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ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITIES OF *ALOE VERA* L. AGAINST SOME SELECTED PATHOGENS

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ABSTRACT

The aim of the study was to investigate the *Aloe vera* phyto chemical compounds and antimicrobial activity of different extracts. The phyto chemical compound screened by qualitative and GC-MS method. Qualitatively analyzed tannin, saponin, flavonoids and terpenoids gave positive results and phlobactanins and steroids gave negative results. In the GC-MS analysis, 21 bio active phytochemical compounds were identified in the ethanolic extract of *Aloe vera*. Three different solvents such as aqueous, ethanol and chloroform were used to extract the bioactive compounds from the leaves of *Aloe vera* to screen the antibacterial activity selected plant pathogens by agar diffusion method. The maximum anti bacterial activities were observed in ethanol extracts (0.30 ± 0.10 nm, 1.23 ± 0.05nm). Other than aqueous extract chloroform extract. *Aloe vera* plant extract with ethanol can be used as antibacterial agents.

Keywords: *Aloe vera*, Phytochemical, Antibacterial

INTRODUCTION

Plants have been an important source of medicine for thousands of years. Even today, the world Health Organization estimates that up to 80% of people still take 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 medicinal plants. Today, Ayurvedic, Hoemoeo and Unani Physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Material Media **[1]**. 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 **[2]**. *Aloe Vera* is a member of Liliaceae family. *Aloe Vera* (L) Burm. Fil (synonym *A. brobadensis* Miller) (Tamil – Southa kathalai; Hindi – Gikanvar) is a cactus like plant with green, dagger – shaped leaves that are flesh, tapering, spiny, marginated and filled with a clear viscous gel **[3]**. The name was derived from aeabic "alloeh" meaning "bitter" because of bitter liquid found in the leaves. It is present in the arid regions of India and is believed to be effective in treating stomach ailments, skin diseases, anti inflammatory, wound healing, anti ulcer and diabetes. Currently the plant is widely used in skin care, cosmetics and as nutraceutical **[4]**. In the present study *Aloe vera* phyto chemical compounds analysis (Qualitative method) (Screening) and GC – MS analysis) also analyzed antibacterial activity (extracts of aqueous, ethanol and chroloform).

MATERIALS AND METHODS

Collection of Plant Material

The plant of Aloe Vera (leaves) was collected from Herbal Garden of Jamal Mohammed College, Tiruchirappalli. The plant part (leaves) was identified by a taxonomist in the Department of Botany, Jamal Mohammed College, Tiruchirappalli.

Preparation of Plant Extract

The leaf of *Aloe vera* was air died and crushed to small piece using Mortar and pestle and powdered in an electric grinder. 20 gms of powdered plant materials mixed with 100 ml of various solvents (Distilled water, ethanol and chloroform solution). The extracts preparations were done by previously described by Alade and Irobi **[5]**. The plant extracts were prepared by using Soxhlet apparatus collected and stored in a vial for further studies.

Screening of Phyto Chemical Components

Phyto Chemical compounds were analyzed qualitatively **[6, 7]**.

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) (30 nm x 0.25 mm ID x 0.25% μm df) and mass detector turbo mass gold of the company which was operated in EI mode. Helium was the carries gas at a flow rate of 1ml/minute the injector was operated at 200^{o}C and the oven temperature was programmed as follows; 60^{o}C for 15 minutes, then gradually increased to 280^{o}C at 3minutes. The identification of components

was based on comparison of their mass spectra with those of Wiley and NBS libraries and those described by Adams **[8]** as well as on comparison of their retention indices **[9]** with literature.

Disc Preparation

The 6mm (diameter) discs were prepared from whatmann No. 1 filter paper the discs were sterilized by auto clave at 12^{o}C. After the sterilization the moisture discs were dried on hot air oven at 50^{o}C. Then various solvent extract discs and control disc were prepared.

Antibacterial Activity of *Aloe vera*

The antibacterial activity studies were carried out by disc diffusion technique **[11]**. The sterile nutrient agar plates were prepared. The bacterial test organisms like *Shigella sonnei, Klebsiella pneumoniae, Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa, Pseudomonas solanacerum* and *Xanthomonas citri* were spread over the nutrient agar plates by using separate sterile cotton buds. 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^{o}C for 24 hrs. 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 $\pm$ 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 indicates positive and negative results are indicated. In this screening process tannin, saponin, flavonoids, Anthroquinones gave positive results and phlobactnins, steroids, terpenoids and cardiac glycosides gave negative results.

In the GC – MS analysis, 21 bioactive phyto-chemical compounds were identified in the ethanolic extract of *Aloe vera.* The identification of phyto-chemical compounds is based on the peak area molecular formula and molecular weight. Oleic acid (C_{18} H_{34} O_2) with RT 20.22 and 9, 12, 15 – Octode Catrienoic acid methyl ester (Z, Z, Z) (C_{19} H_{33}

O_2) with RT 19.47 ranks next having peak area 22.26% and 1.07% respectively. Squalene (C_{30} H_{50}) with RT 31.20 ranks with peak area 10 – 12% the results were presented in the **Table 2** and **Figure 1**. *Aloe vera* is reported to contain mono and polysaccharides, tannins, sterols, Organic acids, enzymes, saponins, vitamins and minerals **[11]**.

Antibacterial Activity

Antibacterial activity of *Aloe vera* was analysed against *Shigella sonnei, Klebsiella pnenmonia, Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa, Pseudomonas solanacearum and Xanthomonas citri.* The maximum antibacterial activities were observed in ethanol extract (0.30 $\pm$ 0.10), 1.23 $\pm$ 0.05). Other than aqueous extract (1.66 $\pm$ 0.05) and chloroform extract. Among the seven bacterial organisms growth suppression was observed in *Pseudomonas solanacearum, Xanthomonas citri and Klebsiella pneumonia*. Results are presented in Table 3. Ferro *et al.*, **[14]** have shown that *Aloe vera* leaf gel can inhibit the growth of two Gram – positive bacteria *Shigella* and *Streptococcus.* Specific plant compounds such as authraquinones **[12]** and di-hydracy anthraquinones as well as saponins **[13]** have been proposed to have direct antimicrobial activity.

CONCLUSION

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

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Table 1: Qualitative Analysis of the Phytochemicals in *Aloe vera* Leaves

S. No.	Secondary Metabolites	Results	Inference
1.	Tannin	Brownish green colour	+
2.	Phlobactanins	No Red colour precipitation	-
3.	Saponin	Emulsion formed on the top	+
4.	Flavonoids	Yellow colour change	+
5.	Steroids	No colour change	-
6.	Terpenoids	No Reddish brown colour	-
7.	Cardic glycosides	No Brown ring formed	-
8.	Anthroquinones	Formation of cloudiness	+

Table 2: Phyto Components Identified in *Aloe vera* Using Ethanol Extract

S. No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	3.06	p-Xylene	C_8H_{10}	106	4.88
2	3.78	Cyclohexane, nitro-	$C_6H_{11}NO_2$	129	2.67
3	4.13	Decane	$C_{10}H_{22}$	142	1.19
4	4.63	Limonene	$C_{10}H_{16}$	136	0.44
5	5.44	Undecane	$C_{11}H_{24}$	156	0.70
6	7.03	1-Heptanol, 2-propyl-	$C_{10}H_{22}O$	158	5.86
7	9.47	7-Tetradecene, (E)-	$C_{14}H_{28}$	196	0.33
8	9.59	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	198	0.49
9	10.87	Hexadecane	$C_{16}H_{34}$	226	0.56
10	11.77	Dodecanoic acid	$C_{12}H_{24}O_2$	200	1.37
11	12.14	Nonadecane	$C_{19}H_{40}$	268	0.66
12	13.49	Eicosane	$C_{20}H_{42}$	282	0.62
13	14.44	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.60
14	15.87	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	1.41
15	17.42	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	27.63
16	19.38	9,12-Octadecenoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	1.07
17	19.47	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.03
18	20.22	Oleic acid	$C_{18}H_{34}O_2$	282	22.26
19	27.15	Eicosane	$C_{20}H_{42}$	282	5.18
20	28.71	Heptacosane	$C_{27}H_{56}$	380	9.94
21	31.20	Squalene	$C_{30}H_{50}$	410	10.12

Table 3: Antibacterial Activity of *Aloe vera* Leaves (Disc Diffusion Method)

S. No.	Test Bacteria	Zones of Inhibition (diameter in cm)				Positive control with Chlorompenicol (30µg/disc)
		Ethanol extract		Chloroform		
		Experimental	Negative control	Experimental	Negative control	
1.	*Shigella sonnei*	-	-	-	-	3.66 ± 0.05
2.	*Klebsiella pneumonia*	-	1.66 ± 0.05	-	-	3.66 ± 0.05
3.	*Bacillus subtilis*	-	-	-	-	2.13 ± 0.02
4.	*Salmonella typhi*	-	-	-	-	2.20 ± 0.10
5.	*Pseudomonas aeruginosa*	-	-	-	-	2.10 ± 0.03
6.	*Pseudomonas solanacearum*	0.30 ± 0.10	-	-	-	2.20 ± 0.10
7.	*Xanthomonas citri*	1.23 ± 0.05	-	-	-	2.11 ± 0.08

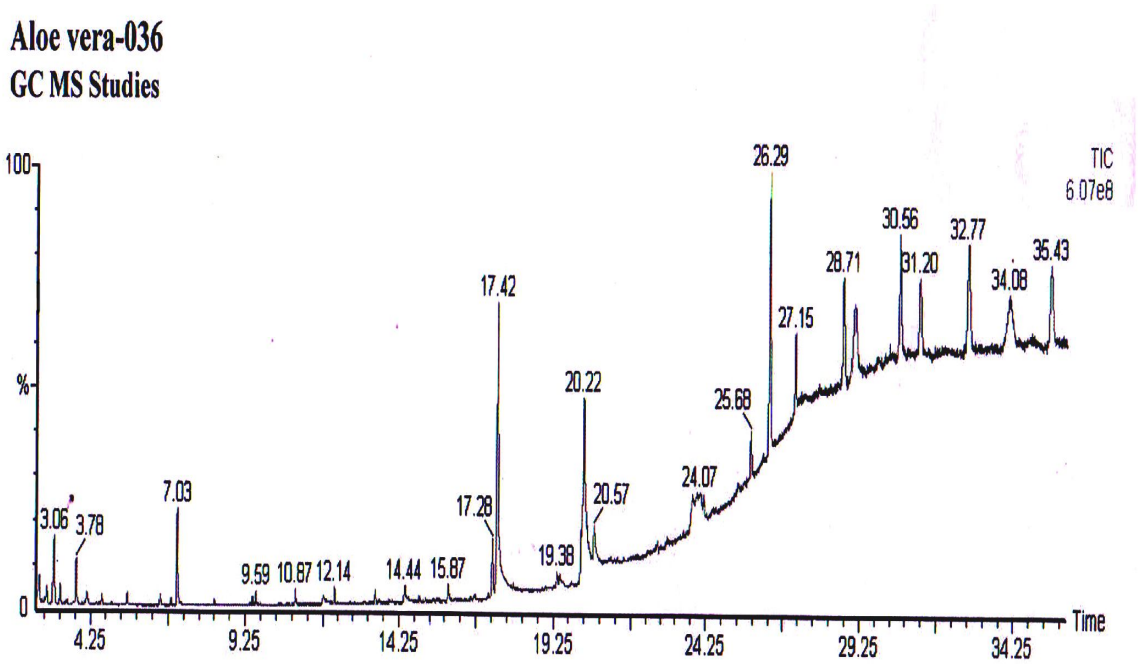

Figure: 1 Phytochemical Components Identified in *Aloe vera* Using Ethanol Extract