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Phytochemical Analysis and Antifungal Activity of Fruit Leaves Extracts on the Mycelial Growth of Fungal Plant Pathogens

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Abstract

Methanolic extracts of leaves of thompson seedless grape, flame seedless grape, zizyphus, pomegranate and fig were screened for their phytochemical constituents and also investigated for their antifungal activity *in vitro* against phytopathogenic fungi, *Alternaria solani, Botrytis cinerea, Botrytis fabae, Fusarium oxysporum* and *Fusarium solani.* Phytochemical screening of crude methanolic extracts revealed the presence of terpenes, tannins, flavonoids, alkaloids, carbohydrate or glycosides, phenolic glycosides and resins in all extracts, while saponins were not detected from any extracts except zizyphus and fig. Zizyphus leaves had the highest concentration of total polyphenols and total flavonoids content which were 147.47 mgGAE/g and 16.35 mgQE/g, respectively. HPLC analysis identified twelve polyphenolic compounds; pyrogallic acid, gallic acid, protocatechuic, catechin, *p*-hydroxy benzoic acid, *p*-coumaric acid, phenol, *o*-coumaric acid, salicylic acid, coumarin, quercetin and cinnamic acid. All extracts contained protocatechuic, catechin, *p*-hydroxy benzoic acid, *p*-coumaric acid, *o*-coumaric acid and coumarin with different concentrations. The methanolic extract of zizyphus leaves had the greatest inhibitory effect on mycelial growth of *B. fabae* by 95.56% at 4 mg/ml. Also, extract of pomegranate caused remarkable reduction on the fungal growth (94.44%) of *B. fabae* at 4 mg/ml, while fig extract caused 91.11% inhibition against the same fungus at the same concentration. *F. oxysporum* and *F. solani* were the most resistant fungi against all methanolic extracts tested.

Keywords: Antifungal activity; Plant extracts; Phytopathogenic fungi; Phytochemicals; HPLC

Introduction

The damage to crops caused by fungal plant pathogens has required the use of range of antifungal control agents. Among pesticides used to protect crops, fungicides were perceived until recently as relatively safe. However, the 1986 National Academy of Sciences (NAS) report on pesticide residues on food indicated that fungicides pose more of a carcinogenic risk than insecticides and herbicides together [1]. Furthermore, the use in crop protection of many synthetic fungicides that have various degrees of persistence has now been cautioned due to their carcinogenicity, teratogenicity and other residual toxicities. Several of the synthetic fungicides are reported to cause adverse effects on treated soil ecosystems because of their non-biodegradable nature [2,3]. Synthetic fungicide residues are suspected to present a significant health risk to consumers, and demand is increasing to find safe alternatives. Additionally, continued use of fungicides leads to an increase in resistance by plant pathogens, creating a need for finding biological alternatives with these pesticides. Present activities to find both natural and synthetic fungicides focus on finding compounds that are safe to humans, environment and delicate ecosystems [4,5].

New antifungal compounds with distinct modes of action need to be identified because of increasing incidence of fungal resistance to existing antibiotics [6]. Plant secondary metabolites have great potential as a source of effective antifungal agents [7]. Plant-derived compounds such as hydroquinones and naphthoquinones (lapachol, juglone), sesquiterpenes (cinnamodial, capsidiol) and alkaloids (berberine) have shown diverse activities as antimicrobial and antifungal. An advantage to the approach of using ethnobotanical leads to identify compounds with antimicrobial activity [8]. For instance, leaves of *Vitis vinifera* are rich in tannins, flavonoids, procyanidins and also contain organic acids, lipids, enzymes and vitamins [9-12]. Furthermore, the quantitative analysis of compounds found in leaves has also been evaluated by Monagas et al. [13]. Grape-vine leaves possesses a resistance towards several fungus diseases as *Plasmopara viticola, Oidium tukeri* and *Botrytis cinerea* which cause downy mildew, powdery mildew and fruit rot, respectively [14]. Flavonoids including quercetin and quercetin-3-O-[β-xylosyl-(1-2)-α-rhamnoside] 4'-O-α- rhamnoside as bioactive compounds were secluded from *Zizyphus spina-christi* leaves [15]. Also, various gallic acid derivatives were isolated from the leaves of *Punica granatum* such 1,2,3-tri-O-galloyl-β-glucopyranose, 1,2,4-tri-O-galloyl-β-glucopyranose, 1,3,4-tri-O-galloyl-β-glucopyranose, 1,2,6-tri-O-galloyl-β-glucopyranose and 1,4,6-tri-O-galloyl-β-glucopyranose [16-18]. The main objective of the present study was to evaluate the antifungal activity of leaf extracts of various plant species against five economically important plant disease organisms under *in vitro* conditions with phytochemical screening and HPLC analysis.

Materials and Methods

Plant materials

Leaves of thompson seedless grape (*Vitis vinifera* cv. Sultana), flame seedless grape (*Vitis vinifera* cv. Roumy Ahmer), zizyphus (*Zizyphus spina-christi* cv. Willd), pomegranate (*Punica granatum* cv. Baladi) and fig (*Ficus carica* cv. Sultani), belong to families Vitaceae, Rhamnaceae,

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Punicaceae and Moraceae, respectively, were used in this study. Samples were collected, cutting the leaves of plants, from the Experimental Station of the Faculty of Agriculture within the campus of the University of Mansoura, which located in the north of Egypt. Leaves were cleaned, dried in the shade, and then grounded to a fine powder using a grinding mill. Leaves powder (3 Kg) of each plant was extracted by soaking at room temperature for six times with methanol (30 L). Extracts were concentrated to nearly dryness under reduced pressure by using rotary evaporator at 45°C to achieve the crude methanolic extracts.

Plant pathogens

Five plant pathogenic fungi, *Alternaria solani, Botrytis cinerea, Botrytis fabae, Fusarium oxysporum* and *Fusarium solani,* were isolated from diseased potato plants, diseased fruits of tomato, diseased broad bean plants, naturally infected tomato and artichoke, respectively. All the diseased plants and the diseased fruits were collected from Mansoura, Egypt. All fungal strains were grown on potato dextrose agar (PDA) or czapek agar (CZA) media and purified using single spore or hyphal tip techniques. Identification of the pure cultures was accomplished according to Barnett and Hunter [19]; Leslie and Summerell [20]; Elad et al. [21] by cultural properties, morphological and microscopical characteristics. Stock cultures of each strain were maintained on PDA at 5°C. For use in antifungal activity assay, the fungi were subcultured onto PDA in Petri dishes (9 cm diameter), and incubated at 25°C for 7–10 d.

Preliminary phytochemical tests of crude plant extracts

Crude extracts were analyzed to detect the presence of terpenes, tannins, flavonoids, saponins, alkaloids, carbohydrate and/or glucosides, phenolic glucosides and resins according to Harborne [22].

Determination of total polyphenols and total flavonoids content

Total phenolics content of air dried leaves were determined by Folin–Ciocalteau method according to Lin and Tang [23]. Gallic acid was chosen as standard of total phenolics for making the standard curve (200-1600 mg/l). Concentration of total phenolics content expressed as milligram gallic acid equivalents GAE/g. Total flavonoids content of air dried leaves were determined using aluminum chloride colorimetric method, which described by Chang et al. [24]. Quercetin was chosen for making standard curve of flavonoids (0–50 mg/l). Concentration of total flavonoids content was expressed as milligram quercetin equivalents QE/g.

Identification of polyphenols by HPLC

Phenolic compounds of plant samples were extracted according to Ben-Hammouda et al. [25]. Identification of individual phenolic compounds of plant samples was performed on a Hewlett-Packard HPLC (Model 1100), using a hypersil C_{18} reversed phase column (250×4.6 mm) with 5µm particle size. Injection by means of Rheodyne injection valve (Model 7125) with 50 µm fixed loop. Phenolic compounds of each sample were identified by comparing their relative retention times with those of the standards mixture chromatogram. The concentration of an individual compounds was calculated on the basis of peak area measurements.

Antifungal activity of plant extracts on the mycelial growth of test fungi

Extracts were dissolved in dimethyl sulphoxide (DMSO) and added to PDA medium immediately before it was poured into Petri dishes (9 cm diameter) at 40–45°C to obtain a series of concentrations (1, 2 and 4 mg/ml). Control plates were treated with DMSO alone, and three replicates per treatment were used. Plates were incubated at 25 ± 2°C. Colony growth diameter was measured after the fungal growth in the control treatment had completely covered Petri dishes. Percentage of mycelial growth inhibition was calculated from the formula: Mycelial growth inhibition=[(diameter of control–diameter of sample)/ diameter of control]×100

Statistical analysis

Statistical analyses of all experimental data were done using the statistical software package CoStat [26]. All comparisons were first subjected to one way analysis of variance (ANOVA) and significant differences between treatment means were determined using Duncan's multiple range test at $P<0.05$ as the level of the significance [27].

Results and Discussion

Preliminary phytochemical tests of crude methanolic extracts

All crude methanolic extracts of leaves were rich in terpenes, tannins, flavonoids, alkaloids, carbohydrate or glycosides, phenolic glycosides and resins. In contrast, all leaves extracts was poor in saponins except zizyphus and fig, (Table 1). Results for thompson and flame seedless leaves were agreed with Bombardelli and Morazzonni [9]; Hebash et al. [10] and Hmamouchi et al. [11]. Felicio et al. [12] found that the *Vitis vinifera* leaves were rich in tannins, flavonoids, procyanidins and contain organic acids, lipids, enzymes and vitamins. Present data for zizyphus leaves extract was agreed with Ikram et al. [28]; Higuchi et al. [29]; Nawwar et al. [30]; Han et al. [31]; Barboni et al. [32]; Abu-Zarga et al. [33]; Cheng et al. [34] and Shahat et al. [15]. Tripathi et al. [35] identified peptide and cyclopeptide alkaloids, flavonoids, sterols, tannins, betulinic acid and triterpenoidal saponin glycosides from the different species of the genus *Zizyphus*. Furthermore, Mahran et al. [36] isolated four triterpenoidal saponin glycosides namely christinin-A (the major saponin), B, C and D from butanol extract of *Zizyphus spinachristi* leaves growing in Egypt.

Results of pomegranate leaves were in agreement with Nawwar et al. [17] and Hussein et al. [18]. They isolated gallotannins and ellagitannins namely; brevifolin carboxylic acid, corilagin, brevifolin, ellagic acid, granatin-B and punicafolin from the leaf extract of Egyptian *Punica granatum*. Data of methanolic extract of fig leaves was

Plant	Terpens	Tannins	Flavonoids	Saponins	Alkaloids	Carbohydrate or Glycosides	Phenolic glycosides	Resins
Thompson seedless	+ +	+	+ +	-	+	+ +	+ +	+ +
Flame seedless	+ + +	+	+ +	-	+	+ +	+ +	+ +
Zizyphus	+ + +	+	+ +	+ + +	+	+ +	+ +	+ +
Pomegranate	+ + +	+	+ +	-	+	+ +	+ +	+ +
Fig	+	+ + +	+ +	+	+	+ +	+	+ +

(+) detected, (-) not detected

Table 1: Preliminary phytochemical tests of crude methanolic extracts.

in accordance with Ahmed et al. [37]. They fractionated two triterpenes from ethanolic extract of fresh leaves of *Ficus carica* L. into hexane soluble and insoluble fractions. Also, Ayinde et al. [38] found that the aqueous extract of *Ficus exasperata* vahl leaves showed the presence of flavonoids, saponins and tannins, with no traces of alkaloids or anthraquinones.

Total polyphenols and total flavonoids content

Total polyphenols include several classes of phenolic compounds that are secondary plant metabolites and integral part of human and animal diets. Flavonoids are a large group of the phenolic compounds consisting mainly of flavonols, flavanols and anthocyanins. Phenolic compounds can play an important role in preventing body cells and organs from injuries by hydrogen peroxide, damage by lipid peroxides and scavenging or neutralizing free radicals [39]. It has been reported that free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, diabetes, tissue inflammatory and cardiovascular diseases [40]. Also, it was found that high total phenols content increase antioxidant activity and there is a linear correlation between phenolic content and antioxidant activity in fig leaves extract [41].

Zizyphus leaves had the highest concentration of total polyphenols (147.47 mgGAE/g). Also, pomegranate and fig leaves had high values, which were 122.52 and 123.51 mgGAE/g, respectively. For total flavonoids, zizyphus leaves contained the highest amount (16.35 mgQE/g) followed by flame seedless leaves by 15.93 mgQE/g. Whereas, thompson seedless and pomegranate leaves contained 13.25 and 10.95 mgQE/g, respectively (Table 2).

These results are in agreement with Monagas et al. [13]. They found that the total polyphenols was 112 ± 18 mgGAE/g of methanolic extract of *Vitis vinifera* leaves. Additionally, Orhan et al. [42] suggested that the total phenolic contents of *V. vinifera* leaves were 205.79 ± 8.89, 57.17 ± 6.5 and 37.97 ± 0.90 mgGAE/g for ethyl acetate, butanol and remaining aqueous fractions, respectively. Also, Orha et al. [43] suggested that the total phenolic contents of *V. vinifera* leaves were 89.4, 216.0, 91.2 and 68.6 mgGAE/g for chloroform, ethyl acetate, butanol and remaining aqueous fractions, respectively. Although, chloroform and ethyl acetate fractions contain 59 and 206 mgQE/g of total flavonoids, respectively, total flavonoids were not detected in butanol fraction and remaining aqueous fraction had a traces of total flavonoids. While, Aseri et al. [44] represented that the total phenols content was 1.81 mg/g in control *Punica granatum* leaves on fresh weight basis. Pari and Suresh [45] showed that the ethanolic extract of grape leave contain phenolic compounds in a concentration of 98.84 ± 9.29 mgGAE/g. Allam et al. [46] found that 20, 40 and 60 mg of fig leaves aqueous extract contain 3.05, 6.10 and 9.14 mg/g extract of total phenols, respectively. While, the previous amounts of the extracts contain 0.22, 0.45 and 0.67 mg/g extract of total flavonoids, respectively.

Leaves extract	Total polyphenols (mgGAE/g)	Total flavonoids (mgQE/g)
Thompson seedless	119.82	13.25
Flame seedless	117.51	15.93
Zizyphus	147.47	16.35
Pomegranate	122.52	10.95
Fig	123.51	9.57

Table 2: Total polyphenols (mgGAE/g) and total flavonoids (mgQE/g) contents of leaves extracts.

Identification of polyphenols by HPLC

Twelve polyphenolic compounds were available as authentic samples namely: pyrogallic acid, gallic acid, protocatechuic, catechin, *p*-hydroxy benzoic acid, *p*-coumaric acid, phenol, *o*-coumaric acid, salicylic acid, coumarin, quercetin and cinnamic acid. These standard samples were used to identify the corresponding components in leaves polyphenols. Results revealed that the twenty four, twenty six, thirty three, twenty seven and twenty one compounds with different retention times were recognized in HPLC chromatogram of thompson seedless, flame seedless, zizyphus, pomegranate and fig leaves, respectively. All leaves samples contained protocatechuic, catechin, *p*-hydroxy benzoic acid, *p*-coumaric acid, *o*-coumaric acid and coumarin with different concentrations comparing with standard compounds. Also, *p*-coumaric acid was the predominant identified component in both thompson seedless and flame seedless leaves in concentrations of 14.761 and 44.732%, followed by phenol (10.031 and 4.179%), whereas, phenol was the main component of polyphenols in zizyphus leaves in concentration of 9.896% followed by *p*-coumaric acid (5.253%), pyrogallic acid (5.089%) and *p*-hydroxy benzoic acid (2.592%). Although, *p*-hydroxy benzoic acid was the major identified phenolic component in both pomegranate and fig leaves in concentrations of 27.587 and 7.736%, while, catechin had the second level (15.243%) in pomegranate leaves and pyrogallic acid had the second rank (4.775%) in fig leaves, (Table 3). Gallic acid and quercetin was not found in thompson seedless, flame seedless, zizyphus and fig leaves, while, pomegranate leaves had 2.264 and 0.286% of gallic acid and quercetin. Cinnamic acid was absent in pomegranate and fig leaves but was identified in thompson seedless, flame seedless and zizyphus at concentrations of 0.086, 0.176 and 0.071%, respectively.

Several authors identified some polyphenoilc derivatives from leaves of *V. vinifera*. For example, Hmamouchi et al. [11] isolated ten hydroxy flavonoids including quercetin-3-rhamnogalactoside, quercetin and caffeic acid, while, Wirth et al. [47] analyzed the released aglycons to identify five aglycon chemical groups including methyl salicylate, 1-phenylethanol, 2-phenylethanol, benzyl alcohol, phenol, 3,4,5-trimethoxyphenol and 4-(4-hydroxyphenyl) butan-2-ol. In addition, Monagas et al. [13] found that phenolic composition of commercial dietary ingredients of V. vinifera leaves contain anthocyanins including delphinidin-3-(6-P-coumaroyl) glucoside, cyanidin-3-(6-P-coumaroyl) glucoside, petunidin-3-(6-P-coumaroyl) glucoside, peonidin-3-(6-P-coumaroyl) glucoside and malvidin-3-(6-P-coumaroyl) glucoside. Also, flavonols as well as quercetin-3-O-galactoside, quercetin-3-O-glucuronide, quercetin-3-O-glucoside and quercetin were identified while, trans-caftaric acid was the only hydroxycinnamic acid derivative identified. Furthermore, Shahat et al. [15] secluded the flavonoids including quercetin, and quercetin-3-O-[β-xylosyl-(1-2)-α-rhamnoside] 4'-O-α-rhamnoside as bioactive compounds of *Z. spina-christi* leaves. Also, various gallic acid derivatives were isolated from the leaves of *Punica granatum* such as 1,2,3-tri-O-galloyl-β-glucopyranose, 1,2,4-tri-O-galloyl-β-glucopyranose, 1,3,4-tri-O-galloyl-β-glucopyranose, 1,2,6-tri-O-galloyl-β-glucopyranose and 1,4,6-tri-O-galloyl-β-glucopyranose [16-18]. Kash [48] found that mulberry leaves (family Moraceae) contain pyrogallic acid, protocatechuic, catechin, p-hydroxy benzoic acid, p-coumaric acid, O-coumaric acid, salicylic acid and coumarin with concentrations of 5.683, 1.089, 11.238, 2.741, 13.874, 1.541, 0.778 and 0.610%, respectively. Results revealed that fifteen, sixteen, twenty three, eighteen and thirteen unknown compounds with different retention times were appeared in HPLC chromatogram of thompson seedless, flame seedless, zizyphus, pomegranate and fig leaves, respectively.

Compounds	Thompson seedless		Flame seedless		Zizyphus		Pomegranate		Fig	
	Rt	%	Rt	%	Rt	%	Rt	%	Rt	%
Pyrogallic acid	22.298	1.722	20.890	2.849	20.993	5.089	-	-	22.199	4.775
Gallic acid	-	-	-	-	-	-	23.541	2.264	-	-
Protocatechuic	27.959	3.140	29.698	1.186	27.878	2.136	29.050	0.607	28.797	1.433
Catechin	32.981	0.824	31.482	1.487	32.685	0.541	31.996	15.243	32.204	1.272
p-hydroxy benzoic acid	35.493	1.983	34.293	1.017	35.412	2.592	35.126	27.587	34.477	7.736
P-coumaric acid	38.311	14.761	37.585	44.732	38.354	5.253	37.796	5.570	37.669	3.042
Phenol	40.183	10.031	40.270	4.179	39.469	9.896	40.582	3.687	-	-
O-coumaric acid	42.874	8.730	43.399	1.028	42.921	1.256	42.852	2.429	42.393	1.109
Salicylic acid	-	-	45.346	0.543	44.197	1.652	-	-	45.460	0.928
Coumarin	46.402	3.854	47.072	1.136	46.423	2.281	46.101	1.126	47.783	0.204
Quercetin	-	-	-	-	-	-	49.862	0.286	-	-
Cinnamic acid	53.337	0.086	51.450	0.176	51.675	0.071	-	-	-	-

Table 3: Polyphenols compounds determined by HPLC in crude methanolic extracts.

Leaves extract	Inhibition %														
	Alternaria solani			*Botrytis cinerea*			*Botrytis fabae*			*Fusarium oxysporum*			*Fusarium solani*		
	1 mg/ml	2 mg/ml	4 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml
Thompson seedless	44.44 d1	53.33 d	61.11 e	42.22 d	50.00 e	63.33 e	52.22 d	68.89 d	78.89 d	15.56 d	24.44 d	37.78 e	17.78 e	25.56 e	40.00 d
Flame seedless	44.44 d	57.78 d	70.00 d	47.78 c	60.00 d	72.22 d	55.56 d	71.11 c	84.44 c	17.78 d	27.78 d	41.11 d	20.00 d	33.33 d	51.11 c
Zizyphus	63.33 a	75.56 a	91.11 a	67.78 a	77.78 a	92.22 a	64.44 a	81.11 a	95.56 a	45.56 a	55.56 a	64.44 a	50.00 a	57.78 a	70.00 a
Pomegranate	61.11 b	71.11 b	86.67 b	66.67 a	75.56 b	90.00 b	62.22 b	77.78 b	94.44 a	38.89 b	47.78 b	57.78 b	42.22 b	48.89 b	58.89 b
Fig	56.67 c	66.67 c	77.78 c	61.11 b	70.00 c	82.22 c	58.89 c	72.22 c	91.11 b	31.11 c	38.89 c	46.67 c	34.44 c	42.22 c	51.11 c

[1]Values within a column followed by a different letter are significantly different according to Duncan's multiple range test (*P*=0.05)

Table 4: Effect of methanolic leaves extracts on mycelial growth of tested fungi.

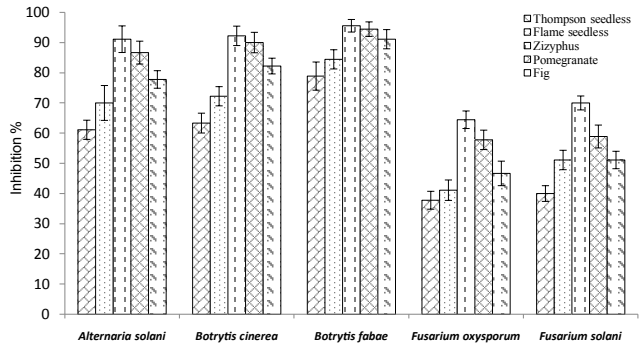

Figure 1: Effect of methanolic leaves extracts on mycelial growth of tested fungi (mean ± s.e) at the concentration of 4 mg/ml.

There were no available authentic samples to identify these unknown compounds which may be some polyphenolic derivatives.

Antifungal activity of plant extracts on the mycelial growth of test fungi

All methanolic leaves extracts showed an extensive antifungal activity against the different fungal pathogens tested (Table 4). However, fungal sensitivity varied according to the species. Extract of zizyphus significantly reduced the mycelial growth of all tested fungi from 64.44 (for *F. oxysporum*) to 95.56% (for *B. fabae*) at the concentration of 4 mg/ml (Figure 1). These were followed by pomegranate extract, which caused a significant decrease on the fungal growth ranged from 57.78 (*F. oxysporum*) to 94.44% (*B. fabae*) at the same concentration (Figure 1). There was no significant difference between inhibitory effects of zizyphus and pomegranate on the growth of *B. fabae* at 4 mg/ml. Also, extracts of zizyphus followed by pomegranate were the most effective among the plant species tested against the growth of the fungi at the concentrations of 1 and 2 mg/ml (Figures 2 and 3). Extract of flame seedless had more effectual antifungal than thompson seedless at all concentrations. *F. oxysporum* and *F. solani* showed high resistance for all methanolic extracts (Table 4). In general, There were positive relationships between the concentration of the methanol extracts and the inhibition rate on mycelia growth of all tested fungi.

According with the obtained results for HPLC analysis, ten polyphenols compounds have been identified in the methanolic extract of zizyphus leaves, besides; the main component found in this extract was phenol. The high antifungal activity of zizyphus extract is possibly due to phenolic compounds. Phenolic compounds are one of the major families of secondary metabolites in plants, and they are of nearly 10,000 individual compounds [49]. Phenolic or polyphenol can be defined chemically as a substance which possesses a benzene ring with one or more hydroxyl groups, with evidence that increased hydroxylation results in increased toxicity [50]. These polyphenols are very important for plants to contribute to resistance to microorganisms, herbivores and insects [51]. Generally, the active antifungal compounds of most plant extracts are phenolic compounds. This may be due to that their antifungal mode of action might be related to that of other compounds.

These results were agreed with Brown and Morra [52] and Nita-Lazar et al. [53]. They suggested that the activation of the phenolic pathway is known in the plant physiology to be part of the defense response against phytopathogenic microorganisms. Also, Sisti et al. [54] showed that the phenolic agents are active against pathogenic microorganisms for humans and animals.

Many potential modes of action by which phenolics counteract envelopment of pathogenic agents have been suggested, from the impairment of enzymatic processes involved in energy production and structural component synthesis by weakening or destroying the

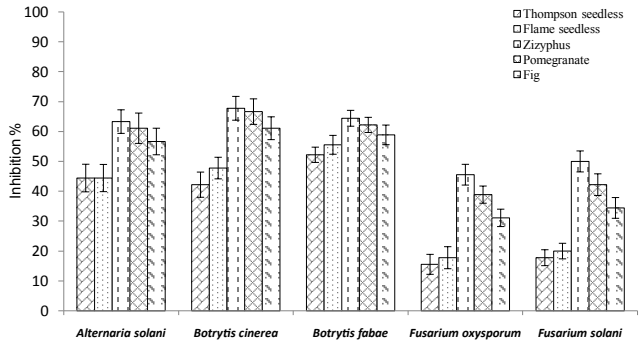

Figure 2: Effect of methanolic leaves extracts on mycelial growth of tested fungi (mean ± s.e) at the concentration of 1 mg/ml.

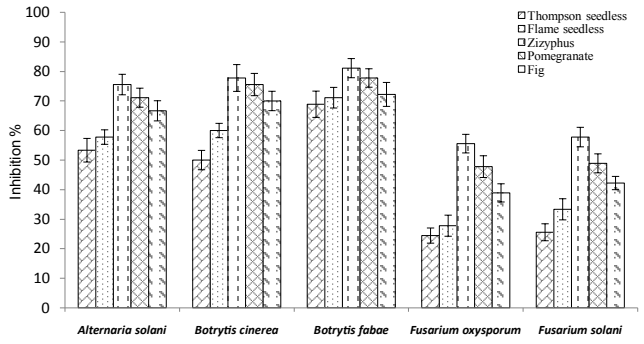

Figure 3: Effect of methanolic leaves extracts on mycelial growth of tested fungi (mean ± s.e) at the concentration of 2 mg/ml.

permeability barrier of the cell membrane by altering the physiological status of the cells or affecting nucleic acids synthesis [55]. In the same way, Galvan et al. [8] mentioned that plant secondary metabolites have great potential as a source of effective antifungal agents, for example, plant-derived compounds such as hydroquinones, naphthoquinones, alkaloids and flavonoids, have shown diverse antimicrobial activities including antifungal activities. Batovska et al. [14] found that the grape-vine leaves possess a resistance towards several fungi, *Plasmopara viticola, Oidium tukeri* and *Botrytis cinerea* as biomarkers for the fungal resistance.

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