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Original paper

Evaluation of antioxidant and antifungal activities of several plants against agents of postharvest citrus sour rot and green mould rot

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Abstract

The antifungal activities of chloroform extracts of 10 plants species belonging to Lamiaceae family, which were collected from Kırklareli (Turkey), against *Geotrichum candidum*, theagent of postharvest citrus sour rot and Penicillium digitatum, the agent of postharvest citrus green mould rot, were researched. The lowest Minimum Inhibitory Concentration (MIC) values against *G. candidum* and *P. digitatum* were obtained in the extract of *Marrubiumperegrinum* L. (250 and 125 µg/ml). In 1000 µg/ml, the extracts of *Melissa officinalis* showed 100% inhibition on the spore germination of *G. candidum* and *P. digitatum*. In the Scanning Electron Microscope (SEM) observations of G. candidum and *P. digitatum* that was subjected to *M. peregrinum extract* (4MIC) degenerative changes in the hyphal morphology were seen in the form of cell wall degradation, lysis and collapsing. The highest values of total phenolics were obtained from *Mentha pulegium extracts* (739.57 mg GAE/g). The lowest EC_{50} values (0.08 mg/ml) were found in the extracts of *M. peregrinum* and *Sideritis montana*. The highest flavanol content was determined from *M. officialis exctracts* (12.71 mg CE/mg). This study demonstrates *M. peregrinum extracts* may possess high antifungal activity against *G. candidum* and *P. digitatum*.

Keywords *Geotrichum candidum, Penicillium digitatum, Antifungal activity, Plant extract, SEM*

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Introduction

The wounds occurring on the fruits and vegetables during harvest, transportation and/or processing may be infected with pathogenic fungi during storage to cause microbial decay, the primary problem for the food industry (AMEZIANE & al [1]; PHILLIPS & al [2]). Due to the infections the fungi caused on the foods, the nutritional value of the food decrease and they become unfit for consuming. Furthermore, the lipid peroxidation caused by the free radical oxidation also damages the foods greatly (PRAKASH & al [3]). Additionally, the fact that free radicals cause degenerative diseases has been provenby many studies (CHEN & al [4]). Turkey is an important growing ground for citrus fruits, producing more than 3.5 million tons of citrus fruits annually, and holds 9th place in the world citrus production (YEŞİLOĞLU & al [5]). Due to their low pH, high water concentration and nutritional components citruses are very sensitive to attacks from pathogenic fungi in the period between harvest and consumption (TRIPATHI & al [6]). The most common and severe diseases in citruses worldwide are sour rot caused by *Geotrichum candidum* (Link ex Pers) and green mould rot caused by *Penicillium digitatum* (Pers.:Fr.). The decay in fruits and vegetables after harvest can be reduced by 90-95% using fungicides. However, the losses may increase by 50% without fungicide treatment (PHILLIPS & al [2]). The green mouldrot in fruits and vegetables after harvest may be controlled by intensive usage of chemical fungicide pre and post-harvest in many countries including Turkey (SOYLU & al [7]). For decades, benomyl, thiabendazole, imazalil, and sodium ortho-phenylphenate (SOPP), which belong to the benzimidazole (BZI), sterol demethylation inhibitor (DMI), and phenyl-phenol chemical classes, respectively, were the most commonly used fungicides until the recent introduction to the market of pyrimethanil (anilinopyrimidine) and fludioxonil (phenylpyrrole) (AMIRI AND BOMPEIX [8]). Sour rot is only controlled by Guazatine, a chemical not authorized in several countries, and not by imazalil and thiabendazole, mainly used in post-harvest chemical treatments (TALIBI & al [9]). The usage of these fungicides are restricted because of their high and acute toxicity, long degradation periods, residues in the food chain, and the chronic poisoning through continuous intake in small qualities(SHUKLA & al [10]). However, the increased resistance of fungi against synthetic chemicals causes problems in the effective uses of fungicides (AL-REZA & al [11]). In addition to the loss of activities of the fungicides, their negative environmental effects force people (PASSONE & al [12]) to discover new crop protection methods and new types of selective control alternatives that does minimal damage to the environment and human health (SOYLU & al [13]). It is essential to optimize alternative methods that have minimal environmental and human damage and have a different effect mechanism in order to avoid the fungicide resistance in microorganisms (PASSONE & al [12]). Therefore, researchers are screening the plant extracts for an alternative method (ABDEL-MONAIM & al [14]). Plants are seen as the source for effective chemical materials and provide reversible sources for harmless fungicides. A typical characteristic of plants is that they produce nitrogen free and nitrogen containing secondary metabolites. In most cases, these secondary metabolical products act as a defensive mechanism against microorganisms, insects and herbivores. Such products from higher plants are relatively broad-spectrum, bio-efficacious, economical, and presumably safer for humans and the environment and can beideal candidates for use as agrochemicals (PASSONE & al [15]). Meanwhile, plants act as singlet and triplet oxygen quenchers, free radical scavengers and enzyme inhibitors. These protective biological effects are attributed to the polyphenol contents of plants (CHEN & al [4]). The systematic screening of the interaction between micro-organisms and plant products is one of the main ways in researching the biological active substances of plants. Plant products whose antimicrobial spectrum has been recognized can be used as antimicrobial compounds or as an adjuvant to other antimicrobial compounds in order to improve their action (DE SOUZA & al [16]). The goals of this study were; (I) The testing of the antifungal activities of chloroform extracts of 10 plants species belonging to Lamiaceae family which were collected from Kırklareli (Turkey) against *G. candidum* and *P. digitatum* using Clinic and Laboratory Standards Institute (CLSI, [17]) procedures, (II) Examining the effects on spore germination and hyphal morphology of these fungi (III). The analyzing of the total phenolic, flavanol contents, antiradical activities and antioxidant capacities.

Materials and Methods

Plant materials

Ten plant materials were collected from different locations in Kırklareli (Turkey) in the months of June and July 2014. All of the samples were identified by Dr.Hüseyin ERSOY and were deposited in the herbarium laboratory in faculty of sciences, Trakya University, Edirne, Turkey. Plant samples were cleaned and dried in the shade, then grinded to a fine powder using a laboratory grinding mill (A11B, IKA) and stored in the dark at 4°C until use.

Pathogens

Cultures of the fungal pathogens *Geotrichum candidum* and *Penicilium digitatum* used in the study were isolated from naturally decayed fruits. A pure culture of these fungi were maintained on potato dextrose agar (PDA) and stored at 4°C and sub-cultured once a month.

Preparation of plant extracts

The powdered plant materials were separately extracted thrice at room temperature with chloroform solvent (500 ml/100 g of plant material each run). The final extracts of each plant part were filtered using filter paper (Whatman) and were evaporated under vacuum at 40°C using rotary vacuum evaporator. All extracts were stored in sterile glass bottles at room temperature.

Determination of minimum inhibitory concentration (MIC)

The pathogen inoculum consisted of aqueous spore suspension obtained from 7-days-old culture incubated at 25°C. Spores were harvested by flooding with 5 ml of sterile distilled water containing 0.05% (v/v) Tween 80, and passing the suspension through two layers of sterile cheesecloth to remove hyphal fragments. The spore concentration was determined with the aid of a heamacytometer and adjusted to 10^6 spores per ml with sterile distilled water. Using as spectrophotometer optic density (OD) and transmittance (T%) of the suspension at 530 nm were recorded. The spectrophotometric values of the inoculum suspension were stabilized for further studies. The 10^6 spore/ml suspension was later watered down to 1:50 ratio with sterile distilled water. Mean while the 1:100 rationed suspensions was added to the PDA plate by the amount of 0.01 ml to confirm 10^4 CFU/ml.

MIC of each extract was determined by using broth microdilutiontechniques according to the guidelines for filamentous fungi (M 38 A) (CLSI, [17]). MIC valueswere determined in RPMI-1640 (Sigma, St. Louis, MO, USA)buffered to pH 7.0 with MOPS. Microtiter trays were incubated at 25°C. MICs were visuallyrecorded at 96 h. For the assay, 40 µL of a stock solution of each extract in DMSO (50 mg/ml) was diluted with 960 µL of RPMI-1640 giving a solution of 2 mg/ml. 200 µL of this new solution was poured into thefirst well and then, 100 µL were transferred to the next well containing 100 µL of RPMI-1640. The same procedure was performedfor all wells of the same file obtaining twofold dilutions of theextract. A volume of 100 µL of inoculum suspension was added toeach well (with the exception of the sterility control where sterilewater was added to the well instead) giving extract's concentrationsfrom 1000 to 0.98 µg/ml and a final DMSO concentration ≤1%. End-points were defined as the lowestconcentration of extract resulting in total inhibition (MIC) of visual growth compared to the growth in the control wells containing no antifungal (SVATEZ & al [18]). Tests were carried out in duplicate.

Effect of plant extracts on spore germination

The germination of spores of *G. candidum* and *P. digitatum* was determined in concentrations of 125, 250, 500 and 1000 µg/ml of chloroform extract for the plants. Aliquots (40 µL) of a spore suspension (10^6 spores/ml) were aseptically transferred in triplicate to sterile depression slides containing 40 µL with different concentrations of chloroform extracts, and then incubated at 25°C for 96 h. Spore germination was estimated under a microscope using a micrometer. At least 100 spores of each replicate were observed. A spore was scored as germinated if the germ tube length was equal or exceed that of the spore (ASKARNE & al [19]; ASKARNE & al [20]). The data were expressed as percent spore germination inhibition and calculated by using the following formula:

GI (%) = $[(G_c-G_t)/G_c] \times 100$, where G_c and G_t represent the mean number of germinated spores in control and treated slides, respectively. Each treatment included three replicates and the experiment was conducted twice.

Effect of plant extract on hyphal morphology

For the determination of the effect of plant extracts on hyphal morphology, a mycelial agar disc from a 7-day old culture was first placed in the centre of PDA plate and incubated at 25°C for 2 days to allow mycelium to grow into the medium. After 2 days of pre-incubation, different concentrations (4MIC) of plant extract used in vitro studies were dropped (onto covers of Petri dishes) and incubated at 25°C for 3 days (SVATEZ & al [18]). For SEM analysis, mycelial discs (1 cm in diameter) were fixed with 2.5% glutaraldehyde in 0.1 M phosphate-buffer (pH=7.2) for 2 h at room temperature. They were washed twice, each time for 10 min, in the same buffer. After fixation, the samples were dehydrated in a graded ethanol series (70%, 80%, 90%, and three times at 100%) for a period of 30 min in each series. The samples were examined and digital images captured using SEM (Quanta FEG 250) at an accelerating voltage of 5 kV.

Determination of total phenolic and flavanol content

Spectrophotometric measurements were performed by spectrophotometer (Shimadzu, UV mini-1240). Total phenolic contents (TPC) of the extracts were determined by the Folin–Ciocalteu colorimetric method (SINGLETON AND ROSSI, [21]). The estimation of phenolic compounds in the extracts was carried out in five replications and calculated by a calibration curve obtained with Gallic acid. Total phenolics were expressed as Gallic acid equivalents (mg GAE/g).

Total flavanol contents (TFC) were assayed colorimetrically by the DMAC method (ARNOUS & al [22]). The absorbance of the extracts was measured at 640 nm and TFC in the extracts were expressed as catechin equivalents (mg CE/g).

Determination of antiradical activity

Different concentrations of extracts in methanol were added to 1 ml methanolic solution of DPPH (0.2 mM) in volumes of 1 ml. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm against methanol as the blank in a spectrophotometer (BAYDAR AND

BAYDAR, [23]). DPPH radical free scavenging activity (%) was calculated according to the formula:

Antiradical activity (%) = [(Absorbance of control − Absorbance of sample) / Absorbance of control] x 100

Extract concentrations providing 50% inhibition (EC_{50}) was calculated from the graph plotting inhibition percentage against extract concentrations. Gallic acid was used as a positive test control.

Statistical analysis

All data were subjected to statistical analysis of variance (ANOVA) using STATISTICA software and Tukey's multiple comparison tests. For these procedures, "SPSS for Windows, v 15.0" statistical package program was used and the values lower than $p<0.05$ were considered significant.

Results and Discussion

Determination of minimum inhibitory concentration (MIC)

One of the technical variables in MIC studies is the amount of inoculums. The colour, shape and size of the spores can affect the spectrometric readings. The idea of fungal species having different optic density values for each genus is documented in M-38A. This is why the OD of inoculum amount must be standardized for each species. The OD and T% values of *G. candidum* and *P. digitatum* inoculum suspensions (10^6spores/ml) at 530 nm were standardized at 0.039, 91.3% and 0.017, 96%, respectively (Table 1).

Table 1. Optical Density and Transmittance (%) values of the spore suspension (10^6 spores/ml) of *Geotrichum candidum* and *Penicillium digitatum* at 530 nm.

Fungi	Optical Density	Transmittance (%)
G. candidum	0.039	91.3
P. digitatum	0.017	96

Chloroform extracts of all plants were evaluated for antifungal properties with the microbroth dilution method. It was reported that the plant chloroform extracts showed effective results in antifungal studies (ASHRAF & al [24]; VOGT & al [25]). A plant with MICs ≤ 1000 µg/ml was considered active. Except *Lamium amplexicaule*, other plant extracts showed antifungal activity against tested fungi. In all of the tested plant species the MIC values were the lowest in *M. peregrinum* against *G. candidum* and *P. digitatum* (250 and 125 µg/ml), something which indicates this plant has more antifungal substances against *G. candidum* and *P. digitatum* than other plants (Table 2). The antifungal compound can lead to the inhibition of the fungus by suppressing the early stages of mycelium growth. Hydrophobicity is an important characteristic of the plant extracts and their components, which renders them more permeability and disturb cell structures by enabling partition of the lipid on the mitochondria and the cell membrane (RASTOGI AND MEHROTRA [26]). The death of the cell can be from the extensive leakage from the living cells or the exit of the critical molecules and ions (DERESSA AND WAKJIRA [27]).

Effects of plant extracts on spore germination

The chloroform extracts of *L. maculatum, M. peregrinum, M. officinalis* and *M. pulegium* have high fungistatic effect (100% inhibition) against the spore germination of *G. candidum*at 1000 µg/ml. At 500 µg/ml, the chloroform extracts of *M. peregrinum* and *M. officialis* inhibited the spore germination by 94.66% and 86%, respectively (Table 3).

Table 2. Antifungal activities (MIC in µg/ml) of the some plant chloroform extracts investigated against *Geotrichumcandidum* and *Penicillium digitatum*.

Plant species	*G. candidum*	*P. digitatum*
Ajuga laxmannii (Murray) Benth.	1000	1000
Lamium amplexicaule L. var. *amplexicaule*	i	i
Lamium maculatum L. var. *maculatum*	500	500
Marrubium peregrinum L.	250	125
Melissa officinalis L. subsp. *officinalis*	500	1000
Mentha pulegium L.	500	1000
Salvia verticillata L. subsp. *verticillata*	1000	500
Sideritis montana L. subsp. *montana*	1000	1000
Teucrium chamaedrys L. subsp. *chamaedrys*	500	1000
Teucrium polium L. subsp. *polium*	500	500

i; inactive (MIC> 1000 µg/ml)

Table 3. Effect of chloroform extracts on spore germination of *Geotrichumcandidum.*

Plant species	Spore germination inhibition (%)[a]			
	Concentration of chloroform extracts (µg/ml)			
	125	250	500	1000
Ajuga laxmannii	0[a]	0[a]	0[a]	50[b]
Lamium maculatum	0[a]	0[a]	20.66[c]	100[d]
Marrubium peregrinum	0[a]	4[e]	94.66[fd]	100[d]
Melissa officinalis	0[a]	0[a]	86[f]	100[d]
Mentha pulegium	0[a]	0[a]	43.66[b]	100[d]
Salvia verticillata	0[a]	0[a]	0[a]	59[b]
Sideritis montana	0[a]	0[a]	0[a]	91[f]
Teucrium chamaedrys	0[a]	0[a]	0[a]	81.66[f]
Teucrium polium	0[a]	0[a]	12.66[c]	62[b]
Control	0[a]	0[a]	0[a]	0[a]

[a] Each value represents the mean of three replicates. Means followed by different letters in each column are significantly different at $P < 0.05$ according to Tukey's multiple comparison tests.

At 1000 µg/ml, the chloroform extract of *M. officinalis* inhibited the spore germination of *P. digitatum* by 100%. *L. maculatum, M. peregrinum* and *M. pulegium* extracts showed 96.83%, 94% and 83% inhibition, respectively (Table 4). The antifungal activity of the metabolites produced by the plant can show differences based on the stages of fungal development (CARVALHO & al [28]). Some plant metabolites act upon the mycelium growth of the fungus while some are effective on the spore germination (TALIBI & al [9]; ASKARNE & al [20]; GARDUNO-PIZANA & al [29]). Cell wall altering its permeability or inhibiting the spore germination may be the general effects of the antifungal compounds acting (AMSALU & al [30]).

Table 4. Effect of chloroform extracts on spore germination of *Penicillium digitatum.*

Plant species	Spore germination inhibition (%)[a]			
	Concentration of chloroform extracts (µg/ml)			
	125	250	500	1000
Ajuga laxmannii	0[a]	0[a]	0[a]	10.33[b]
Lamium maculatum	0[a]	0[a]	56.88[c]	96.83[d]
Marrubium peregrinum	21.66[b]	26.66[eb]	80.66[f]	94[d]
Melissa officinalis	0[a]	9[b]	85.33[f]	100[d]
Mentha pulegium	0[a]	0[a]	29.66[e]	83[f]
Salvia verticillata	0[a]	0[a]	0[a]	6[b]
Sideritis montana	0[a]	0[a]	0[a]	35.33[e]
Teucrium chamaedrys	0[a]	0[a]	0[a]	22[b]
Teucrium polium	5.33[b]	7[b]	8[b]	9.66[b]
Control	0[a]	0[a]	0[a]	0[a]

[a]Each value represents the mean of three replicates. Means followed by different letters in each column are significantly different at $P < 0.05$ according to Tukey's multiple comparison tests.

Effects of extract on hyphal morphology

Important morphological differences were found when comparing the control to the hyphal morphologies of *G. candidum* and *P. digitatum* that have been treated with the 4MIC extract of *M. peregrinum* in PDA plates. When compared to hyphal structures that have a linear, orderly and homogenous cell wall, the SEM observations showed that the chloroform extract of *M. peregrinum* cause lysis, collapsing and cell wall degradation in *G. candidum*; cell wall degradation and collapsing in *P. digitatum* (Fig. 1 and Fig. 2). These results confirm that the chloroform extract of *M. peregrinum* is phytotoxic. The degradations in the cell wall may be related to the chloroform extract of *M. peregrinum* reacting with the enzymes regulating the cell wall, which in turn can destroy the fungal morphology and inhibit hyphal growth. Similar results were obtained by SOYLU & al [13] at recording the effect of essential oil against cell wall growth of *Botrytis cinerea*.

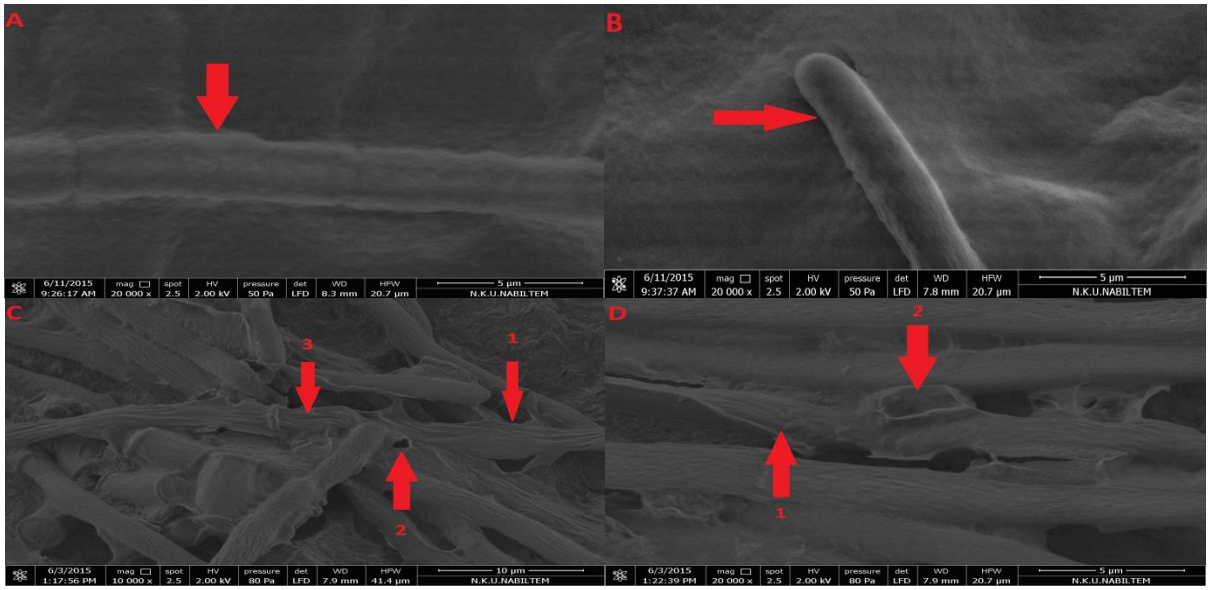

Fig. 1. Scanning electron microscopy of *G. candidum* hyphae exposed to *M. peregrinum* choloroform extract (1000 µg/ml). (A and B) Healthy hyphae in control petri plates. (C and D) Effectsof the extract on hyphal morphology. Note alterations in hyphal morphology including cell wall degradation (1.arrow), lysis (2.arrow), collapsing (3.arrow) (C) and cell wall degradation (1.arrow), lysis (2.arrow) (D).

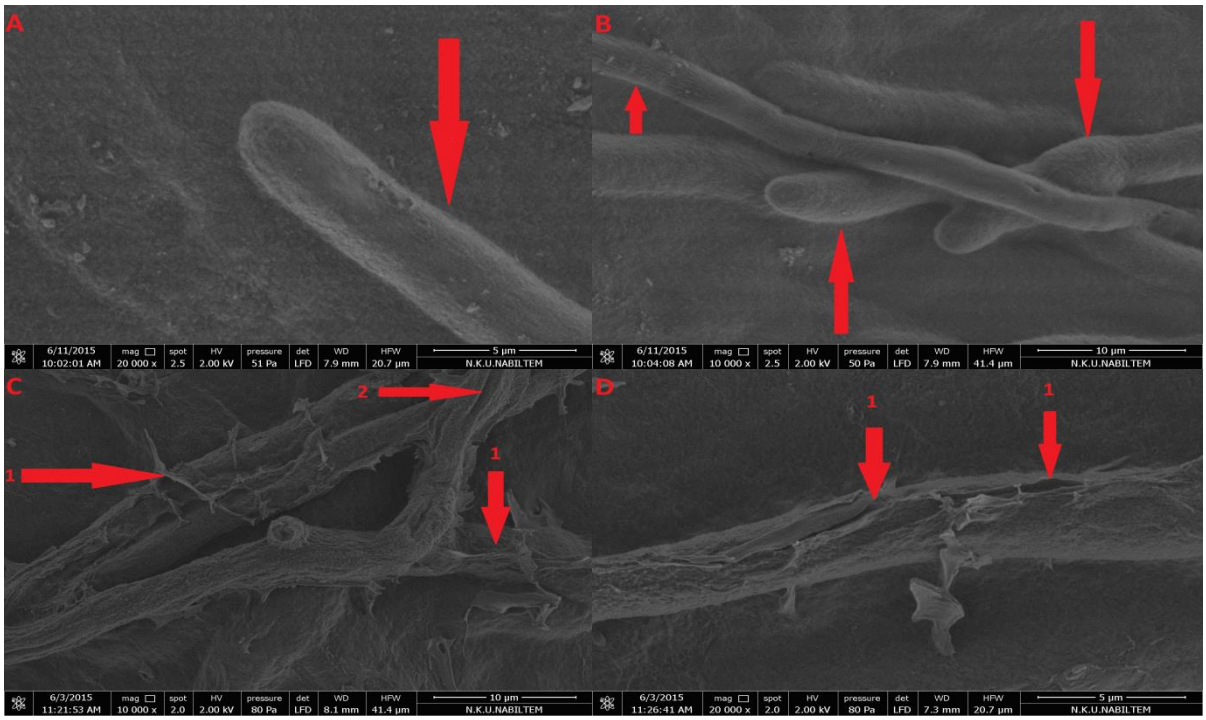

Fig. 2. Scanning electron microscopy of *P. digitatum* hyphae exposed to *M. peregrinum* chloroform extract (500 µg/ml). (A and B) Healthy hyphae in control petri plates. (C and D) Effects of the extract on hyphal morphology. Note alterations in hyphal morphology including cell wall degradation (1.arrows), collapsing (2.arrow) (C) and cell wall degradation (1.arrows) (D).

Total phenolic and flavanoid contents

All plants make phenolic substances as secondary metabolites, whose roles are not known enough. This is why there are phenolic substances in varying amounts and quality in plant base foods. Phenolic substances are one of the biggest secondary metabolite groups which act as antimicrobial and antioxidant. The hydroxyl group's position and amount determine the antimicrobial properties, while the ability to bond with metals and scavenge free radicals determine the antioxidant properties of phenol groups.

It has been suggested that phenolics have many effect types in preventing the growth of pathogenic agents. Because of these effects the enzymatic processes in energy production gets destroyed, the permeable membrane of the cell gets damaged or weakened, the physicochemical structure of the cell changes or nucleic acid synthesis gets affected (CUTTER [31]). These bioactive compounds, interfere with the life process of the fungi, whether singly or in combination, by changing the physiological status of the cells, weakening or destroying the permeability barrier of the cell membrane, altering structural component synthesis, and acting as chelating agents (RONGAI & al [32]). The highest TPC was found in *M. pulegium* (739.57 mgGA/g) while the lowest TPC was determined to be in *L. amplexicule* (87.51 mgGA/g) (Table 5). The TPC amounts found in the extracts of *S. verticillate* in methanol (TOSUN & al [33]); *S. montana* in methanol, acetone and ethyl acetate (RADOJEVIĆ & al [34]); *T. chamaedrys* in methanol, acetone, ethyl acetate and petrol ether (STANKOVIC & al [35]); *T. polium* in methanol (STANKOVIC & al [36]) were reported as 50.3, 97.85, 84.55, 49.05, 168.46, 169.50, 143.95, 30.39, 42.64 and 157.84 mgGA/g. These results are different from what we found. The reason for this may be because of the difference in the solvents used in extractions as well as the difference of the regions and the geographical conditions where the plants grew (TRIGUI & al [37]).

The antioxidant activities of the extract were evaluated by the TFC as well. Colourless compounds called flavanols are the intermediate products in flavonoid biosynthesis. Flavanols easily react with the oxygen in the air by both chemical and enzymatic reactions. After the reactions they form proanthocyadin by condensing. Proanthocyadins are powerful antioxidants, they reduce free radicals. The highest flavanol content (12.71 mg CE/g)was found in the extract of *M. officinalis* (Table 5).

Table 5. Total phenolic, total flavanol contents and EC_{50} values of chloroform extracts.

Plant species	Total phenolic (mg GA/g)*	Total flavanol (mg CE/g)*	EC_{50} (mg/ml)*
Ajuga laxmannii	150.23^{a}	5.73^{a}	58.20^{a}
Lamium amplexicaule	87.51^{b}	4.29^{b}	19.18^{b}
Lamium maculatum	377.90^{c}	8.32^{c}	0.32^{c}
Marrubium peregrinum	504.90^{d}	3.78^{d}	0.08^{c}
Melissa officinalis	379.90^{c}	12.71^{e}	2.28^{c}
Mentha pulegium	739.57^{e}	3.43^{fd}	0.15^{c}
Salvia verticillata	352.23^{c}	3.73^{d}	0.11^{c}
Sideritis montana	501.57^{d}	3.97^{d}	0.08^{c}
Teucrium chamaedrys	511.90^{d}	5.76^{a}	2.81^{c}
Teucrium polium	519.57^{d}	3.95^{d}	3.08^{c}
Gallic acid			1.86^{c}

***** Each value represents the mean of three replicates. Means followed by different letters in each column are significantly different at $P < 0.05$.

DPPH free radical scavenging activity

The antioxidant activity of the extracts can also be evaluated by the determination of the EC_{50} values corresponding to the amount of extract required to scavenge 50% of DPPH radicals present in the reaction mixture. High EC_{50} values indicate low antioxidant activity (BAYDAR AND BAYDAR, [23]). EC_{50} values of *L.maculatum, M. peregrinum, M. pulegium, S. verticillate* and *S. montana* were less than the EC_{50} value obtained for Gallic acid used as positive control (Table 5). Among the extracts, *M. peregrinum* and *S. montana* extracts were the most potent radical scavengers. STANKOVIC & al [35] reported EC_{50} values of 0.56 and 1.16 mg/ml in petrol ether extracts of *T. chamaedrys* leaves and flowers, respectively; RADOJEVIĆ & al [34] reported EC_{50} values of 0.031, 0.064 and 0.52 for methanol, acetone and ethyl acetate extracts of *S. montana*, respectively. The idea of the plant extract that has more total phenolics also shows more antiradical activity (BAYDAR AND BAYDAR, [23], OANCEA et al [38]) is shown again as in our study the high correlation ($r=0.8981$; $p<0.01$) between total phenolic amount and antioxidant capacities can be seen.

Conclusion

A. laxmannii, L. amplexicaule, L. maculatum, M. peregrinum, M. officinalis, M. pulegium, S. verticillate, S. montana, T. chamaedrys and *T. polium* species belonging to Lamiaceae family which can be found abundantly and grown easily in Kırklareli (Turkey), were tested with microdilution method against the primary fungi pathogen of citruses postharvest, *G. candidum* and *P. digitatum*. We can say that due to its high antifungal activity against *G. candidum* and *P. digitatum*, the chloroform extract of *M. peregrinum* has a high potential to be used as an alternative natural control agent instead of chemical fungicides. Also, among the tested plants, *L. maculatum, M. peregrinum, M. pulegium, S. verticillate* and *S. montana* have a lower EC_{50} value than Gallic acid which shows that they have high antioxidant activity.

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