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EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF ALOE VERA GEL

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Abstract:

In our present work we are going to find out weather Ethanol and DMSO extracts of A.Vera gel has antibacterial and anti fungal activity and to test it on few species of bacteria and fungi by measuring their zone of inhibition by cup plate method. The bacterial strains which we have used were.Bacillus subtilis and Sallmonella typhi. The fungal strains which we have used were Aspergillus fumigates and Pencillium notatum.The ethanolic extract showed greater antibacterial and antifungal activity compared to DMSO extract of aloe vera. The study suggests the antimicrobial and antifungal activity of the A. vera gel extract to be dependant on the synergistic effect of different compounds. With the broad spectral antimicrobial and antifungal effect of A. vera gel, it could be further recommended in the treatment of bacterial and fungal diseases.

Keywords: *Aloe vera, Dimethyl sulphoxide(DMSO),Synergistic effect.*

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INTRODUCTION:

The Aloe vera plant[1] has been known and used for centuries for its health, beauty, medicinal and skin care properties. The name Aloe vera derives from the Arabic word "Alloeh" meaning "shining bitter substance," while "vera" in Latin means "true." 2000 years ago, the Greek scientists regarded Aloe vera as the universal panacea. The Egyptians called Aloe "the plant of immortality." Today, the Aloe vera plant has been used for various purposes in dermatology. Aloe vera has been used for medicinal purposes in several cultures for millennia: Greece, Egypt, India, Mexico, Japan and China. Egyptian queens Nefertiti and Cleopatra used it as part of their regular beauty regimes. Alexander the Great, and Christopher Columbus used it to treat soldiers' wounds. The first reference to Aloe vera in English was a translation by John Goodyew in A.D. 1655 of Dioscorides' Medical treatise De Materia Medica.By the early 1800s, Aloe vera was in use as a laxative in the United States, but in the mid-1930s, a turning point occurred when it was successfully used to treat chronic and severe radiation dermatitis. *Aloe barbadensis* Miller (*Aloe vera)* belongs to the Liliaceae family, of which there are about360 species. It is a cactus-like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities. The gel of *A. vera* was used to treat stomach ailments, gastrointestinal problems, skin disease, constipation, radiation injury, inflammatory effect, healing wounds and burns,ulcer and diabetes. A number of reports are available on the microbial and fungal activity of the hexane, acetone, petroleum ether; ethyl acetate extracts*A. vera* gel and leaves the present study focuses on weather ethanol and DMSO extract show antibacterial and antifungal activity on selected strains of bacteria and fungi.

MATERIALS AND METHODS:

Collection of Plant Material

Aloe vera leaves were collected from the Professor Jayashankar Telangana state agricultural university,Hyderabad

Test Organisms

Reference strains *viz*., *Bacillus subtilis* (MTCC 441), *Salmonella typhi* (MTCC 531), Pencillium notatum(MTCC 5108) Aspergillus fumigates(MTCC 9657) were obtained from Shantha Biotech Hyderabad.

Extraction

Mature, healthy and fresh leaves of *A. vera* were washed in the running tap water for 5 min and rinsed with sterile distilled water, then dissected longitudinally and the colourless parenchymatous tissue (aloe gel) was scraped out using a sterile knife without the fibres.

Preparation of Ethanolic extract

For the preparation of ethanol extract, fresh leaf gel was dried in the oven at 80 0C for 48 h. and then powdered. Twenty grams of this powder was filtered in 200ml. of of the solvent namely ethanol through Whattman filter paper no. 1 and the filtrate was evaporated to dryness.This dried extract was further powdered and then dissolved in distilled water. Different concentration of *A. vera* gel extract was subjected to antimicrobial and anti fungal studies.

Preparation of Ethanolic stock solution

1 gm of Ethanolic extract powder was dissolved in 100ml of distilled water to get a concentration of 0.01gm i.e 10mg /1ml which is labeled as stock solution 1.

Again from the above solution 1ml was dissolved in 100ml distilled water to get a concentration of 1mg /1ml which is labeled as stock solution 2.

From the above stock solution 2 different concentrations such as 100,200,300,400and500 µg were prepared

S.No	ML of solution taken from stock solution 2.	Concentration (µg)
1.	0.1ml	100
2.	0.2ml	200
3.	0.3ml	300
4.	0.4ml	400
5.	0.5ml	500

Preparation of DMSO extract

The gel was ground with DMSO using the mortar and pestle. The extracts were filtered using Whatman filter paper No. 1and the filtrate was centrifuged at 5000 rpm for 5 min. The supernatant liquid was evaporated to dryness.Different concentration of *A. vera* gel extract was subjected to antimicrobial and anti fungal studies.

Preparation of DMSO stock solution

1 gm of DMSO extract powder was dissolved in 100ml of distilled water to get a concentration of 0.01gm i.e 10mg /1ml which is labeled as stock solution 1.

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5.	0.5ml	500

Preparation of nutrient agar medium

28gms of nutrient agar powder was dissolved in 1000ml of distilled water in conical flask and was sterilized in auto clave at 121 °C at 15 lbs pressure for 15min

Inoculation of bacterial and fungal cultures on nutrient medium

Species of bacteria and fungi were inoculated on 5 petri plates each by cup plate method in Laminar air flow chamber and incubated for 24hrs at 37°c for proper growth of bacteria and fungi.

Inoculation of Ethanolic and DMSO extract of aloe vera gel extract

Several concentrations of ethanol and DMSO in the range of 100,200,300,400 and 500 µg were inoculated on incubated petri plates with bacterial and fungal species and kept in incubator for 24hrs at 37°c for measuring zone of inhibition.

RESULTS AND DISCUSSION:

Table 1: Anti bacterial activity of ethanolic extract (Bacillus subtilis)

Concentration in µg/ml	Zone of inhibition in mm
100	4
200	4.9
300	5
400	6.1
500	7

Table 2: Anti bacterial activity of DMSO extract (Bacillus subtilis)

Concentration in µg/ml	Zone of inhibition in mm
100	3
200	4.5
300	5
400	5.2
500	5.8

Table 3 : Anti bacterial activity of ethanolic extract (Salmonella typhi)

Concentration in µg/ml	Zone of inhibition in mm
100	1.8
200	2.1
300	3
400	3.4
500	4.0

Table 4: Anti bacterial activity of DMSO extract (Salmonella typhi)

Concentration in µg/ml	Zone of inhibition in mm
100	3
200	3.2
300	4
400	4.1
500	5

Table 5: Anti Fungal activity of Ethanolic extract (Aspergillus fumigatus)

Concentration in µg/ml	Zone of inhibition in mm
100	2
200	2.9
300	4
400	6
500	7.9

Table 6: Anti Fungal activity of DMSO extract (Aspergillus fumigates)

Concentration in µg/ml	Zone of inhibition in mm
100	5
200	5.3
300	5.9
400	6.3
500	7.9

Table 7: Anti Fungal activity of Ethanolic extract (Pencillium notatum)

Concentration in µg/ml	Zone of inhibition in mm
100	3.1
200	4
300	4.1
400	6.2
500	8.1

Table 8: Anti Fungal activity of DMSO extract (Pencillium notatum)

Concentration in µg/ml	Zone of inhibition in mm
100	6.1
200	6.4
300	7
400	7.2
500	7.9

All the inoculated cultures showed good antibacterial and antifungal activity with significant zone of inhibition.

Ibrahim *et al[* ***2]*** investigated the phyto constituents and antimicrobial activity of aqueous, ethanol and acetone extracts of the *A.vera* gel against some human and plant pathogens by disc diffusion method. Among the three extracts, ethanol and acetone extracts recorded significant antimicrobial all test pathogens. Antibacterial and antifungal activity of the acetone extract was found to be quite impressive as compared to ethanol and aqueous extracts

Cock [**3]** studied the antimicrobial activity of *A. barbadensis* leaf gel components. Methanolic extracts of *A.barbadensis* inner leaf gel were fractionated byRP-HPLC and the resultant fractions were tested for inhibitory activity against a panel of bacteria and fungi. Five fractions were identified as having antimicrobial activity. Of which fraction1 had the broadest antibacterial activity

Agarry et al [**4]**compared the antimicrobial activities ofethanolic extracts of *A. vera* gel and leaf against*S. aureus*, *P. aeruginosa*, *Trichophytonmentagraphytes*, *T. schoeleinii*, *M.canis*and*C.albicans*. Antimicrobial susceptibility test showed that both the gel and the leafinhibitedthe growth of *S. aureus*. Only the gel inhibitedthe growth of *T. mentagrophytes*, while the leafpossesses inhibitory effects on both *P.aeruginosa* and *C. albicans*

.Thiruppathi *et al [5]* conducted a study to determine the antimicrobial activity of *A. vera* juice with different solvents viz., hexane, ethyl acetate, petroleum ether and ethanol against Gram positive bacteria(*B. subtilis*, *S. aureus*), Gram negative bacteria(*E. coli*, *K. pneumoniae*, *P. aeruginosa*). The result showed that more antimicrobial activity inethyl acetate (1-9 mm) and ethanol extract (7-12mm). The least inhibitory effect on petroleum ether extract was 2 mm

Thiruppathi *et al* prepared the *A. vera* gel crude extracts according to the method described by Ahmad et al 21 with minor modifications. 1gm gel extract was mixed in 5 mL of ethanol and mixed well and kept it under shaker for overnight 22. After overnight incubation the mixture was filtered through Whatmann No. 1paper and it was evaporated at room temperature. After evaporation, pellet was resuspended with 0.5 mL of Di Methyl SulphoOxide (DMSO) using micro syringe and recollect it for further use. Plant powder residue left after ethanol extraction was sequentially extracted with ethyl acetate, hexane and petroleum ether. They studied the antibacterial activity of hexane, ethyl acetate, petroleum ether and ethanol extract of *A. vera* gel against Grampositive bacteria (*B. subtilis*, *S. aureus*), Gramnegative bacteria (*E. coli*, *K. pneumoniae*, *P.aeruginosa*).

Arunkumar and Muthuselvam[**6**] used three different solvents aqueous, ethanol and acetone to extract the bioactive compounds from the leaves of *A. vera* to screen the antimicrobial activity against selected human clinical pathogens by agar diffusion method. They observed the maximum antibacterial activities in acetone extracts(12±0.45 nm, 20±0.35 nm, 20±0.57 nm and15±0.38 nm) other then aqueous and ethanol extracts. The maximum antifungal activity was observed in acetone extracts (15±0.73 nm and8±0.37 nm) when weighed against other extracts.
Pawar et al[**7**] prepared the crude *A.vera* gel extract by hot extraction with acetone, ethanol and methanol in the oven at 80°C for 48 h. In the present study, we prepared the cold *A.vera* extracts by using Dimethyl sulfoxide as a solvent.

Pugh et al[8]and Lawless and Allan screened the antimicrobial activity of *A. vera* gel against the pathogens viz., *Saureus, B. subtilis,K. pneumonia, Streptococcus pyogenes, Pseudomonas, E. coli, Helicobacter pylori* and *S. typhi.* They observed the maximum zone of inhibition against *Bacillus* with 23 mm. They observed the minimum inhibition activity against the pathogen *E. coli*. In contrary in the present study we observed maximum zone of inhibition against the pathogen *E.coli* (13 mm).
.Alemdar and Agaoglu[9] conducted a study to determine the antimicrobial activity of the *A.vera* juice against Gram-positive bacteria(*Mycobacterium smegmatis, S. aureus,Enterococcus faecalis, M. luteus* and *B.sphericus*), Gram-negative bacteria (*P.aeruginosa, K. pneumoniae, E. coli* and *S.typhimurium*) and *C. albicans* as *in vitro*. Thestudy showed that *A. vera* juice hasantimicrobial activity against *M. smegmatis*, *K.pneumoniae*, *E. faecalis*, *M. luteus*, *C.albicans* and *B. sphericus*, but has no inhibitory effect against the other bacterial strains. The result of the present study supplemented the previous observations on the antimicrobial activity of the *A. vera* gel extracts.

CONCLUSION:

As global antibiotic and fungalresistance by bacteria is becoming aninteresting public health concern and the race to discover thenew antibacterial agent is on, *Aloe vera* gel along with itsidentified compounds with promising antibacterial activitycould be used as an alternative herbal remedy. Further, thesecompounds also have been reported to have a number ofother advantages on human health along with little sideeffects in the overdoses. Hence, it can be concluded that the
compounds isolated from *Aloe vera* gel extracts could berecommended for human trials (in proper dosage) against different bacterial and fungal pathogens

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