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Antifungal Activity of *Aloe Vera* Extracts Against Phytopathogenic Fungus *Aspergillus* Spp.

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ABSTRACT

Medicinal plant contains many natural products to perform antifungal activity. The present study was undertaken to determine the antifungal activity of *Aloe vera* gel with ethyl acetate and methanol extracts against the *Aspergillus* spp. The fungus was isolated using standard potato dextrose agar. After identifying the fungus based on their morphology they were subjected to various biochemical tests. Gas chromatography-mass spectrometry (GC-MS) analysis was also performed to determine the content of *Aloe vera* gel with ethyl acetate and methanol extractions. The *Aloe vera* gel with methanol extract was found to have high yield as compared to ethyl acetate extract. The methanol extraction of *Aloe vera* also showed high inhibition rate of fungal flora as depicted by colony forming unit (CFU) method. Further studies are recommended to ascertain the role of *Aloe vera* with other solvent extracts as a potent pharmacological and therapeutic agent

Keywords: Antifungal activity, *Aloe vera*, extracts, GCMS

INTRODUCTION

Soil harbors several pathogenic fungi causing diseases and deaths worldwide. It becomes challenging to manage fungal infections as the underlying pathogen is difficult to diagnose and treat them with available anti-fungal drugs. Owing to the less adverse effect of herbal formulas, the natural products have emerged as the single most powerful strategy for treating fungal infections. *Aloe vera* has been found to exhibit antifungal, anti-inflammatory, antiviral and immune-stimulatory activity.[(1)] Numerous studies have documented the efficacy of *Aloe vera* gel extractions as a potent antimicrobial. [(2)] Keeping the pharmacological activity of *Aloe vera* in mind, the present study was designed to screen the important phytochemicals present in its methanol and ethyl acetate extract and to reveal their antifungal activity against *Aspergillus* spp. The current study documents the effect of *Aloe vera* gel with two different extracts in mitigating fungal growth.

MATERIALS & METHODS

Soil sample collection: The soil samples were collected from 8-10 cm depth of ground using a sterile spatula from Gopalpura road, Kalyanpuri colony and RIICO industrial area of Jaipur district, Rajasthan and were transferred to sterilized polybags immediately.

Isolation of the fungi: Potato Dextrose Agar was used to isolate fungi using standard soil dilution method. [(3)] The selected dilution factor taken for further experiments was 10^3.

Morphology observation by staining with cotton blue: Shape and size of fungal mycelia were observed under microscope after routine staining procedure using cotton blue and lactophenol.

Biochemical tests:

(1) Phosphate solubilizing screening test: Pikovskaya's media was used for the phosphate solubilising screening test. [(4)] The prepared medium was adjusted to pH 7.0. Halo zones around the fungal colonies were considered positive cultures.

The index of solubilization (SI) was considered by the given formula

$$SI = \frac{colony\ diameter + halo\ zone\ diameter}{colony\ diameter}$$

(2) Starch hydrolysis test: Starch agar medium was inoculated with isolated fungal cultures. [(5)] The plates were incubated at 25˚C in the inverted position for 5 to 7 days. The surface of the plates was flooded with iodine solution for 30 seconds. Positive results showed a clear zone around the fungal growth.

(3) Cellulose hydrolysis test: The isolated fungus was cultured on Czapek Dox agar medium under aseptic conditions and incubated at 35˚C in an inverted position for 5 days. The plated surface was flooded with 1% aqueous solution of hexadecyl trimethyl ammonium bromide for 30 seconds. The positive cultures were observed by the halo zone around the fungus.

(4) Lipase hydrolysis test: Tributyrin agar was used to examine lipase hydrolysis-producing fungi. Isolated fungal cultures were inoculated on the TBA plates and allowed to incubate for 10 -15 days at 27˚ C. The fungal colonies producing lipase showed a clear zone around them.

(5) Protein content estimation by Lowry's method: The Lowry method was used to determine protein content. [(6)] For this, 0.5 ml of protein solution was taken in glass test tube and 2.5 ml alkaline solution which was prepared by mixing 2% Na_2CO_3 (in 0.1N NaOH), 2% sodium potassium tartrate with 1% $CuSO_4.5H_2O$ in 100: 1: 1 ratio was added to determine protein content.

Plant extracts used:

The plant *Aloe vera* was used for determining antifungal activity with two extracts-methanol and ethyl acetate. The *Aloe vera* gel with both extracts weighed 800 grams. The weight of extract was 18.50 gm and 19.30 gm for ethyl acetate and methanol, respectively. The gels were kept at 4˚C for further requirements.

GC-MS analysis:

For GCMS analysis, the plant Hewlett Packard 6890/5973 GCMS, which operates at 1000ev ionization energy using the standard samples and extract, were determined through Agilent equipped at 7890 A/ 5975C GCHP-5. The source of carrier gas helium (He) was used along with capillary column (phenyl, methyl, siloxane) and 25m*0.23 mm through spilled ratio 1:5. The temperature of the oven was 100º C for 3 minutes to 28º C at 1 to 40º C min; the detector was 250 to 2, carrier gas He (0.9ml/ mm). Maintenance time was used to analyze maintenance indices of samples, which were injected beneath a similar chromatographic condition. Mass comparison of spectra along with retention time, given in prose, was a way to the recognize component of the standard and plant sample and by comparing mass spectra of the Wiley library or with the existing mass spectra.

RESULTS

Isolation of fungus: Potato dextrose agar (PDA) media was used to isolate fungi by serial dilution method. Serial dilution was carried out by using 1 gm soil sample from each selected region in 9 ml sterilized distilled water to make (10^{-1} to 10^{-10}) dilution factor of each soil sample. Microbial suspension of 10^{-3} was used to isolate fungi and incubated at 28±2˚C for seven days. The fungal colonies emerged after seven days.

The colonies were further microscopically screened for their morphology.

Biochemical tests:

The result of Phosphate solubilization screening test showed that all three fungi- *Aspergillus niger*, *Aspergillus flavus and Aspergillus fumigatus* showed a formation of halo zone. The Solubilization Indices (SI) was also recorded. All three diverse fungal flora showed negative response to starch hydrolysis test whereas cellulose hydrolysis test came positive as the colonies showed clear zone formation. In lipase hydrolysis test only *A. flavus* was negative for the results while *A. niger* and *A. fumigates* were positive for results. (Table 1)

Fungal species	Phosphate solubilization screening test	Starch hydrolysis test	Cellulose hydrolysis test	Lipase hydrolysis test
Aspergillus niger	1.4 ± 0.18	-	+	+
Aspergillus fumigatus	1.1 ± 0.35	-	+	+
Aspergillus flavus	1.2 ± 0.13	-	+	-

Table 1- Result for biochemical tests

Protein content determination by Lowry's Method: *A. flavus* showed least protein content 0.137) followed by *A. fumigatus* (0.250) while *A. niger* showed the maximum protein content (0.634).

Antifungal activity of *Aloe vera* with both extracts: The antifungal activity of both *Aloe vera* gel extracts on three different *Aspergillus* spp. is shown in table 2

Fungal flora of soil sample	CFU % in PDA (Control)	CFU % in medium containing Aloe vera with ethyl acetate extract	CFU % in medium containing Aloe vera with methanol extract
Aspergillus niger	5.74	5.64	5.56
Aspergillus fumigates	5.73	5.64	5.62
Aspergillus flavus	5.49	5.39	5.34

Table 2- Colony forming unit of isolated *Aspergillus sp.*

GC-MS Report of *Aloe vera* plant extract:

The GS-MS of ethyl acetate and methanol extract is shown in Table 3 and Table 4.

RT	Compound Name	Area	Area%
4.53	Dimethyl sulfone	413969744	3.96
12.26	9Octadecene,(E)	20732134	0.2
13.46	Bicyclo-[7.1.0]-decane	16805361	0.16
14	Phenol, 2,4bis(1,1dimethylethyl)	35676912	0.34
14.61	Dodecanoic acid	104472017	1
14.83	Fumaric acid, ethyl 2methylallyl Ester	22019358	0.21
15.04	3-Eicosene,(E	39115128	0.37
15.14	Hexadecane	35920265	0.34
15.85	10-Undecenoic acid, ethyl ester	56788187	0.54
16.41	10-Methylnonadecane	21236020	0.2
17.13	Tetradecanoic acid	234968059	2.25
17.54	E15-Heptadecenal	193063143	1.84
17.62	Hexadecane	97545848	0.93
17.92	Isopropyl myristate	23797401	0.23
18.1	3,7,11,15 Tetramethyl2hexadecen1ol	58493193	0.56
18.31	Pentadecanoic acid	97907782	0.94
18.5	1,2Benzenedicarboxylic acid, bis (2methylpropyl) ester	96172147	0.92
18.56	1-Hexadecanol	43824080	0.42
18.77	Hexadecane	16156379	0.15
19.06	Hexadecanoic acid, methyl ester	52610307	0.5
19.24	n-Hexadecanoic acid	25410653	0.24
19.48	n-Hexadecanoic acid	3690247030	35.26
19.8	3Eicosene,-(E)	284001515	2.71
19.87	Eicosane	52424005	0.5

Table 3 To Be Continued…			
20.23	9-Hexacosene	12690240	0.12
20.31	10-Heneicosene-(c,t)	25382175	0.24
20.52	Tetradecanoic acid	114645939	1.1
20.77	n-Nonadecanol-1	40193529	0.38
21.35	Cis-Vaccenic acid	2018671527	19.29
21.56	Octadecanoic acid	495633059	4.74
21.87	1-Heneicosanol	156450768	1.49
22.02	2,6,10,14Tetramethylpentadecan-2ol-	15151469	0.14
22.55	Oxalic acid, allyl tetradecyl ester	27941791	0.27
22.89	Eicosane	42223428	0.4
23.49	11-Tricosene	82677700	0.79
23.77	n-Tetracosano-l1	68599136	0.66
23.81	Eicosane	73206945	0.7
24.11	1-Hexyl 2- nitrocyclohexane	19895103	0.19
24.7	Eicosane	227412640	2.17
24.85	Hexadecanoic acid, 2-hydroxy1 (hydroxymethyl) ethyl ester	40044914	0.38
25.01	Hexadecanal	12438108	0.12
25.21	Phthalic acid, di (2- propylpentyl) Ester	222232151	2.12
25.3	1-Octanol,2-butyl	65681867	0.63
25.56	Eicosane	180615758	1.73
26.49	1-Dodecanol,2-octyl	354843383	3.39
27.07	1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	52927962	0.51
27.44	1-Hexyl 2- nitrocyclo hexane	34092230	0.33
27.56	Eicosane	130547200	1.25
28.01	Squalene	71214439	0.68
28.83	1-Dodecanol,2-octyl-	146721983	1.4

Table- 3 Gas chromatography-mass spectrometry examination of *Aloe vera* ethyl acetate gel extract

RT	Compound Name	Area	Area%
4.52	5-Thio d-glucopyranose	12037708	1.06
4.63	Carbonic acid, ethyl 2-mercaptoethyl Ester	5070499	0.45
4.75	3-(1'pyrrolidinyl)-2-butanone	25426631	2.24
5.19	5-Thio-d-glucopyranose	11938796	1.05
5.35	Benzene-1,4-diol monophosphate	22331862	1.97
5.59	2-Butene,1-bromo	46241735	4.07
5.85	Decane, 2,5,6-trimethyl	19389795	1.71
5.94	Cyclobutanone, 2-(2,6-dimethylheptyl)	12180718	1.07
6.71	Benzene acetaldehyde	20539633	1.81
6.78	[1,4]-Dioxino-[2,3b] 1,4-dioxin, hexahydro 2,3,6,7-tetramethyl	14398598	1.27
7	Cyclopropane, 1,1-dichloro-2-hexyl	8865328	0.78
7.09	Propylamine, N,N,2,2-tetramethyl, Noxide	2813352	0.25
7.3	l-Alanine,Ncyclobutylcarbonyl,heptyl ester	49231654	4.33
7.43	2,2-DiethylNethylpyrrolidine	27994097	2.46
7.71	1,3-Dioxolane, 2-cyclohexyl 4,5 –dimethyl	41911678	3.69
8.11	2,6,10,14-Tetramethylpentadecan-2-ol	3755929	0.33
8.29	Propane, 1,2-bis (difluoroamino)2-methyl	7490264	0.66
8.46	4H-Pyran-4 one, 2,3-dihydro 3,5 dihydroxy6methyl	162595524	14.31
9.28	Isosorbide Dinitrate	6752384	0.59
9.37	Isosorbide Dinitrate	5075505	0.45
9.65	à-D-Glucopyranoside, O-à-D-glucopyranosyl (1.fwdarw.3) á-D-fructofuranosyl	7376603	0.65
9.85	5- Hydroxymethylfurfural	281603265	24.78
10.2	1-HImidazole,-2 (diethoxymethyl)	3846937	0.34
10.4	4,6-dimethyl2-propyl-1,3,5-dithiazinane	2854020	0.25
10.9	a-D-Galactopyranosiduronic acid, methyl, methyl ester	91398223	8.04
12.4	6,10,14-Trimethyl-pentadecan-2-ol	6766622	0.6
12.4	6,10,14-Trimethyl-pentadecan2-ol	12870027	1.13
14.6	2,2-Dimethylpropyl 2,2-dimethyl –propanesulfinyl Sulfone	3739047	0.33
14.9	aD-Glucopyranoside, O-à-D-glucopyranosyl (1.fwdarw.3)-á-D-fructofuranosyl	3061283	0.27
16.6	1,1-Diisobutoxy –butane	5499531	0.48
16.7	a-D-Glucosylo xyazoxymethane	4817663	0.42
16.8	a-D-Glucopyranoside, methyl 3,6-anhydro-	4014738	0.35
17.2	Z-2-Dodecenol	13014367	1.15
18.3	Sucrose	3229198	0.28
19.1	Dodecanoic acid, 2methyl	2873579	0.25
19.4	17-Octadecene-9,11-diynoic acid, 8-hydroxy, methyl ester	9519432	0.84
19.5	-n Hexadecanoic acid	57406872	5.05
19.6	-Phthalic acid, 2-chloropropyl isobutyl ester	8990137	0.79
20.9	Cyclohexanemethyl propanoate	2991435	0.26
21	Methyl 12-oxo-9-dodecenoate	3626717	0.32
21.4	1-Hexyl-2-nitrocyclohexane	33305775	2.93

Table 4 To Be Continued…			
23.8	Eicosane	2166153	0.19
24.7	Sulfurous acid, butyl nonyl ester	4583680	0.4
25.2	Phthalic acid, di (2propylpentyl) Ester	5342782	0.47
25.6	Sulfurous acid, butyl heptadecyl ester	8484968	0.75
26.5	Sulfurous acid, butyl nonyl ester	16489019	1.45
27.6	Sulfurous acid, butyl heptadecyl ester	14064142	1.24
28	Squalene	4746861	0.42
28.8	2- methyloctacosane	7294293	0.64
29.7	Propennitrile, 2-isopropyl sulfonyl-3-(3pyridylamino)-	4403939	0.39

Table 4- Gas Chromatography-Mass Spectrometry examination methanol *Aloe vera* gel extract

DISCUSSION

Despite the presence of various nutrients including vitamins, sugars, enzymes, minerals, lignin, saponins, tannins, sterols, anthraquinones, salicylic acid and amino acids in *Aloe vera*, a little is documented about its role in treating fungal infections. Many studies have confirmed the presence of phytochemicals, alkaloids and glycosides in *Aloe vera*. [(7)] A similar study has also documented the greater effectiveness of methanolic extract as compared to ethanolic extract of *Aloe vera* in inhibiting the *Aspergillus* growth. [(8)] The report of antifungal activity of *Aloe vera* in our study is in complete sync with one finding where different *Aloe vera* extracts (chloroform, ethanol and petroleum) showed antifungal properties. [(9)] Similarly the *Aloe vera* extract of Methanol was found to contain maximum antimicrobial activity as compared to other solvents like ethanol and distilled water. [(10)] Our results also corroborate with one study where greater effectiveness of antimicrobial activity of methanolic extracts of *Aloe vera* were documented. [(11)] Our results are also suggestive of methanolic extracts to be more effective than ethyl acetate. The difference found in the antimicrobial effects of methanol and ethyl acetate of *Aloe vera* can be attributed to different solubility of both various compounds.

CONCLUSION

The study revealed the presence of numerous phytochemicals in *Aloe vera* plant. All three isolated *Aspergillus* spp. showed a clear growth inhibition with both extracts as compared to standard. The methanol extract showed strong antifungal activity as compared to ethyl acetate. The study proved that the 1.0 mg/ml concentration of *Aloe vera* extract can significantly reduce fungi expansion and can be traditionally used as antifungal.

Recommendations

The current study strongly recommends both methanolic and ethyl acetate extractions over aqueous solvent of *Aloe vera* as a natural potent antifungal. The antifungal properties of *Aloe vera* with different solvents need to be investigated further.

Declaration by Authors

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