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EVALUATION OF ANTIMICROBIAL POTENTIAL OF ALOE VERA PLANT LEAVES

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ABSTRACT
The problem of microbial resistance towards antimicrobial drugs is becoming a major problem for humankind as it leads to the death of millions of people. To tackle this problem, medicinal plants with ethnobotanical importance can be act as a source for the identification of the new drugs. Therefore, in the current study we aimed for phytochemical screening and evaluation of antimicrobial potential of leaf extracts *Aloe vera* plant. Leaves of *A. vera* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 50% methanol and double distilled water. Results depicted that 50% methanolic leaf extract of *A. vera* showed maximum zone of inhibition (ZOI) of 24mm for bacterial strain *Staphylococcus aureus* and it has also showed maximum ZOI of 18mm for fungal strain *Candida albicans.* Aqueous leaf extract of *A. vera* showed maximum ZOI of 16mm against bacterial strain *Klebsiella pneumonia* and it has also showed maximum ZOI of 13mm against fungal strain *Candida albicans.* The secondary metabolites like alkaloids, flavonoids, glycosides, steroids, phenolic compounds/tannins were detected in both 50% methanol and aqueous leaf extracts of *A. vera*. In conclusion, methanolic and aqueous leaf extracts *A. vera* have been demonstrated to possess antimicrobial activity, and hence they could be exploited in the development of antimicrobial drugs against various pathogenic microorganisms.

Keywords: Aloe vera, Leaf extracts, Methanol, Aqueous, Antimicrobial, Microbial resistance

INTRODUCTION
Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicines. Its civilization is very ancient and the country as a whole has long been known for its rich resources of medical plants. Today, Ayurvedic, Homoeo and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Material Media.[1]

Furthermore, medicinal plants have played important role in the traditional and orthodox system of medicine in the curing of different types of diseases. Analysis of different species of medicinal plants for biologically active components known to have pharmacological properties have been conducted and most of the studied plants have shown antimicrobial property.[2-4]

The resistance of microorganisms against antimicrobial drugs is a major problem of recent times which is increasing day by day.[5] As synthetic antimicrobials or antibiotics have considerable side effects over natural antimicrobial agents it is compulsory need to search for drugs which are effective against a wide range of microorganisms with minimal or no side-effects. To tackle this problem, medicinal plants with ethnobotanical importance can be act as a source for the identification of the new drugs. Medicinal plants are considered as the greatest pharmaceutical stores existing on the earth as they can produce eternal secondary phytochemicals having bioactive properties. These phytochemicals work efficiently to cure various diseases and illnesses since ancient times.[6]

Aloe vera is a member of liliaceae family. *Alove vera* (L.) Burm. Fil is a cactus like plant with green, dagger-shaped leaves that are fleshy, tapering, spiny, marginated and filled with a clear viscous gel (Figure 1).[7] *Aloe vera* is as old as civilization and throughout history it has been used as a popular folk medicine. It is present in the arid regions of India and is believed to be effective in treating stomach ailments, gastrointestinal problems, skin diseases, constipation for radiation injury, for its anti-inflammatory effect, for wound healing and burns, as an anti-ulcer and diabetes. Currently the plant is widely used in skin care, cosmetics and as nutraceuticals.[8]

Figure 1. Showing *Aloe vera* plant

With this scenario, in the present study we aimed for phytochemical screening and evaluation of antimicrobial potential of *Aloe vera* plant leaves.

MATERIALS AND METHODS

Collection Leaves of *A. vera*

The leaves of *A. vera* were collected in and around district headquarter places of Karnataka. The leaves were gently and thoroughly washed with running tap water to remove the dirt particles and wiped off, and sprayed with ethanol, and then shade dried. The dried leaves were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.[9]

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *A. vera* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of 50% methanol (Extract A) and double distilled water (Extract B). The extracts were concentrated by distilling the solvents in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.[9]

Phytochemical Screening

Phytochemical screening was carried out on the leaf extracts of *A. vera* by using standard procedures to detect phytoconstituents as described by sofora,[10] Trease and Evans[11] and Harborne.[12]

Test for alkaloids
Approximately 0.2g of leaf extracts of *A. vera* were warmed with 2% H_2SO_4 (2.0mL) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for tannins and phenolic compounds
The leaf extracts of *A. vera* in **s**mall quantity were mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins and phenolic compounds.

Test for glycosides
About 0.6g of leaf extracts of *A. vera* were hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for saponins
About 0.2g of leaf extracts of *A. vera* were shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for flavonoids
0.2g of leaf extracts of *A. vera* were dissolved in diluted 10%NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for steroids
2 mL of acetic anhydride was added to 0.5g of leaf extracts of *A. vera* and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for terpenoids
0.3g of leaf extracts of *A. vera* were mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Formation of reddish-brown coloration at the interface indicates positive results for the presence of terpenoids.

Evaluation of Antimicrobial Activities
The disc diffusion technique was used for assay of antibacterial and antifungal activity as describe by Ferro et al. Briefly, sterile nutrient agar plates and potato dextrose agar plates were prepared. The bacterial test organisms like *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia,* and *Bacillus aereus* were spread over the nutrient agar plates by using separate sterile cotton buds. Then the fungal test organisms like *Aspergillus niger,* Aspergillus *flavus, and Candida albicans* were spread over the potato dextrose agar plates. After the microbial lawn preparation three different extracts of plant disc were placed (50 mg/ml) on the organism inoculated plates with equal distance. All bacterial plates were incubated at 27°C for 24 hrs and fungal plates at 24°C for 72hrs. The diameter of the minimum zone of inhibition (mm) was measured.[13]

RESULTS
The major phytochemicals found in Extract A (50% methanol) and Extract B (Aqueous) of *A. vera* were found to be alkaloids, flavonoids, glycosides, steroids, phenolic compounds. Whereas, phytochemicals *viz.* saponins and tannins were found to be present only in Extract B and Extract A respectively. However, terpenoids were found to be absent in both Extract A and Extract B (Table 1).

Table 1: Photochemical screening of leaf extracts of *A. vera*

Phytochemical Components	**Extract A**	**Extract B**
Alkaloids	+	+
Flavonoids	+	+
Glycosides	+	+
Saponins	+	-
Steroids	+	+
Phenolic compounds	+	+
Tannins	-	+
Terpenoids	-	-

+: Present; -: Absent; Extract A: 50% Methanol extract; Extract B: Aqueous extract

The results of Zone of inhibition (ZOI) of *A. vera* extracts *viz.* Extract A and Extract B against selected bacterial strains were represented in Table 2 and plotted in Figure 2. Results depicted Extract A showed maximum ZOI of 24mm for bacterial strain *Staphylococcus aureus* followed by 21mm, 15mm, and 12mm for *Klebsiella pneumonia, Bacillus aereus,* and *Escherichia coli*. Similarly, Extract B showed maximum ZOI of 16mm against bacterial strain *Klebsiella pneumonia* followed by 15mm, 11mm, and 8mm for *Staphylococcus aureus, Bacillus aereus,* and *Escherichia coli.*

Table 2: Antibacterial activity of leaf extracts of *A. vera*, zones of inhibition of the extractsagainst the selected bacterial strains (mm)

Microorganisms	**Extract A ZOI (mm)**	**Extract B ZOI (mm)**
Staphylococcus aureus	24	15
Escherichia coli	12	8
Bacillus aereus	15	11
Klebsiella pneumonia	21	16

Extract A, 50% Methanol extract; Extract B, Aqueous extract; ZOI, Zone of inhibition Values are expressed as Mean; n=3

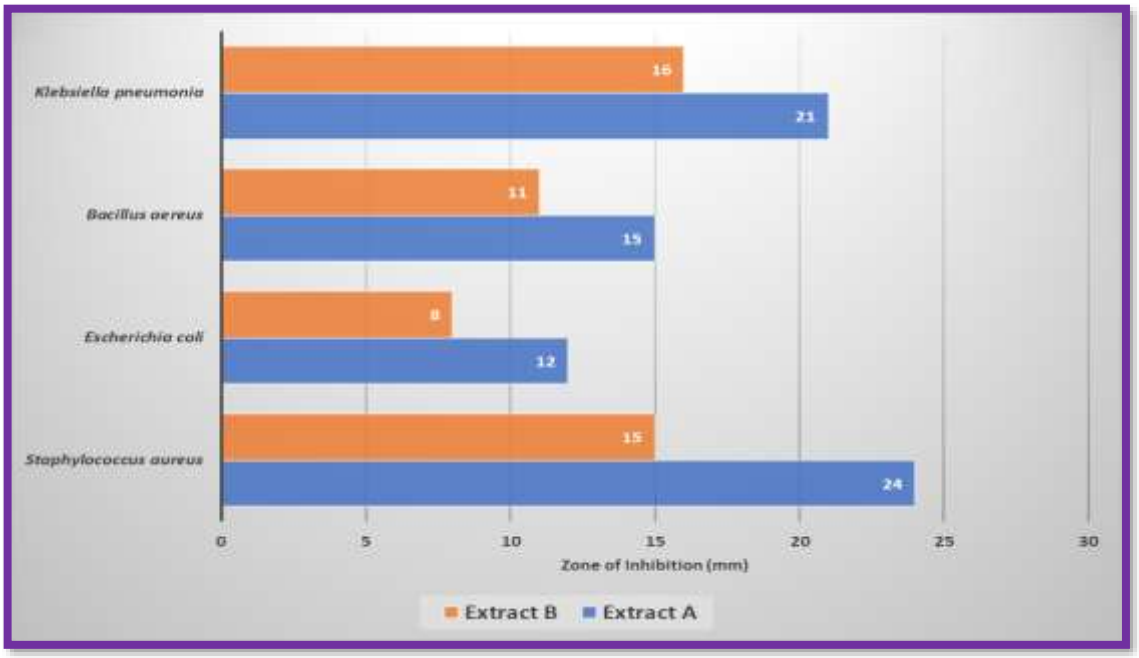

Figure 2: Zones of inhibition of the leaf extracts of *A. vera* against the selected bacterial strains (mm)

Extract A, 50% Methanol extract; Extract B, Aqueous extract

The results of Zone of inhibition of *A. vera* extracts *viz.* Extract A and Extract B against selected fungal strains were represented in Table 3 and plotted in Figure 3. Results portrayed Extract A showed maximum ZOI of 18mm for fungal strain *Candida albicans* followed by 12mm and 10mm for *Aspergillus flavus* and *Aspergillus Niger*. Similarly, Extract B showed maximum ZOI of 13mm against fungal strain *Candida albicans* followed by 9mm and 8mm for *Aspergillus flavus* and *Aspergillus niger.*

Table 3: Antifungal activity of leaf extracts of *A. vera*, zones of inhibition of the extracts against the selected fungal strains (mm)

Microorganisms	**Extract A ZOI (mm)**	**Extract B ZOI (mm)**
Aspergillus Niger	10	8
Aspergillus flavus	12	9
Candida albicans	18	13

Extract A, 50% Methanol extract; Extract B, Aqueous extract; ZOI, Zone of inhibition Values are expressed as Mean; n=3

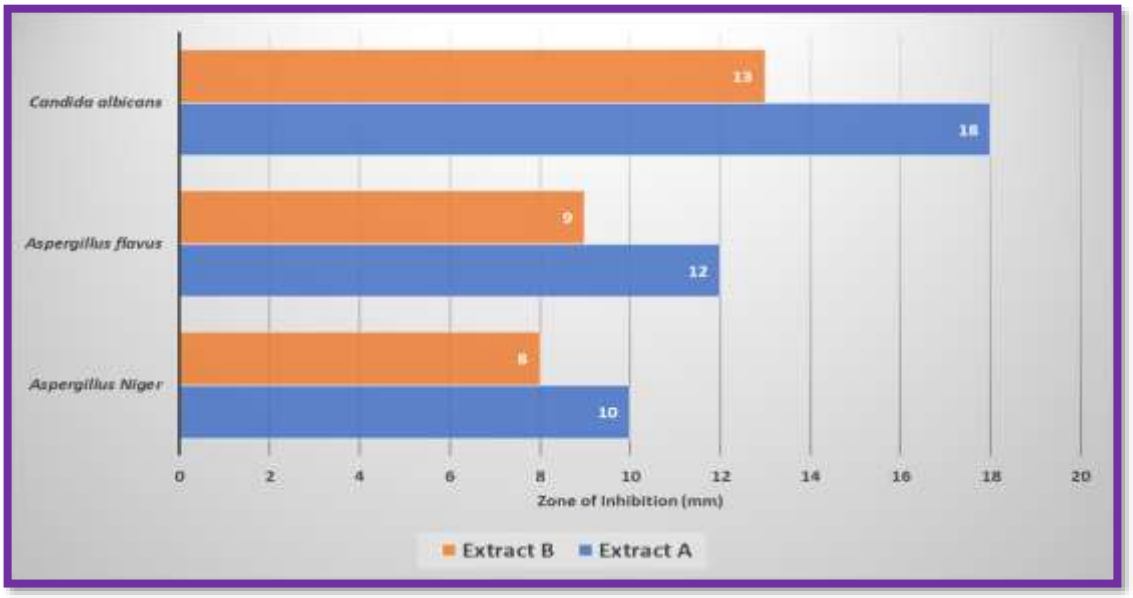

Figure 3: Zones of inhibition of the leaf extracts of *A. vera* against the selected fungal strains (mm)

Extract A, 50% Methanol extract; Extract B, Aqueous extract

DISCUSSION

The problem of microbial resistance towards antimicrobial drugs is becoming a major problem for humankind as it leads to the death of millions of people.[5] Most of the world's population relies on plant derived traditional medicines for the need of their primary health care.[14] Plants can be a very important source of newer drugs or antimicrobial compounds as they exhibit a vast range of phytochemicals. Various Aloe species are found all over the world which are used in cosmetics, medicine/pharma and food industry.[15] Hence in the current study we aimed for phytochemical screening and evaluation of antimicrobial potential of 50% methanolic and aqueous leaf extracts *Aloe vera* plant.

Our study results on antimicrobial activities screening revealed that 50% methanolic leaf extract of *A. vera* showed maximum ZOI of 24mm for bacterial strain *Staphylococcus aureus* followed by 21mm, 15mm, and 12mm for *Klebsiella pneumonia*, *Bacillus aereus*, and *Escherichia coli* respectively. Whereas, aqueous leaf extract of *A. vera* showed maximum ZOI of 16mm against bacterial strain *Klebsiella pneumonia* followed by 15mm, 11mm, and 8mm for *Staphylococcus aureus, Bacillus aereus,* and *Escherichia coli* respectively. These findings were comparable with the results of various research investigators reported in the literature.[16,17]

50% methanolic leaf extract of *A. vera* showed maximum ZOI of 18mm for fungal strain *Candida albicans* followed by 12mm and 10mm for *Aspergillus flavus* and *Aspergillus niger* respectively. While, aqueous leaf extract of *A. vera* showed maximum ZOI of 13mm against fungal strain *Candida albicans* followed by 9mm and 8mm for *Aspergillus flavus* and *Aspergillus niger* respectively. These findings were comparable with results of various other research studies reported in the literate by various other research investigators.[16,18-20] Furthermore, results of inhibiting effect on *C. albicans* also established that the *A. vera* leaf extracts of methanol and water, though share certain components, are distinct from one another.[21]

Literature reports evidenced that secondary metabolites identified in plant material have been reported as having inhibitory action against pathogenic microorganisms.[22,23] *Aloe vera* leaves contain various chemicals from different classes which have antimicrobial activity.[17] In our study secondary metabolites like alkaloids, flavonoids, glycosides, steroids, phenolic compounds were detected in both 50% methanol and aqueous extracts of *A. vera*. These secondary metabolites could be responsible for antibacterial and antifungal activities possessed by leaf extracts *A. vera*. Recent

reports revealed that these secondary metabolites exert antimicrobial activity through different mechanisms: Tannins have been found to form irreversible complexes with proline-rich protein resulting in the inhibition of cell protein synthesis.[24]

CONCLUSION

In conclusion, results of this study portrayed that methanolic and aqueous leaf extracts of *A. voera* have been demonstrated to possess antimicrobial activity. The antimicrobial activity of *A. vera* leaf extracts could be accredited to phytochemicals such as alkaloids, flavonoids, glycosides, steroids, phenolic compounds present in the *A. vera* leaf extracts. Hence, methanolic and aqueous leaf extracts of *A. vera* could be explored in the development of antimicrobial drugs against various pathogenic microorganisms.

REFERENCES

1. Rao N, Thammanna K. Medicinal Plants of Ritual Hills. Department of Garden, Tirupati Devasthanams, Tirupati, India. 1987:297.
2. Rabe T, Van Staden J. Antibacterial activity of South African plants used for medicinal purposes. Journal of ethnopharmacology. 1997;56(1):81-7.
3. Ongsakul M, Jindarat A, Rojanaworarit C. Antibacterial effect of crude alcoholic and aqueous extracts of six medicinal plants against *Staphylococcus aureus* and *Escherichia coli*. J. Health Res. 2009;23(3):153-6.
4. Ahsan MR, Islam KM, Haque ME, Mossaddik MA. In vitro antibacterial screening and toxicity study of some different medicinal plants. The world J. Agric. Sci. 2009;5(5): 617-621.
5. Cohen ML. Changing patterns of infectious disease. Nature. 2000;406(6797):762-7.
6. Jigna P, Rathish N, Sumitra C. Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. Indian journal of pharmacology. 2005;37(6):408-9.
7. Guide YA. Harper Collins Australia. Australia. Retrieved from https://Yates-Garden-Guide/dp. 2002;207198209.
8. Klein AD, Penneys NS. *Aloe vera*. Journal of the American Academy of Dermatology. 1988;18(4):714-20.
9. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. Journal of ethnopharmacology. 1993;39(3):171-4.
10. Sofora A. Medicinal plants and Traditional Medicine in Afric. John Wiley Son Ltd. 1993:150-3.
11. Trease GE, Evans WC. Pharmaccology, 11th Edtn. London: Brailliar Tiridel and Macmillian Publishers; 1989.
12. Herborne JB. Phytochemical methods. 3rd ed D.E. and Hall Ltd. London; 1973. p. 135-203.
13. Ferro VA, Bradbury F, Cameron P, Shakir E, Rahman SR, Stimson WH. In vitro susceptibilities of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of *Aloe barbadensis* Miller. Antimicrobial agents and chemotherapy. 2003;47(3):1137-9.
14. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC complementary and alternative medicine. 2006; 6:1-7.
15. Park YI, Jo TH. Perspective of industrial application of *Aloe vera*. In New perspectives on *Aloe vera*. 2006 (pp. 191-200). Boston, MA: Springer US.
16. Alemdar S, Agaoglu S. Investigation of in vitro antimicrobial activity of *Aloe vera* juice. J. Animal and Veterinary Adv.2009;8(1):99-102.
17. Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. World journal of agricultural sciences. 2009;5(5):572-6.
18. Olaleye MT, Bello-Michael CO. Comparative antimicrobial activities of *Aloe vera* gel and leaf. African journal of biotechnology. 2005;4(12):1413-14.

19. De Rodrıguez DJ, Hernández-Castillo D, Rodrıguez-Garcıa R, Angulo-Sánchez JL. Antifungal activity in vitro of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. Industrial Crops and Products. 2005;21(1):81-7.
20. Rosca-Casian O, Parvu M, Vlase L, Tamas M. Antifungal activity of *Aloe vera* leaves. Fitoterapia. 2007;78(3):219-22.
21. Foster S. *Aloe vera*: The succulent with skin soothing cell protecting properties. Herbs for Health magazine. 1999; 1999:59-60.
22. Freeman B, Beattie G. An overview of plant defenses against pathogens and herbivores.2008.
23. Wiley J. Medicinal Chemistry of Bioactive Natural Products.2006.
24. Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). African journal of pharmacy and pharmacology. 2009;3(2):058-62.