International Journal of Scientific Research and Engineering Development-– Volume 5 Issue 4, July-August 2022

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

A Comparative Study of Antifungal Activities and Chemical of Bluish-Black and Yellowish-White Fruits of *Myrtus communis* L.

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Abstract:

The work was conducted on Bluish-Black and Yellowish-White Fruits of *Myrtus communis* harvested in North-West Tunisia. Fruits were stored over six months using to two methods; frozen fruits and dried fruits.

Total phenol content was conducted using Folin-Ciocalteu reagent. The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method. The antifungal activity of myrtle extracts was tested against four fungal strains: *Alternaria alternate, Penicillium olsonii, Ulocladium atrum, Phytophtora nicotianae* and *Aspergillus fumigates*. Results of total phenol content showed that there is significant difference between the studied samples. Black dried fruits exhibited the highest phenol content (2.40 mg EGA/ml).

Black frozen fruits showed the highest rate of flavonoids (1.95 mg RE/ml).

Alternaria alternate was shown to be the most resistant strain, while *Ulocladium atrum* and *Phytophtora nicotianae* were the most sensitive strains to the extracts studied..

Keywords **— *Myrtus communis*, black fruits, white fruits, antifungal activity, phenols, flavonoids.**

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I. INTRODUCTION

Over the past twenty years, a strong progression of the fungal diseases, which affect a very wide range of hosts, has been observed. These diseases are caused by a surprisingly large number fungal species [1].

These fungal species cause significant damage on humans as well as on plants species [2]. To address these issues, chemicals substances were the most commonly used. Their fight against plant pathogens essentially concerns the fungi responsible for fungal diseases of plants. Most fungicides directly affect essential functions, such as respiration, sterol biosynthesis or cell division. Unfortunately, this mode of action can lead to risks for humans and non-target organisms and to the development of resistant fungal strains. However, this treatment quickly meets its limitations due to many disadvantages related to the phenomena of pollution, phytotoxicity, biological imbalance and especially risk of selecting resistant fungal strains [2, 3].

Faced with this problem of resistance, the researchers have adopted new axes of research to develop new molecules that are not yet affected by the resistance of microorganisms and with minimum risk

for human and environment [4]. One of these axes is the exploitation of plants as a source of bioactive compounds.

In this context, our study focused on *Myrtus communis*, in order to study the effect of their extracts against four fungal phytopathogens.

II. MATERIAL AND METHODS

A. Plant material

Bluish-Black and Yellowish-White Fruits of *Myrtus communis* were harvested in Nefza locations (North-West Tunisia). The plant was identified by Dr A. Khaldi from I.N.R.G.R.E.F-Tunisia and certified specimens (VS1-MC2009) were deposited at the Herbarium run by I.N.R.G.R.E.F.

Fruits were stored over six months using to two methods; frozen fruits and dried fruits.

B. Extracts preparation

20 g of berries was soaked in 200 ml of methanol/water 80% for 24 hours with intermittent shaking. The extracts were filtered through Whatman filter paper into pill vials. The obtained filtrates were used for the experiments.

C. Total phenol content

Total phenol content was conducted according to the method using Folin-Ciocalteu reagent [5].

500 µl of the extracts of each sample was mixed with 100 µl of the Folin–Ciocalteu reagent (10 times diluted) and 2 ml of sodium carbonate Na2CO3. The whole is incubated at room temperature for 30 minutes and the reading is carried out against a blank using a spectrophotometer at 755 nm.

From an aqueous stock solution of gallic acid, with a concentration of 0.5 g/l, a standard range of solutions in an aqueous medium was prepared.

100 µl of 10% folin-Ciocalteu reagent (10 times diluted in distilled water) is added. After two minutes of incubation, 2 ml of 2% Na2CO3 sodium carbonate are added. The tubes are then shaken and placed in the dark for 30 minutes at room temperature.

The reading of the absorbance of each solution prepared using a UV-Visible spectrophotometer, at a wavelength of 755 nm against a blank prepared in the same way except that it does not contain gallic acid but distilled water instead of the test substance. The absorbance values of each concentration allowed us to plot the calibration curve for gallic acid.

D. Total flavonoids content

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method [6]. 1 ml of diluted sample was mixed with 1 ml of 2% aluminum chloride methanolic solution. The mixture was allowed to stand for 15 min, and absorbance was measured at 430 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per mL of juice (mg RE/ml).

E. Antifungal activity

The antifungal activity of myrtle extracts was tested against four fungal strains: Alternaria alternate, *Penicillium olsonii, Ulocladium atrum, Phytophtora nicotianae,* and *Aspergillus fumigates.*

Fungal strains were isolated and identified by Dr. Awatef Slama, a mycologist, following conventional mycological methods.

The preparation of the PDA medium was performed by adjusting with distilled water the aqueous extract of the broth of 200 g of potato to 1 liter and adding 20 g of agar and 20 g of glucose.

The culture was made on a PDA medium at the rate of 20 ml per Petri dish. 2 ml of juice were introduced into the 20 ml of PDA after having been mixed and homogenized with tween 0.1%. After cooling the medium, a 5 mm diameter disk of each fungal strain was placed in the center of the petri dish while placing the mycelial surface down. The dishes were incubated at 22°C for six days. The fungicidal effect was determined by calculating the growth diameter of the strain in question and comparing it to that of a negative control, i.e. a PDA medium without juice [7].

The percentage inhibition was calculated according to the following formula [8]:

$$I(\%) = [(dC-dE)/dC] \times 100$$

Where: dC: witness diameter (mm)
dE: diameter in the presence of oil tested (mm)

F. Statistical analysis

The statistical processing of the data was carried out using the SAS GLM (General Linear Models) procedure. An analysis of variance relative to the parameters studied was carried out.
Results are presented as the mean of three replicates ± standard deviation.

III. RESULTS AND DISCUSSION

Results of total phenol and flavonoids content of extracts from white and black fruits of *Myrtus communis* are summarized in table1.

Results of total phenol content showed that there is significant difference between the studied samples. Black dried fruits exhibited the highest phenol content (2.40 mg EGA/ml).

When considering only the storage conditions, it was demonstrated that dried fruits contained the highest amount of phenols, while frozen fruits showed the lowest phenols rates. This finding was supported by several studies on the effect of frozen on phenol content. Numerous researches reported that frozen decrease the amount of phenols in fruits during storage [9, 10, 11].

Significant differences were shown between the total flavonoids of the sample studied. Black frozen fruits showed the highest rate (1.95 mg RE/ml). The lowest value was reached by White dried fruits. It was reported that flavonoids content decrease with in hot air dried samples. This is probably due to activation of oxidative enzymes like polyphenol oxidase during hot air drying [12, 13].

Table1. Total phenol and flavonoids content of extracts from white and black fruits of *Myrtus communis*

		Total phenol content (mg EGA/ml)	Total flavonoids content (mg RE/ml)
White	Frozen	2,17±0.2	1,68±0.1
	Dried	2,33±0.1	1,55±0.2
Black	Frozen	2,07±0.3	1,95±0.3
	Dried	2,40±0.2	1,77±0.1

The percentages of fungi growth inhibition are presented in table 2.

All the tested samples showed an antifungal activity against the four studied strains.

Alternaria alternate was shown to be the most resistant strain, while *Ulocladium atrum* and *Phytophtora nicotianae* were the most sensitive strains to the extracts studied.

The highest percentage of inhibition was recorded by black frozen fruits against *Ulocladium atrum* (68%). This extract exhibited the highest phenol content and this may explain its antifungal power. The effect of phenols on fungal strains has long been studied. Several studies highlighted the effectiveness of phenols against phytopathogenic fungi [14, 15, 16].

Table2. Antifungal activity of extracts from white and black fruits of *Myrtus communis*

		A. alternate	*U. atrum*	*P. nicotianae*	*A. fumigates*
White	Frozen	9,18±0.1	29,66±0.05	30,87±0.05	24,12±0.23
	Dried	17,63±0.1	33,88±0.2	27,30±0.2	17,07±0.1
Black	Frozen	24,78±0.1	68,07±0.21	63,37±0.01	24,12±0.3
	Dried	29,74±0.2	28,44±0.02	54,92±0.03	19,51±0.2

IV. CONCLUSIONS

The present study describes antifungal activity of white and black fruits of myrtle against four phytopathogenic strains, for the first time, which indicates a preliminary antifungal activity. Further studies, especially in the pharmacological area, are necessary to confirm these results.

REFERENCES

[1] D. Bitar, O. Lortholary, F. Dromer, B. Coignard and D. Che, "Mycoses invasives et France Metropolitaire, PMSI, incidence, létalité et tendances 2001– 2010". *Bul épidém hebdom*, vol. 5, pp. 109-114, 2013.

[2] B. Arias-rivas, DC. Megee and JS. Burris. "Corn seed treatment with polymers for controlling Pythium spp." *Fi-topatol.Venez.* vol. 11, pp. 10-15, 1998.
[3] A.E. Dorrance, S.A Berry, P. Bowen and P.E. Lipps, Characterization of *Pythium* spp. From three Ohio fields for pathogenicity on corn and soybean and metalaxyl sensitivity. Plant Health Progress. 2004. Available: www.plantmanagementnetwork.org/pub/php/research/ 2004 /pythium
[4] F. Zossoungbo, "Etude de l'effet synergique des huiles essentielles sur l'activité antibiotique d'un aminoside : la streptomycine". Master Sciences et Techniques, Université Sidi Mohammed Ben Abdellah, Fès. 2013.
[5] VL.Singleton and JA. Rossi, "Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents", *Amer J Enol Viticul*, vol. 16, pp. 144-158, 1965.
[6] C. Quettier-Deleu, B. Gressier, J. Vasseur, T. Dine, J. Brunet and M. Luyck, "Phenolic Compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour", *J Ethnophar*, vol. 72, pp. 35-40, 2000.
[7] A. Cakir, S. Kordali, H. Zengin, S. Izumi and T. Hirata. "Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*". *Flavour Frag J*. vol. 19, pp. 62-68, 2004.
[8] S. Singh and M. Kulshreshtha. "Mathematical modelling of juice expression from carrots under uniaxial compression", *J Food Eng*, vol. 27, pp. 323-336, 1996.
[9] C. Turkben, E. Sarıburun, C. Demir and V. Uylaser, "Effect of Freezing and Frozen Storage on Phenolic Compounds of Raspberry and Blackberry Cultivars". *Food Anal Met*, vol. 3, pp.144-153, 2010.
[10] A. Khattaba, GB. Celli, A. Ghanem and M.S.L. Brooks. "Effect of frozen storage on polyphenol content and antioxidant activity of haskap berries (*Lonicera caerulea* L.) Rabie", *J Ber Res*, vol. 5, pp. 231–242, 2015.
[11] L. Neri, M. Faieta, C. Di Mattia, G. Sacchetti, D. Mastrocola and P. Pittia, "Antioxidant Activity in Frozen Plant Foods: Effect of Cryoprotectants", *Freez Proc Froz Stor Foods,* vol. 9, pp. 1886-1892, 2020.
[12] A.R. Hirsch, K. Förch, S. Neidhart, G. Wolf, and R. Carle. "Effects of thermal treatments and storage on pectin methylesterase and peroxidase activity in freshly squeezed orange juice". *J. Agric. Food Chem.* vol.56, pp. 5691–5699, 2008.
[13] DS. Kim and SB. Lim, "Extraction of favanones from immature *Citrus unshui* fruits: Process optimization and antioxidant evaluation". *Sci. Rep*. vol. 10, pp.1–13, 2020.
[14] M.J. Salvador, OL. Zucchi, RC. Candido, IY. Ito and DA. Dias, "in vitro antimicrobial activity of crude extracts and isolated constituents of *Alternanthera maritime*". *Pharm. Biol*. vol. 42, pp. 138–148, 2004.
[15] N. Rangkadilok, S. Tongchusak, R. Boonhok, SC. Chaiyaroj, VB. Junyaprasert and W. Buajeeb. "In vitro antifungal activities of longan (*Dimocarpus longan* Lour.) seed extract". *Fitoter*. vol.83, pp.545–553, 2012.
[16] G. Simonetti, E. Brasili and Gabriella Pasqua, "Antifungal Activity of Phenolic and Polyphenolic Compounds from Different Matrices of *Vitis vinifera* L. against Human Pathogens", *Molec*, vol. 25, pp. 3748-3754, 2020.

