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Study on Effect of *Aloe vera* Leaf Extracts on Growth of *Aspergillus flavus*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AB, MM and HT participated in the study design, data analysis and the experimental work. Author MM drafted the manuscript and carried out the manuscript writing and participated in the critical review of the manuscript. Authors AB and HT coordinated in the field sample collection. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The aim of this study is to evaluate and compare the antifungal activity of different extracts of *Aloe vera* plant on the growth of *Aspergillus flavus*.
Study Design: Considering the wide dispersal of *Aspergillus flavus* across the globe and its massive contamination on feed and food stuff of animal and human beings, it is inevitable to find a solution to inhibit the growth of this fungus and subsequently production of aflatoxin.
Place and Duration of Study: Department of Animal Science, Faculty of Agricultural Science, Malayer University, between August 2012 and March 2013.
Methodology: Six different solvents such as acetone, ethanol, water, methanol, chloroform and ethyl ether were employed for extraction from *Aloe Vera* fresh leaves. Antifungal activity of the extracts was evaluated by Agar Plate Diffusion Plate method.
Results: The maximum antifungal activity (100%) observed was in acetone extract group in concentration of 2000µL; the complete inhibition of fungus growth was found (100%).
Conclusion: The acetone extract of *Aloe vera* can be used as an effective antifungal agent to inhibit the growth of *A. flavus* compared to other solvents.

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Keywords: Aloe vera; A. flavus; antifungal; aflatoxin.

1. INTRODUCTION

Medicinal plants are a rich source of antimicrobial agents and normally produce bioactive secondary metabolites and many of them exhibit activity, hence can be used in antimicrobial drugs [1,2]. *Aloe vera* is a stemless or very short-stemmed succulent plant belongs to family Liliaceae. The leaves are hard edges, thick, fleshy and green to grey-green– in color- with some varieties showing white flecks on the upper and lower stem surfaces [3]. *Aloe vera* contains various components including phenol, saponin, anthraquinones, which are classified as anti-bacterial, antiviral and antifungal agents. One of the microorganisms engaged in oxidation and spoilage of feed and food stuff (especially those are stored before use) are fungi, which decreases the value of feed stuff. Among all, the dominant form of fungus that could normally grow on stored products in favored conditions are *Aspergillus*, *Fusarium* and *Penicillium* species [4]. *Aspergillus flavus* and *Aspergillus parasiticus* have a global distribution to contaminate agricultural products and are found on many types of perishable organic materials [5]. These fungi produce toxic and carcinogenic secondary 40 metabolites, such as aflatoxin group mycotoxins. Aflatoxin is considered as the most potent mycotoxin that can have several adverse effects which needs a tight control on growth the pathogenic strains of *A. flavus* and *A. parasiticus* fungi and subsequently aflatoxin production [6,7,8]. Consumption of feed and food stuff contaminated with aflatoxin causes acute or chronic diseases such as liver cancer or can be fatal, if consumed in high quantities [9,10,11]. One of the strategies to reduce the adverse effects of aflatoxin problems is preventing mold growth on the substrate [12]. Natural herb components are widely used in countries like, Japan, India and Russia to preserve the feed, traditionally [13]. In several studies it has been reported that extracts and powders from various herbs and oils have antifungal activity and some of them are even inhibit the production of aflatoxin [14,15,16] as demonstrated against the mycelium growth of *Botrytis gladiolorum*, *Heterosporium pruneti*, *Fusarium oxysporium* and *Penicillium gladioli.* Especially hydro alcoholic extracts of fresh *Aloe vera* leaves strongly controlled aflatoxin production [17]. Similarly the antifungal activity of this plant has been reported [18]. Various herbs such as *Centella asiatica, Areca catechu, Piper betle, Momordica charantia, Cirtrus reticulate* and *Cassia bakeriana* have been studied for growth inhibition of *A. flavus* and results showed that raw ethanol extracts of some medicinal plants can inhibit the fungi growth [19]. Aqueous extracts of plants such as *Lupinus albus*, *Ammi visnagaand Xanthium pungens* could cause the growth of mycelia and aflatoxin production by *A. flavus* [4]. It has been found that extracts of *Argemone Mexicana* and *Cyperus rotundus* could control the aflatoxin production through inhibition of *A. flavus* growth [20]. The aim of this study is to evaluate and compare the antifungal activity of different extracts of *Aloe vera* plant on the growth of *A. flavus*.

2. MATERIALS AND METHODS

2.1 Initial Preparation of Plant

Freshly collected *Aloe vera* leaves were washed with distilled water, followed by disinfecting with ethanol 70%. Later, they were chopped into the small pieces and were exposed to 60ºC for 3 days to get dried. After complete drying, leaf parts were powdered using Electric Grinder.

2.2 Preparation of Extracts

30g of powdered plant material was mixed with 100ml of each of acetone, ethanol, water, methanol, chloroform and ethyl ether (total 22 samples) and kept in room temperature for 72h. Later, each mixture was filtered through Whatman No.1 paper filter and then separated part evaporated at 65ºC in Water Bath till complete dryness. Dried extracts were powdered and again dissolved in small portion with respected solvent and distilled water (50:50) and kept at 4ºC for further analysis.

2.3 Fungus Strain

In this study, *Aspergillus flavus* (ATCC5004) strains were obtained from the Department of Mycology, Pasteur Institute of Iran. Fungi were cultured on Potato Dextrose Agar (PDA) medium. The fungus was cultured in the laboratory and preserved. It has been sub-cultured on PDA for 7 days at 28ºC and slants were keptin 4ºC for further study after 7 days of growth.

2.4 Evaluation of Antifungal Activity of Extracts Obtained by Agar Plate Diffusion Plate Method

To evaluate the antifungal activity of extracts obtained by different solvents, Agar Plate Diffusion Plate method was employed. Various concentrations (0, 2, 20, 200 and 2000µL in 20mL) of each extract is added into hot PDA (60-70ºC) and mixed. After solidification of plates, produced fungi plaques (0.4 mm) of standard strain were spotted in the center of the plate of each concentrates and incubated at 28ºC for 7 days. There were 3 replicated for each treatment. At the end of day 7, the growth of fungi (cm in radial) is measured and statistically analyzed. The fungi growth inhibition is calculated using below formula [17].

$$\text{Mycelium growth inhibition (\%)} = \frac{(\text{colony diameter in control group} - \text{colony diameter in treatment})}{\text{colony diameter in control group}} \text{X100}$$

3. RESULTS AND DISCUSSION

As shown in Table 1 and Figs. 1 & 2, the antifungal activity of different extracts of Aloe vera on radial growth of A. flavus fungus in 0, 2, 20, 200, and 2000µL in 20µL assessed using Agar Plate Diffusion method. Statistical analysis revealed that, a significant ($P<0.05$) reduction in fungal growth and antifungal activity of in 2000µL of acetone extract and had a positive impact on inhibition of *A. flavus* production (100%). It has also showed the minimum inhibitory effect in 2µL of methanol extract of *Aloe Vera* leaves (6.25%). Results showed that acetone extract of *Aloe vera* leaves in concentration of 2000µL could significantly inhibit (100%) the growth of *A. flavus* and at the lowest concentration of this extract (2µL), the growth inhibition was found to be 51.72%. In *Aloe vera* extracts with solvents ethanol, ethyl ether, water, chloroform and methanol – at the highest concentration used in the extraction – the growth prevention of 37.5%, 48%, 58.3%, 61.53% and 76.72% were found respectively. In these extracts, the minimum concentration of 2µL was found to have the inhibitory effect 6.25%, 20%, 28.33%, 33.84% and 35.34%,

respectively. However, the maximum and the most significant impact on growth inhibition in different concentrations of acetone extract turned to the other extracts (Table 1).

Table 1. Antifungal activity of *Aloe vera* extracts on *A. flavus* growth at 28ºC after 7 days incubation

Extracts	Concentrations µl in 20ml	Colony average (cm)	Growth Inhibition (%)
Acetone Extract	0	5.8	0
	2	2.8	51.72
	20	2.3	60.34
	200	1.6	72.4
	2000	0	100
Ethanol Extract	0	5.8	0
	2	3.75	35.34
	20	3.25	44
	200	2.25	61.2
	2000	1.35	76.72
Methanol Extract	0	4	0
	2	3.75	6.25
	20	3.25	18.75
	200	2.75	31.25
	2000	2.5	37.5
Diethyl Ether Extract	0	6.5	0
	2	4.3	33.84
	20	4.25	34.61
	200	4	38.46
	2000	2.5	61.53
Chloroform Extract	0	6.25	0
	2	5	20
	20	4	36
	200	3.75	40
	2000	3.25	48
Water Extract	0	6	0
	2	4.3	28.33
	20	3.75	37.5
	200	3.25	45.8
	2000	2.5	58.3

Considering the ability of rapid growth rate of the fungus *A. flavus* on feed and food stuff, inhibiting fungal growth can greatly help the human community and animal health. Results obtained from this study can provide the basic information about the usefulness and effectiveness of different extracts of *Aloe vera* plant to reduce the fungal growth. Studies on the antifungal activity of *Aloe vera* have been conducted by different researchers across the globe. It has been proved that extracts of *Aloe Vera* have anti-fungal effects on *A. flavus, A. glaucus*, *Candida albicans, C. tropicalis, Trichophyton mentagrophytes* and *T. rubrum* [21,22]. In 2009, the highest antifungal activity of acetone extract of *Aloe vera* plant was reported on the growth of *A. niger* and *A. flavus,* which are in agreement with the findings of current study [23]. It has been reported that *Aloe vera* gel at 0.35%, showed effective antifungal potential by inhibiting the growth of *Drechslera*

hawaiensis and *Penicillium digitatum* were completely inhibited and in concentration of 0.15%, 0.25% and 0.35% of the *Aloe vera* gel, a significant reduction in production rate of *A. niger, A. flavus* and *Alternaria alternata* were seen [24,25]. It is found that Hydro-alcoholic extracts from the leaves of *Aloe vera*, had the inhibitory effect on mycelia growth of *Botrytis gladiolorum, Fusarium oxysporium, Heterosporium pruneti* and *Penicillium gladioli* [17]. Also, its antifungal activity against *Rhizoctoma solani, Fusarium oxysporium* and *Collectotrichum coccodes* was observed at a concentration of 105µL/L [19]. Similarly, the inhibitory effect on fungal growth by *Aloe vera* extract on *Alternaria alternate, A. citri* and *A. tenuissima* was studied [19].

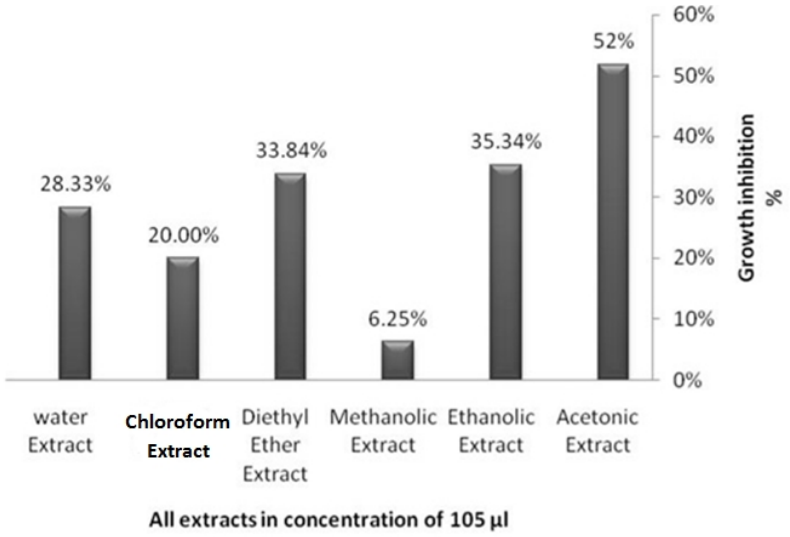

Fig. 1. Comparison of *A. flavus* growth inhibition potential of different extracts of *Aloe vera*

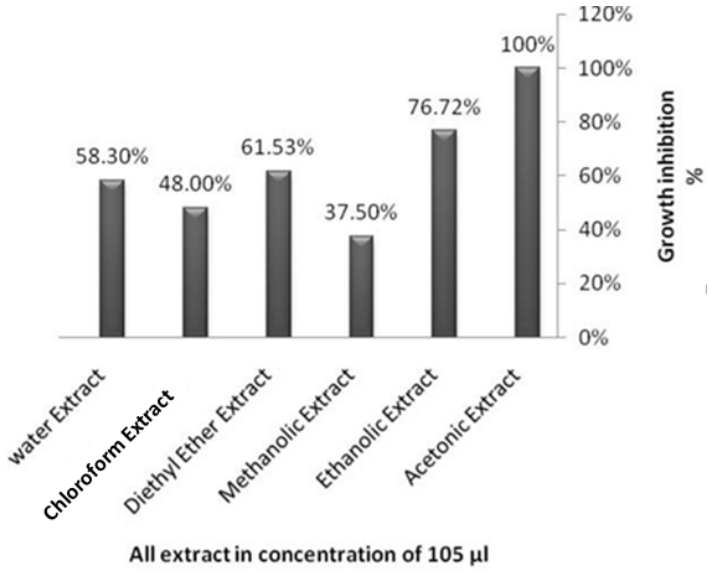

Fig. 2. Comparison of *A. flavus* growth inhibition in concentration of 10^2 in all extracts

Due to the different solubility of various compounds found in *Aloe vera*, in any particular solvent some specific compounds are isolated from this plant. Thus, each of the extracts obtained from different solvents, have a specific antifungal or antimicrobial activities. Data analysis shows that in the Agar Plate Diffusion method, antifungal effect of different extracts of *Aloe vera* on fungal growth was significant ($P<0.05$) at all tested concentrations. In this study, among all tested extracts, acetone extract at concentrations of 2000µL on the *A. flavus* growth was found to have the highest antifungal activity. Therefore it is expected that the *Aloe vera* extracted by acetone solvent can be considered and used as a more effective antifungal agent than other solvents. Hence, the advantage of the compounds produced by plants as a source of safe, harmless and more effective controlling agents than synthetic antimicrobial agents must be considered.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Muhammad BI. Anti-microbial effects of extract leaf stem and root bark of Anogeissus leiocarpus on *Staphylococcus aureaus*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus vulgaris*. J. Pharma. Devpt. 1997;2:20-30.
2. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World J. Agri. Sci. 2008;4:5, 839- 843.
3. Surjushe A, Vasani R, Saple DG. Aloe vera: a short review. Indian J Dermatol. 2008;53(4):163-6.
4. Thanaboripat D. Control of Aflatoxins in Agricultural Products using plant extracts. KMITL Sci. Tech. J. 2011;11:1-35.
5. Dvorackova I. Aflatoxins and Human Health. Informa Health Care, CRC press, Florida; 1990.
6. Ehrlich KC, Kobbeman K, Montalbano BG, Cotty PJ. Aflatoxin- producing aspergillus species from thailand. Int. J. Food Microbiol. 2007;114:153–159.
7. Hesseltine CW. A millennium of fungi, food and fermentation. Mycologia. 1965;57:149-197.
8. Narasaiah KV, Sashidhar RB, Subramanyam C. Biochemical analysis of oxidative stress in the production of aflatoxin and its precursor intermediates. Mycopathologia. 2006;162:179–189.
9. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW. Aspergillus flavus: Human pathogen, allergen and mycotoxin producer. Microbiology. 2007;153:1677-1692.
10. Pitt JI, Hocking AD. Fungi and Food Spoilage. 2nd Edition, Blackie Academic & Professional, London, United Kingdom; 1977. (http://www.springer.com/978-0-387-92206-5).

11. Sergent T, Ribonnet L, Kolosova A. Molecular and cellular effects of food contaminants and secondary plant components and their plausible interactions at the intestinal level. Food & Chem. Toxicol. 2008;46:813–841.
12. Moreno-Martinez E, Vazquez-Badillo M, Facio-Parra F. Use of propionic acid salts to inhibit aflatoxin production in stored grains of maize. Agrociencia. 2000;34(4):477-484.
13. Bullerman LB, Lieu Y, Sieier SA. Inhibition of growth and aflatoxin production by Cinnamon and Clove oils, Cinnamic aldehyde and eugenol. J. food Sci. 1977;46:1107-1109.
14. Krishnamurthy YL, Shashikala J. Inhibition of aflatoxin B1 production of *Aspergillus flavus* isolated from soybean seeds by certain natural plants products. Lett Appl Microbiol. 2006;43:469-474.
15. Thanaboripat D, Mongkontanawut N, Suvathi Y, Ruangrattametee V. Inhibition of aflatoxin production and growth of *Aspergillus flavus* by citronella oil. KMITL Sci. J. 2004;4(1):1-8.
16. Casian OR, Parvu M, Vlase L, Tamas M. Antifungal activity of *Aloe vera* leaves. Fitoterapia. 2007;78(3): 219-222.
17. Kawai K, Beppu H, Simpo K, Chihara T, Yamamoto N, Aggatsu T, Ueda H, Yamada Y. In vivo effects of Aloe arborescens Miller var natalensis Berger (Kidachi aloe) on Experimental Tinea Pidis in guinea pig feet. Phytotherapy Res. 1998;12:178-182.
18. Thanaboripat D, Prugcharoen P, Ruangrattanametee V. Inhibitory effect of some medicinal plant extracts on the growth and aflatoxin production of *Aspergillus flavus*. In Yang Q, and Yu Z, eds. 2005. Study on Plant Pest and Disease Biological Control and Bio-technology, Harbin: Heilongjiang Science and Technology Press. 2005;52-62.
19. Masood A, Ranjan KS. The effect of aqueous plant extracts on growth and aflatoxin production by *Aspergillus flavus*. Lett Appl Microbiol. 1991;13:32- 34.
20. Coopoosamy RM, Magwa ML. Traditional use, antibacterial activity and antifungal activity of crude extract of Aloe excelsa. Afr. J. Biotechnol. 2007;6(20):2406-2410.
21. Manafi, M. Counteracting Effect of High Grade Sodium Bentonite during Aflatoxicosis in Broilers. J. Agr.Sci. Tech, 2012;14:539-547.
22. Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L. against clinical pathogens. World J. Agri. Sci. 2009;5(5):572- 576.
23. Sitara U, Hassan N, Naseem J. Antifungal activity of Aloe vera gel against plant pathogenic fungi. Pak. J. Bot. 2011;43(4):2231-2233.
24. Jasso de Rodriguez D, Hernandez-Castillo, R. Rodriguez-Gracia, J. L. Angulo-Sanchez. Antifungal activity in vitro of Aloe vera pulp and liquid fraction against plant pathogenic fungi. Industrial Crops and Products. 2005;21(1):81-87.
25. Ali MI, Shalaby NMM, Elgamal MHA, Mousa ASM. Antifungal effects of different plant extracts and their major components of selected Aloe species. Phytother Res. 1999;13:401-407.

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