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Antifungal Effects of Mature and Immature Fruit Extracts of *Cucumis melo* L. on *Aspergillus flavus*

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Abstract: Aflatoxins are of the most important fungi toxin which is produce by *Aspergillus flavus* on animal's feedstuff. *Cucumis melo L.* is one of medicinal plants which has few use and is harmless. In this research after collecting, drying and milling of the tested plant by percolation method by water, ethanol 80% and methanol 80% it was extracted and effects of aqueous and alcoholic extracts of this plant on *A. flavus* was determined by disc and well diffusion methods and determination of MIC and MFC was verified. Methanolic and ethanolic extracts of mature and immature fruit of *C. melo* by disc and well diffusion methods have effect on fungi. By increasing extract amount, anti-growth halo diameter around disc and well was increased. The aqueous extract was without effect. Results were analyzed by t-student test and SPSS 16 showing that the antifungal effect of ethanolic extract is more than that of methanolic one.

Key words: *Aspergillus flavus* · *Cucumis melo* · Mycotoxin

INTRODUCTION

Aspergillus flavus is the most important fungi species which produces fungi toxin on human food, animal and birds. The fungi can produce aflatoxin on many plant yields like peanut, pistachio, coconut, soybean, maize, rice, cotton and grains [1]. When animals use feedstuff which is poisoned by aflation, toxin metabolizes on liver and excretes like aflatoxin M_1 AFM_1 of milk, urine and feces. AFM_1 is transmitted from milk and dairy products to human [2, 3]. Aflotoxin is so dangerous for human health and animal because it creates liver cancer, anti-immunology activities, reducing growth and reducing weight. The main target organ for toxicity and carcinogenicity is liver [4]. *A. flavus* causes aspergillosis in animal and birds in which there are respiratory, digestive and skin disease complications and abortion.

Wild melon, *C. melo*, is of Cucurbitaceous family. Annual, herbaceous, prostrates and creeping plant and its fruit is as large as 2-40×8-24 mm, ellipsoid, yellow, dark green-veined with is bitter pulped [5]. In different cucurbitaceous plants, bitter material by the name of cucurbitacine on different types is found (C,A,B,D,F,E,I,G,H,J,K,L). Cucurbitacine glycosides are tetra cycle three terpenic and have anti-tumor effect. This plant has medicinal use in common medicine exclusively for curing disease. The plant fruit is used as vomitive but on few amount by honey is tonic for stomach. Paste of crushed grain of the plant by *Cynodon dactylon* juice is used for curing and removing herpes grains and boils [6]. In order to eliminate aflatoxin impurity from foods and food resources, use of chemical, biological and physical methods have to be performed [7]. The most important mycotoxin absorbents applied on hens, chameleon, birds and pigs and inhibit absorption from digestive system include: alominosilicates like natural and synthetic zeolite, clinoptiololite, natural bentonite and montmorillonite, diatomite or diatom herd and active coal [8, 9]. Sweeny and Dobson [10] reported that they could use bacteria, yeast, mould, actinomyces and algae for reducing aflatoxin amount on human and animal food. Saprophytic algae like *Picha anomala*, *Candida krusi* and others could inhibit producing aflatoxin. *Achromobacter xylosoxidans* bacteria could inhibit aflatoxin production by impeding pre-producing aflatoxin by the name of norsolorinic acid from *A. parasiticus* [11]. Biologic control has been determined by non-aflatoxigenic *A. flavus* strain competition [12]. So many samples of different plants in reducing growth of *A. flavus* and

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producing aflatoxin have been reported [13, 14]. Chinese grass root, extract *scutellaria baicalensis* has protective property against gene toxin of AFB_1. Its flavonoid has anti-mutagenic liver [15]. Aqueous and methanol extract of *Agave asperrima* and *Agave striata* plants inhibit growth of mycotoxin *A. flavus* and *A. paraziticus* producers. *Agave lecheguilla* plant has anti-fungi activity [16]. Essential oils of thyme species like *Thymus eriocalyx* and *thymus x-poriock* has anti-fungus activity against growth of *Aspergillus* and aflatoxin producing [17]. On carpinella and cooperative study, ethanol extract of mature *M. azadirachta* has influenced *A. flavus* [18]. Hadizadeh *et al.* [3] were found aflatoxin B_1 in animal food between 10.4 to 68.8%. Also, in northern Iran AFM_1 was found in 100% of the yogurt samples with concentrations of 2.1-61.7 ng/l and 7-53 ng/l, respectively [19]. Due to importance of aflatoxin on animal and bird industry and access to plant extract on destroying generating fungi on feedstuff, in this research; anti-fungal properties of mature and immature fruit extract of *C. melo* by disc and well diffusion methods on *A. flavus* was studied.

MATERIALS AND METHODS

Preparation of Plant Extracts: After collecting and washing layers of the plant on hot and shade atmosphere, dry it then mill it to extract it better and easily. Then extract it by percolation method by water, ethanol and methanol 80% [20].

Aqueous Extract: Twenty five gm of plant added to 250 ml water were extracted by heating and then put on stove at 45°C for eliminating water fully and drying extract [21].

Ethanol and Methanol Extracting: Plant alcohol extracting by percolation method has been done. In this case, 100ml ethanol and methanol alcohol 80% for every 10 gm of the plant and 100ml of D-ethyl ether and N-hexane were added and then was closed by aluminum foil for 72 h. Then extract was purified by filtration and by rotary evaporator separated methanol solvent from extract. Omit fat on two phases.

Preparing Extract Solution: 0.5gm of different dried extract was added to 4.5cc distilled water to prepare 1.10 solutions in which every cc 10^5 µg of effective material was present.

Determining Antifungal Activity of the Extracts by Disk Diffusion Method: In every extract, 40, 50, 60 and 70? dilutions were absorbed by standard discs, after drying, put them on Sabouraud?? dextrose agar (SDA) medium inoculated with *A. flavus* suspension, after 48-72h incubation at 25-30°C, the presence of zone of growth inhibition was recorded [22-24].

Determining Antifungal Activity of the Extracts by Well Diffusion Method: For wells made on SDA medium inoculated with *A. flavus* suspension were filled with 80, 90, 100 and 110 ? of extract. And incubated at 25-30°C for 48-72 h and the presence of growth inhibition was determined [25].

Determination of MIC of Prepared Extracts: Eleven tubes were used; one ml of 1:10 dilution was poured in the first tube, therefore amount of effective material in the first tube was 5×10^4 µg/ml. 1:2 dilution in other tube was prepared adding 1000λ extract solution from the first tube and thus the effective material amount in the second tube reached to 25×10^3 µg/ml, this serial dilution was carried out till tenth tube. On preparing relevant serial dilutions, nothing was done to the eleventh tube and it was only observer of growth. Then fixed number (50 λ) of test fungi was added to the tubes [22].

Determination of MFC of the Extracts: For determining the minimum fungicidal concentration of the prepared extracts, the tube showing no growth turbidity was cultured on SDA medium the minimum concentration at which there was no growth of organisms was determined [20]. All statistical analysis has been done by SPSS 16.

RESULTS

Aqueous Extract Effect of *C. melo* on *A. flavus*: *A. flavus* showed no sensitivity to the *C. melo* mature and immature extract by disc and well diffusion method and grows all around the plate.

Methanol Extract Effect of *C. melo* on *A. flavus*: Mature and immature methanol extract of the *C. melo* had less effect by disc method than well method and by increasing extract amount in the disc and wells, anti-growth halo amount around disc was increased (Table 1).

Table 1: Diameter zone (mm) created against different amounts of *C. melo* extracts by disc and well diffusion methods

Kind fruit	Methods	Cons.	Methanolicc extract	Ethanolic extract
Mature	Disc	40 λ	9	22
		50 λ	10	24
		60 λ	11	36
		70 λ	12	37
	Well	80 λ	25	22
		90 λ	26	26
		100 λ	28	30
		110 λ	29	31
Immature	Disc	40 λ	17	11
		50 λ	23	14
		60 λ	24	15
		70 λ	26	16
	Well	80 λ	18	26
		90 λ	19	30
		100 λ	20	32
		110 λ	26	40

Cons. = Concentration, Result has been determined by eye which is the mean of three tests.

Table 2: MIC and MFC determination of the *C. melo* extracts on *A. flavus*

	Methanolic extract		Ethanolic extract	
Kind fruit	MIC	MFC	MIC	MFC
Mature	25×10^3	25×10^3	25×10^3	25×10^3
Immature	125×10^2	25×10^3	25×10^3	25×10^3

Ethanol Extract Effect of the *C. melo* on *A. flavus*: In this case by increasing extract amount in discs and wells, the amount of anti-growth halo around well was increased too. But ethanol extract (1:10 dilutions) of immature wild melon by disc method had a good effect on *A. flavus.*

MIC and MFC Determination of *C. melo* Ethanol and Methanol Extract on *A. flavus*: MIC of methanol mature wild melon extract was equal 25×10^3 µg/ml. MFC of methanol mature *C. melo* extract was equal to 25×10^3. MIC and MFC of ethanol mature *C. melo* extract were to those of methanolic extract (Table 2).

Data Analysis: In table 3 different amounts of ethanol and methanol immature extracts tested by well method (Put mean and standard deviation) showed that effect of methanol extract was more than ethanol.

Table 3: The mean and standard deviation diameter zone of *A. flavus* created against different amounts of *C. melo* extracts by disc and well diffusion methods

			Mature		Immature	
Methods	Cons.	Extract	m	S	m	S
Disc	40 λ	Methanolic	9	1	17	1.73
		Ethanolic	22	1	11	1.73
	50 λ	Methanolic	10	2	23	1.73
		Ethanolic	24	1	14	1.73
	60 λ	Methanolic	11	1	24	1
		Ethanolic	36	2.65	15	1
	70 λ	Methanolic	12	1.73	26	2.65
		Ethanolic	37	1.73	16	2.65
Well	80 λ	Methanolic	25	2	18	1
		Ethanolic	22	2	26	1.73
	90 λ	Methanolic	26	2.65	19	2
		Ethanolic	26	3	30	2.65
	100 λ	Methanolic	28	2.65	20	2
		Ethanolic	30	3.46	32	2.65
	110 λ	Methanolic	29	2	26	1.73
		Ethanolic	31	1.73	40	1

S= Standard deviation, m = Mean

DISCUSSION

Aflatoxin potential danger for human from milk and dairy product consumption has been proved by many researchers [26, 27]. Uncorrected method of culture, maintenance harvest and store and transformation increase fungal growth on grains. Anyway, these yields may be contaminated and are dangerous for human. We should applied regulation for reducing contamination of animal feedstuff from aflatoxigenic moulds. Different plant extracts like *Trametes versicolor*, *Lentinula edodes*, *Grifola frondosa* and *Ganoderma lucidum*, inhibit production of the aflatoxin of *A. parasiticus* about 40 to 90% and growth of the *A. paraziticus* too [28, 29]. *Rhodococcus erythropolis* inhibits growth of *A. flavus* and aflatoxin production [30]. Irkin and Korukluoglu [31] reported effects of different plant extracts; garlic, onion and leek on *A. niger*. *Garcinia* chloroform extract has anti aflatoxigenic and antioxidant effect. The plant immature extract of low viscosity has anti-fungal and antioxidant activities. The lowest concentration inhibits growth of *A. flavus*. 300 ppm concentration inhibits fully aflatoxin production maize flour [32]. Matasyoh *et al.* [33] detected the activity of the cymbopogon citrates oil against the mycotoxingenic fungi (*Aspergillus* species) with MIC value ranging from 15 to118 mg/ml. Adekunle and Oluwo [6] have reported anti-fungus effect of *cucumis melon var agrestis*, on *A. flavus*, *A. niger*, *A. wenti*,

Botrodiplodia theobromae, *penicillium pinophylum*, *Mucor sp*., *Phycomyces sp*. and *Rhizopus sp*. Whereas in our study methanolic and ethanolic extracts of mature and immature fruit of *C. melo* by disc and well diffusion methods have effect on *A. flavus*. By increasing extract amount, diameter zone around disc and well would increase too. Aflatoxins are common contaminators of food, especially in the developing countries. These poisons are created as the result of fungus action on production producing, harvest and store procedure [34, 35]. Fungal contamination begins before harvest and increases from production and harvest conditions. As the extract of *C. melo* has some effect on *A. flavus*, it is possible to add the extract in feedstuff for decreasing the growth of A. *flavus*. As a result, aflatoxin production will decrease.

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