

Analysis of Phytochemical Constituents and Antimicrobial Activities of *Aloe vera* L. Against Clinical Pathogens

S. Arunkumar and M. Muthuselvam

Muthaiyah Research Foundation, Thanjavur, Tamilnadu, India-613 005

Abstract: The aim of the study was to investigate the *Aloe vera* phyto chemical compounds and antimicrobial activity of different extracts. The phytochemical compound screened by qualitative and GC-MS method. Qualitatively analyzed Tannin, Saponin, Flavonoids and Terpenoids gave positive results and phlobactanins and Steroids and Steroids gave negative results. In the GC-MS analysis, 26 bioactive phytochemical compounds were identified in the ethanolic extract of *Aloe vera*. Three different solvents such as aqueous, ethanol and acetone were used to extract the bioactive compounds from the leaves of *Aloe vera* to screen the antimicrobial activity selected human clinical pathogens by agar diffusion method. The maximum antibacterial activities were observed in acetone extracts ($12\pm 0.45\text{nm}$, $20\pm 0.35\text{nm}$, $20\pm 0.57\text{nm}$ and $15\pm 0.38\text{nm}$) other than aqueous extracts and ethanol extract. Antifungal activity of *Aloe vera* was analyzed against *Aspergillus flavus* and *Aspergillus niger*. The maximum antifungal activity was observed in acetone extracts ($15\pm 0.73\text{nm}$ and $8\pm 0.37\text{nm}$) when compared other extracts. *Aloe vera* plant extract with acetone can be used as antimicrobial agents.

Key words: *Aloe vera* % Phyto chemical % Antibacterial % Antifungal activity

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, Homeo and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Material Media [1]. *Aloe vera* has been used to treat various skin conditions such as cuts, burns and eczema. It is alleged that sap from *Aloe vera* eases pain and reduces inflammation. Evidence on the effects of *Aloe vera* sap on wound healing, however, is contradictory [2]. Screening techniques of biologically active medicinal compounds have been conducted on well-known species of plants used in traditional medicines and most plants have shown antibacterial activity [3]. *Aloe vera* is a member of liliaceae family. *Aloe vera* (L.) Burm. Fil (Synonym *A. brobadensis* Miller) (Tamil- Southakathalai, Hindi- Gikanvar) is a cactus like plant with green, dagger-shaped leaves that are fleshy, tapering, spiny, marginated and filled with a clear viscous gel [4]. The name was derived from the Arabic

‘alloe’ meaning ‘bitter’ because of bitter liquid found in the leaves. It is also known as ‘lily of the desert’ the plant of immortality and the medicine plant with qualities to serve as alternate medicine.

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 [5]. In this present study *Aloe vera* phyto chemical compounds analysis (Qualitative method (Screening) and GC-MS Analysis), also analyzed antibacterial and antifungal activity (extracts of Aqueous, Ethanol and Acetone).

MATERIALS AND METHODS

Collection of Plant Material: The plant of *Aloe vera* (leaves) was collected from Herbal Garden of Ponnaiyah Ramajayam College, Thanjavur. The plant part (leaves) was identified by a taxonomist in the Department of Botany, PRIST University, Thanjavur.

Preparation of Plant Extract: The leaves of *Aloe vera* was air dried and crushed to small piece using Mortar and Pestle and powdered in an electric grinder. Twenty grams of powdered plant materials mixed with 100ml of various solvents (Distilled water, Ethanol and Acetone solution). The extracts preparations were done as previously described by Alade and Irobi [6]. The plant extracts were prepared by using soxhlet apparatus collected and stored in a vial for further studies.

Screening of Phytochemical Components: Phytochemical components were analyzed qualitatively [7,8].

GC-MS Analysis: The GC-MS analysis of the *A. vera* was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% Diphenyl 95% dimethyl poly siloxane) (30nm×0.25mmID×0.25µm) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carries gas at a flow rate of 1 ml/min. the injector was operated at 200°C and the oven temperature was programmed as follows; 60°C for 15min, then gradually increased to 280°C at 3min. the identification of components was based on comparison of their mass spectra with those of Wiley and NBS Libraries and those described by Adams [9] as well as on comparison of their retention indices [10] with literature.

Disc Preparation: The 6mm (diameter) discs were prepared from whatmann No. 1 filter Paper the discs were sterilized by autoclave at 12°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. Then various solvent extract discs and control discs were prepared.

Antibacterial and Antifungal Activity of Aloe Vera: The antibacterial and antifungal activity studies were carried out by disc diffusion technique [12]. The sterile nutrient agar plates and potato dextrose agar plates were prepared. The bacterial test organisms like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* were spread over the nutrient agar plates by using separate sterile cotton buds. Then the fungal test organism like *Aspergillus flavus* and *Aspergillus niger* were spread over the potato dextrose agar plates. After the microbial lawn preparation three different extracts of plant disc were placed on the organism inoculated plates with equal

distance control discs were also prepared. 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 was measured in mm. For each test, three replicates were performed.

Statistical Analysis: Data were expressed as mean±standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference between extract used and also between the lengths of incubation.

RESULTS AND DISCUSSION

The present study carried out on the *Aloe vera* revealed the presence of medicinal active constituents. The phytochemical active compounds of *Aloe vera* were qualitatively analyzed and the results are presented in Table 1. In analysis of Tannin compounds brownish green colour developed to indicate the presence of Tannin. Similarly based on the presence or absence of colour change indicate positive and negative results are indicate. In this screening process Tannin, Saponin, Flavonoids and Terpenoids gave positive results and phlobactanins and Steroids gave negative results.

In the GC-MS analysis, 26 bioactive phytechemical compounds were identified in the ethanolic extract of *Aloe vera*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. J. Sitosterol (C₂₉ H₅₀O) with RT 38.78 has peak area 13.19% Oleic Acid (C₁₈ H₃₄O₂) with RT (21.85) and 9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z). (C₁₉H₃₃O₂) with RT 22.06 ranks next having peak area 11.74% and 11.36% respectively. Phytol (C₂₀ H₄₀O) with RT 21.07 ranks with peak area 10.01% the results are presented in Table 2. *A. vera* is reported to contain mono- and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins and minerals [12].

Table 1: Qualitative analysis of photochemical al components

Sl. No.	Phytochemical components of qualitative analysis	<i>Aloe vera</i>
1.	Tannin	+
2.	Phlobatannins	-
3.	Saponin	+
4.	Flavonoids	+
5.	Steroids	-
6.	Terpenoids	

+ = Presence, - = Absence

Table 2: Phytochemicals identified in the plant sample extract

No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	16.05	1-Tetradecyne	C ₁₄ H ₂₆	194	0.54
2	17.67	Tridecanoic acid, methyl ester	C ₁₄ H ₂₈ O ₂	228	0.79
3	18.70	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	5.07
4	18.93	Hexadecanoic acid, ethyl ester	C ₁₈ H ₄₀ O	284	8.81
5	21.07	Phytol	C ₂₀ H ₄₀ O	296	10.01
6	21.85	Oleic Acid	C ₁₈ H ₃₄ O ₂	282	11.74
7	22.06	9,12,15- Octadecatrienoic acid methyl ester, (ZZZ)	C ₁₉ H ₃₂ O ₂	292	11.36
8	24.13	Oxalic acid, allyl pentadecyl ester	C ₂₀ H ₃₆ O ₄	340	0.29
9	25.73	Oxalic acid, allyl hexadecyl ester	C ₂₁ H ₃₈ O ₄	354	0.52
10	27.11	9-Octadecenal	C ₁₈ H ₃₄ O	266	2.28
11	27.28	1-Octanol, 2-butyl-	C ₁₂ H ₂₆ O	186	1.92
12	28.08	Didodecyl phthalate	C ₂₂ H ₄₂ O ₄	502	0.56
13	28.48	1-Octadecyne	C ₁₈ H ₃₄	250	0.29
14	28.77	Sulfurous acid, hexyl pentadecyl ester	C ₂₁ H ₄₄ O ₃ S	376	1.58
15	30.21	1-Iodo-2-methylundecane	C ₁₂ H ₂₅ I	296	1.78
16	31.60	Eicoane	C ₂₀ H ₄₂	282	2.48
17	31.90	Squalene	C ₃₀ H ₅₀	410	1.24
18	32.95	Octadecane, 2-methyl-	C ₁₉ H ₄₀	268	3.45
19	34.26	Nonadecane, 2-methyl	C ₂₀ H ₄₂	282	3.97
20	34.91	â-Tocopherol	C ₂₈ H ₄₈ O ₂	416	4.24
21	36.09	Vitamin E	C ₂₉ H ₅₀ O ₂	430	2.39
22	36.80	Sulfurous acid, butyl heptadecyl ester	C ₂₁ H ₄₄ O ₃ S	376	2.48
23	37.38	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	C ₂₅ H ₃₈ O ₂	370	1.15
24	38.25	Tetracontane, 3,5,24-trimethyl-	C ₄₃ H ₈₈	604	1.60
25	38.78	-Sitosterol	C ₂₉ H ₅₀ O	414	13.19
26	40.28	Lupeol	C ₃₀ H ₅₀ O	426	6.7

Table 3: Antibacterial Activity

S.No.	Extract	Zone of Inhibition (mm in diameter) (Mean±SD) (n=3)			
		<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
1	Aqueous	-	9±0.54	-	-
2	Ethanol	7±0.38	20±0.36	15±0.53	-
3	Acetone	12±0.45	20±0.35	20±0.57	15±0.38

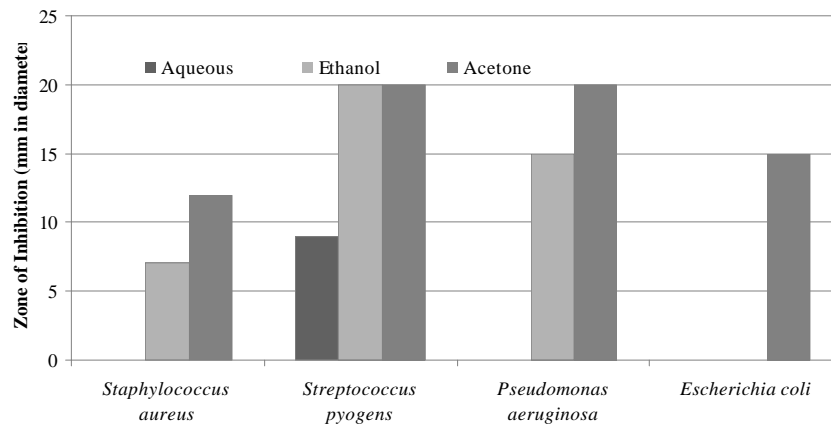


Fig. 1: Antibacterial Activity

Antibacterial Activity: Antibacterial activity of *Aloe vera* was analyzed against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*. The maximum antibacterial activities were observed in acetone extract (12±0.45, 20±0.35, 20±0.57, 15±0.38) other than aqueous extract (0.00, 9±0.54, 0.00, 0.00) and ethanol extract (7±0.38, 20±0.36, 15±0.53, 0.00). Among the three bacterial organisms maximum

growth suppression was observed in *Streptococcus pyogenes* (20±0.35) and *Pseudomonas aeruginosa* (20±0.57) when compared with *Staphylococcus aerues* (12±0.45) and *Escherichia coli* (15±0.38). Results are presented in Table 3 and Fig. 1. Ferro *et al.* [15] have shown that *Aloe vera* leaf gel can inhibit the growth of the two Gram-positive bacteria *Shigella flexneri* and *Streptococcus pyogenes*. Specific plant compounds such

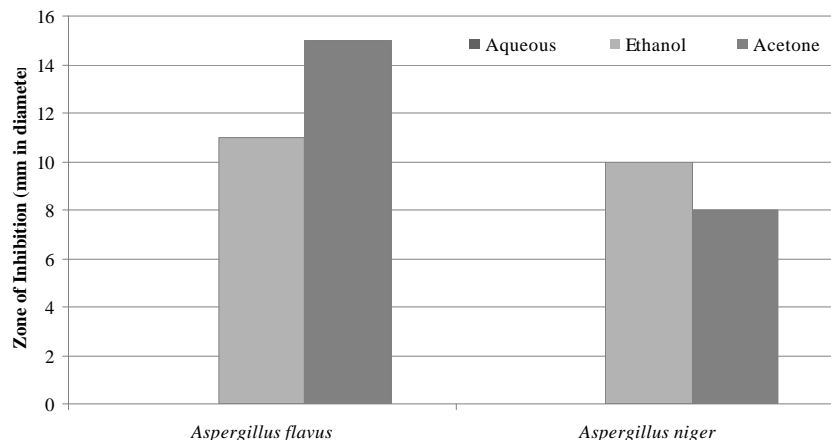


Fig. 2: Antifungal Activity

Table 4: Antifungal activity

		Zone of Inhibition (mm in diameter) (Mean±SD) (n=3)	
S.No.	Extract	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>
1	Aqueous	-	-
2	Ethanol	11±0.53	10±0.32
3	Acetone	15±0.73	8±0.37

as anthraquinones [13] and dihydroxyanthraquinones as well as saponins [14] have been proposed to have direct antimicrobial activity.

Antifungal Activity: Antifungal activity of *Aloe vera* was analyzed against *Aspergillus flavus* and *Aspergillus niger*. The maximum antifungal activities were observed in acetone extract disc (15±0.73 and 8±0.37) other than aqueous extract (0.00 and 0.00) and ethanol extract (11±0.53 and 10±0.32). Among the two fungal organisms maximum growth suppression was observed in *Aspergillus flavus* (15±0.73) than *Aspergillus niger* (8±0.37). Results are presented in Table 4 and Fig 2. Many previous studies such as that of Ferro *et al.*, [15] have focused on the antimicrobial activity of *Aloe vera* whole gel. Other reports have attempted to determine the antimicrobial activity of purified fractions such as anthraquinones [13] and saponins [14]. *Aloe vera* extracts have been shown to inhibit the growth of fungi that cause tinea, however evidence for control beneath human skin remains to be established. For bacteria, inner-leaf gel from *Aloe vera* was shown to inhibit growth of *Streptococcus* and *Shigella* species in vitro. In contrast, *Aloe vera* extracts failed to show antibiotic properties against *Xanthomonas* species [15].

CONCLUSION

This study has revealed the presence of many secondary metabolites in the leaves of *Aloe vera*. It has further confirmed that the plant extracts could be used for the treatment of various infections including skin transmitted infections. The results lend credence to the folkloric use of this plant in treating microbial infection and shows that *Aloe vera* could be exploited for new potent antimicrobial agents.

ACKNOWLEDGMENTS

The authors are thankful to Muthaiyah Research Foundation, Thanjavur for offering facilities to carry out this study.

REFERENCES

1. Narayana Rao and K. Thammanna, 1987. Medicinal Plants of Ritual Hills, Department of Garden, Tirupati Devasthanams, Tirupati, India.
2. Vogler, B.K. and E. Enst, 1999. *Aloe vera* a systematic review of its clinical effectiveness. British Journal of General Practice, 49(447): 823-828.
3. Rabe, T. and J. Van Staden, 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology, 56(1): 81-87.
4. Yates, A., 2002. Yates Garden Guide. Harper Collins Australia, Australia.
5. Klein, A.D. and N.S. Penneys, 1988. *Aloe vera*. Journal of the American Academy of Dermatology, 18(1): 714-720.

6. Alade, P.I. and O.N. Irobi, 1993. Antimicrobial activities of crude leaf extracts of *Acalypha wilkensiana*. Journal of Ethnopharmacology, 39: 171-174.
7. Sofowara, A., 1993. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria, pp: 289.
8. Harborne, J.B., 1973. Phytochemical methods London, Chapman and Hall, Ltd., pp: 49-188.
9. Adams, R., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Co., Carol Stream, IL.
10. Vanden Dool, H. and P.D. Kratz, 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography, 11: 463-471.
11. Bauer, R.W., M.D.K. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by standard single disc diffusion method. American Journal of Clinical Pathology, 45: 493-496.
12. Newall, C.A., L.A. Anderson and J.D. Phillipson, 1996. Herbal medicines. The pharmaceutical Press London, pp: 25.
13. Dabai, Y.U., S. Muhammad and B.S. Aliyu, 2007. Antibacterial activity of anthraquinone fraction of *Vitex doniana*. Pakistan Journal Biological Science, pp: 1-3.
14. Reynolds, T. and A.C. Dweck, 1999. *Aloe vera* leaf gel: a revise update. Journal of Ethnopharmacology, 68: 3-37.
15. Ferro, V.A., F. Bradbury, P. Cameron, E. Shakir, S.R. Rahman and W.H. Stimson, 2003. *In vitro* susceptibilities of *Shigella flexneri* and *Streptococcus phygenes* to inner gel of *Aloe barbadensis* Miller. Antimicrobial agent and Chemotheraphy, pp: 1137-1139.
16. Satish, S., K.A. Raveesha and G.R. Janardhana, 2002. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. Letters in applied microbiology, 28: 145-147.