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Comparative Analysis of Phytochemicals and Antimicrobial Activity of Aloe Vera Leaf Extract and Gel

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ABSTRACT

Aloe vera is a well-known medicinal plant and possesses many antimicrobial activities. The present research focuses on the phytochemical analysis of leaf extract and gel and assessing antibacterial and antifungal activity against *Staphylococcus aureus*, *Bacillus subtillis, E. coli, Klebsiella pneumonia*, *Salmonella typhimurium*, *Pseudomonas aeroginosa*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger*. The plant samples were collected from Goniana mandi, Punjab, and the extract was prepared. The extract was further analyzed for physiochemical, phytochemical and antimicrobial susceptibility tests. Results have shown that plant possesses phenols, glycosides, terpenoids, carbohydrates, flavonoids, resins, tannins, sterols, and saponins. For 100mg of concentration Aloe vera, the zone of inhibition of test organisms *Pseudomonas aeruginosa* MTCC-2488 was 10.5mm, *S.aureus* MTCC-737 showed 14mm inhibition zone, *E.coli* showed 13 mm inhibition zone. *Bacillus subtillis* have shown the least inhibitory effect. Aspergillus *niger* MTCC-281 have shown inhibition zone of 11mm. *Candida albicans* shows an inhibition zone of 16mm by disc diffusion method and 4 mm by well diffusion method. *S.aureus* MTCC-737, *Salmonella typhi, Kleibseilla sp., Proteus vulgaris* have shown minimum inhibitory concentration of 25mm. Both fungal cultures *Candida albicans* and *Aspergillus niger* have shown MIC of 25mm. This study has shown that aloe vera leaf and gel complement one another in their medicinal properties. It can be an alternative to chemicals used in medications, food and cosmetic sectors.

KEYWORDS: *Aloe vera, MIC, inhibition Zone, Gel, well diffusion*

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INTRODUCTION

Aloe vera is an ornamental and medicinal plant (Morten,1961) belongs to family Iilaceae. It is very popular with gardeners as a medicinal plant. The species requires well-drained and sandy potting soil and bright sunny conditions The species is resistant to an insect. The gel is a rich source of 75 nutrients and 200 active compounds, including minerals and amino acids (Danish et al., 2020). It also contains various phytochemicals such as Anthraquinones, lignin, sterols ad saponins. Aloe vera is known to possess many bactericidal, fungicidal, and viricidal properties (Sánchez et al., 2017). It also contains many vitamins such as vitamin A, vitamin C, vitamin B, niacin Vitamin B2, vitamin B12, choline, and folic acid. Phenolic compounds Aloe-emodin-9 anthrone, isobarbaloin, Anthron-C-glycosides, and chromes have been known to occur in the plant sap. Aloin and emodin are painkillers and also function as antibacterial (Egbuna et al., 2020). Aloe vera promotes cell growth and provides strength to the whole immune system. It also possesses anti-cancer properties. It is very beneficial in case of cough, ulcers, lesions, diabetes, cancer, headaches, indigestion and asthma. It also promotes mensuration when it is suppressed (Naous et al., 2019).

Studies have shown that Aloe gel has shown antimicrobial activity against pathogenic organisms *Mycobacterium, Trichophyton* and *Bacillus subtillis*. Leaf extract has shown antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus, T.schoeleinni, Microsporium canis,* and *Candida*

albicans(Danish et al., 2020). Methanolic extract of *Aloe barbadensis* have shown inhibitory activity against *Aspergillus niger.* Aloe juice has also shown antibacterial activity against only gram-negative bacteria *Aeromonas hydrophilia* and *E. coli* (Lawrence et al., 2009). The present Research focuses on the phytochemical analysis of Leaf extract and gel and assessment of antibacterial and antifungal activity against *Staphylococcus aureus*, *Bacillus subtillis*, *E. coli*., *Klebsiella pneumonia*, *Salmonella typhimurium*, *Pseudomonas aeroginosa*, *Proteus vulgaris*, *Candida albicans,* and *Aspergillus niger*.

Materials and methods

Host plant collection and sterilization

Fresh samples of Aloe vera leaves were harvested from mature Aloe vera plant Goniana Mandi, Punjab. Plant samples were sterilized with ethanol (70%) for 1 min. Sodium hypochlorite (3%) was used for sterilization. They were again washed with autoclaved water to remove the chemicals. (Madhaiya *et al*. 2015, Dubey et al., 2021).

Extract preparation

Aloe vera gel was drained out. 10 gm of plant material was crushed with mortar pestle and soaked in ethanol for 4 days. fine powder was further subjected methanol extraction by the soxhlet extraction method. Liquid was converted to semi-solid material by rotatory evaporator .The extract was dissolved in sterile water and were analyzed for phytochemicals ((Singh et al., 2018).

Phytochemical and anti-microbial analysis

Aloe vera leaf extract and gel were further tested for their antimicrobial activity. Pure samples of Staphylococcus *aureus*, *Bacillus subtillis*, *E. coli*., *Klebsiella pneumonia*, *Salmonella typhimurium*, *Pseudomonas aeroginosa*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger* were collected from IMTECH, sec 39 Chandigarh. Bacterial strains were grown in Nutrient broth whereas fungal strains were grown in PDA media for overnight. Agar well diffusion method was used for studying the antimicrobial sensitivity test. Muller Hinton agar plates and Sabouraud dextrose agar plates were prepared. The standard cork borer was used to cut the uniform wells on the surface of agar. Dimethyl sulfoxide which serves as control were put in separate well. The wells were filled with 0.32 ml of each leaf extract and gel. Plates were inoculated with microbial culture and incubated at 37^0 C for 24 hrs for bacteria culture and 28^0 C for 48hrs for fungal culture. Clearance zone around each well was measured. Minimum inhibitory concentration was measured. The least concentration of the dilution showing clear zone was taken as minimum inhibitory concentration.

RESULTS AND DISCUSSION

The phytochemical screening of Aloe vera samples showed the presence of flavonoids, glycosides, phenols, tannins and reducing sugars**.** Aloe vera leaf extract and gel were further tested for their antimicrobial activity. Pure samples of Staphylococcus *aureus*, *Bacillus subtillis*, *E. coli*., *Klebsiella pneumonia*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger.*

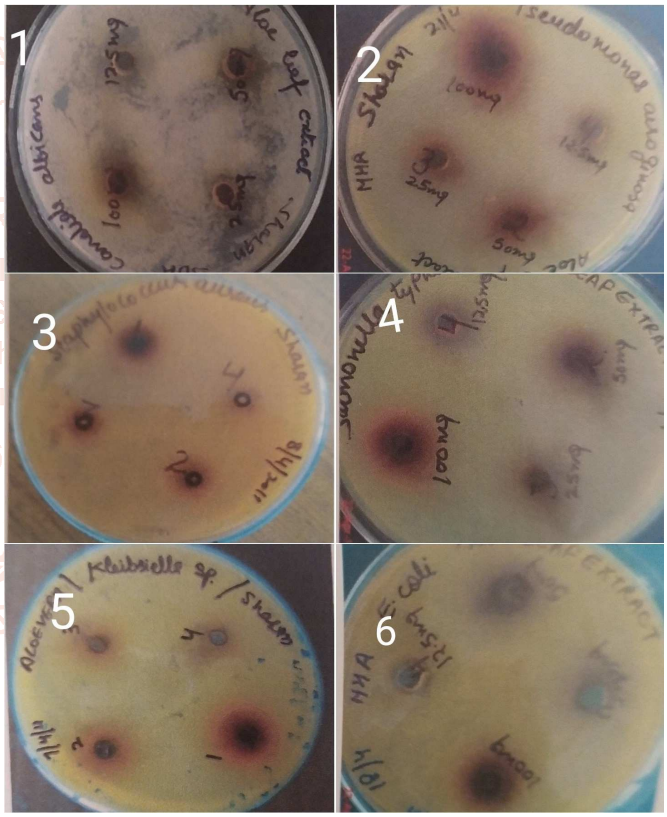

Fig. I) Antibacterial activity of Aloe vera leaf extract on *Candida albicans* 2) *Pseudomonas aeruginosa* 3) *Staphylococcus aureus* 4) *Salmonella typhii* 5) *Klebsiella* 6) *E.coli.* by well diffusion method

Table.1 Phytochemical screening of Aloe vera gel and leaf extract

Chemical constituents	Tests performed	Aloe vera gel	Aloe vera extract
Carbohydrates	Molisch's test Fehling's test Benedict's test	(+) (+) (+)	(+) (+) (+)
Proteins and amino acids	Biuret's test	(-)	(-)
Alkaloids	Mayer's test	(-)	(-)

Terpenoids	Libermann Burchards test Salkowiski's test	(+) (+)	(+)
Saponins	Test for saponins	(+)	(+)
Sterols	Libermann Burchard test	(+)	(+)
Tannins	Ferric chloride test	(+)	(+)
Resins	Test for resins	(+)	(+)
Flavonoids	Shinoda test	(+)	(+)
Terpenoids	Libermann burchard test	(+)	(+)
Glycosides	Bortrager's test	(+)	(+)
Anthraquinones	Test for anthraquinones	(+)	(+)
Phenols	Ferric chloride test	(-)	(+)
Cardiac glycosides	Killer Kiliani test	(-)	(+)

(+) indicates the presence of plant constituents
(-) indicates the absence of plant constituents.

The above table shows the presence of phenols, glycosides, terpenoids, carbohydrates, flavonoids, resins, tannins, sterols, and saponins. Alkaloids were absent in the Aloe vera gel sample. Aloe vera gel sample contain cardiac glycosides

Table.2 Antibacterial activity of aloe vera leaf extract on a bacterial and fungal strain by agar well diffusion method

S. No.	Test organisms	Zone of inhibition				MIC
	Bacteria	The concentration of Aloe vera leaf extract(mg)				
		100mg	50mg	25mg	12.5mg	
1.	*P. aeruginosa* MTCC-2488	10.5mm	6mm	4mm	N.Z	25 mm
2.	*Staphylococcus aureus* MTCC-737	14mm	11 mm	9 mm	2 mm	12.5 mm
3.	*Bacillus subtilis* MTCC-736	5mm	3 mm	2 mm	0.5 mm	12.5 mm
4.	*E.coli* MTCC -443	13mm	11 mm	7 mm	2 mm	12.5 mm
5.	*Proteus vulgaris* MTCC-1771	12.5mm	10 mm	6 mm	2 mm	12.5 mm
6.	*Salmonella typhi* MTCC-1255	16mm	11 mm	7 mm	N.Z	25 mm
7.	*Klebsiella spp.*MTCC-432	11mm	9 mm	4 mm	N.Z	25 mm
Fungus						
8.	*Aspergillus niger* MTCC-281	11mm	8mm	5mm	N.Z	25mm
9.	*Candida albicans* MTCC-183	11.5mm	7mm	3mm	N.Z	25mm

N.Z-= No zone of inhibition

The antimicrobial activity of was investigated using the agar disc diffusion method. *Candida albicans* and *Salmonella typhi* gave the largest inhibition zone i.e 11.5 mm and 16mm at 100mg leaf extract of Aloe vera *Salmonella typhi* MTCC-1255, *Klebsiella spp.*MTCC-432, *Aspergillus niger* MTCC-281, and *Candida albicans* MTCC-183 have shown No inhibition zone at 12.5 mg leaf extract. *P. aeruginosa* MTCC-2488, *Salmonella typhi* MTCC-1255, *Klebsiella spp.*MTCC-432, *Aspergillus niger* MTCC-281, and *Candida albicans* MTCC-183 shows MIC 25mm.

Table.3 Antibacterial activity of aloe vera gel on a bacterial and fungal strain by agar well diffusion method and disc diffusion method

S. No.	Test organisms	Zone of inhibition	
	Bacteria	Disc diffusion	Well diffusion
1.	*P. aeruginosa* MTCC-2488	4mm	1.5mm
2.	*Staphylococcus aureus* MTCC-737	2mm	4mm
3.	*Bacillus subtilis* MTCC-736	6mm	1mm
4.	*E.coli* MTCC -443	N.Z	N.Z
5.	*Proteus vulgaris* MTCC-1771	N.Z	N.Z
6.	*Salmonella typhi* MTCC-1255	N.Z	N.Z
7.	*Klebsiella spp.*MTCC-432	N.Z	N.Z

Fungus			
8.	*Aspergillus niger* MTCC-281	2mm	3mm
9.	*Candida albicans* MTCC-183	16mm	4mm

From above, it was observed that only five microorganisms showed susceptibility against Aloe vera gel. *S.aureus* MTCC-737 has shown a 2 mm inhibition zone by the disc diffusion method and 4mm by well diffusion method. *Candida albicans* shows an inhibition zone of 16mm by disc diffusion method and 4 mm by well diffusion method. *E.coli* MTCC -443, *Proteus vulgaris* MTCC-1771,*Salmonella typhi* MTCC-1255,*Klebsiella spp.*MTCC-432 have shown no inhibition zone.

Discussion

In Ayurveda Aloe vera is used as laxatives, antihelminthic, hemorrhoid and uterine stimulator. The present research focuses on the phytochemical analysis of leaf extract and gel and assessing antibacterial and antifungal activity against *Staphylococcus aureus, Bacillus subtillis, E. coli, Klebsiella pneumonia*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger*. All the test organisms were sensitive to 100mg w/v and 50 mg w/v concentrations. Predominance activity was observed for 100mg concentration of Aloe vera. For 100mg of concentration Aloe vera, the zone of inhibition of test organisms *Pseudomonas aeruginosa* MTCC-2488 was 10.5mm, *S.aureus* MTCC-737showed 14mm inhibition zone, *E.coli* showed 13 mm inhibition zone. *Bacillus subtillis* have shown the least inhibitory effect whereas *Candida albicans* MTCC-183 was found to be more sensitive strain and showed an inhibition zone of 11.5 mm.

P. aeruginosa MTCC-2488, *Salmonella typhi* MTCC-1255, *Klebsiella spp.* MTCC-432, *Aspergillus niger* MTCC-281, and *Candida albicans* MTCC-183 have shown a minimum inhibitory concentration of 25mm. Both fungal cultures have shown a MIC of 25mm. Studies have shown that Aloe preparations added to the drinking water of chickens were useful in the treatment of diarrheal conditions with blood droppings caused by *E. coli*(Edeh, 2013). Researchers have observed that the aloe vera inner have shown antimicrobial activity against gram-positive and gram-negative bacteria by different methods(Pellizzoni et al., 2012). The antibacterial activity of emodin against . Acetone and crude extract of Aloe vera extract was found to be effective against *Candida allbicans* with 22mm and 11mm inhibition zone (Kohli et al., 2011) Acetone extract was known to show less zone of inhibition ranging from 6.00nm for *E. coli* to 7.33 mm for *S.pyogens* whereas no response have been observed towards *P. aeroginosa* and *S. typhi* (Lawrence et al., 2018). Studies found that Aloe vera ethanol extract showed an inhibition effect against *C. albicans* with inhibition zone 12.450±0.208 mm (6.25%), 13.975±0.457 mm (12.5%), 15.650±0.420 mm (25%), and 17.225±0.512 mm (50%), respectively. Fluconazole revealed a comparable antifungal effect with MIZ of 11.025±0.478 mm with a significant effect $p<0.005$(Nabila et al, 2020). The antibacterial activity of acetone extract, ethyl acetate, chloroform, n-butanol, and aqueous was evaluated using the Aromatogram method against *B. cereus, E. coli, S.aureus, A. baumanii,* and *P. aeruginosa.* The antibacterial activity of the n-butanol fraction, acetone, and methanol extracts was found to be effective against all microorganisms tested. MIC ranging from 1.25 to 10 mg/mL was observed. The acetone extract had shown the highest antioxidant activity in the phenanthroline assay (A0.50 value: 46.75 0.35 g/mL)(Bendjedid et al., 2021).

Conclusion

This investigation has shown that aloe vera leaf and gel complement one another in their medicinal properties. It was determined that Aloe vera juice had inhibitory effect against pathogenic bacteria, causing food poisoning or different disease in humans.It can be an alternative to chemicals used in medications, food, and cosmetic sectors.

STATEMENT OF COMPETING INTEREST

We have no competing interest

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