ANTIFUNGAL ACTIVITY OF SOME CITRUS EXTRACTS

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This study followed the antimicrobial activity of some citrus extracts obtained from the peel and whole fruit against Rhizopus *sp. and* Alternaria *sp., two genra affecting the growth and the quality of fruits. Citrus originated from both EU and non-EU countries and were collected from different commercial sources. The Kirby and Bauer method was used to evaluate the antifungal activity of citrus extracts. If the antifungal activity against* Rhizopus *sp. is discussed, the diameter of the growth inhibition zone varied largely, between 7.50 ± 1.9148 mm (peel and whole fruit of grapefruit from non-EU countries, respectively peel of mandarins from non-EU countries) and 0 mm (oranges coming from EU countries). Referring to the antifungal activity of extracts against* Alternaria *sp., only the peel exhibited it; the diameter of the growth inhibition zone ranged between 11.66 ± 0.5773 mm (limes from non-EU countries) and 0 mm (oranges and mandarins coming from EU countries, and also lemons coming from non-EU countries). The minimum inhibitory concentration (MIC) of the citrus extracts ranged in large limits, from 1.5 to 6.1 mg/mL, depending on the tested sample and filamentous fungi. Some extracts were inactive against* Rhizopus *sp. and* Alternaria *sp. respectively, within the tested concentration range. In-depth knowledge of the antifungal activity of citrus extracts is located at the confluence of many factors. Although it is a challenging area of study, this one is of huge interest due to the content of phenolics or essential oils in citrus, compounds with promising antifungal activity. The replacement of synthetic fungicides with biofungicides is a horizon of action for lawmakers and a desideratum for people.*

Keywords: citrus; antifungal activity; inhibition; disk diffusion method.

1. Introduction

Postharvest fungal attacks on citrus fruits typically arise from inadequate agricultural and postharvest practices, improper transportation, or unsuitable packaging. Managing postharvest diseases necessitates balanced fertilization of the orchards, prompt cleaning or removal of spoiled fruits, and the application of fungicides during the flowering to fruiting stages [1].

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In the postharvest period of citrus fruits, storing them at low temperatures (4 - 7°C) is recommended to preserve them and delay the development of fungi. A sustainable alternative to postharvest treatment with fungicides (e.g., prochloraz, strobilurin, etc., forbidden in several countries) is natural products, such as fruit-coating biofilms and biofilms containing essential oils [2]. This alternative enhances the quality of the fruits and extends their shelf life. This can be attributed to the fruits' reduced transpiration rate and the fungi's lowered metabolic activity [3]. The study's general objective was to evaluate the antifungal activity of extracts obtained from the peels and whole fruits of various citrus species, aiming to correlate this activity with the chemical composition of the fruits. Based on the established correlations, strategies for valorizing specific food industry waste can be developed (supporting the concept of the (organic) circular economy), and opportunities for replacing synthetic fungicides with natural compounds safe for human health and environmentally friendly. The research aimed at (*i*) isolating certain fungal species from natural environments, (*ii*) testing the antimicrobial activity of the extracts, and (*iii*) selecting promising extracts in line with the study's objectives.

2. Materials and Methods

2.1. Sample and sample preparation

Citrus fruits (Table 1) were collected randomly according to EC/333/2007 [4]. Citrus aqueous extracts were prepared according to the procedure described by Minea et al. (2024) [5].

Table 1

Data related to samples collected in the spring of the year 2024 [5]

Sample cod	Sampling place	The sample	Origin Country
1G -I	Warehouse	Grapefruit	non-EU
1Li-I	Storage	Limes	non- EU
1L-SI (organic)	Retail	Lemons (organic)	EU
1M-SI (organic)	Retail	Mandarins (organic)	EU
1P-SI	Storage	Oranges	EU
2G-I	Retail	Grapefruit	non-EU
2L-I	Storage	Lemons	non-EU
2Li-I	Retail	Limes	non-EU

Sample cod	Sampling place	The sample	Origin Country
2M-I	Storage	Mandarins	non-EU
2M-SI	Retail	Mandarins	EU
2P-SI	Storage	Oranges	EU
3G-I	Retail	Grapefruit	non-EU
3L-I	Storage	Lemons	non-EU
3LI-I	Retail	Limes	non-EU
3M-I	Retail	Mandarins	non-EU
3P-SI	Retail	Oranges	EU
4L-I	Retail	Lemons	non-EU

EU – European Union; non-EU - countries that are not members of the European Union.

2.2 Isolation of microorganisms from natural environments

To assess the antifungal activity of citrus extracts, including the peel and the whole fruit, various fungal species were isolated from natural environments such as fruits, vegetables, cereals, and air (Fig. 1).

Fig. 1. Preliminary stage of fungal selection (i.e., heterogeneous microorganic stage).

The identification of fungi was performed using macroscopic criteria related to colonial morphological features (i.e., colony color, shape, surface texture, margins or edge, and growth rate) and microscopic observations. These fungi were subsequently subcultured on specific culture media, namely, malt agar (MMA) and potato dextrose agar (PDA), to obtain pure cultures of the desired strains.

2.3. Disk diffusion method of Kirby and Bauer

The antimicrobial activity of the extracts was evaluated on solid nutrient media using the diffusion method (Kirby-Bauer) [6].

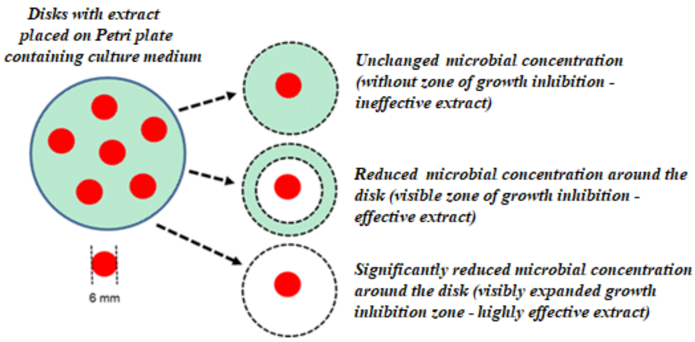


Fig. 2. Disk diffusion method of Kirby and Bauer.

This approach enabled the determination of the sensitivity spectrum of microorganisms. Discs impregnated with the extracts were used to assess the antifungal activity, i.e., the disk diffusion method (Fig. 2), a standardized method recommended by the National Committee for Clinical Laboratory Standardization (NCCLS document M2-A7) [7]. The density of the mold spore suspension was standardized (0.5 McFarland equivalent to approx. 10^6–10^7 spores/ml. The surface of the PDA plate was seeded with 100 µl spore suspension using the “spread plate” method. Subsequently, sterile paper discs (6mm diameter) were impregnated with the citrus extracts and placed on the surface of the Petri dishes, leaving a minimum of 3 cm distance between them. The Petri plates were incubated at 25–28°C for 48–96 h, monitoring daily for the appearance of inhibition zones.

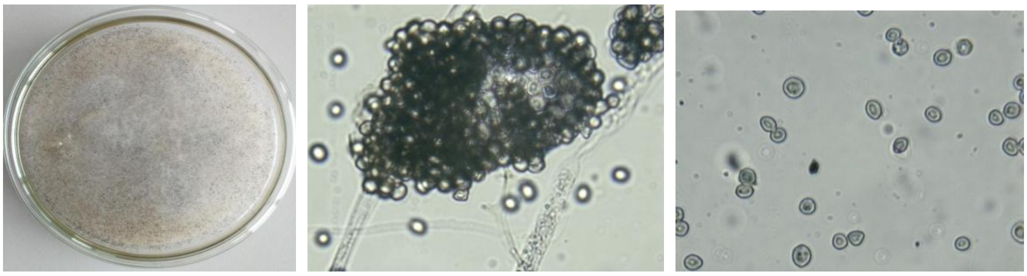
To determine the lowest concentration (MIC) of citrus extract that completely inhibits fungal growth, the test tube macrodilution method in liquid medium was applied, according to Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI document M38-A2) [8]. The mold spores from the mature culture were dispersed in sterile water and Tween 80, then filtered to remove mycelia. The concentration of the fungal solution was adjusted to 10^4 spores/ml. A series of double dilutions of the citrus extracts to be analyzed was prepared, decreasing from a concentration of 25 mg/mL to a final concentration by 1.5 mg/mL. A growth control sample (without citrus extract) and a sterility control sample (without extract and fungal spores) were also prepared. The tubes were incubated vertically, at 25–28°C, for 48–96 hours. The MIC was determined as the lowest concentration at which the medium remained clear, with no visible signs of visual growth. The correlation between the diameter of the inhibition zone and the minimum inhibitory concentration was determined by using IBM SPSS Statistics 26.0.

3. Results and Discussion

3.1. Antifungal activity of citrus extracts against **Rhizopus** *sp.*

Citrus fruit peels are promising bioresources of antifungal agents with potential applications in the food industry, pharmaceutical industry, or packaging production (e.g., for obtaining biofilms). In addition to using peels as biofungicides, the recovery of these wastes can reduce environmental pollution.

The species *Rhizopus nigricans*, part of the *Mucoraceae* family within the *Mucorales* order, is found everywhere and requires moisture along with a carbon source, such as starch or sugars, for its growth and development. Various species of *Rhizopus* contribute to agricultural losses during transport and storage, adversely affecting the quality of fruits and vegetables [9]. *Rhizopus* sp. is responsible for the decay of many plant species. It can be found on the surfaces of fruits and vegetables and in packaging and storage areas. Once it invades plant tissues, it swiftly damages the roots and eventually produces spores on the surface of the affected products. These spores are contained in sporangia, which are borne on sporangiophores, and they can be black in aspect. The spores can spread over long distances, aided by wind or other passive vectors such as insects [10].



a) b) c)

Fig. 3. Macroscopic and microscopic morphology of *Rhizopus* sp. tested for the antifungal activity of citrus extract; a) colony on MMA after 5 days incubation at 25^0C; b) columella; c) sporangia

Although thermal and chemical treatments are commonly used to combat *Rhizopus nigricans* in food products, there is a growing interest in the search for new plant-based antimicrobial agents due to this fungus's resistance to synthetic products [11]. *Rhizopus stolonifer* (syn. *nigricans*) displays gray mycelium and sporangiophores typically grouped in clusters of 3 to 4. The sporangia are ovoid in shape and brown to blackish in color (Fig. 3). Within the group of samples subjected to analysis, the values of the diameter of the zone of growth inhibition varied within relatively wide limits, illustrating differences in the antifungal activity of the extracts against *Rhizopus* sp. Although these values are not very high compared to those specific to antibiotics, they provide valuable information to identify new compounds/natural extracts with antifungal activity. The obtained extracts can be concentrated under conditions that preserve the compounds of interest, allowing the achievement of the lowest possible values for the minimum inhibitory concentration (MIC). This process enhances the valorization of citrus fruit peel. The highest values of the diameter of the inhibition zone (D_i), i.e., 7.5 ± 1.9148 mm, were recorded in the case of samples 1G-I peel, 3M-I peel, and 3G-I whole fruit, respectively (Table 2).

Table 2

Antimicrobial activity of citrus extracts against *Rhizopus nigricans*.

Sample code	Peel extract		Whole fruit extract	
	Di [mm]	**MIC [mg/mL]**	**Di [mm]**	**MIC [mg/mL]**
1M-SI(organic)	5.25 ± 0.9574	4.8	0.00	> 25
1L-SI(organic)	0.00	> 25	6.00 ± 0.00	4.6
1G-I	7.50 ± 1.9148	3.2	0.00	> 25
1Li-I	6.00 ± 0.00	4.5	6.00 ± 0.00	4.6
1P-SI	0.00	> 25	6.00 ± 0.00	4.4
2G-I	7.00 ± 1.15	3.5	0.00	> 25
2Li-I	4.00 ± 0.00	5.6	4.75 ± 0.9574	5.5
2L-I	6.00 ± 0.00	4.5	7.00 ± 1.15	3.8
2M-I	6.00 ± 0.00	4.5	7.00 ± 1.15	4.2
2M-SI	3.75 ± 1.50	6.2	3.75 ± 1.5	6.1
2P-SI	0.00	> 25	5.25 ± 0.9574	5.1
3G-I	0.00	> 25	7.50 ± 1.9148	3.6
3Li-I	0.00	> 25	7.00 ± 1.15	4.1
3L-I	3.75 ± 1.50	6	6.00 ± 0.00	4.5
3M-I	7.50 ± 1.9148	3	5.00 ± 0.00	5.2
3P-SI	0.00	> 25	6.50 ± 1.9148	4.2
4L-I	4.50 ± 1.00	5.5	6.00 ± 0.00	4.5

Regarding the peel of citrus fruits, a high antimicrobial activity (Di ≥ 6.00 mm) was determined as follows: 1G-I = 3M-I > 1Li-I = 2L-I = 2M-I. The samples 1G-I, 2G-I, and 3M-I exhibited growth inhibition zone values of at least 7.00 mm, supporting the antifungal potential of the extracts. The minimum inhibitory concentration (Table 2) is an essential indicator for the next phase of research. In this stage, it will utilize extracts that exhibit antifungal activity, either in their original form or integrated into different matrices. This approach aims to preserve specific food products and explore potential health benefits.

Extracts obtained from the peel of the samples 1L-SI (organic), 1P-SI, 2P-SI, 3G-I, 3Li-I, and 3P-SI did not show antimicrobial activity against *Rhizopus* sp. On the other hand, the samples 2L-I, 2M-I, 3G-I, 3Li-I, and 3P-SI stand out due to their zone of inhibition against the growth of *Rhizopus sp.*, with diameters of approximately 7.00 mm, from extracts obtained from whole fruits. At the opposite extreme are samples 1M-SI (organic), 1G-I, and 2G-I, for which no inhibition level of the growth of the tested fungal species was determined. Figure 4 shows clearly that the mandarins were the only fruits from the EU whose extracts obtained from peels exhibited moderate activity against *Rhizopus* sp.

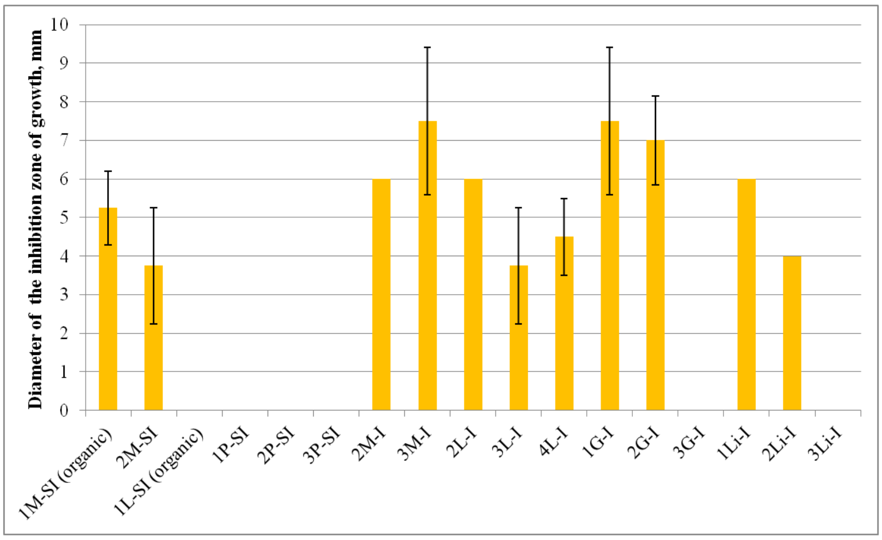


Fig. 4. Antimicrobial activity of peel citrus extracts against *Rhizopus* sp.
(M – mandarins; L – lemons; P – oranges; G – grapefruit; Li – limes)

The extracts obtained from the peel of the fruits from non-EU countries, except grapefruits and limes from retail (sample no. 3), displayed antifungal activity, very likely due to the chemical compounds with which the fruits were treated at the time of their placement on the market (namely synthetic fungicides) and not the chemical compounds in the peel. The comparative analysis (peel/whole fruit) of the diameter values of the zone of inhibition of the growth of *Rhizopus nigricans* highlights that in the peel of the samples 1M-SI (organic), 1G-I, and 2G-I, there are compounds with the antifungal role, compounds that were not found in the whole fruits. This may be due to the extraction method applied or to the dilution of the samples resulting from the whole fruits (depending on their mass). The contribution of antifungal compounds originating only from the peel in the case of the previously mentioned samples seems insufficient in inhibiting *Rhizopus nigricans*. In contrast, for the extracts obtained from the whole fruits 1L-SI (organic), 1P-SI, 2P-SI, 3G-I, 3Li-I, and 3P-SI, values of the diameter of the growth inhibition zone were determined (Fig. 5), which underlines the fact that the compounds with the antimicrobial role are found in the fruit, and not in the peel of these citrus fruits. Interestingly, in this case, it can discuss fruits (i.e., lemons and oranges) coming mainly from EU countries; their antifungal activity can be attributed to chemical compounds from pulp, such as phenolics.

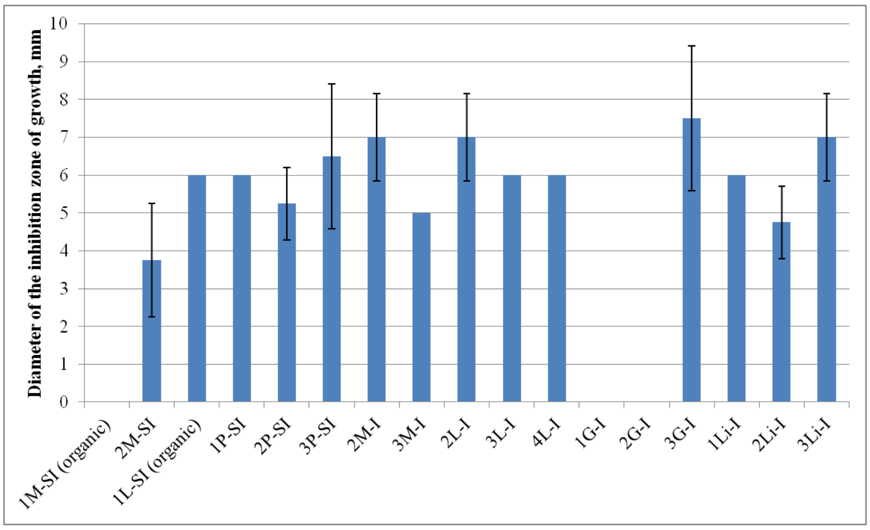


Fig. 5. Antimicrobial activity of citrus extracts (whole fruit) against *Rhizopus* sp.
(M – mandarins; L – lemons; P – oranges; G – grapefruit; Li – limes)

Compounds with antifungal activity were present both in the peel and in the whole fruit of the samples from which the extracts 1Li-I, 2Li-I, 2L-I, 2M-I, 2M-SI, 3L-I, 3M-I, and 4L-I were obtained. The mandarin (sample no. 2) was the only citrus fruit coming from the EU that exhibited, although weak, antifungal activity against *Rhizopus* sp., both for peel and whole fruit. A synergistic effect seemed to be significant in the case of the samples 2L-I, 2M-I, 3L-I, and 4L-I (all of which ones obtained from non-EU countries), samples for which higher values of the growth inhibition diameter were determined in the whole fruit compared to the citrus peel. This synergy could be due to the fungicides from the peel and chemical compounds, for instance, phenolics, from the pulp of lemons and mandarins.

3.2. Antifungal activity of citrus extracts against Alternaria sp.

Alternaria sp. (Fig. 6) is a primary post-harvest pathogen affecting fruits and vegetables, including oranges and lemons.

Fig. 6. Microscopic aspect (10 x 100) of the *Alternaria* sp. tested for the antifungal activity of citrus extracts.

Alternaria brown spot (ABS) is a significant disease of mandarins and their hybrids. The causative agent is a pathotype of *Alternaria alternata* (Fr.) Keissl is prevalent in humid citrus production areas (e.g., Brazil, Argentina) and Mediterranean regions with cool, wet winters and warm, dry summers, such as Spain, Italy, Greece, Turkey, and Israel. Control of *Alternaria sp.* relies on the application of fungicides; however, their use encounters challenges due to the development of resistant pathogenic strains and adverse effects on animals, humans, and the environment [12].

Table 3

Antimicrobial activity of extracts obtained from citrus peels against *Alternaria* sp.

Sample code	Di [mm]	MIC [mg/mL]	Sample code	Di [mm]	MIC [mg/mL]
1M-SI(organic)	9.33 ± 0.5773	2.5	2M-SI	0.00	> 25
1L-SI(organic)	7.66 ± 0.5773	3.5	2P-SI	0.00	> 25
1G-I	9.33 ± 1.1547	2	3G-I	6.00 ± 0.00	4.5
1Li-I	7.66 ± 1.5275	3.2	3Li-I	6.00 ± 1.00	4.5
1P-SI	0.00	> 25	3L-I	0.00	> 25
2G-I	6.66 ± 0.5773	4	3M-I	6.00 ± 0.00	4.5
2Li-I	11.66 ± 0.57735	1.5	3P-SI	0.00	> 25
2L-I	0.00	> 25	4L-I	0.00	> 25
2M-I	6.00 ± 0.00	4.5			

Among the samples, the antifungal activity against *Alternaria* sp. of sample 2Li-I was highlighted at the tested concentrations of extracts derived from the citrus peel (Fig. 7). High inhibitory efficiency values (≥ 6 mm) were determined for 1M-SI (organic), 1L-SI(organic), 1G-I, 1Li-I, 2G-I, and 3Li-I, respectively. Samples 1L-SI (organic), 3G-I, and 3Li-I (peel) showed antifungal activity against *Alternaria* sp., but not against *Rhizopus* sp. In contrast, samples 1P-SI, 2P-SI, and 3P-SI (peel) did not show antimicrobial activity against any of the tested fungal species at the extract concentration used (Table 3). No values of *Alternaria* sp.'s inhibition efficiency at the tested concentrations were determined for extracts obtained from whole fruits. Liu et al. (2021) investigated the antifungal activity of aqueous (hot water) and alcoholic extracts obtained from citrus peels, which resulted in by-products [13]. Aqueous extracts from mandarin peels were found to be more effective in terms of the growth of *Aspergillus flavus* compared to extracts from orange and lemon peels, respectively. A total of 12 polyphenols (10 flavonoids and 2 phenolic acids) were identified by high-performance liquid chromatography with diode-array detection (HPLC-DAD), narirutin and hesperidin being the most abundant. The authors highlighted that the magnitude of inhibition of the studied fungus also depends on the extraction solvent, with alcoholic extracts being more effective than aqueous ones in their research [13].

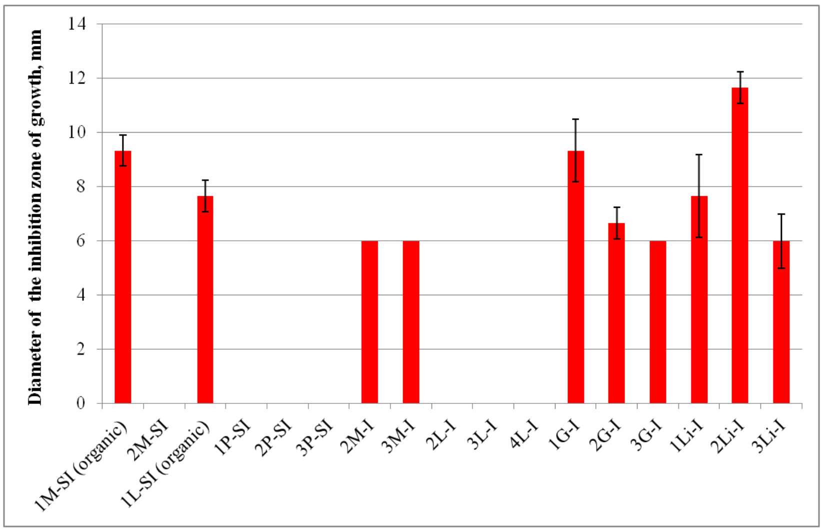


Fig. 7. Antimicrobial activity of peel citrus extracts against *Alternaria* sp.
(M – mandarins; L – lemons; P – oranges; G – grapefruit; Li – limes)

The antifungal properties of polyphenols can be attributed to their functionality, such as molecular size and functional groups [14, 15]. Various mechanisms underlying the antifungal action of polyphenolic compounds have been suggested, including 1) inhibition of glycan and chitin biosynthesis, which results in deformation of the fungal cell wall; 2) disintegration of the plasma membrane and impairment of its biosynthesis, which results in the release of intracellular components; 3) suppression of fungal nucleic acid metabolism by inhibiting mitochondrial processes; 4) inhibition of metabolic enzymes [15,16]. Plants are thought to store various phytochemicals with antifungal, antibacterial, and antiviral activity in the peel, leaves, and exocarp of fruits, particularly to preserve the fruits and protect the seeds from microbial attack [17]. Ramírez-Pelayo et al. (2019) analyzed the chromatographic profile of peels from different citrus varieties (oranges, mandarins, and limes) grown in Colombia [18]. The isolated compounds' antifungal activity (mycelial growth and spore germination) showed that furanocoumarins were more active than coumarins. Bergapten and limettin showed the highest inhibitory effects against *Colletotrichum sp.*, even higher than phytoalexins. Also, the mixture of bergapten and limettin demonstrated an antifungal effect even higher than the individual compounds [18]. Hernández et al. (2021) investigated the antifungal capacity of the polyphenolic extract from orange peel against three post-harvest pathogens, namely *Monilinia fructicola, Botrytis cinerea*, and *Alternaria alternata*, emphasizing that the inhibitory activity can be attributed to an additive effect of phenolic acids [19]. Phytochemical screening of extracts derived from the peel of Citrus sinensis L. revealed the presence of alkaloids, saponins, tannins, flavonoids, and phenols- constituents that may be responsible for the antifungal activity of this orange species [17]. The extracts inhibited the growth of *Rhizopus stolonifer*, with the highest percentage of inhibition determined at a concentration of 200 mg/mL (32% for aqueous extracts and 40% for alcoholic extracts) [17].

Referring to the tested fruits, the grapefruits, mandarins, and limes coming from non-EU countries exhibited a large antifungal activity against both tested microbial strains. The oranges from EU countries showed antimicrobial activity only against *Rhizopus* sp. and only in the case of the extracts obtained from the whole fruit. The organic mandarins proved to be efficient against *Rhizopus* sp. and *Alternaria* sp., but only for peel extracts. The data obtained for the lemon extracts also varied depending on the anatomic part of the fruits and the tested strains. In the case of the lemons coming from non-EU countries, the growth of *Rhizopus* sp. was affected, while the peel extracts of organic lemons from EU countries showed antifungal activity against *Alternaria* sp. Organic mandarins and lemons are promising sources of biofungicides; their biological activity is, most probably, the result of the chemical compounds present naturally in the peel and pulp.

The antimicrobial activity of aqueous extracts (100 mg/mL) obtained from orange, pineapple, cashew, and banana peels, respectively, against *Aspergillus niger* and *Alternaria alternata* was tested by Olakunle et al. (2019) [20]. The diameters of the inhibition zones of *Aspergillus niger* ranged from 0.33±0.33 cm (orange peels) to 0.87±0.33 cm (banana peels). The diameters of the inhibition zones of *Alternaria sp*. ranged from 0.5±0.5 cm (orange peels) to 1.37±0.67 cm (banana peels). The antifungal activity of these extracts could be due to the different classes of compounds present in the peels, such as alkaloids, flavonoids, tannins, cardiac glycosides, saponins, and phlobatannins [20]. The efficacy of phenolic compounds in controlling *Alternaria* blight was tested *in vitro* and *in vivo* at a laboratory scale and then under semi-commercial conditions [12]. Among the compounds tested, umbelliferon was the most effective phenolic compound (86%) in controlling the disease in mandarins under semi-commercial conditions. Its effect seems to be related to the ability to induce resistance in the host (i.e., *Alternaria* sp.). Essential oils have lower persistence and toxicity than synthetic fungicides, which may help reduce their potential negative environmental impact [21]. For this purpose, the chemical composition and *in vitro* antifungal effect of essential oils obtained by hydrodistillation from the peel of two *Citrus sinensis* orange varieties on the mycelial growth of *Rhizopus stolonifer* were evaluated [9]. Limonene was the major monoterpene in the composition of orange peels, identified by chromatographic techniques. At the highest dose evaluated (i.e., 100 µL), the authors determined percentages of inhibition of the *R. stolonifer* mycelium of 91.95% (lima oranges) and 80.05% (bahia navel oranges), respectively [9]. In another study, essential oils obtained by hydrodistillation of the epicarp of four varieties of *Citrus sinensis* (i.e., Thomson navel, Jaffa sweet, Sanguine blood, and Valencia) were tested for their inhibitory effects against *Alternaria sp*. This pathogen affects the quality of oranges. The activity of enzymes responsible for cell wall degradation, enzymes produced by the pathogens, was significantly reduced in the experimental variants with essential oils, used in concentrations ranging from 0 to 1000 µg/mL. The reduction in disease index was observed in all four orange varieties, similar to the effect of the fungicide [21]. Essential oils obtained from citrus fruits could also be suitable alternatives to chemical additives used in the food industry, satisfying the consumer demand for natural compounds [22]. The authors studied the effect of lemon, tangerine, grapefruit, and orange essential oils on the growth of molds commonly associated with food spoilage: *A. niger, A. flavus, P. chrysogenum*, and *P. verrucosum*. All essential oils exhibited antimicrobial activity against these molds. The mechanisms of action of essential oils, either to inhibit microbial proliferation or even to induce cell lysis, are not yet fully understood [9].

4. Correlation between the diameter of the inhibition zone and the minimum inhibitory concentration

Some limitations, such as diffusion of the extract in agar or molecular weight of active compounds, are known when the correlation between Di and MIC is discussed. The Pearson correlation and the regression analysis were performed taking into account the results obtained for all tested extracts and fungal strains.

If the dependent variable was considered Di, the Pearson coefficient showed an inverse (as expected) and strong correlation between Di and MIC (r = - 0.980, p < 0.01). The regression analysis led to the following regression equation:

Di = 12.794 – 0.002*MIC

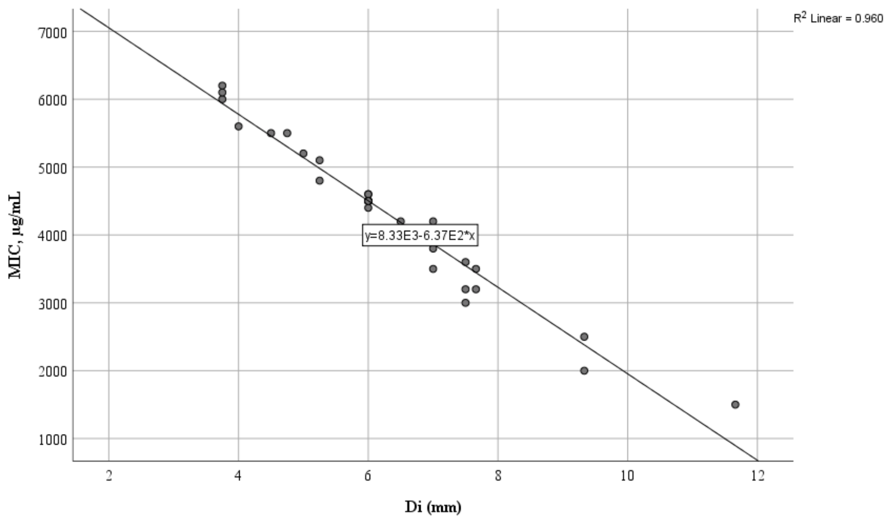


Fig. 8. Correlation between the diameter of inhibition zone and the minimum inhibitory concentration of the citrus extracts against *Rhizopus* sp. and *Alternaria* sp.

The R-squared value (R^2 = 0.960) showed a very good model according to the regression analysis. This is also observed in Figure 8, where the data is useful for further studies involving the same strains of filamentous fungi and citrus extracts. The R-squared value was higher for the cubic regression line than for the linear one (R^2 = 0.974), indicating that the cubic model fits the data better and explains a greater proportion of the variability of the inhibition diameter. However, the linear model can be used for easy interpretation of data. If some potential phenomena, such as the plateau effect, should be discussed, the cubic model could be useful.

4. Conclusions

Extracts obtained from citrus peels and whole fruits demonstrated satisfactory antifungal activity against *Rhizopus sp*. and *Alternaria sp*., fungi recognized for their action of deteriorating the quality of food, in particular, fruits. In the subsequent research stage, the extracts' concentration and the extractant's nature will be optimized, respectively; the antimicrobial activity of the extracts against other mold species of interest for ensuring the quality and shelf life of citrus fruits will be evaluated *in vitro*. A comparative analysis between the antimicrobial activity of the extracts obtained from citrus coming from non-EU and EU countries against the two fungal tested strains emphasizes that the citrus coming from the EU countries exhibited a moderate or a lack of activity within the group of samples. The antifungal activity of citrus coming from non-EU countries could be due, in the case of the extracts obtained from the peel, to the fungicides with which they were treated. A further comprehensive analysis will take into account the chemical composition of citrus in relation to its antimicrobial activity.

The influence of the sampling place of citrus fruits (warehouse, retail, or storage) on their antifungal activity against the two tested strains was not conclusive; subsequent research is also needed in this direction. In addition to the previously mentioned influencing factors on the antifungal activity, the time spent in the transportation chain and also in the commercial network by citrus fruits cannot be neglected. The samples analyzed in this paper were different in these terms, too. Finally, besides the chemical composition and concentration of citrus extracts, the antifungal activity is strain-dependent. To conclude, this study laid the basis for exhaustive research of the antifungal activity of citrus extracts against microorganisms with an interest in their preservation and quality.

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