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ANTIFUNGAL EFFECT AND CYTOTOXIC AVTIVITY AGAINST HUMAN TUMORS OF SOME MEDICINAL PLANTS ADDED TO COOKIES FORMULA

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ABSTRACT

Three medicinal plants namely carob, tamarind, and doum were investigated for their chemical composition, the most acceptable level added to cookies formula, antifungal effect, and cytotoxic activity against human tumors cell lines. The most acceptable level added to the cookies formula was 10% for each plant. The three medicinal plant-extracts had an antifungal effect against the three tested fungi *Aspergillus parasiticus, A. ochraceus* and *Fusarium moniliform* but the doum extract had the strongest effect. Carob extract had cytotoxic activity against breast carcinoma cell line (MCH7) but both tamarind and doum had cytotoxic activity against breast carcinoma cell line (MCH7) and human hepato cellular carcinoma cell line (Hepg2). These results suggest that carob, tamarind, and doum could be utilized as functional foods or food ingredients.

INTRODUCTION

There are 25000 phytochemicals and >150000 edible plants on earth. Modern humans eat only 150–200 of these plants worldwide (Heber, 2002).In humans, phytochemicals can have complementary and overlapping actions, including antioxidant effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of steroid metabolism and antibacterial and antiviral effects. Embracing a cuisine rich in spice, as well as in fruit and vegetables, may further enhance the chemopreventive capacity of one's diet (Lampe, 2003).

The carob (*Ceratonia siliqua L.*) tree has been widely cultivated in the Mediterranean countries for years. This tree was distributed by Arabs in the Mediterranean area. The two principal components of the fruit of carob tree are the pods and the seeds. The seeds, which comprise about 10% of the weight of the fruit, are used as food stabilizer, and they have other applications in the textile, food, cosmetic, and pharmaceutical industries (Kumazawa *et al.,* 2002). Nowadays, the main application of the pods is as animal feed. For humans, the carob pods have been used mainly as a cocoa substitute in a few countries because of its low price and the absence of caffeine (Yousif and Alghazawi, 2000).

Tamarind (*Tamarindus indica L.*) is evergreen tree, which may have orientated in Africa but now widespread throughout the tropics. The *Tamarindus* comes from the Arabic, Tamar-Hindi, "date of India," and refers to the datelike pulp inside the pods (Thoreau, 1995). Tamarind is characterized by a sour, fruity flavor and pleasant aroma, so it can be used to enhance the flavor of many dishes. Besides, it has many medicinal uses such as the pulp of the ripe fruits and the poultice of the leaves is applied externally to inflammatory swelling to relieve pain, juice expressed from the flowers is given orally for bleeding piles, decoction of the leaves is used as gargle in throat

affections and also used as a wash for indolent ulcer to promote healthy action. The bark is used topically for loss of sensation in paralysis (Kapoor, 1990). Recently, Babu *et al.* (2003) found that using the solid mixture of tamarind polysaccharides from tamarind kernel powder as a carrier improved the dissolution rate of poorly water-soluble drugs, celecoxib. Moreover, Raimondi *et al.* (2003) verified the protective effect of a new viscosising agent from tamarind polysaccharides in reducing the damage of eye cells exposed to UV-B rays.

Doum palm (*Hyphaene thebaica*) is a native of Upper Egypt, Sudan, Kenya, and Tanzania, and it was considered sacred by ancient Egyptians. Seeds of doum nuts have been found in the pharos' tombs. The doum palm, also known as the gingerbread palm, grows a red-orange, apple-size fruit that tastes like gingerbread. It is used in cooking in various ways, and varieties differ in their edibility. The nut is eaten raw. The fruit' hard white nut is used to make buttons, rind from doum nuts is used to make molasses and the ground nuts is used to dress wounds. The palms' leaves are used to make mats bind parcels and writing papers (Martin, 1999 and Anonymous, 2003).

In the present study, carob, tamarind, and doum were examined for their potential to inhibit the fungal growth and their cytotoxic activity. In addition, cookies with the three previous plants were prepared using different levels of each and then, the cookies were evaluated for organoleptic characteristics.

MATERIALS AND METHODS

The raw materials used throughout the current study such as carob, tamarind, doum, flour, shortening, sugar, sodium bicarbonate, vanilla and salt were purchased from the local market. The plants were grounded to the same size of wheat flour. *Aspergillus parasiticus* NRRL 2999, *A. ochraceus* NRRL 3174 and *Fusarium moniliform* were obtained from Standard Association of Australia 80 Arthur St., North Sydney, NSW.

Gross chemical composition: moisture, protein, fat, ash, fiber and minerals contents of grounded carob, tamarind and doum were determined according to the method described in A.O.A.C. (2000). Carbohydrates were calculated by difference. Total dietary fibers were carried out by the method of Prosky *et al.* (1985).

The cookies were prepared according to Finny & Gaines (1989) and Sanchez *et al.* (1995). The formula was 100g flour, 45g sugar, 35g shortening, 0.8g sodium bicarbonate, and 0.1g vanilla. The amount of water was added as needed. The medicinal plants powders (carob, tamarind, and doum) were added at concentrations of 5, 10, and 15% for each medicinal plant to produce the different cookies.

Organoleptic evaluation: The different cookies (carob cookies, tamarind cookies and doum cookies) were evaluated for their physical and organoleptic properties as described by Claughton and Pearce (1989). Randomly selected panelists evaluated the cookies for surface appearance, color, odor, taste, texture and overall acceptability.

Statistical analysis according to Gomez and Gomez (1984) was made to determine the most acceptable level of each added medicinal plant powder.

To prepare the different extracts, each plant powder was extracted with boiling water (50g/500ml) for 15 min, and then the decocted liquids were centrifuged at 3000 r.p.m. for 15 min. The water extract was directly lyophilized (Miayamura *et al.,* 1997). The ethanolic extract was carried out using 250 g of each plant powder for 24 hr. The roots were subsequently removed by centrifugation at 3000 r.p.m. for 15 min.The ethanolic extracts were evaporated to dryness under vacuum at 40°c (Diallo *et al.,* 2001).

The antifungal effect was carried out according to Hegazi (1999). 10 ml. of sterile Potato Dextrose Agar (PDA) medium was added in each 100 mm in diameter Petri dish; the medium was slightly shaken then allowed to harden. Also, 10 ml. of warmed PDA medium was incubated with 0.5 ml. broth of fungal culture containing 1×10^6 cell/ml. of each organism, then was shaken well and put on the hard PDA in the Petri dish (seed layer) and allowed to harden. Pores were made in the hard medium. Different concentrations of the extracts were poured in the pores (0.2 ml of each concentrate) in the plates. The plates were placed in refrigerator for one hr., and then incubated at optimal temperature (25°c). After 96 hr. the inhibition zones were measured in mm.

The potential cytotoxicity of water plant extracts was tested at the National Cancer Institute, Cairo University, Egypt. The cell lines were obtained from the International Agency for Research on Cancer (IARC), U.S.A. Two cell lines were used in the present work: breast carcinoma cell line (MCF7) and human hepato cellular cell line (Hepg2). The experiment was carried out as described by Skehan *et al.* (1990). The cytotoxicity was measured by Sulforhodamine B stain (SRB) assay. Cells were plated in 96-multiwell plate for 24hr. before treatment with the plant extracts to allow attachment of cell to the wall of the plate. Different concentrations of plant extracts were added to the cell monolayer and then incubated for 48 hr at 37° C in atmosphere of 5% CO_2. After 48 hr cells were fixed, washed, and stained with SRB stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and plant extracts concentrations is plotted to get the survival curve of each tumor cell line. For the evaluation of the cytotoxic activity of each extract, the 50% growth inhibition (GI_{50}) and 10% growth inhibition (GI_{10}) parameters were used.

RESULTS AND DISCUSSION

Table (1) shows the chemical composition of different medicinal plant powders. The highest amount of protein (6.13%) was for the carob powder, followed by the tamarind (5.23%) then the doum powder (4.09%). However, fat contents revealed that the tamarind contained the highest concentration of fat (3.70%). Regarding the ash content of different powders, the same table shows that the highest value, 8.31 %, was for the doum powder. Also, the doum powder recorded the highest amount of fiber (15.29%), while the tamarind recorded the lowest amount (10.37%). The contents of dietary fiber show that the doum powder had the highest level 38.15%. Concerning the mineral contents, it is noticeable in the same table that the doum powder had

the highest contents of calcium (908.24 mg/100gm), iron (13.76 mg/100gm), magnesium (156.79 mg /100gm), phosphorus (123.69 mg/100gm), and zinc (3.94 mg/100gm). However, the tamarind contained the highest levels of potassium (263.54 mg/100mg) and sodium (132.09 mg/100ml). These results are in agreement with those reported by Kapoor (1990) and Kumazawa *et al.* (2002).

Table (1) :Proximate chemical composition of different medicinal plant powders*

Chemical Composition	Different medicinal plant powders		
	Carob	Tamarind	Doum
Gross chemical composition (%)			
Moisture	6.30	9.70	8.08
Protein	6.13	5.23	4.09
Fat	1.67	3.70	2.09
Ash	4.60	5.10	8.31
Fiber	11.09	10.37	15.29
Total dietary fiber	31.24	29.45	38.15
Carbohydrate**	76.51	75.60	70.22
Mineral contents (mg/100g)			
Calcium	812.64	786.24	908.24
Iron	11.32	9.81	13.76
Magnesium	145.73	131.54	156.79
Phosphorus	106.25	97.81	123.69
Potassium	209.92	263.54	257.31
Sodium	112.35	132.09	121.51
Zinc	2.54	3.06	3.94

* On dry weight basis ** By difference

Organoleptic evaluation: different plants-added cookies were organolepticlly evaluated. Data were statistically analyzed and the results are tabulated in table (2). As shown in the table the acceptability scores of all tested parameters (surface appearance, color, texture, odor, taste and overall acceptability) indicate that at the level of 15% all products recorded lower scores which indicated less acceptability. Therefore, the addition of different medicinal plant' powders to the cookies' formula at a concentration of 10% was assigned the most acceptable concentration.

Table (2) : Organoleptic evaluation of different cookies

Different cookies	Surface appearance (10)	Color (10)	Texture (10)	Odor (10)	Taste (10)	Overall acceptability (10)
Control	8.600 ^{a}	8.400 ^{a}	8.800 ^{a}	8.600 ^{a}	8.200 ^{a}	8.520 ^{a}
Carob						
5 %	8.400 ^{a}	8.600 ^{a}	8.400 ^{a}	8.200 ^{a}	8.200 ^{a}	8.360 ^{a}
10 %	8.400 ^{a}	8.000 ^{a}	8.200 ^{a}	8.400 ^{a}	8.400 ^{a}	8.280 ^{a}
15 %	6.600 ^{b}	6.400 ^{b}	6.100 ^{b}	5.500 ^{b}	5.200 ^{b}	5.960 ^{b}
LSD*	1.269	1.328	1.194	1.269	1.284	0.634
Tamarind						
5 %	8.200 ^{a}	8.600 ^{a}	8.400 ^{a}	8.200 ^{a}	8.000 ^{a}	8.280 ^{a}
10 %	8.000 ^{a}	8.400 ^{a}	8.400 ^{a}	8.200 ^{a}	8.200 ^{a}	8.240 ^{a}
15 %	5.000 ^{b}	5.000 ^{b}	4.800 ^{b}	5.000 ^{b}	5.000 ^{b}	4.960 ^{b}
LSD*	1.401	1.703	1.370	1.460	1.460	0.512
Doum						
5 %	8.400 ^{a}	8.200 ^{a}	8.200 ^{a}	8.200 ^{a}	8.000 ^{a}	8.200 ^{a}
10 %	8.400 ^{a}	8.000 ^{a}	8.200 ^{a}	7.800 ^{a}	7.400 ^{a}	7.960 ^{a}
15 %	5.200 ^{b}	4.800 ^{b}	4.800 ^{b}	5.00 ^{b}	4.600 ^{b}	4.880 ^{b}
LSD*	1.728	1.489	1.431	1.752	1.401	0.690

***Values within column followed by the same letters are not significantly different at 0.01 levels.**

The antifungal effect of both water extract and ethanolic extract of medicinal plant powders is presented in table (3). The table shows the antifungal activity of the extracts at different concentrations, i.e. 100, 200, 300, 400, and 500 mg/ml, against three fungal strains *Aspergillus parasiticus*, *A. ochraceus,* and *Fusarrium moniliform*. For the water extract, the carob extract had no inhibition effect until 300mg/ml against the three strains but at a concentration of 400mg/ml the inhibition zones were 5.1, 4.3, and 0.0 mm for *Aspergillus parasiticus*, *A. ochraecus,* and *Fusarrium moniliform* respectively. At a concentration of 500mg/ml the inhibition zones were 6.2, 6.7, and 6.5 mm, respectively. The tamarind extract did not show any inhibition zones until 200mg/ml, but at a concentration of 300mg/ml it showed an inhibition zone 5.3 mm in diameter against *A. ochracus* only. Meanwhile, at a concentration of 400mg/ml the inhibition zones were 3.5, 8.1, and 4.2 mm in diameters against *Aspergillus parasiticus*, *A. ochraceus,* and *Fusarrium moniliform,* respectively. The inhibition zones were7.3, 9.6, and 8.3 mm in diameters against the three strains respectively. The doum extract had the strongest effect among the three water extract powders especially against *A. ochraceus* since the inhibition zones were 4.8, 6.5, 8.5, 10.5, and 18.1 mm in diameters at concentrations of 100, 200, 300, 400, and 500mg/ml, respectively.

Table (3): Antifungal effect of different medicinal plant powder extracts (inhibition zones in mm)

Tested fungi / Different plants	Different plants powder extracts									
	Water extract concentrations (mg/ml)					Ethanolic extract concentrations (mg/ml)				
	100	200	300	400	500	100	200	300	400	500
Aspergillus parasiticus										
Carob	?	?	?	5.1	6.2	?	?	2.1	7.4	10.1
Tamarind	?	?	?	3.5	7.3	?	4.2	5.3	7.4	11.2
Doum	?	?	?	7.1	9.2	?	6.1	9.1	14.4	19.1
Aspergillus ochraceus										
Carob	?	?	?	4.3	6.7	?	?	5.7	6.9	9.8
Tamarind	?	?	5.3	8.1	9.6	?	5.7	8.2	10.2	13.7
Doum	4.8	6.5	8.5	10.5	18.1	5.4	7.2	10.2	15.2	20.1
Fusarrium moniliform										
Carob	?	?	?	?	6.5	?	?	?	9.1	13.5
Tamarind	?	?	?	4.2	8.3	?	3.2	5.7	9.2	15.3
Doum	?	?	4.5	6.5	8.5	?	7.2	10.2	12.5	16.1

The antifungal effect of ethanolic extracts followed the same trend as it could be seen that the doum extract caused the highest inhibition effect comparing to the other two extracts in particularly against *A. ochraceus* which had inhibition zones of 5.4, 7.2, 10.2, 15.2, and 20.1mm in diameters at concentrations of 100, 200, 300, 400, and 500mg/ml, respectively. In this respect, Sone and Sato (1994)and Mastromarino *et al.,* (1997) reported that natural polysaccharides of carob and tamarind showed an antiviral effect as a result of blocking a step in viral replication subsequent to viral attachment, such as internalization and/or uncoating. Moreover, Bidlack (1998) reported that oligosaccharides were shown to enhance intestinal flora resulting in anti-metastatic, anti-viral, anti-microbial, and anti-inflammatory activities. In

addition, the antimicrobial effect of tamarind was confirmed by De *et al.* (1999) who reported that the tamarind had potent antimicrobial activity against the microorganisms and established the traditional use as food preservatives, disinfectants, and antiseptics. Recently, Chhabra *et al.* (2002) mentioned that an extracellular mannanase which is an enzyme expressed on carob galactomannan has been shown in vitro to degrade specific glycosides that presumably serve as carbon and energy sources for the microorganisms.

Regarding the cytotoxic activity of the three water plant extracts against human breast carcinoma cell line (MCH7) and human hepato cellular carcinoma cell line (Hepg2), the results are illustrated in table (4) and figs. (1, 2 and 3). It could be observed that the carob extract showed a cytotoxic activity only against human breast carcinoma cell line (MCH7) which was reduced to 50% survival at concentration of 24.0 ىg/ml. The tamarind extract was proven to reduce the survival to 50% for both human breast carcinoma cell line (MCH7) and liver tumor cell line (Hepg2) at concentration of 25.0 and 25.3 ىg/ml, respectively. The doum extract showed similar trend as it reduced the survival to 50% for both human breast carcinoma cell line at concentration of 25.0 ىg/ml and human hepato cellular carcinoma cell line at concentration of 25.8 ىg/ml. In this concern, Heber (2002) stated that the scientific community and the public are becoming increasingly aware of the cancer-preventive potential of phytochemicals. Phytochemicals influence gene expression in the multistep pathway of carcinogenesis via direct effects on cell signaling, proliferation, and apoptosis; on induction or suppression of enzyme systems that affect hormone and xenobiotic metabolism; and on angiogenesis. In addition, Kumazawa *et al.* (2002) reported that the total polyphenol content in carob pods was 19.2% and the quantity of flavanol was about 23% of the total polyphenol (4.3%). Furthermore, they confirmed the antioxidant activity of the polyphenol in carob pods (tannins, catechins, gallic acid and procyanidins) in scavenging the free radicals which is due to their hydrogen-donating ability.

Table (4): The cytotoxic activity of plant extracts against human breast tumor cell line (MCF7) and liver tumor cell line (Hepg2)

Plant extracts	MCF7 (breast)		Hepg2 (liver)	
	IC_{50}	IC_{10}	IC_{50}	IC_{10}
Carob	24.0 ىg/ml	Nd	Nd	Nd
Tamarind	25.0 ىg/ml	Nd	25.3 ىg/ml	Nd
Doum	25.0 ىg/ml	Nd	25.8 ىg/ml	Nd

IC_{50} : Dose of plant extracts which reduces survival to 50%
IC_{10} : Dose of plant extracts which reduces survival to 10%
Nd : Not detected

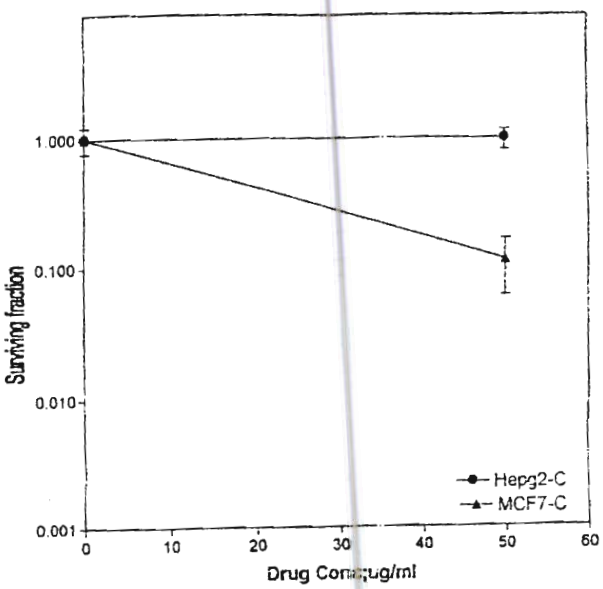

Fig.1: The cytotoxic activity of carob extract against human breast carcinoma cell line(MCH7)and human hepato carcinoma cell line (Hepg2)

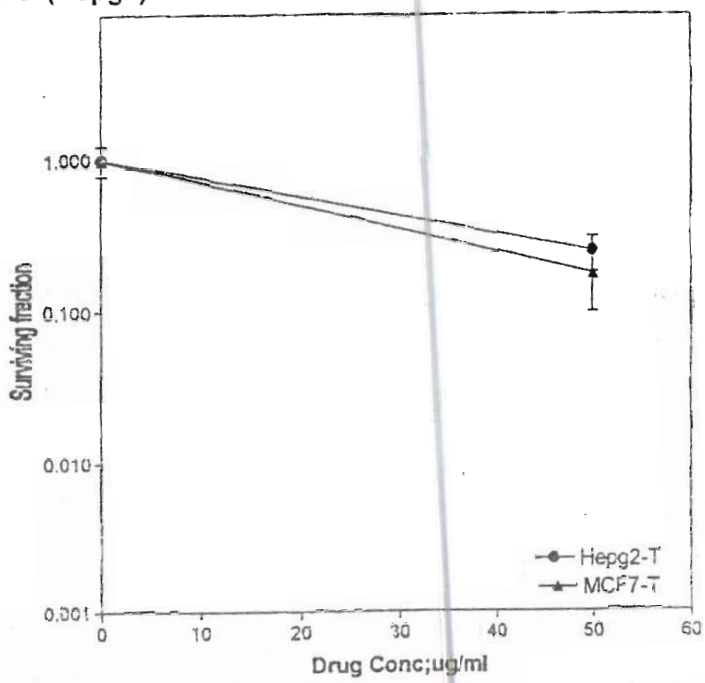

Fig.2: The cytotoxic activity of tamarind extract against human breast carcinoma cell line (MCH7) and human hepato carcinoma cell line (Hepg2)

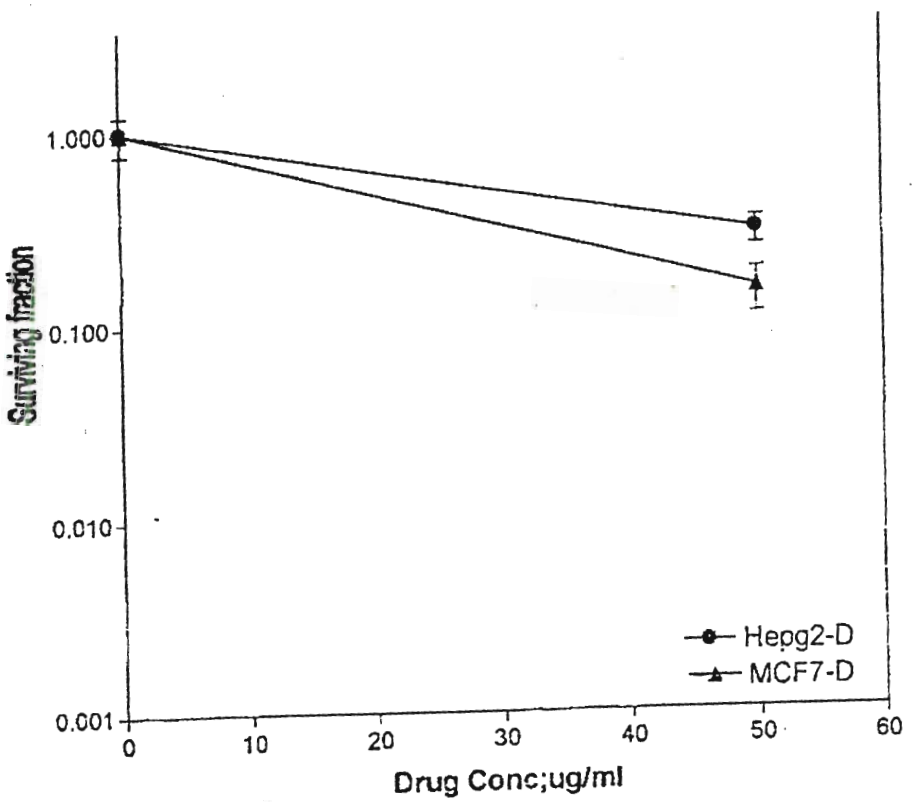

Fig.3: The cytotoxic activity of doum extract against human breast carcinoma cell line(MCH7)and human hepato carcinoma cell line (Hepg2)

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التأثيرات المضادة للفطريات و السامة للخلايا السرطانية فى الإنسان لبعض النباتات الطبية المضافة للبسكويت

بدوية حمزة

معهد بحوث تكنولوجيا الأغذية- مركز البحث الزراعية-الجيزة-مصر

تم دراسة التركيب الكيماوي للخروب و التمر الهندي و الدوم ثم إضافة كل منهم للبسكويت بتركيزات مختلفة و دراسة الصفات الحسية للبسكويت الناتج لتحديد النسبة المثلي لإضافة هذه النباتات والتعرف على مدى تقبل

المستهلك العادي للمنتج وقد أثبتت النتائج أن ١٠% كانت أفضل النسب و أن تجاوز هذا التركيز يخفض درجة تقبل المستهلك.

تم أيضا دراسة تأثير كل من النباتات السابقة على نمو فطريات

Aspergillus parasiticus و *A. ochraceus* و *Fusarrium moniliform*

و أثبتت النتائج أن كل منهم له تأثير مثبط عل نمو هذه الفطريات ولكن كان الدوم الأكثر تاثيرا.

وقد أظهرت النتائج عند دراسة تأثير كل من النباتات السابقة على نوعين من الأورام هما سرطان الثدي وسرطان الكبد أن الخروب له تأثير مثبط لنمو خلايا سرطان الثدي فقط أما التمر الهندى والدوم كان لهما تأثير مثبط لنمو خلايا سرطان الثدى و الكبد.