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EXTRACT OF *ALOE VERA* LEAVES AS ANTIBIOTIC FOR SOME FUNGI AND YEASTS CAUSING DISEASES FOR HUMAN AND CONTAMINATION OF FOOD STUFFS

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ABSTRACT

The present study aimed to use the extract *Aloe vera* leaves as natural antibiotic against *Candida albicans* which causes respiratory and dermal diseases, some fungi such as *Trichophyton rubrum* which attacks skin, hair and nails and some fungi such as *A. niger*, *A. flavus*, and *A. candidus* which cause contamination of foods and produce aflatoxins.

The extract of *Aloe vera* leaves was added at 1, 2, 3, 4 and 5 % to liquid and solid Sabouroud dextrose to examine its effect on the above mentioned fungi. It is found that all the tested concentrations inhibited the fungal growth and dry weight and the inhibition was dependent on the extract concentration.

The potent antifungal effect of the extract was found at 5 % where the radial growth values for *A. flavus*, *A. niger*, *C. albicans* and *A. candidus* were 95, 21, 33, 33, 52, 34, 4 % and 37 %, respectively compared with the control. The least affected was *A. flavus*. It was observed that spraying the extract on/ some fruits resulted in reducing the growth of contaminating fungi where the extract prevented the fungi to penetrate to inside the fruits.

Key words: Natural extracts, *Aloe, Candida, Trichophyton, Aspergillus.*

INTRODUCTION

Aloe is a desert plant. Its stem varies in length according to the various species. Its leaves are broad, fleshy and green. The margin is spiny and the epidermis is waxy. The flowers of *Aloe* are large in size and the colour varies from yellow to purple. There are three types of *Aloe* used in medicine. The first is *Aloe vera* with purple leaves. The leaf length ranges between 20- 30 cm and its width ranges between 4- 7 cm. The second type is the African one (*Aloe ferox*) which is cultivated in dry regions and resistant to dryness. The third type is Asian *Aloe* which is known as *Aloe ferox. Aloe* plants could be cultivated at any time of the year in most of the lands and it is abundant in Saudi Arabia **[Marey & Hany (2006)].**

The fungi have a widespread in nature. They are found in every place where their nutritional needs are existed. Their spores can spread quickly particularly those which contaminate food and produce mycotoxins such as the spores of *A. niger, A. flavus, A. candidus, Claviceps, Alternaria, Fusarium* and *Stachyobotrys.* These mycotoxins cause many diseases such as cancer, paralysis and others **[Tournas & Katsoudas (2005)].**

Fungicides are used to get ride of fungi, however, they cause many diseases such as kidney failure, tumors and others. In addition, when fungicides reach animal feeds they can be associated with the milk and animal meat then transfer to human body **[Abuo- Arqoob & Mosa (2002)].** Some of these fungicides can remain in the soil and persist for longer time and causes harmful effects in the environment **[Abo-Arqoob (2002)].** Therefore, it was important and urgent to find an alternative way to avoid the harmful effect of fungicides through finding out natural products such as *Aloe* extract that can alleviate fungal effects **[Alawadat & Bashy (1985)].**

Trichophyton causes skin diseases such as *Tinea capitis* and *Tinea barbae. Candida albicans* causes inflammation of eyes **[El- Fadel (1986) and Abuo- Arqoob & Mosa (2002)].** The extract of *Aloe arborescens* at concentration of 25 mg ml^{-1} inhibited the growth of *Trichophyton metagraphyte* **[Keisuke *et al.*, (1978)].** Also, it has been reported the *Aloe* extract is successful as antibiotic for some bacteria such as

Klebsiella, Serratia, Streptocococcus, Staphylococcus and *E. coli* and some fungi such as *Candida albicans* and *Trichophyton* **[David & Willams (2000)].**

Aloe extract is found to be effective as antibiotic against Gram positive bacteria such as *Streptococcus pyogenes* and *S. flexneri* **[Valerie *et al.*, (2003)]**. *Aloe* extract was effective to maintain grape fruits on storing for 35 days against oxidative activity and protecting fruits from contamination by fungal spores.

Therefore, the present investigation aimed to investigate the effect of *Aloe* extract on the growth of some fungi which contaminate foods such as *A. candidus*, *A. flavus* and *A. niger*. In addition, it is aimed to study the effect *Aloe* extract on fungi attacking human skin e.g. *T. rubrum* and *C. albicans*.

MATERIALS AND METHODS

Preparation of Aloe vera extract:

The isolated fresh leaves were washed with distilled water and then dried in air for 48 hr. The leaves were cut into small pieces and homogenized manually to obtain viscous sap which was filtered using Whatman Gf/A paper No. 2 **[Yamada & Azuma (1977)].** The obtained filtrate represented the crude extract of *Aloe* which is used for analysis.

Culture medium:

Sabouroud dextrose agar was used in this investigation. About 65 gm of the medium and dissolved in 1000 ml of distilled water. After complete dissolution the prepared medium was sterilized at 121 ℃ for 15 min. Also, the liquid Sabouroud dextrose is used where 65 gm of medium was taken into 1000 ml of distilled water, sterilized as done for the solid medium.

Isolation and identification of examined fungi:

The fungi were isolated from some contaminated fruits according to **[Abdel-Hafez (1984)]** on Glucose–Czapek's-Dox modified medium by **[Naguib (1968)]** and Rosbengal with the addition of 1/15000 L^{-1} to prevent bacterial growth **[Smith & Dawson (1944)].** The plates were

incubated at 28± 2 ℃ for 7 days. The colonies were counted and identified according to many authors **[Raper & Thom (1949); Gilman (1957); Raper & Fennel (1965); Ellis (1971 & 1976) and Sutton *et al.*, (1998)].**

Investigated fungi:

The more contaminating fungi isolated form the fruits were *A. candida*, *A. flavus* and *A. niger*. *Candida albicans* and *Trichophyton rubrum* were obtained from the laboratory of Azizyia hospital. They were pure and the identified isolates were isolated from the patients in this hospital.

Cultivation of fungi in medium contains Aloe vera extract:

Sabouroud dextrose agar was distributed into 250 conical flasks; each flask contains 50 ml, followed by sterilization, cooling at 45 ℃. Before solidification *Aloe* extract with different concentrations (1, 2, 3, 4, 5 %) was added with continuous stirring. This medium was poured in plates and left to solidify and incubated with fungal samples according to **[Bollen (1972)]**. Control examples were used. The same work was carried out for liquid medium. The area of fungal growth and dry weight was estimated according to **[Yamada & Azuma (1977)].**

Antagonistic tests:

250-conical flask containing 50 ml of Sabouroud dextrose agar was sterilized and cooled to 45 ℃. Before the medium being solidified it was incubated with spore suspension of the fungi under investigation. The medium with fungal spores were poured at plates with stirring and left for solidification. After being solidified, 50 ml of *Aloe* extract (5 %) was added to a hole within the medium in the plates. Control samples were prepared. The plates were incubated for 7 days at 28 °C and then photographed.

Testing the effect of Aloe vera leaves extract on some fruits infected by fungi:

The best concentration of *Aloe* extract was 5%. The extract at 5% was sprayed on fruits under investigation, and then incubated with *A. flavus* on their surface. Control samples to which no extract used were carried out. The treated and non-treated fruits were left 7 days at room temperature and photographed.

Statistical analysis:

The results obtained are expressed as percentages and analyzed using T-test.

RESULTS AND DISCUSSION

The results in Table (1) demonstrate that *Aloe* extract caused apparent reduction in the growth of *A. candidus, A. flavus, A. niger, C. albicans* and *T. rubrum*.

Table (1): Effect of different concentrations of *Aloe vera* extract on radial growth (cm) of the various tested fungi for 7 days of growth on solid Sabouroud medium 28 ±2 °C.

Fungi	Control	Concentration (%)				
		1%	2%	3%	4%	5%
A.candidus	8.20 ± 0.00	7.46* ± 0.04	4.60* ± 0.13	٣,٨٣* ± ٠,٠٤	٢,٦٠* ± ٠,٠٧	١,٨٠* ± ٠,٠٥
A. flavus	8.10 ± 0.00	6.90 ± 0.58	4.17* ± 0.13	٣,٥٧* ± ٠,٠٨	٣,٣٧ ± ٠,٢٩	٣,٠٠ ± ٠,٠٠
A. niger	7.33 ± 0.21	6.37* ± 0.11	5.53* ± 0.34	٤,٤٣* ± ٠,٠٤	٣,١٧* ± ٠,١١	٢,٥٣* ± ٠,٠٤
C. albicans	3.60 ± 0.07	2.37* ± 0.08	2.00* ± 0.00	١,٦٣ ± ٠,١٧	١,٤٠ ± ٠,٠٧	١,٢٠ ± ٠,٠٠
T .rubrum	7.9 ± 0.07	7.63* ± 0.11	5.70* ± 0.07	٥,٧٠* ± ٠,٠٧	٠,٠٠ ± ٠,٠٠	١,٠٠ ± ٠,٠٠

The results in Fig. 1 indicate the effect of various concentrations of *Aloe vera* extract on relative radial growth of the tested fungi. The results indicate that fungi were not able to resist the suppressive effect of *Aloe vera* extract were the relative radial growth was decreased with increasing the concentration of *Aloe vera* extract.

The reduction of the fungal growth (Fig. 2) was dependent on concentration of *Aloe* extract and this is in agreement with the results of **[Mohammed *et al.*, (1999)]**, who found the same effect of *Aloe* extract on the growth of *A. niger, Cladosporium herbaqrum* and *Fusarium moniliforme*. The effect of *Aloe* extract could be attributed to presence of some inhibitory substances such as anthraquinones and dihydroxyanthraquinones **[Boateng (2000) and Wang *et al.*, (1998)]**.

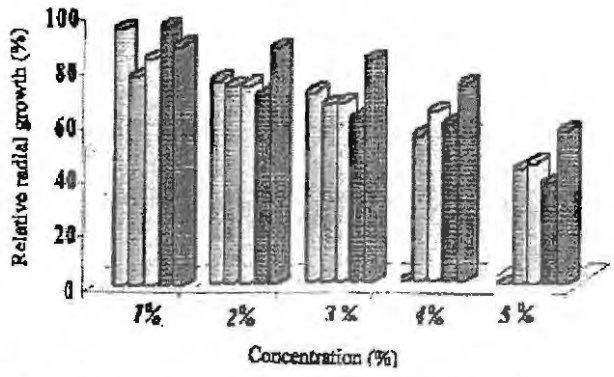

Fig. 1: Effect of various concentrations of *Aloe vera* on the relative radial growth of the tested fungi after 7 days of growth on solid Sabouroud medium at 28 ±2 °C.

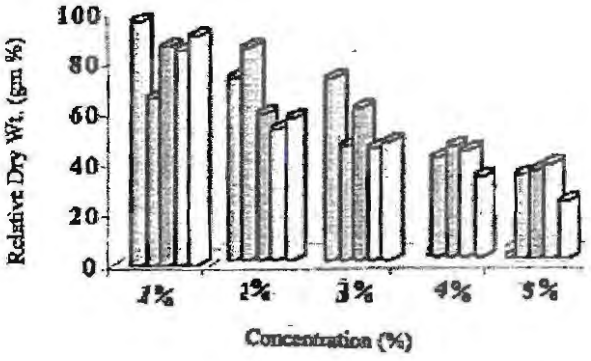

Fig. 2: Effect of various concentrations of *Aloe vera* on the relative dry weight of the tested fungi after 7 days of growth on liquid Sabouroud medium at 28 ±2 °C.

In addition, **[Valeria *et al.*, (2003)]** reported that *Streptococcus pyogenes* was largely affected by extracts from leaves of *Aloe vera* and *A. barbadensis*. On comparing the effectiveness of these extracts with the effect of ampicillin, it was found that the extracts were more effective than the ampicillin. **[Davis *et al.*, (1994)]** referred the effectiveness of these extracts to inhibit *Shigella flexneri* to the presence of acemannan which considered as antibacterial agent.

Keisuke *et al.*, (1978) reported that that extract of *Aloe* leaves proved to be strong inhibitor for *T. mentagraphytes*. This is confirming our results in the present investigation.

The results in Table (2) show that effects of the various concentrations of *Aloe* extract on the dry weight of fungi after 7 days of incubation. These results indicate that the tested fungi were unable to resist the inhibitory effect of *Aloe* extract on liquid medium. The inhibition was dependent on the concentration. *T. rubrum* was the most affected fungus where its growth was completely inhibited at 4% and 5%. On the other hand *A. cadidus* was the least affected fungus.

Thus the above results indicate that *Aloe* extract may contain alkaloids which are antifungal and these results are in harmony with the results of other scientists **[Murthy & Bagyaraj (1978); Pieta (1985); Parashar *et al.*, (1990); Metha *et al.*, (1992); Andrew (1996) and Baghestani *et al.*, (1999)].**

Table (2): Effect of different concentrations of *Aloe vera* extract on dry weight (gm) of the various tested fungi for 7 days of growth on liquid Sabouroud medium 28 ±2 °C.

Fungi	Control	Concentration (%)				
		1%	2%	3%	4%	5%
A.candidus	٠,٦٢ ± ٠,٠٢	0.55 ± 0.01	0.54 ± 0.00	٠,٥١* ± ٠,٠١	٠,٤٥* ± ٠,٠١	٠,٣٥* ± ٠,٠١
A. flavus	٠,٤٣ ± ٠,٠٢	0.41 ± 0.02	0.30* ± 0.00	٠,٢٦ ± ٠,٠٠	٠,٢٥ ± ٠,٠١	٠,١٦* ± ٠,٠١
A. niger	٠,٥٦ ± ٠,٠٣	0.47 ± 0.11	0.41* ± 0.02	٠,٣٧ ± ٠,٠٠	٠,٣٥ ± ٠,٠١	٠,٢٥* ± ٠,٠١
C. albicans	٠,٢٦ ± ٠,٠٢	0.20* ± 0.01	0.19* ± 0.02	٠,١٧ ± ٠,٠١	٠,١٤ ± ٠,٠٢	٠,١١ ± ٠,٠١
T. rubrum	٠,٢٠ ± ٠,٠١	0.19 ± 0.02	0.15* ± 0.02	٠,١٤ ± ٠,٠١	٠,٠٠ ± ٠,٠٠	٠,٠٠ ± ٠,٠٠

The results in Fig. 2 show the effect of different concentrations of *Aloe vera* extract on relative dry weight of the tested fungi on liquid Sabouroud medium. It was observed that the relative dry weight decreased with increasing the concentration of *Aloe vera* extract.

The above mentioned results indicate that 5% extract was the best and the most effective concentration on the growth of the different tested fungi (Fig. 3 and Fig. 4).

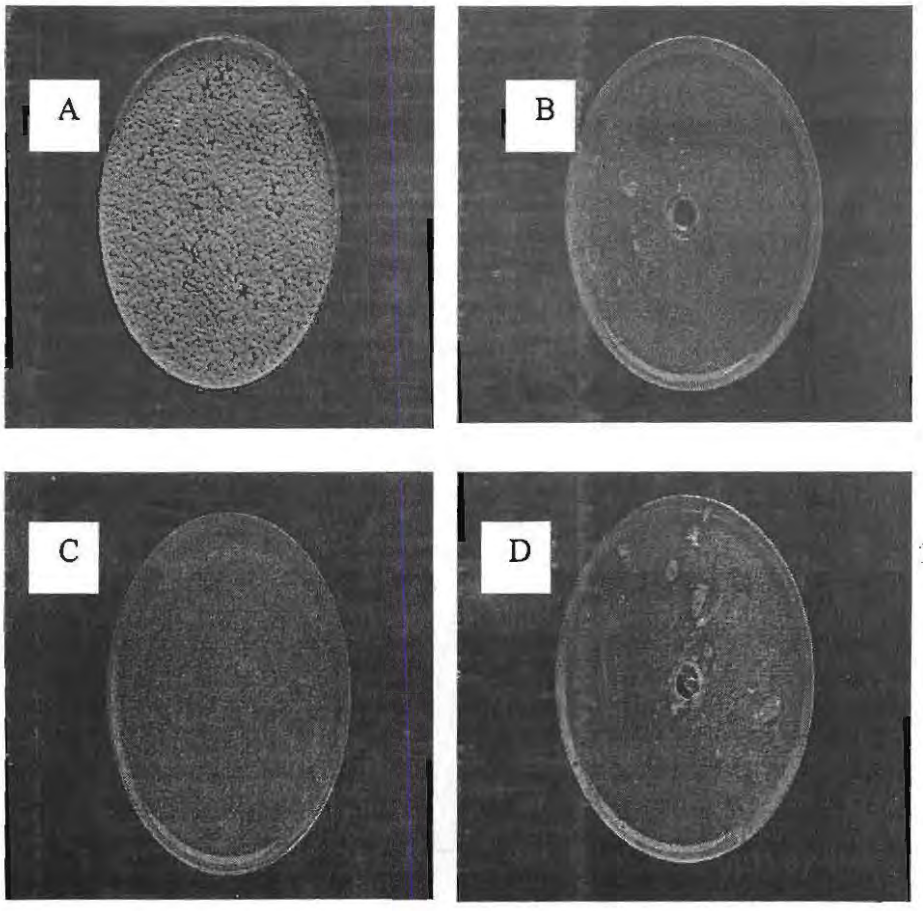

Fig. (3): A and B represent control sample and inhibition zone for *Trichophyton rubrum* by *Aloe* extract. C and D represent control sample and inhibition zone for *Candida albicans*.

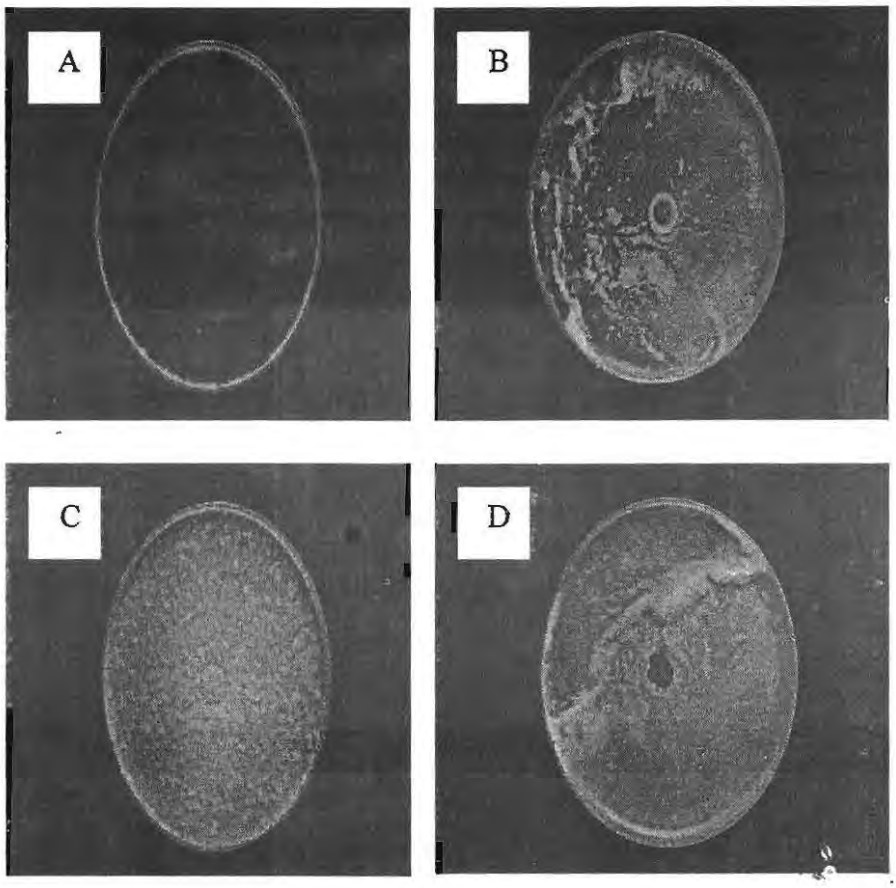

Fig. (4): A and B represent control sample and inhibition zone for *A niger* by *Aloe* extract. C and D represent the control sample and inhibition zone for *A. flavus* by *Aloe* extract.

The comparison is based on the appearance of inhibition zone around the hole which contained the extract. Generally, all the tested fungi were inhibited by the extract compared to the control to which no extract was added (photos from 1- 8). These results are in agreement with those of other investigators who studied the effect of phenolic compounds; isolated from higher plants, on the growth of fungi and yeasts **[Mitscher *et al.*, (1980 & 1983); D'Arcy & Kay (1987); Millard *et al.*, (1987); Vancura (1988); El-Naghy *et al.*, (1989); Dahiya (1991); Marley & Hillocks (1993); Hillocks *et al.*, (1997); Raja & Kurucheve (1998) and He & Woylon (2000)].**

The inhibitory effect of *Aloe* extract could be attributed to interference of some *Aloe* components with anabolic enzymes within fungi and lead to inhibition of their growth. Therefore, it is important to find out antifungal compounds without side- effects beside their effective role in treatment. For the present results to be applicable, the 5% extract was sprayed on some fruits such as peach, guava and Orange (Fig. 5) and left 7 days at room temperature.

We could see from the picture that spraying the fruits with *Aloe* extract protected them from infection with fungi compared with those left without spraying with extract. These results suggest that the seeds of fruits could be treated with the extract before cultivation to avoid infection of seedlings with fungi from the soil. These fungi can cause wilting, seedling death and root rotting and consequently lead to economic disaster. Also, these results suggest to use *Aloe* to rape the food stuffs as safe method instead of chemicals which are pollutants for the environment.

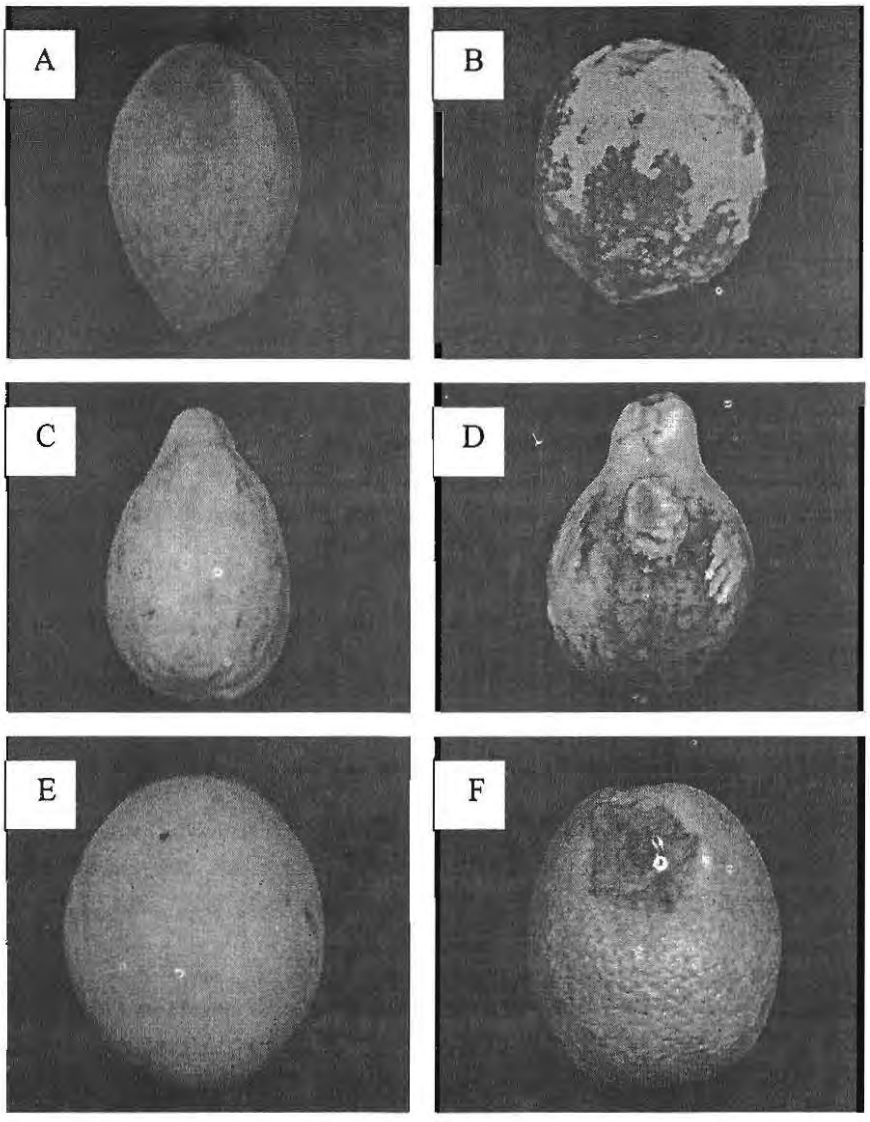

Fig. (5): A and B sprayed fruit of peach with *Aloe* extract and non-sprayed infected fruit by *A. flavus*. C and D sprayed fruit of guava with *Aloe* extract and non sprayed infected fruit by *A. flavus*. E and F sprayed fruit of orange with *Aloe* extract and non-sprayed infected fruit by *A. flavus*.

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مستخلص أوراق الصبار كمضاد حيوي لبعض الفطريات والخمائر المسببة للأمراض في الإنسان والتلوث في الأغذية

نهاد محمود مصطفى قمقمجي

جامعة الملك عبد العزيز

فرع كلية التربية للبنات للأقسام العلمية بجدة

هدفت الدراسة إلى استخدام مستخلص أوراق الصبار *Aloe vera* كمضاد حيوي طبيعي في مقاومة الخميرة *Candida albicans* المسببة للأمراض التناسلية، التنفسية والجلدية وبعض الفطريات الممرضة مثل *Trichophyton rubrum* الذي يصيب الجلد والشعر والأظافر وبعض الفطريات الممرضة مثل *Aspergillus A.flavus, A.niger, candidus* والمسببة لتلوث الأغذية والمفرزة للسموم الفطرية .تم إضافة مستخلص أوراق الصبار *Aloe vera* بتركيزات 1، 2 ، 3، 4 ، 5 ٪ إلى منبت سابورود دكستروز السائلة والصلبة لدراسة تأثيرها على نمو الفطريات المختبرة *A.flavus, Aspergillus candidus A.niger،T.rubrum C.albicans* وقد وجد أن جميع التركيزات المستخدمة للمستخلص أظهرت قصور في كل من النمو القطري أو الوزن الجاف أثناء تكوين الأغزال الفطرية وبزيادة تركيز المستخلص لوحظ التثبيط التام لنمو فطر *T. rubrum* عند التركيزات 4 ، 5 ٪ سواء على المنبت الغذائي الصلب أو السائل .وجد أن أفضل تأثير تضادي للمستخلص عند تركيز 5 ٪ حيث كانت نسبة النمو القطري للفطريات الأخرى *A. flavus , A .niger , C. albicans , A .candidus* بنسبة نمو قطري (95 , 21، 33 , 52 ، 34 ، 04، 37 ٪) على التوالي مقارنة بالعينة الضابطة كما لوحظ أن أقلهم تأثراً هو *A. flavus* بعد 7 أيام من التحضين. وعند رش المستخلص على بعض الفواكه لوحظ عدم قدرة الفطريات الملوثة على النمو حيث أن المستخلص حال دون نفاذ الفطريات إلى داخل الثمار بالإضافة إلى الحيلولة دون نمو الفطر على القشرة الخارجية للثمرة ، وقد أظهرت النتائج إيجابية كبيرة .