JJBS

Volume 1, Number 3, September. 2008
ISSN 1995-6673
Pages 89 - 99

Jordan Journal of Biological Sciences

In vitro Antifungal Activities of Various Plant Crude Extracts and Fractions Against Citrus post-harvest Disease Agent *Penicillium digitatum*

Ghassan. J. Kanan*, Rasha. A. Al-Najar

Dept of Biological Sciences; Mu'tah University Karak – Jordan

Abstract

The aim of the present study is to in vitro evaluate various plant extracts and liquid fractions against citrus post-harvest disease agent Penicillium digitatum. Crude extracts of seven plant materials (fenugreek seeds, harmal seeds, garlic cloves, cinnamon bark, sticky fleabane leaves, and nightshade leaves and fruits). In addition to their methanolic, hexane, and aqueous - fractions were assayed by agar well diffusion and amended agar methods. Regression analysis of results was carried out by using Microsoft Excel and SPSS program. Results indicated that crude extracts of nightshade fruits cinnamon bark have completely inhibited the growth of tested fungal isolates (IC_{50} = 57.5 µg ml^{-1}, 190-252.5 µg ml^{-1}) respectively. Methanolic (except fenugreek), hexane and aqueous fractions of all tested plants have resulted in complete inhibition of tested isolates. The methanolic fraction of cinnamon bark extract has shown the highest antifungal activity as compared with the same fraction from other plants (IC_{50} in the range of 5-23 µg ml^{-1}). Moreover, cinnamon bark hexane fraction was the most effective hexane fraction in all tested plants (IC_{50} range: 12.25-14.5 µg ml^{-1}). Concerning fractions of garlic extract, only methanolic fraction has resulted in complete inhibition of fungal growth (IC_{50} range: 3.75-18 µg ml^{-1}). However, the nightshade leaves aqueous fraction (IC_{50} range: 6.75-10.5 µg ml^{-1}) was the most effective over other fractions of the same plant.

الملخص

تهدف هده الدراسه الى تقييم فاعلية بعض المستخلصات النباتيه بالكحول وأجزائها العضويه و المائية للسيطره على فطر البنسيليوم الاخضر المهاجم لثمار الحمضيات. تم دراسة سبعة أنواع من المستخلصات النباتيه ومجزءات الميثانول، الهكسين والماء على سلالات الفطر في المختبر سواء باستخدام طريقة وضع المستخلص في حفر داخل الوسط الغذائي أو بتوزيع المستخلص على سطح الوسط الغذائي ثم زراعة الفطر. دلت النتائج أن مستخلص ثمار النبات nightshade أدت الى تثبيط نمو الفطر بشكل كامل حيث كان التركيز المثبط للنمو بنسبة 50% يساوي 57.5 ميكروجرام/مل. مستخلص لحاء القرفه أدى كذلك الى تثبيط كامل لنمو الفطر. مجزء أو مستخلص الميثانول لجميع النباتات المدروسه (باستثناء الحلبة) أدى الى تثبيط كامل لنمو سلالات الفطر. كذلك مستخلص الهكسين و المحلول المائي لجميع النباتات قيد الدراسه أدت الى تثبيط النمو في العزلات المستعملة. مستخلص الميثانول للحاء القرفه أظهر أعلى فاعليه مثبطة للفطر مقارنة مع نفس مستخلص من النباتات الاخرى. اضافه لذلك مستخلص الهكسين للحاء القرفه كان الأكثر فاعليه مقارنة مع نفس النوع من المستخلصات في النباتات الأخرى. مستخلص الميثانول من الثوم كان الوحيد من جميع مستخلصات الثوم القادرة على تثبيط نمو الفطر بشكل كامل بينما المستخلص المائي لنبات nightshade كان الاكثر فاعلية مقارنة مع بقية المستخلصات من نفس النوع من النبات.

Keywords: green mold, citrus fruits, post-harvest diseases.

1. Introduction

Post-harvest green mold caused by *Penicillium digitatum* [(Pers: Fr) Sacc.] is considered to be a universal disease that leads to the spoilage of almost all kinds of mature citrus fruits (Plaza *et al*., 2004). This disease is currently controlled through the massive use of chemical fungicides (Pramila and Dubey, 2004). However, consumer demands for fungicide-free products, development of resistant fungal strains as a result of continuous use of fungicides, and the effectiveness of applied fungicides necessitate the search for alternative control options (Obagwu and Korsten, 2003; Soylu *et al*., 2005). Plant's extracts are one of several non-chemical control options that have recently received attention. However, actual use of these extracts to control post-harvest pathogens of fruits and citrus pathogens in

* Corresponding author. e-mail: gkanan@mutah.edu.jo

particular is still limited (Obagwu and Korsten, 2003). In this study, the antifungal activity of crude extract and fractions of various medicinal, commercial, and wild-type plants were investigated against *P. digitatum* isolates. The studied plant materials include fenugreek seeds (*Trigonella foenum-graecum* L.), harmal seeds (*Peganum harmala* L.), garlic cloves (*Allium sativum* L.), cinnamon bark (*Cinnamomum cassia* L. Presl), sticky fleabane leaves (*Inula viscose* L. Aiton), and nightshade leaves and fruits (*Solanum nigrum* L.). Fenugreek is an annual Mediterranean herb with aromatic seeds that contain a number of steroidal sapogenins, (saponin fenugrin B) as well as alkaloids (Zargar *et al*., 1992; Zhao *et al*., 2002). The crude extract of fenugreek has shown to be highly specific for the dermatophytes (Shtayeh and Abu Ghdeib, 1999). In addition, the methanolic extract was potent in inhibiting *Candida albicans* (Olli and Kirti, 2006). Extracts from the seeds, as well as the roots of harmal, were found to contain a mixture of active alkaloids such as harmine, harmaline, and tetrahydroharmine. Harmaline was the most active (Kartal *et al*., 2003; Telezhenetskaya and Dyakonov, 2004). The crude extract of freshly crushed garlic cloves has shown strong inhibitory activity on several microbial growth systems including bacteria, fungi, and viruses (Yoshida *et al*., 1999; Elsom, 2000; Sokmen, 2001). Cinnamon bark crude extract has consistently been reported to have antifungal activity. This activity was attributed mainly to the presence of cinnamaldehyde, as well as to the presence of eugenol (He *et al*., 2005). Sticky fleabane is a perennial wild plant that has a wide range of distribution in the Mediterranian region (Curadi *et al*., 2005). The leaf extract of *Inula viscose* proved to have a significant antifungal efficacy against dermatophytes, *Candida* spp, and downy mildew. This high activity may be attributed to the high concentration of sesquiterpene compounds presence (Cafarchia *et al*., 2002; Cohen *et al*., 2006). Nightshade is an herbaceous annual wild plant that has a globular and smooth skinned green fruits (turned black or red at maturity); and are borne in small clusters (Dafni and Yaniv, 1994, Dhellot *et al*, 2006). The plant, due to the presence of steroidal alkaloids, showed antifungal activity against eleven agronomically important fungi (Al-Fatimi *et al*., 2007). The objective of this study is to "*In vitro*" evaluate antifungal activity of crude extracts of seven plants and their liquid fractions against green mould rot of citrus, *P. digitatum*.

2. Materials and Methods

This study was conducted during the year 2007 in laboratories of biological sciences department at Mu'tah University, Jordan.

2.1. Penicillium Digitatum Tested Isolates

Conidiospores of four *P. digitatum* isolates (dg2, dg4, dg5, and dg6) were obtained from spoiled citrus orange (*Citrus sinensis* L.), and lemon (*Citrus limon* L.) fruits were collected from two Jordanian cities: Irbid and Al-Karak.

2.2. Media Used

The *Aspergillus nidulans* complete medium (CM) described previously by Cove (1966) was used (gave maximum zone of growth as compared to potato dextrose agar (PDA) media) with slight modification (i.e. pH 5.5, supplemented with 10 mM glutamic acid, and 10 gl^{-1} fructose as C- source).

2.3. Purification of Isolates

Conidiospores suspensions in a 5 ml solutions of normal saline/Tween 80 (0.05%) were made from each tested isolate at a concentration of approximately 1×10^8 spores per milliliter. Aliquot of 100 µl from a dilution of 10^{-6} or 10^{-7} were plated again on complete media to confirm the identity of each single pure colony as a source of pure culture (Zhang *et al*., 2004).

2.4. Optimal Growth Conditions of Tested Fungal Isolates

Nine replicates (for each tested condition) of conidiospores suspension (20 µl) from each tested isolate were inoculated into complete media, having different pH regimes (i.e. 3, 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, and 9) for optimal pH (5.5). At pH 5.5, L-glutamic acid at a concentration of 10 mM was the best N-source used among the following materials: Urea; L-proline, L-lysine, L-arginine, L-adenine, L-glutamine, NH_4^+, NO_3^-, and L-histidine. In addition, inoculated plates of complete media adjusted to optimal pH of 5.5, and tehn supplemented with 10 mM glutamic acid and then were incubated at five temperature regimes (i.e. 10 °C, 20 °C, 25 °C, 30 °C, 37 °C) in order to determine the optimal temperature of growth (20 °C). Also, various C-sources (glucose, sucrose, sorbitol, fructose, and maltose) were tested at a final concentration of 10 gl^{-1} to determine best serving C-source (fructose). Each group of nine replicates were incubated for 5 days, at 20°C or at the tested temperature, and then the radius of each growing colony was measured in two directions at right angles to each other.

2.5. Plant Material

Six crude extracts, out of seventy plant species, have shown antifungal activity against tested isolates of *P. digitatum* and these are fenugreek seeds (*Trigonella foenum-graecum* L.), harmal seeds (*Peganum harmala* L.), garlic cloves (*Allium sativum* L.), cinnamon bark (*Cinnamomum cassia* L. Presl), sticky fleabane leaves (*Inula viscose* L. Aiton), and nightshade leaves and fruits (*Solanum nigrum* L.). The former four plant materials were brought from traditional medicine shops in Irbid city whereas the latter two species were collected from wild-type populations occupying orchard fields and road sides of Mu'tah and Al-Iraq towns within Al-Karak area. Plant species were named and classified with the help of the plant taxonomist Dr. Saleh AL- Quran, Mu'tah University, Jordan.

2.5.1. Extracts Preparation

The plant material was dried in the shade, and then it was ground by using liquid nitrogen and extracted (48 h) with absolute ethanol in a soxhlet apparatus (Ndukwe *et al*., 2006). The solvent was removed using rotary evaporator (Heidolph, VV2000) under reduced pressure at temperature below 50 ºC. The resulting crude extracts were stored at 20 ºC until assayed. Stock solutions and serial dilutions of extracts and fractions were prepared in dimethylsulphoxide (DMSO) (Ambrozin *et al*., 2004). Control experiments were performed by using DMSO with identical concentration used to test the extracts. Extracts

were dissolved in DMSO and evaluated for their ability to inhibit the growth of *P. digitatum* isolates.

2.5.2. Fractionation of Plant Crude Extracts

Each crude extract sample was fractionated with (1:1) ratio of water /dichloromethane (v/v). The resultant aqueous fraction was further extracted with dichloromethane, and then combined and concentrated to dryness using rotary evaporator and kept in sterile containers at 4°C until used. The dichloromethane fraction was concentrated to dryness using rotary evaporator, and then portioned with (1:1) n-hexane/90% methanol. The hexane and methanol fractions were concentrated to dryness using rotary evaporator and kept in sterile containers 4°C until used. Each fraction was dissolved in dimethylsulphoxide (Ambrozin *et al*., 2004).

2.6. Antifungal Assay by Agar Well Diffusion Method

Aliquot of 100 µl spores suspension ($1x10^8$ spores/ml) of each tested isolate was streaked in radial patterns on the surface of complete media plates. Wells of 6 mm in diameter were performed in the media, and then each was filled with certain concentration (0.65, 1.3, 13, 32, 65, and 97 µg) of each tested crude extract. DMSO was used as control for the ethanolic extracts. The cultured plates were incubated at 20ºC for 3-5 days. The radius for the zone of inhibition was measured in two directions at right angles to each other. Experiments were carried out with three replicates per treatment and each treatment was repeated at least twice (Ndukwe *et al*., 2006).

2.7. Antifungal Assay of Crude Extracts and Their Fractions by Amended Agar Method

Each crude extract was fractionated into aqueous, hexane, and methanolic fractions. Also the emulsion that may form between layers sometimes was tested. Stock solutions of each fraction were sterilized through a 4 µm Millipore filter (Soylu *et al*., 2005). Each fraction was used in this experiment with different concentrations, depending on its inhibitory activity. Each concentration (25, 50, 130, 260, 390, and 520 µg ml^{-1}) of crude extracts or their fractions (5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100,120, and 150 µg ml^{-1}) from the included plant species was amended (streaked in radial patterns) on the surface of solidified complete media prepared as described above. To ensure time was adequate for the diffusion of the extract into media, the plates were incubated at room temperature for at least two hours. 20 µl of conidiospores suspension ($1x10^8$) from each of the isolates were pipetted and left as drops on the surface of the amended media, and then were kept at room temperature for at least one hour until it became completely absorbed. Three inocula per isolate/plate and three replicate Petri plates were used per treatment, where each treatment was repeated at least twice. A long with each treatment, 20 µl of dimethylsulfoxide (DMSO) was replicated as mentioned above and used as controls for the ethanolic extracts. The inoculated Petri dishes were incubated for 3-5 days at optimal temperature (20 °C). Colony diameter was determined by measuring the average radial growth of each isolate. The radius of the growing colonies was measured in two directions at right angles to each other. The MIC was defined as the lowest concentration of the extract inhibiting visible growth of each isolate (Obagwu and Korsten, 2003).

2.8. Determination of Plant Crude Extract or Fraction Sensitivity

The percentage of mycelial growth inhibition by each extract or fraction concentration was calculated from the mean colony diameter (mm) on medium without plant fraction (control), and from the mean colony diameter (mm) on each fraction amended plate (zone of growth). A linear regression of percent inhibition versus plant fraction concentration, estimated produce 50% (IC_{50}), was determined from the regression equation or by interpolation from the regression line. Percentage of inhibition of mycelial growth was determined by using the following formula (Nwachukwu and Umechuruba, 2001).

$$\% \text{ MGI} = \frac{\overline{x} - xi}{\overline{x}} \times 100\%$$

Where:

% MGI denotes for: % of mycelial growth inhibition.

$\overline{x}$: refers to diameter (mm) of control colony on non-amended medium.

xi: refers to diameter (mm) of tested colony replicates on a single crude extract or fraction amended plate (zone of growth).

2.9. Statistical Analysis

The concentration of plant crude extract or fraction, producing 50% growth inhibition (IC_{50}), was calculated by regression analysis for the relationship between the size of inhibition zone (mm) and the concentration (µg) of crude extract or fraction (Log value). Both Microsoft Excel 2003 and SPSS (version 10) were used in such analysis.

3. Results

3.1. Antifungal Activity of Crude Extracts by Agar Well Diffusion Method

Regression analysis of the relationship between size of inhibition zone (mm) and plant crude extract concentration (Log value) showed that there was a significant correlation between concentrations of tested plant extracts and the mean inhibition zone of *P. digitatum* isolates (Table 1). However, such correlation was not significant when crude extracts of garlic cloves, sticky fleabane leaves, and harmal seeds were tested against isolates: dg2; dg4 and dg6; dg5 and dg6, respectively. In addition, none of the extracts has completely inhibited the growth of the four isolates within a range of concentrations from 0.65 to 97 µg ml^{-1}. Moreover, sticky fleabane and harmal extracts have shown the least inhibitory activity against fungal isolates whereas fenugreek seeds, followed by nightshade fruits and leaves extracts, have shown the highest antifungal activity (Table 1).

3.2. Antifungal Activity of Crude Extracts by Amended Agar Method

A linear regression of inhibition percentage versus plant crude extract concentration, estimated to produce 50% (IC_{50}) inhibition, was determined by interpolation from the regression line (Table 3). As clearly seen in (Table 2), the nightshade fruits crude extract has completely inhibited the growth of the four tested *P. digitatum* isolates with an MIC equal to 130 µg ml^{-1} (IC_{50} = 57.5). In addition, cinnamon bark extract has completely inhibited the growth of isolates dg2 (IC_{50}=252.5), dg4

(IC_{50}= 222.5) and dg6 (IC_{50}= 190). Furthermore, fenugreek seeds extract has completely inhibited the growth of dg6 isolate at an MIC value of 390 µg ml^{-1} (IC_{50}= 142). Moreover, results indicated that the tested concentrations of nightshade leaves extract (in the range of 25-520 µg ml^{-1}) has reflected significant correlation in the percentage of inhibition of isolates: dg2 and dg4 (r= 0.999; *P*= 0.001); dg2 and dg5 (r= 0.976; *P*= 0.024); dg2 and dg6 (r= 0.960; *P*= 0.040); dg4 and dg5 (r= 0.967; *P*= 0.033); dg5 and dg6 (r= 0.983; *P*= 0.017). Concentrations of garlic cloves crude extract (50-520 µg ml^{-1}) have also reflected significant correlation in the percentage of inhibition of isolates: dg2 and dg4 (r= 0.992; *P*= 0.008); dg2 and dg5 (r= 0.996; *P*= 0.004); dg4 and dg5 (r= 0.984; *P*= 0.016). In addition, harmal seeds extract has reflected significant correlation between isolates: dg2 and dg4 (r= 0.995; *P*= 0.005); dg4 and dg6 (r= 0.993; *P*= 0.007) percentage of inhibition.

3.3. Antifungal Activity of Plants Extracts Fractions

Results presented in (Table 3) show the *In vitro* antifungal activities of methanolic, hexane, and aqueous fractions of plant materials. The methanolic fractions of all plants (with the exception of fenugreek) have completely inhibited the growth of all fungal isolates. Hexane and aqueous fractions of all plants (not including fenugreek and garlic) have resulted in complete inhibition of fungal growth in the four isolates. The methanolic fraction of cinnamon bark has shown the highest antifungal activity against four *P. digitatum* isolates - followed by methanolic fractions of garlic, nightshade fruits, sticky fleabane, harmal seeds, and nightshade leaves respectively (indicated by the IC_{50} values). The hexane fraction of cinnamon was the most effective fraction against all tested isolates followed by sticky fleabane, harmal, nightshade fruits and leaves, and hexane fraction respectively (Table 3). Moreover, the aqueous fraction of nightshade leaves was the most effective against all isolates, followed by the aqueous fraction of nightshade fruits and cinnamon. Concerning the efficacy of fractions in each particular plant, results indicated that cinnamon methanolic fraction was the most effective - followed by hexane and aqueous fraction of cinnamon extract respectively. However, the fenugreek fractions have not caused complete inhibition of fungal growth in all tested isolates. In garlic, the methanolic fraction was the only fraction that has caused complete inhibition of all tested fungal isolates (Table 3). Moreover, methanolic fractions of both sticky fleabane leaves and harmal seeds were more effective, followed by hexane fraction of sticky fleabane, in controlling the growth of tested isolates although complete inhibition of fungal growth was generated with both fractions (Table 3). Concerning the effect of fractions in nightshade fruits, methanolic fraction was the most effective, and followed by the aqueous then hexane fraction. In contrast, the aqueous fraction of nightshade leaves was the most effective fraction, then followed by hexane and finally methanolic fraction as the least effective in all nightshade leaves fractions.

4. Discussion

Results indicated that growth of *P. digitatum* isolates was completely inhibited by all fractions of cinnamon bark and most effectively with the methanolic fraction. However, garlic methanolic fraction was the only fraction of garlic extract that generates complete inhibition in all isolates. Furthermore, both methanolic and hexane fractions of sticky fleabane leaves, harmal seeds, and nightshade fruits have generated complete inhibition of fungal growth. The aqueous fraction of nightshade leaves was the most effective fraction. A comparative study between these findings and previously obtained results (Al-Najar, 2007) against blue mold *P. italicum* indicated that *P. digitatum* isolates were more susceptible to the same plant extracts, where complete inhibition or higher percentage of inhibition was obtained within the same range of concentrations. Similarly, when results on fractions of tested plants were compared with those obtained against *P. italicum* (Al-Najar, 2007); all fractions of cinnamon bark have completely inhibited the growth of blue mold isolates. However, such fractions were more effective in controlling the growth of *P. digitatum* isolates. In contrast to what it is obtained in this study, the aqueous fraction of garlic cloves has caused complete inhibition to the blue mold isolates (IC_{50} values in the range of 49.5-70 µg ml^{-1}). However, methanolic fraction has shown higher efficacy to *P. digitatum* isolates although complete inhibition to *P. italicum* was obtained (IC_{50} values in the range of 30.5-31.5 µg ml^{-1}) (Al-Najar, 2007). Furthermore, the sticky fleabane methanolic fraction has shown almost the same activity to isolates from both species. Quite the opposite, hexane fraction of the same plant was much less effective against *P. italicum* isolates, where no complete inhibition was achieved (Al-Najar, 2007). Naturally, none of the harmal seeds or the nightshade fruits and leaves fractions (except for nightshade leaves hexane fraction) has caused complete inhibition of *P. italicum* isolates, as compared to their high efficacy against *P. digitatum* isolates in the present study. This indicates that *P. digitatum* isolates are more susceptible to plant extracts than isolates of *P. italicum*. Moreover, when results of this study were compared with those obtained from the *In vivo* use of crude extracts of the same plant species against *P. digitatum* isolates infecting orange and lemon fruits (Kanan 2007, data not presented), crude extracts of the nightshade fruits, cinnamon, and garlic were the most effective especially to isolate dg6 (MIC values within the range of 130-390 µg ml^{-1}). When results were compared to the *In vivo* results of the same crude extracts tested against *P. italicum* isolates (Al-Najar, 2007), all extracts have shown complete inhibition of growth to all isolates infecting orange rather than lemon fruits. Based on above results, it is suggested that high efficacy of cinnamon extract or fractions may be related to cinnamaldehyde, eugenol, cinnamic acid, as well as to various organic acids that have consistently been reported by different workers to show antifungal activities (Inouye *et al*., 2000; Gill and Holly, 2004). Yet, this activity may be traced mainly to cinnamaldehyde, which acts as a specific inhibitor for enzymes such as ß-(1, 3)-glucansynthase that participate in the biosynthesis of chitin and ß-glucans cell wall components(Cowan,1999).

Table 1. In vitro activity of different concentrations of various plant crude extracts (conc. range 0.65-97 µg ml^{-1}) against P. digitatum isolates using agar well diffusion method.

Source of plant crude extracts	Fungal Isolate	Mean size zone of inhibition (mm) ± SD/ Range	Corr. Value(r)	Sig-value	*Regression Equation*
Nightshade fruits	dg2	0.0 - 27.33 ± 4.41	0.954**	0.001	*y=9.72x+6.17*
Nightshade fruits	dg4	0.0 - 27.33 ± 13.04	0.901**	0.006	*y=8.19X+5.55*
Nightshade fruits	dg5	0.0 – 30.67± 9.97	0.890**	0.007	*y=9.15x+6.59*
Nightshade fruits	dg6	0.0 – 27.5± 1.22	0.974**	0.000	*y=12.93x+1.96*
Nightshade leaves	dg2	0.0-31.33± 3.44	0.957**	0.001	*y=10.68x+5.76*
Nightshade leaves	dg4	0.0-22.5± 0.55	0.941**	0.002	*y=8.02x+5.75*
Nightshade leaves	dg5	0.0-22.8± 1.72	0.925**	0.003	*y=7.64x+5.97*
Nightshade leaves	dg6	0.0-25.17± 0.98	0.902**	0.006	*y=8.02x+6.97*
Cinnamon	dg2	0.0-24.67± 1.97	0.896**	0.006	*y=10.31x-1.73*
Cinnamon	dg4	0.0-19.83± 7.08	0.780*	0.039	*y=7.60x-2.16*
Cinnamon	dg5	0.0-24.67± 4.18	0.783*	0.037	*y=10.03x-2.77*
Cinnamon	dg6	0.0-23.67± 7.03	0.783*	0.037	*y=9.48x-2.68*
Garlic	dg2	0.0-11.5± 1.05	0.628	0.131	*y=3.56x-1.28*
Garlic	dg4	0.0-20.5± 0.55	0.783*	0.037	*y=8.73x-2.39*
Garlic	dg5	0.0-22.0± 2.13	0.781*	0.038	*y=9.85x-2.65*
Garlic	dg6	0.0-25.33± 0.52	0.784*	0.037	*y=10.47x-2.90*
Sticky fleabane	dg2	0.0-26.33± 1.03	0.900**	0.006	*y=11.53x-1.68*
Sticky fleabane	dg4	0.0-0.0			*Y=0.0*
Sticky fleabane	dg5	0.0-19.00± 1.27	0.781*	0.038	*y=7.48x-2.09*
Sticky fleabane	dg6	0.0-0.0			*Y=0.0*
Fenugreek	dg2	0.0-39.83± 1.97	0.987**	0.000	*y=17.94x+1.33*
Fenugreek	dg4	0.0-39.17± 3.66	0.864*	0.012	*y=17.29x-3.72*
Fenugreek	dg5	0.0-38.17± 2.22	0.989**	0.000	*y=17.69x+1.75*
Fenugreek	dg6	0.0-41.17± 1.47	0.987**	0.000	*y=18.39x+1.78*
Harmal	dg2	0.0-25.17± 0.75	0.775*	0.041	*y=9.34x-2.74*
Harmal	dg4	0.0-11.0± 1.26	0.782*	0.038	*y=4.82x-1.33*
Harmal	dg5	0.0-14.17± 0.75	0.629	0.130	*y=4.55x-1.62*
Harmal	*dg6*	*0.0-13± 0.0*	*0.626*	*0.133*	*y=3.88x-1.41*

Values are means ± SD of at least two independent experiments.

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed).

Table 2. *In vitro* antifungal activity of crude plant extracts against four *P. digitatum* isolates using amended agar method.

Source of plant crude extracts	Conc (Range) (µg ml-1)	Fungal Isolate	IC50	% of inhibition (range)	Corr. Value (r)	Sig-value	*Regression Equation*
Nightshade fruits	25-125 130-520	Dg2,dg4 dg5, dg6	57.5	21-98 100			*Y=100*
Nightshade leaves	25-520	dg2		11-63.11	0.915	0.085	*y=0.022x+52.68*
Nightshade leaves	25-520	dg4		9.5-62.70	0.931	0.069	*y=0.038x+44.57*
Nightshade leaves	25-520	dg5		6.5-54.71	0.809	0.191	*y= 0.05x+30.29*

Nightshade leaves	25-520	dg6		9.5-67.92	0.773	0.227	*y= 0.041x+49.74*
Cinnamon	50-390	dg2	252.5	14.5-74.33	0.993**	0.007	*y=0.166X+11.4*
	520			100			
Cinnamon	50-390	dg4	222.5	11.5-56.48	0.939	0.061	*y=0.165X+6.49*
	520			100			
Cinnamon	50-520	dg5		3.5-59.42	0.913	0.087	*y=0.113X+4.99*
Cinnamon	50-260	dg6	190	14-67.38	0.944	0.056	*y=0.176X+18.46*
	390-520			100			
Garlic	50-520	dg2		9.0-55.62	0.972*	0.028	*y=0.091X+10.97*
Garlic	50-520	dg4		7.5-54.41	0.956*	0.044	*y=0.096X+9.08*
Garlic	50-520	dg5		5.5-43.53	0.952*	0.048	*y=0.086X+2.06*
Garlic	50-520	dg6		10-56.15	0.991**	0.009	*y=0.081X+12.58*
Sticky fleabane	50-520	dg2		7.5-35.84	0.962*	0.038	*y=0.051X+11.77*
Sticky fleabane	50-520	dg4		17-50.78	0.962*	0.038	*y=0.027X+38.09*
Sticky fleabane	50-520	dg5		18-59.99	0.975*	0.025	*y=0.041X+37.94*
Sticky fleabane	50-520	dg6		16-50.81	0.772	0.228	*y=0.027X+39.58*
Fenugreek	50-520	dg2		12.5-42.26	0.922	0.078	*y=0.029X+24.87*
Fenugreek	50-520	dg4		8.5-48.19	0.959*	0.041	*y=0.065X+16.33*
Fenugreek	50-520	dg5		9.5-58.23	0.996**	0.004	*y=0.091X+10.59*
Fenugreek	50-260	dg6	142	20.5-54.55	0.920	0.080	*y=0.155X+25.41*
	390-520			100			
Harmal	130-520	dg2		0.0-22.47	0.878	0.122	*y= 0.051X-8.56*
Harmal	50-520	dg4		1.0-34.72	0.950	0.050	*y= 0.076X-1.81*
Harmal	25-520	dg5		2.5-30.59	0.995**	0.005	*y=0.052+4.12*
Harmal	25-520	dg6		4.0-25.68	0.905	0.095	*y=0.014X+18.99*
Benomyla	0.01-20	dg2	20.7	10.0-29.5	0.875**	0.000	*y=19.86x+34.10*
	25-520			100			
Benomyl	0.01-35	dg4	37	0.0-13.5	0.837**	0.000	*y=23.29x+17.30*
	40-520			100			
Benomyl	0.01-250	dg5	262	10.5-27.0	0.796**	0.000	*y=18.9x+16.01*
	300-520			100			
Benomyl	0.01-35	dg6	36	0.0-30.5	0.884**	0.000	*y=21.88x+23.8*
	40-520			100			
DMSOb	*5.0-520*	*dg2,dg4 dg5, dg6*		*0.0*			

Values are means ± SD of at least two independent experiments.
** Correlation is significant at the 0.01 level (2-tailed),
* Correlation is significant at the 0.05 level (2-tailed).
[a] :positive control fungicide, [b] :negative control.

The high antifungal activity noticed with the crude extract and fractions of nightshade leaves and fruits could be related to the presence of steroidal alkaloids including solamargine, solasonine, solanine and saponin (Zhou *et al*., 2006).

Table 3. *In vitro* activity of different concentrations of methanolic, hexane and aqueous fractions from various plant crude extracts against *P. digitatum* isolates.

Source of plant extract Fraction	Fungal isolate	Conc (Range) (µg)	% of inhibition (range)	IC50	Corr. Value(r)	Sig-value	*Regression Equation*
Night shade/fruits methanolic	dg2	5.0	33.33	8.75	0.559		
		20-100	100			0.093	*y=0.38x+72.71*
	dg4	5	24.59	10	0.559	0.093	*y=0.43x+69.14*
		20-100	100				
	dg5	5.0	29.03	9.5	0.559	0.093	*y=0.403x+70.95*
		20-100	100				
	dg6	5.0-70	21.67-65.0	24.75	0.944**	0.000	*y=0.799x+22.12*
		80-100	100				
Night shade/fruits hexane	dg2	5-80	8.36-57.38	56	0.896**		
		90-100	100			0.001	*y=0.80x+10.82*
	dg4	5-80	11.21-56.45	62	0.892**	0.001	*y=0.81x+10.21*
		90-100	100				
	dg5	5-80	13.64-85.0	36.5	0.969**	0.000	*y=0.77x+22.17*
		90-100	100				
	dg6	5-80	10.91-53.33	70	0.858**	0.003	*y=0.80x+8.11*
		90-100	100				
Night shade/fruits aqueous	dg2	5-20	22.31-67.21	14.8	0.548		
		30-100	100			0.127	*y=0.219x+83.24*
	dg4	5-20	19.64-66.13	15.25	0.548	0.127	*y=0.226x+82.69*
		30-100	100				
	dg5	5-30	16.73-60.0	21	0.730*	0.025	*y=0.544x+57.15*
		40-100	100				
	dg6	5-80	12.94-66.67	39.5	0.870**	0.002	*y=0.669x+24.09*
		90-100	100				
Nightshade /leaves methanolic	dg2	20-70	9.24-36.11	72	0.894	0.106	*y=2.11x-87.78*
		80-90	100				
	dg4	20-70	11.63-36.11	72	0.883	0.117	*y=2.04x-81.91*
		80-90	100				
	dg5	20-70	17.94-67.24	44	0.739	0.261	*y=1.20x-6.11*
		80-90	100				
	dg6	20-90	0.0-8.33		0.878	0.122	*y=0.21x-12.06*
Nightshade leaves hexane	dg2	20-50	4.64-13.89	58.5	0.878		*y=2.21x-82.06*
		70-90	100			0.122	
	dg4	20-50	6.32-16.67	58	0.878	0.122	*y=2.14x-76.19*
		70-90	100				
	dg5	20-80	18.94-60.35	55	0.850	0.150	*y=1.15x-16.40*
		90	100				
	dg6	20-80	7.31-27.78	83.75	0.767	0.233	*y=1.74x-83.65*
		90	100				

Nightshade	dg2	5.0	43.33	6.75	0.581		
leaves aqueous		20-90	100			0.131	*y=0.39x+74.24*
	dg4	5.0	35.0	8.5	0.581	0.131	*y=0.45x+70.45*
		20-90	100				
	dg5	5.0	33.33	8.75	0.581	0.131	*y=0.46x+69.69*
		20-90	100				
	dg6	5.0	21.67	10.5	0.581	0.131	*y=0.54x+64.39*
		20-90	100				
Cinnamon methanolic	dg2	5.0-10	49.18-59.02	5.0	0.819*	0.046	*y=1.123x+55.7*
		20-50	100				
	dg4	5.0-20	15.25-20.34	9.5	0.900*	0.015	*y=2.35x-2.20*
		30-50	100				
	dg5	5.0-20	10.0-30.0	23	0.920**	0.009	*y=2.44x-4.66*
		30-50	100				
	dg6	5.0-10	40-51.66	9.25	0.819*	0.046	*y=1.33x+47.71*
		20-50	100				
Cinnamon hexane	dg2	5-10	32.79-36.07	12.25	0.817*	0.047	*y=1.59x+37.16*
		20-50	100				
	dg4	5-10	27.11-28.81	13	0.815*	0.048	*y=1.74x+31.05*
		20-50	100				
	dg5	5-10	5.0-5.0	13	0.814*	0.049	*y=2.29x+9.16*
		20-50	100				
	dg6	5-10	16.66-26.66	14.5	0.819*	0.046	*y=1.91x+24.68*
		20-50	100				
Cinnamonaa queous	dg2	20	38.33	25.5	0.926	0.074	*y=1.32x+18.51*
		50-70	100				
	dg4	20	16.66	32.5	0.926	0.074	*y=1.79x-10.12*
		50-70	100				
	dg5	20	21.66	31	0.926	0.074	*y=1.68x-3.51*
		50-70	100				
	dg6	20-60	23.33-51.66	47.5	0.881	0.119	*y=1.30x-8.21*
		70	100				
Garlic methanolic	dg2	5-10	18.03-60.66	8.5	0.784	0.065	
		20-50	100				*y=1.53x+40.19*
	dg4	5-10	64.52-64.52	3.75	0.814*	0.049	*y=0.86x+66.07*
		20-50	100				
	dg5	5-10	63.33-71.67	3.75	0.818*	0.046	*y=0.79x+68.57*
		20-50	100				
	dg6	5-20	33.33-55.0	18	0.929**	0.007	*y=1.73x+26.48*
		30-50	100				
Garlic hexane	dg2	50-100	8.20-16.39		0.959**	0.010	*y=0.16x-0.47*
	dg4	50-100	1.61-12.90		0.938*	0.019	*y=0.233x-11.09*
	dg5	50-100	0.0-8.33		0.824	0.086	*y=0.164x-10.16*
	dg6	50-100	1.67-18.33		1.000**	0.000	*y=0.33x-15*
Garlic aqueous	dg2	50-100	6.56-14.75		0.959**	0.010	*y=0.157x-2.11*
	dg4	50-100	9.68-19.36		0.882*	0.048	*y=0.18x+2.68*
	dg5	50-100	13.33-20.0		0.885*	0.046	*y=0.13x+5.65*

	dg6	50-100	11.67-18.33		0.814	0.094	*y=0.126x+7.16*
Sticky fleabane methanolic	dg2	20	34.43	27.25	0.874	0.053	
		50-80	100				*y=1.114x+24.53*
	dg4	20	25.81	30	0.874	0.053	*y=1.26x+14.61*
		50-80	100				
	dg5	20	20	31.75	0.874	0.053	*y=1.36x+7.93*
		50-80	100				
	dg6	20	26.68	29.75	0.874	0.053	*y=1.25x+15.60*
		50-80	100				
Sticky fleabane hexane	dg2	20-80	22.63-81.97	38	0.926		
		90	100			0.074	*y=0.86x+16.96*
	dg4	20-80	27.94-72.58	38.5	0.825	0.175	*y=0.82x+16.27*
		90	100				
	dg5	20-80	31.76-65.0	36.5	0.683	0.317	*y=0.70x+23*
		90	100				
	dg6	20-80	19.46-66.67	48	0.864	0.136	*y=1.07x-8.10*
		90	100				
Fenugreek methanolic	dg2	20-90	0.0-13.88		0.969	0.160	*y=0.233x-5.43*
	dg4	20-90	6.52-25.42		0.955	0.192	*y=0.42x-6.72*
	dg5	20-90	0.0-23.33		1.000*	0.015	*y=0.50x-16.71*
	dg6	20-90	0.0-16.66		0.945	0.212	*y=0.496x-21.03*
Fenugreek hexane	dg2	20-90	0.0-13.89		0.899	0.101	*y=0.19x-2.14*
	dg4	20-90	0.0-13.89		0.841	0.159	*y=0.37x-18.5*
	dg5	20-90	0.0-6.90		0.775	0.225	*y=0.21x-11.72*
	dg6	20-90	0.0-6.67		0.990**	0.010	*y=0.233x-11.83*
Fenugreek aqueous	dg2	20-90	0.0-24.59		0.870	0.055	*y=0.33x-2.95*
	dg4	20-90	8.33-32.26		0.945*	0.015	*y=0.323x+1.29*
	dg5	20-90	0.0-30.0		0.877	0.051	*y=0.42x-11.83*
	dg6	20-90	0.0-18.33		0.866	0.058	*y=0.15x+5.83*
Harmal methanolic	dg2	20-80	0.0-59.02	66.5	0.811*	0.027	*y=0.821x-1.41*
		90-150	100				
	dg4	20-80	0.0-58.07	68.5	0.815*	0.026	*y=0.882x-8.91*
		90-150	100				
	dg5	20-90	0.0-61.67	85	0.876**	0.010	*y=0.809x-7.78*
		100-150	100				
	dg6	20-120	0.0-83.33	81.5	0.984**	0.000	*y=0.830x-19.57*
		150	100				
Harmal hexane	dg2	20-90	23.38-65.57	47	0.825	0.086	*y=0.79x+6.34*
		100	100				
	dg4	20-80	19.67-61.29	40.5	0.819	0.090	*y=0.93x+3.71*
		90-100	100				
	dg5	20-90	12.86-46.67	90.5	0.705	0.184	*y=0.93x-18.06*
		100	100				
	dg6	*20-100*	*9.74-51.67*		*0.953**	*0.012*	*y=0.244x+25.41*

This is in agreement with previous results (Al-Fatimi et al, 2007), which indicate that steroidal alkaloids have shown antifungal activity against eleven agronomically important fungi including Aspergillus spp, Rhizopus spp, Fusarium spp, Alternaria brassicicola. Garlic extracts have shown significant effect on the growth of P. digitatum isolates. This finding agrees with earlier reports that stated extracts of garlic can inhibit mould growth. And the effectiveness of this inhibition is related to the solvent used in the extraction (Irkin and Korukluoglu, 2007). The antifungal activity of garlic is related to allicin, which is the main biologically active component of garlic extract inhibiting essential enzymes for pathogen infection (Miron et al., 2000). Similarly, ajoene (allicin derivative) has shown also strong inhibitory activity against several fungal species including the black mold (Aspergillus niger), Candida albicans, and Paracoccidiodes brasiliensis (Naganawa et al., 1996). Ajoene was superior to allicin in the severity of inhibiting fungal growth by disrupting the cell wall (Yoshida et al., 1987). Moreover, leaf extracts of I. viscose proved to have a significant antifungal activity against dermatophytes and downy mildew. This may be attribuatble to high concentration of sesquiterpene as well as phenolic compounds present in the methanolic or aqueous fractions (Cafarchia et al., 2002; Cohen et al., 2006; Al-Najar, 2007). These findings confirm results obtained earlier (Shtayeh and Abu Gheleib, 1999; Cohen et al, 2002). Yet, results disagreed with the findings of Wang and his co-workers (2004), which indicate poor activity of I. viscosa water extract against plant diseases. The strong inhibitory activity of methanolic and hexane fractions of harmal may be related to the high content of alkaloids (harmine, harmaline and tetrahydroharmine), and the presence of phenolic compound. This is well-matched with earlier reports (Kartal et al., 2003; Telezhenetskaya and Dyakonov, 2004; Al-Najar, 2007). Although fenugreek fractions are rich in alkaloids, as well as phenolic compounds (Al-Najar, 2007), reduced activity was detected in this study against Penicillium isolates. And this may be linked to the fractionation process. However, these findings disagreed with that obtained by Olli and Kirti (2006), who stated that the methanolic extract of Fenugreek was highly specific for dermatophytes.

Acknowledgement

This work was funded by Mu'tah University (Deanship of Scientific research, process number S. R/120/14/180). The authors would like to thank Dr. Saleh Al-Quran for helping in classifying the tested plant species

References

AL-Fatimi M, Wurster, M, Schroder, G and Lindequist, U. 2007. Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J. Ethnopharmacol. 111(3): 657-666.

Ali-Shtayeh MS and Abu Ghdeib SI. 1999. Antifungal activity of plant extract against dermatophytes. Mycoses. 42(11-12): 665-672.

Al-Najar RA. 2007. Selection and evaluation of alternatives to synthetic fungicides for the control of post-harvest citrus fruits rot caused by Penicillium italicum (blue mold) in Jordan. (MSc thesis). Mu'tah (Jordan): Mu'tah University.

Ambrozin ARP, Vieira PC, Fernandes JB, Da Silva MFGF and Albuquerque S. 2004. Trypanocidal activity of Meliaceae and Rutaceae plant extracts. Mem Inst Oswaldo Cruz. 99(2): 227-231.

Cafarchia C, De Laurentis N, Milillo MA, Losacco V and Puccini V. 2002. Antifungal activity of essential oils from leaves and flowers of Inula viscosa (Asteraceae) by Apulian region. Parassitologia. 44(3-4): 153-156.

Cohen Y, Wang W, Ben-Daniel BH and Ben-Daniel Y. 2006. Extracts of Inula viscosa control downy mildew of grapes cause by Plasmopara viticola. Phytopath. 96(4): 417-424.

Cohen Y, Baider A, Ben-Daniel BH and Ben-Daniel Y. 2002. Fungicidal preparations from Inula viscosa. Plant Prot. Sci. 38: 629-630.

Cove DJ. 1966. The induction and repression of nitrate reductase in the fungus Aspergillus nidulans. Biochem. Biophys. Acta. 113: 51-56.

Cowan MM. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-582.

Curadi M, Graifenberg A and Magnani GG. 2005. Growth and element allocation in tissues of Inula viscosa in sodic-saline conditions: A candidate for programs of desertification control. Arid Land Res. Manag. 19: 257-265.

Dafni A and Yaniv Z. 1994. Solanaceae as medicinal plants in Israel. J. Ethnopharmacol. 44(1): 11-18.

Dhellot JR, Matouba E, Maloumbi MG, Nzikou JM, Dzondo MG, Linder M, Parmentier M and Desorbry S. 2006. Extraction and nutritional properties of Solanum nigrum L seed oil. Afri. J. Biotech. 5(10): 987-991.

Elsom GK. 2000. An antibacterial assay of aqueous extract of garlic against anaerobic/microaerophilic and aerobic bacteria. Microb. Ecol. Health. Dis. 12: 81-84.

Gill AO and Holly RA. 2004. Mechanisms of bactericidal action of Cinnamaldehyde against Listeria monocytogenes and of eugenol against L. monocytogenes and Lactobacillus sakei. Appl. Environ. Microbiol. 70: 5750-5755.

He ZD, Qiao CF, Han QB, Cheng CL, Xu HX, Jiang RW, Pui-Hay BP, and Shaw PC. 2005. Authentication and quantitative analysis on the chemical profile of Cassia bark (cortex cinnamomi) by high pressure liquid chromatography. J. Agri. Food Chem. 53: 2424-2428.

Inouye S, Tsuruoka M, Watanabe M, Takeo K, Akao M, Nishiyama Y and Yamaguchi H. 2000. Inhibitory effect of essential oils on apical growth of Aspergillus fumigatus by vapour contant. Mycoses. 43:17-23.

Irkin R and Korukluoglu M. 2007. Control of Aspergillus niger with garlic, onion and leek extract. Afri. J. Biotech. 6(4): 384-387.

Kartal M, Altun ML and Kurucu S. 2003. HPLC method for the analysis of harmol, harmalol, harmine and harmaline in the seeds of Peganum harmala L. J. Pharmaceu. Biomed. Anal. 31: 263-269.

Lopez-Malo A, Alzamora SM and Palou E. 2005. Aspergillus flavus growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. Int. J. Food. Microbiol. 99: 119-128.

Marino M, Bersani C and Comi G. 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. Int. J. Food Microbiol. 67: 187-195.

Miron T, Rabinkov A, Mielman D, Wilchek M Weiner L. 2000. The mode of action of allicin. Bioch. Biophys. Acta. 1463: 20-30.

Naganawa R, Iwata N, Ishikawa K, Fukuda H, Fujino T and Suzuki A. 1996. Inhibition of microbial growth by ajoene, a sulfur containing compound derived from garlic. Appl. Environ. Microbiol. 62: 4238-4242.

Ndukwe IG, Habila JD, Bello IA and Adeleye EO. 2006. Phytochemical analysis and antimicrobial screening of crude extracts from the leaves, stem bark and root bark of Ekebergia senegalensis A. Juss. Afri. J. Biotech. 5(19): 1792-1794.

Nwachukwu EO and Umechuruba CI. 2001. Antifungal activities of some leaf extracts on seed -borne fungi of Africa Yam bean seeds, seed germination and seedling emergence. J. Appl. Sci. Environ. Mgt. 5(1): 29-32.

Obagwu J and Korsten L. 2003. Control of citrus green and blue molds with garlic extracts. Eur. J. Plant Path. 109: 221-225.

Olli S and Kirti PB. 2006. Cloning, characterization and antifungal activity of Defensin Tfgd1 from Trigonella foenum-graecum L. J. Biochem. Mole. Biol. 39: 278-283.

Plaza P, Sanbruno A, Usall J, Lamarca N, Torres R, Pons J and Vinas I. 2004. Integration of curing treatments with degreening to control the main postharvest diseases of Clementine mandarins. Post. Biol. Technol. 34(1): 29-37.

Pramila T, and Dubey NK. 2004. Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Post. Biol. Technol. 32: 235-245.

Rasooli I and Abyaneh MR. 2004. Inhibitory effect of thyme oils on growth and aflatoxin production by Apergillus parasiticus. Food Cont. 15: 479-483.

Sokmen A. 2001. Antiviral and cytotoxic activities of extract from the cell cultures and respective parts of some Turkish medicinal plants. Turk. J. Biol. 25: 343-350.

Soylu EM, Tok FM, Soylu S, Kaya AD and Evrendilek G.A. 2005. Antifungal activities of essential oils on post harvest disease agent Penicillium digitatum. Pakistan J. of Biol. Sci. 8(1): 25-29.

Telezhenetskaya MV and D'yakonov AL. 2004. Alkaloids of Peganum harmala. Unusual reaction of peganine and vasicinone. Chem. Nat. Comp. 27: 471-474.

Wang WQ, Ben-Daniel BH and Cohen Y. 2004. Extracts of Inula viscosa control downy milew caused by Plasmopara viticola in grape-vines. Phytoparasitica. 32: 208-211.

Yoshida H, Katsuzaki H, Ohta R, Ishikawa K, Fukuda H, Fujino T and Suzuki A. 1999. An organosulfur compound isolated from oil-macerated garlic extract, and its antimicrobial effect. Biosci. Biotechnol. Biochem. 63(3): 588-590.

[35] Yoshida S, Kasuga S, Hayashi N, Ushiroguchi T, Matsuura H and Nakagawa S. 1987. Antifungal activity of Ajoene derived from garlic. Appl. Environ. Microbiol. 53: 615-617.

Zargar AH, Laway ANBA and Dar FA. 1992. Effect of consumption of powdered Fenugreek seeds on blood sugar and HbAIc levels in patients with type II diabetes mellitus. Intel. J. Diab. Dev. Countries. 12: 49-51.

Zhang HY, Fu CX, Zheng XD, He D, Shan LJ and Zhan X. 2004. Effect of Cryptococcus laurentii (Kufferath) skinner in combination with sodium bicarbonate on biocontrol of post harvest green mold decay of citrus fruit. Bot. Bull. Acad. Sinica. 45:159-164.

Zhao HQ, Qu Y, Wang XY, Zhang HJ, Li FM and Masao H. 2002. Determination of trigonelline in Trigonella foenum-graecum by HPLC. Zhongguo. Zhong. Yao. Za. Zhi. 27(3): 194-196.

Zhou X, He X, Wang G, Gao H, Zhou G andYe W. 2006. Steroidal saponins from Solanum nigrum . J. Nat. Prod. 69(8): 1158-1163.