 |
| Petroleum ether | 8.66±1.15 | 13±1 | - | 7.66±0.57 | 8±1 |
| Aqueous solution | 6.66±0.57 | 9±2 | - | - | - |

No inhibitory zone was observed in the petroleum ether extracts as well as in aqueous solutions of *Aloe juvenna* and *Gasteria pulchra* against *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhimurium*.

Table IV: Measurements of zone of inhibition of *Gasteria pulchra* in different extracts against human pathogens in terms of Mean and Standard Deviation (mm).

| EXTRACTS | BACTERIAL STRAINS | | | | |
|------------------|-------------------|--------------------|-----------------------|-----------------|-------------------|
| | <i>E coli</i> | <i>Klebshiella</i> | <i>Staphylococcus</i> | <i>Bacillus</i> | <i>Salmonella</i> |
| Ethanol | 12.33±0.57 | 15.33±1.15 | 13±1 | 10±2 | 14±2 |
| Methanol | 12.66±1.52 | 12.66±0.57 | 13±1 | 14±2 | 13.33±2.51 |
| Petroleum ether | 8.33±1.52 | 10.66±0.57 | - | 9.66±2.08 | 11±1 |
| Aqueous solution | - | 10±1 | - | - | - |

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Antifungal activity of different extracts of *Aloe species* was evaluated on the basis of radial growth of *Aspergillus niger* and *Candida albicans* by disc diffusion method. Statistical analysis revealed that *Candida albicans* showed higher inhibition zones than *Aspergillus niger*. The results of antifungal activity of *Aloe species* of *Aspergillus niger* was illustrated in Table V and *Candida albicans* in Table VI.

Table V: Measurements of zone of inhibition of *Aloe species* in different extracts against *Aspergillus niger* in terms of Mean and Standard Deviation

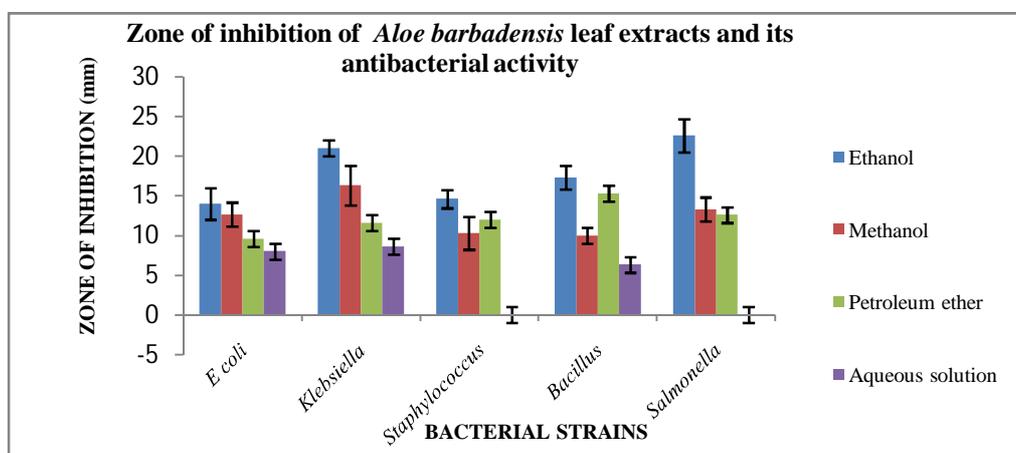
| <i>Aspergillus niger</i> | | | | |
|--------------------------|-------------------------|-----------------------|---------------------|-------------------------|
| EXTRACTS | <i>Aloe barbadensis</i> | <i>Aloe rupestris</i> | <i>Aloe juvenna</i> | <i>Gasteria pulchra</i> |
| Ethanol | 11.33 ± 0.57 | 11.33 ± 0.57 | 11.66 ± 0.57 | 9.66 ± 1.15 |
| Methanol | 8 ± 0 | 7.6 ± 0.57 | 8 ± 1 | 9.66 ± 0.57 |
| Petroleum ether | 7.33 ± 0.57 | 9 ± 0 | 7.33 ± 0.57 | 8 ± 1 |
| Aqueous solution | - | - | - | - |

Table VI: Measurements of zone of inhibition of *Aloe species* in different extracts against *Candida albicans* in terms of Mean and Standard Deviation (mm).

| <i>Candida albicans</i> | | | | |
|-------------------------|-------------------------|-----------------------|---------------------|-------------------------|
| EXTRACTS | <i>Aloe barbadensis</i> | <i>Aloe rupestris</i> | <i>Aloe juvenna</i> | <i>Gasteria pulchra</i> |
| Ethanol | 14.66 ± 0.57 | 12.33 ± 0.57 | 12.66 ± 1.15 | 15.33 ± 1.52 |
| Methanol | 11.66 ± 0.57 | 11.66 ± 1.15 | 11.66 ± 1.15 | 12.66 ± 1.52 |
| Petroleum ether | 9.33 ± 0.57 | 10.33 ± 1.15 | 6.66 ± 0.57 | 10 ± 1 |
| Aqueous solution | 10.5 ± 0.5 | 6.33 ± 0.57 | 8 ± 1 | - |

Among the five different solvents used, ethanol as well as methanol extracts were more efficient in giving maximum zone of inhibition than petroleum ether and aqueous solutions. This difference is due to the varying degrees of solubility of the active constituents in the solvents used for extraction. The varied antibacterial and antifungal activity of *Aloe species* can be illustrated in Fig I, Fig II, Fig III, Fig IV, Fig V and Fig VI.

Fig I: Antibacterial activity of leaf extracts of *A. barbadensis* against human pathogens expressed in terms of mean. Experiments were performed in triplicates with ± standard deviation.



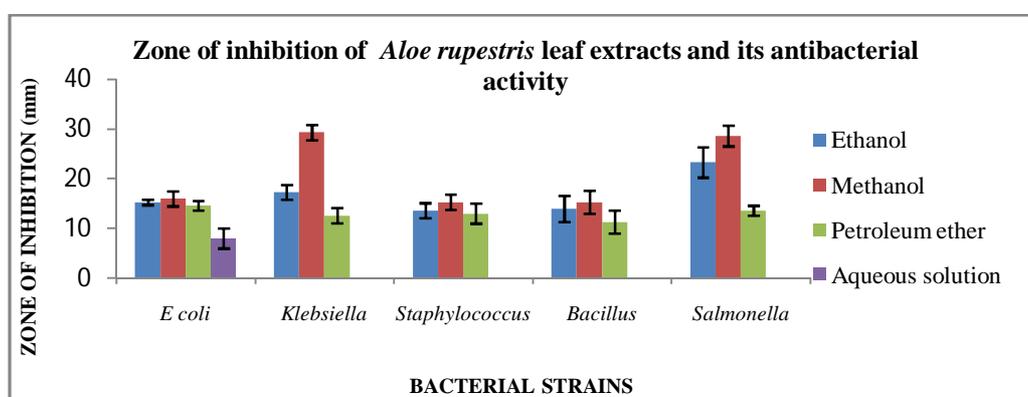
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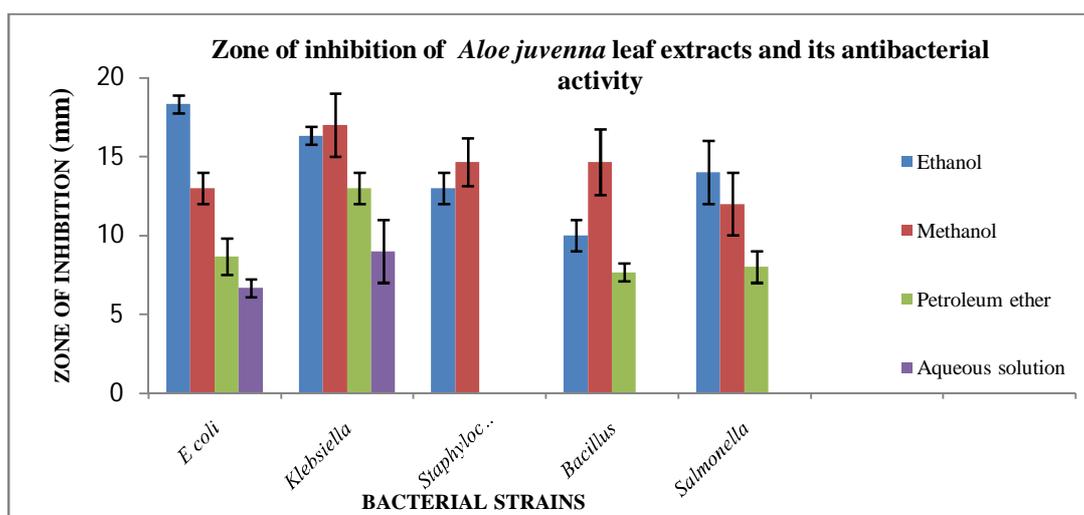
In *Aloe barbadensis*, the ethanolic extracts exerted highest activity on all the bacterial strains. Methanolic extracts of *Aloe rupestris* participated more in resisting the *Klebsiella pneumonia*, *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* giving maximum zone of inhibition than ethanol, petroleum ether and aqueous solution extracts.

Fig II: Antibacterial activity of leaf extracts of *A. rupestris* against human pathogens expressed in terms of mean. Experiments were performed in triplicates with \pm standard deviation.



Ethanolic and methanolic extracts of *Aloe juvenna* showed inhibition with varied activity. Among the bacterial strains *E.coli* had given maximum zone of inhibition in the ethanolic extracts of *Aloe juvenna* followed by *Klebsiella pneumoniae*.

Fig III: Antibacterial activity of leaf extracts of *A. juvenna* against human pathogens expressed in terms of mean. Experiments were performed in triplicates with \pm standard deviation.



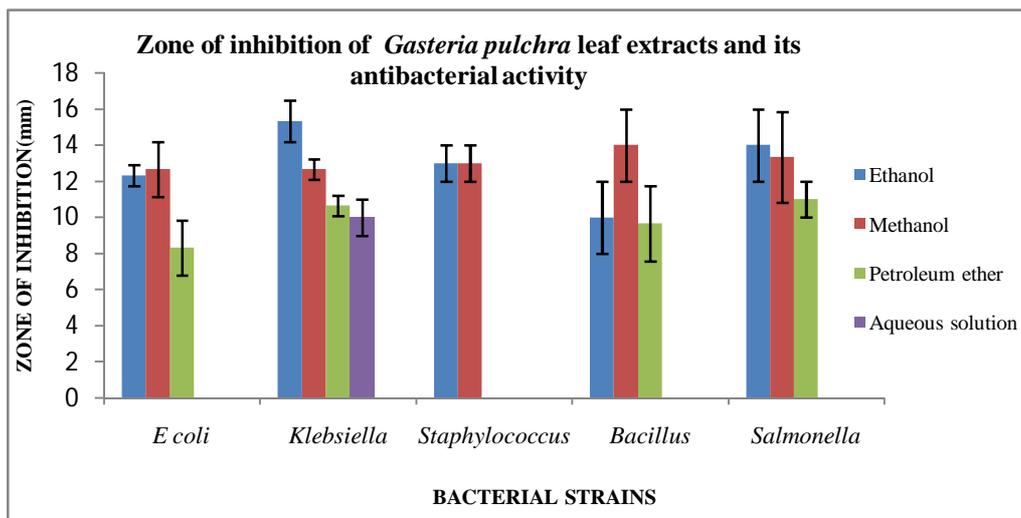
Gasteria pulchra participated effectively in inhibiting the growth of *Klebsiella pneumonia*. Ethanol extracts of *Gasteria pulchra* activated the susceptibility of *Klebsiella pneumonia* by giving higher zone of inhibition.

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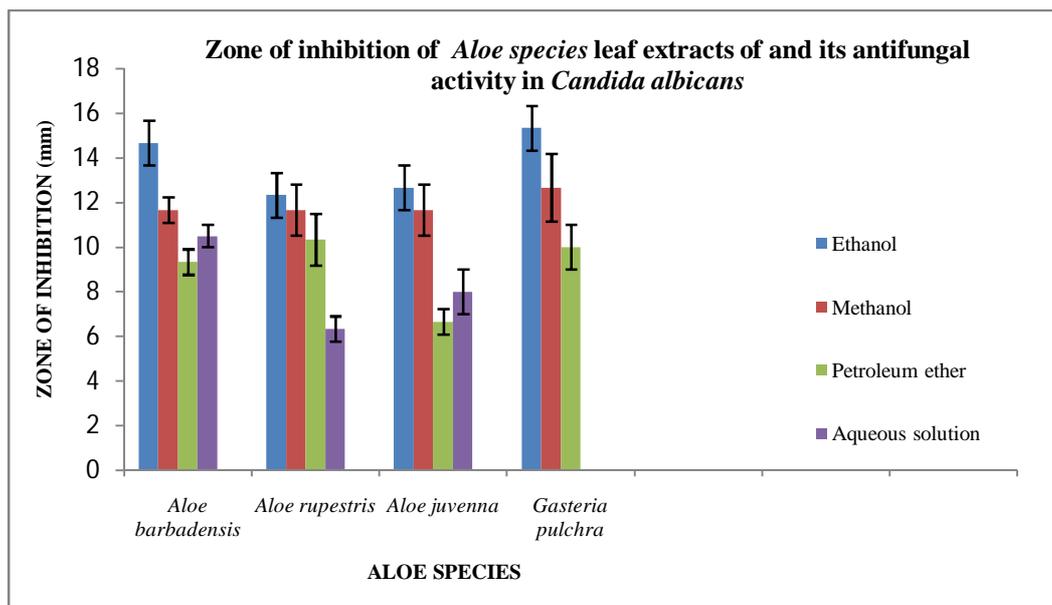
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Fig IV: Antibacterial activity of leaf extracts of *G. pulchra* against human pathogens expressed in terms of mean. Experiments were performed in triplicates with \pm standard deviation.



Aloe leaf extracts displayed antifungal activity in tested *Aspergillus niger* and *Candida albicans*. Some extracts failed to give inhibition zones due to the low activity of extraction to dissolve active constituents that resist the tested pathogen.

Fig V: Antifungal activity of leaf extracts of *Aloe species* against *Candida albicans* expressed in terms of mean. Experiments were performed in triplicates with \pm standard deviation.

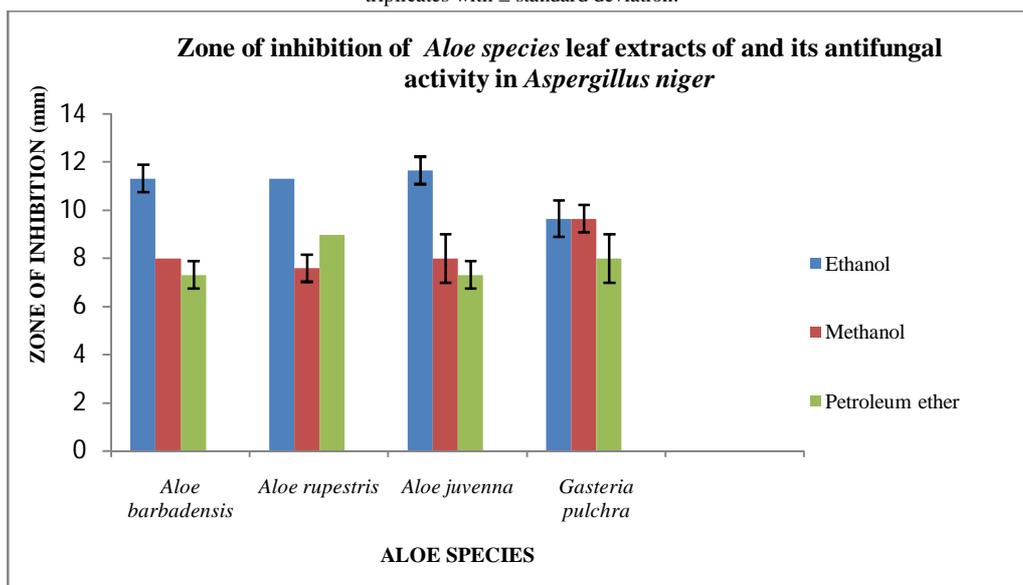


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Fig VI: Antifungal activity of leaf extracts of *Aloe species* against *Aspergillus niger* expressed in terms of mean. Experiments were performed in triplicates with \pm standard deviation.



Methanol, ethanol and petroleum ether is the solvents added to isolate the active components from the plant cells. These phytochemical constituents contribute the antimicrobial activity of plant extracts against the pathogens. The varied activity of these extracts differs due to the difference in the solubility to isolate the active components from the plant cell [6]. Methanol extract of *Aloe rupestris* inhibited *E.coli* showing maximum zone of inhibition conform with the datas of [7] *et al.* 2014 and [8]. The findings on ethanol extract of all species showing 14mm zone of inhibition against *Staphylococcus aureus* resembles with the reports of [9] *et al.* 2011.

Methanolic extracts had given largest inhibition zones for *A. barbadensis* and for *A. chabaudii* against *Klebsiella sp.* and *Salmonellae typhimurium* were the least susceptible. [10] *et al.*, 2010 found that the water extracts had lower activity when compared to the methanol extracts against all test organisms. Studies on the antimicrobial properties revealed ethanol and aqueous extracts of *Aloe vera* were only effective on *E.coli* and *Streptococcus pyrogenes* [11] Many studies have revealed the presence of many biologically active phytochemicals in the ethanolic extracts of *Aloe vera* gel that are responsible for its hypoglycemic and anti-oxidant properties [12]. Methanolic extract was found to be more effective than aqueous extracts against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterobacter aerogens* [13, 14]. The highest antifungal activity of acetone extract of *Aloe vera* plant was reported on the growth of *A. niger* and *A. flavus* [15]. The present study also agreed with the findings of [16] *et al.*, 2011, a study conducted in the DMSO, methanol and aqueous extracts of green rind and leaf pulp and also found to be potentially effective against *Candida albicans*. *Candida albicans* were the most sensitive in the antimicrobial study of *Aloe barbadensis* Miller (*Aloe vera*), *Aloe chabaudii* and *Aloe arborescens* sap extracts by [10] *et al.*, 2010. The study of [6] *et al.*, 2014, revealed that the *Candida albicans* was susceptible to the ethanol extracts of *Aloe vera* gel.

IV. CONCLUSION

All the four species such as *Aloe barbadensis*, *Aloe rupestris*, *Aloe juvenna* and *Gasteria pulchra* exhibited antimicrobial action with varied activity. Due to ever increasing antimicrobial resistance activity; studies became relevant for the identification and development of new natural antimicrobial compounds. The results of the study strengthens the conclusion that besides *Aloe barbadensis*, other three species such as *Aloe rupestris*, *Aloe juvenna* and *Gasteria pulchra* plant extracts also possess antimicrobial properties that can be used in therapeutic purposes which is active against multidrug-resistant organisms.

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REFERENCES

- [1] Hussain, H., Badawy, A., Elshazlyed, A., Krohn, K., Riaz, M., Schulz, B., "Chemical constituents of *Salix subserrata*", *Records of Natural Products*, Vol. 5, pp.133-137, 2011.
- [2] Pavithra, P. S., Janani, V. S., Charumathi, K. H., Indumathy, R., Potala, S., Verma, R. S., "Antibacterial activity of the plant used in Indian herbal medicine", *Int. J. Green Pharm*, Vol. 10, pp.22-28, 2010.
- [3] Boudreau, M.D., Beland, F.A., "An Evaluation of the Biological and Toxicological Properties of *Aloe barbadensis* (Miller), *Aloe Vera*", *Journal of Environmental Science and Health Part C*, Vol. 24 Issue 1, pp.3-54, 2006.
- [4] Asma Bashir, Bushra Saeed, Talat, Y., Mujahid and Nayar Jehan, "Comparative study of antimicrobial activities of *Aloe vera* extracts and antibiotics against isolates from skin infections" *African Journal of Biotechnology*, Vol. 10, Issue 19, pp.3835-3840, 2011.
- [5] Karpagam, T., Aruna Devaraj, "Studies on the efficiency of *Aloe vera* on antimicrobial activity", *International Journal of Research in Ayurveda and Pharmacy*, Vol. 2, Issue 4, pp.1286-1289, 2011.
- [6] de Boer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I., Levenfors, J. J., "Antifungal and antibacterial activity of some herbal remedies from Tanzania", *J. Ethnopharm*, Vol. 9: pp. 461-469, 2005.
- [7] Mbajiuaka Chinedu Stanley, Obeagu Emmanuel Ifeanyi and Okwandu Godson Eziokwu, "Antimicrobial effects of *Aloe vera* on some human pathogens", *Int.J.Curr.Microbiol.App.Sci*. Vol. 3, Issue 3, pp.1022-1028, 2014.
- [8] Cock, I., "Antimicrobial Activity of *Aloe barbadensis* Miller Leaf Gel Components", *The Internet Journal of Microbiology*, Vol. 4, Issue 2, pp 1-9, 2007.
- [9] Pratibha, P., Sakshi, G., Vatsala, K. G., Sahil, S., "In vitro antimicrobial activity of ethanolic extract of some bacterial and fungal species, used in the India", *Advanced Biotechnology*, Vol. 11, Issue 3, pp.32-33, 2011.
- [10] Alemdar, S., Agaoglu, S., "Investigation of *In vitro* Antimicrobial Activity of *Aloe vera* Juice" *J. Animal Veter. Advances*, Vol. 8, Issue 1, pp.99-102, 2009.
- [11] Joshua Mbanga, Ngonidzashe Mangoma and Bamusi Saidi, "An Evaluation of the Antimicrobial Activities of *Aloe barbadensis*, *Aloe chabaudii* and *Aloe arborescens* Leaf Extracts Used in Folklore Veterinary Medicine in Zimbabwe", *Journal of Animal and Veterinary Advances*, Vol. 9, Issue 23, pp.2918-2923, 2010.
- [12] Nwankwo, J. I., Nwankwo, B. N., "A Preliminary comparative study on the Antimicrobial properties of *Cassia alata*, *Jatropha curcas*, *Aloe barbadensis* and *Allium sativum*", *Journal of Pharmacy and Biological Sciences*, Vol. 9, Issue 4, pp.37-40, 2014.
- [13] Rajasekaran, S., Sivagnanam, K., Subramanian, S., "Antioxidant effect of *Aloe vera* gel extract in *Streptozotocin*-induced diabetes in rats", *Pharmacology Republic*, Vol. 57, pp.90-96, 2005.
- [14] Krishna Kumar, H. N., E. Chandana, H. N., Preethi, S. D., Jyothi Bala Chauhan, "Invitro antimicrobial activity and phytochemical screening of *Aloe vera* Linn." *International Journal of Current Pharmaceutical Research*. Vol. 4, Issue 3, pp.45-47, 2012.
- [15] Sitara, U., Hassan, N., Naseem, J., "Antifungal activity of *Aloe vera* gel against plant pathogenic fungi", *Pakistan Journal of Botany*, Volume 43, Issue 4, pp.2231-2233, 2011.
- [16] Yebpella, G. G., Adeyemi Hassan, M. M., Hammuel, C., Magomya, A. M., Agbaji, A. S., Okonkwo, E. M., "Phytochemical screening and comparative study of antimicrobial activity of *Aloe vera* various extracts", *African Journal of Microbiology Research*, Vol. 5, Issue 10, pp. 1182-1187, 2011