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Isolation of Tyrosol the main phytotoxic metabolite produced by the edible fungus *Agaricus litoralis*

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Abstract

The phytotoxicity activity of EtOAc extract obtained from *Agaricus litoralis* showed high phytotoxicity on tomato (*Lycopersicon esculentum* L.) leaves. A well-known phytotoxic metabolite: tyrosol [2-(4-hydroxyphenyl)-ethanol], was isolated and identified from *A. litoralis* by spectroscopic methods. Phytotoxic activity of tyrosol was tested on seven agrarian plants: eggplant (*Solanum melongena* L.), fig (*Ficus carica* L.), apricot (*Prunus armeniaca* L.), peach (*Prunus persica* (L.) *Batsch*), lemon (*Citrus limon* (L.) *Osbeck*), *Cucurbita* (Cucurbita L.), grapevine (*Vitis vinifera* L.) and two wild plants: willow oak (*Quercus phellos* L.), blackberry (*Rubus fruticosus* L.) using the leaf puncture assay, it showed weak phytotoxicity. The highest effect was observed on grapevine, apricot, peach, cucurbita and blackberry at 1 mg/ml; moderately phytotoxicity on eggplant, less phytotoxicity on fig, lemon and no necrosis on willow oak leaves. Tyrosol has been previously isolated from microorganisms, fungi, and plants, this being the first record from *Agaricus litoralis* as a phytotoxic metabolite.

Keywords: phytotoxicity, secondary metabolites, agrarian plants, wild plants, mushrooms, *Agaricus litoralis*.

1. Introduction

Wild edible macro-fungi have a long history of uses for medicinal and nutritional properties [1, 2]. People have consumed for thousands of years [3, 4]. Furthermore, edible macro-fungi have been considered one of the most prominent functional foods. They are collected in the wild or cultivated worldwide [5]. Fungi produce compounds with a broad spectrum of biological activity, including antimicrobial, antifungal, antiviral, anti-inflammatory, antioxidant, antidiabetic, antiviral, antithrombotic, antitumor, anticancer, anticomplementary, anticoagulant, hypolipidemic, hepatoprotective, immunostimulant and immunological activities, which made them suited for use in food, cosmetics, biomedicine, agriculture, environmental protection and wastewater management [6-9].

Phenolics compounds are one of the most important biologically active substances present in fungi. They are secondary metabolites that originate from the pentose phosphate, shikimate, and phenylpropanoid pathways. These metabolites have an aromatic ring bearing one or more hydroxyl groups [10, 11].

If fact, phenolic compounds have essential roles in the ecology of many different organisms such phytotoxins that have a potential application in agriculture as natural herbicides and fungicides. On the other hand, the use of phytotoxins to combat diseases is ideal for sustainable agricultural production with minimal damage to the environment [12-16].

A little or no work has been carried out on the chemical composition and bioactive compounds of the wild edible mushrooms in Algeria, especially the forests of Djebel El ouahech of Constantine region. Thus, the main aim of this work was the isolation of secondary metabolites from wild edible mushroom (*Agaricus litoralis*), and their phytotoxicity of different agrarian and wild plant leaves.

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2. Materials and Methods

2.1. General experimental procedures

^{1}H, NMR spectra were recorded at 400 MHz in $CDCl_3$ on Bruker Karlsruhe, Germany). The same solvent was also used as internal standard. ESI MS and LC/MS analyses were performed using the LC/MS TOF system (AGILENT 6230B, HPLC 1260 Infinity, Milan, Italy). Analytical and preparative TLCs were carried out on silica gel (Kieselgel 60, F_{254}, 0.25 and 0.5 mm respectively) plates (Merck, Darmstadt, Germany). The spots were visualized by exposure to UV radiation, or by spraying first with 10% H_2SO_4 in MeOH, and then with 5% phosphomolybdic acid in EtOH, followed by heating at 110°C for 10 min. Column chromatography was performed using silica gel (Kieselgel 60, 0.063-0.200 mm) (Merck).

2.2. Fungal material

Fresh macro-fungi were collected from the forest of Djebel El Ouahch of Constantine region in the eastern of Algeria, in December 2017 and May 2018 and identified by Roukia Zatout as *Agaricus litoralis* (MW165560) at the Laboratory of Mycology, Biotechnology and Microbial Activity (LaMyBAM), Department of Applied Biology, University of the Mentouri Brothers, Constantine, Algeria. The identification of this mushroom was confirmed by Dr Pierre-Arthur Moreau, Faculty of Pharmacy, Université de Lille, France.

2.3. Extraction and purification of metabolites from *Agaricus litoralis*

The fruiting bodies of the macro-fungi were air-dried. The dried fruiting bodies were cut into small pieces using scalpels and crushed using a pistil and a mortar. The powder obtained was stored in plastic bags until analysis. 500 g of this powder was extracted with MeOH-H_2O (1:1, v/v) under stirred conditions at room temperature for 24 h. The suspension was centrifuged, and the supernatant was evaporated under reduced pressure to eliminate the methanol, the aqueous phase was extracted by EtOAc (3 × 400 mL), dried with Na_2SO_4 and evaporated under reduced pressure.

The organic extract (170 mg) was purified by column chromatography on silica gel, eluted with $CHCl_3$-*i*-PrOH (8:2, v/v) affording 10 groups of homogeneous fractions. The residue (36 mg) of the third fraction was purified by preparative TLC eluted with $CHCl_3$-*i*-PrOH (85:15, v/v), yielding the main metabolite as white solid.

2.4. Phytotoxic activity

2.4.1. Tomato cutting assay

Tomato cuttings were taken from 21-day old seedlings. The organic extract (EtOAc) was assayed at 1mg/mL on tomato (*Lycopersicon esculentum* L.) cutting. The EtOAc extract was dissolved in 40% of MeOH in sterile distilled water (SDW) and tested at 1 mg/mL. MeOH in distilled water (4%) was used as negative control. Three replications were performed for the EtOAc extract. Symptoms were visually evaluated up to 28h and wilting symptoms were evaluated using a visual 0–4 scale (0 = no symptoms; 1 = wilting; 2 = necrotic spots; 3 = severe necrotic; 4 = severe necrosis and shrivelling).

2.4.2. Leaf puncture assay

The phytotoxic activity of tyrosol was assayed by a leaf puncture on seven agrarian plants: eggplant (*Solanum melongena* L.), fig (*Ficus carica* L.), apricot (*Prunus armeniaca* L.), peach (*Prunus persica* (L.) *Batsch*), lemon (*Citrus limon* (L.) *Osbeck*), cucurbita (*Cucurbita* L.) and grapevine leaves (*Vitis vinifera* L.). Two wild plant leaves: willow oak (*Quercus phellos* L.) and blackberry (*Rubus fruticosus* L.).

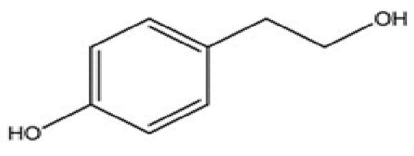

Fig. 1. Chemical structure of tyrosol isolated from *A. litoralis*.

All these plants were tested at 1 mg/mL. The compound was dissolved in MeOH and diluted with distilled H_2O and brought up to a final concentration of 1 mg/mL; (the MeOH concentration was 4%). Droplets of the test solutions (20 µL) were applied on the axial side of leaves that had previously been needle punctured, an additional droplet (20 µL) of

MeOH in distilled H_2O (4%) were applied on leaves as controls. Each treatment was repeated twice. Leaves were observed after 28 h.

3. Results and Discussion

3.1. Purification and identification of tyrosol

The EtOAc extract obtained from *A. litoralis* showed high phytotoxicity on tomato (*Lycopersicon esculentum* L.) leaves. It was purified by a combination of column chromatography and TLC as detailed in the Material and methods section, giving, a known metabolite identified as tyrosol **(Fig. 1)** by comparing their spectroscopic (^{1}H-NMR and ESI MS) data with those previously reported in the literature [17-21].

Tyrosol had: ^{1}H NMR (400 MHz), δ: 7.10 (d, J = 8.2 Hz, H-2 and H-6), 6.79 (d, J = 8.2 Hz, H-3 and H-5), 3.82 (t, J = 6.6 Hz, H2-8), 2.80 (t, J = 6.6 Hz, H2-7); ESIMS (+), *m/z*: 299 [2M + Na]+.

Tyrosol, is a ubiquitous secondary metabolite that has been found in plants [20], bacteria [22], endophytic fungi [23] and macro-fungi [24]. Moreover, tyrosol has been considered a well-known metabolite that has various biological activities [25], including the phytotoxicity.

Recently, tyrosol was also identified as a phytotoxic metabolite produced by several fungi which act as pathogens for agrarian [26], and forest plants [23]. Its phytotoxic activity on faba bean, legume, tomato [21], grapevine [27], marigold, prickly sida and lamb's quarters was previously reported [28, 29].

Tyrosol has showed different biological activity and is reported also as a quorum sensing molecule in *Candida albicans*, controlling growth, morphogenesis, and biofilm formation [30].

3.2. Phytotoxicity activity

In leaf puncture assay, the phytotoxic activity of tyrosol was estimated on seven agrarian plants [eggplant (*Solanum melongena* L.), fig (*Ficus carica* L.), apricot (*Prunus armeniaca* L.), peach (*Prunus persica* (L.) *Batsch*), lemon (*Citrus limon* (L.) *Osbeck*), *Cucurbita* (Cucurbita L.), grapevine (*Vitis vinifera* L.)] and two wild plants [willow oak (*Quercus phellos* L.), blackberry (*Rubus fruticosus* L.)].

All these plants were tested at 1 mg/mL. Tyrosol was active in this assay by causing a necrosis in all plants except willow oak in wild plants leaves.

The highest effect was observed on grapevine, apricot, peach, cucurbita and blackberry, moderately phytotoxicity on eggplant, less phytotoxicity on fig and lemon leaves.

Tyrosol also appeared with low phytotoxicity on fig and lemon leaves **(Table 1)**, **(Fig. 2)**.

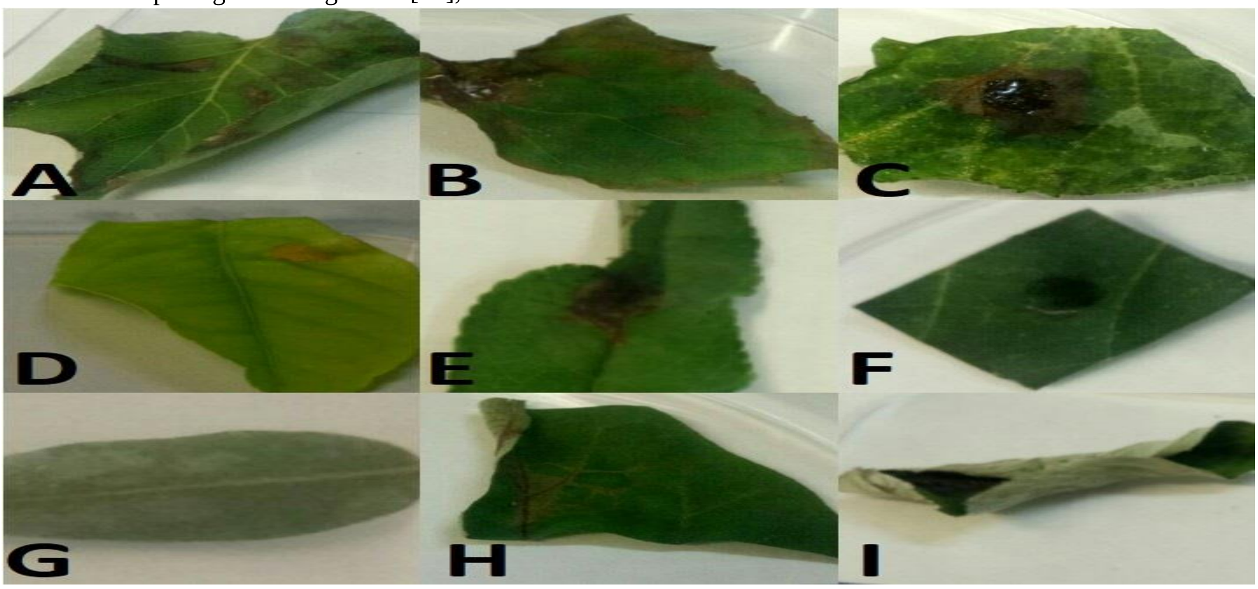

Fig. 2 A-I Phytotoxic effect of tyrosol on leaves of different agrarian and wild plants at concentration of 1 mg/ml after 28 h, (A) apricot, (B) grapevine, (C) cucurbita, (D) lemon, (E) peach, (F) fig, (G) willow oak, (H) eggplant, (I) blackberry.

a Severity scale: (0) no symptoms; (1) wilting; (2) necrotic spots; (3) severe necrotic; (4) severe necrosis and shrivelling.

Table 1: Level of toxicity induced 28 h after treatment on leaves of different agrarian and wild plants by tyrosol compound produced by *A. litoralis*.

Compound	Level of toxicity [a]								
Tyrosol	Apricot	Grapevine	Cucurbita	Lemon	Peach	Fig	Willow-oak	Eggplant	Blackberry
	3	3	3	1	3	1	0	2	3

4. Conclusions

This work represents the first production of known secondary metabolite tyrosol by an edible macro-fungus (*Agaricus litoralis*), adds further information on tyrosol's phytotoxicity effects on different agrarians and wild plants.

5. Conflicts of interest

The authors declare that there are no conflicts of interests.

6. Acknowledgments

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7. References

[1] Wu F., Zhou L.W., Yang Z.L., Bau T., Li T.H. and Y.C. Dai., Resource diversity of Chinese macrofungi: edible, medicinal and poisonous species. Fungal Diversity 98(1), 1-76(2019).

[2] Zeb M. and Lee C.H., Medicinal Properties and Bioactive Compounds from Wild Mushrooms Native to North America. Molecules. 26 (2), 251(2021).

[3] Poucheret P., Fons F. and Rapior S., Biological and pharmacological activity of higher fungi: 20-year retrospective analysis. Cryptogamie Mycologie, 27(4), 311(2006).

[4] Zhang J., Chen Q., Huang C., Gao W. and Qu, J., History, current situation and trend of edible mushroom industry development. Mycosystema, 34 (4), 524-540(2015).

[5] Zhao R., Species of *Agaricus* section *Agaricus* from China. Phytotaxa, 452(1), 001-018(2020).

[6] Miles P.G. and Chang S.T., Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact . CRC press (2004).

[7] Gerhäuser C., Zhang W.D., Ho-Chong-Line N. and Fourasté I., New lanostanoids from *Ganoderma lucidum* that induces NAD (P) H: qui-none oxidoreductase in cultured Hepalclc7 murine hepatoma cells. Planta medica, 66 (07), 681-684(2000).

[8] Loria-Kohen V., Lourenço-Nogueira T., Espinosa-Salinas I., Marín F.R., Soler-Rivas C. and Ramirez de Molina A., Nutritional and functional properties of edible mushrooms: A food with promising health claims. Journal of Pharmacy and Nutrition Sciences, 4, 187-198(2014).

[9] Kozarski M., Klaus A., Jakovljevic D., Todorovic N., Vunduk J., Petrović P. and Van Griensven L., Antioxidants of edible mushrooms. Molecules, 20(10), 19489-19525(2015).

[10] Balasundram N., Sundram K. and Samman, S., Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food chemistry, 99(1), 191-203(2006).

[11] Palacios I., Lozano M., Moro C., D'arrigo M., Rostagno M.A., Martínez J.A. and Villares A., Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chemistry, 128 (3), 674-678(2011).

[12] Cimmino A., Masi M., Evidente M., Superchi S. and Evidente, A., Fungal phytotoxins with potential herbicidal activity: chemical and biological characterization. Natural product reports, 32(12), 1629-1653(2015).

[13] Cimmino A., Cinelli T., Masi M., Reveglia P., da Silva M.A., Mugnai L. and Evidente A., Phytotoxic lipophilic metabolites produced by grapevine strains of *Lasiodiplodia* species in Brazil. Journal of agricultural and food chemistry, 65 (6), 1102-1107(2017).

[14] Cimmino A., Nocera P., Linaldeddu B.T., Masi M., Gorecki M., Pescitelli G. and Evidente A., Phytotoxic metabolites produced by *Diaporthella cryptica*, the causal agent of hazelnut branch canker. Journal of agricultural and food chemistry, 66(13), 3435-3442(2018).

[15] Evidente A. and Motta A., Phytotoxins from fungi, pathogenic for agrarian, forestal and weedy plants. Bioactive compounds from natural sources, 473(2001).

[16] Masi M., Reveglia P., Baaijens-Billones R., Górecki M., Pescitelli G., Savocchia S. and Evidente, A., Phytotoxic metabolites from three *Neofusicoccum* species causal agents of *Botryosphaeria* dieback in australia, luteopyroxin, neoanthraquinone, and luteoxepinone, a disubstituted furo-*α*-pyrone, a hexasubstituted anthraquinone, and a trisubstituted oxepi-2-one from *Neofusicoccum*. Journal of natural products, 83 (2), 453-460(2020).

[17] Kimura Y. and Tamura S., Isolation of l-β-phenyllactic acid and tyrosol as plant growth regulators from *Gloeosporium laeticolor*. Agricultural and Biological Chemistry, 37(12), 2925-2925(1973).

[18] Einhellig F.A. and Rasmussen J.A., Synergistic inhibitory effects of vanillic and p-hydroxybenzoic acids on radish and grain sorghum. Journal of chemical ecology, 4(4), 425-436(1978).

[19] Cole R.J. and Cox R.H., Handbook of toxic fungal metabolites. Academic Press (1981).

[20] Capasso R., Cristinzio G., Evidente A. and Scognamiglio F., Isolation, spectroscopy and selective phytotoxic effects of polyphenols from vegetable waste waters. Phytochemistry, 31(12), 4125-4128(1992).

[21] Evident A., Punzo B., Andolfi A., Cimmino A., Melck D. and Luque J., Lipophilic phytotoxins produced by *Neofusicoccum parvum*, a grapevine canker agent. Mediterranean Phytopathologia , 49 (1), 74-79(2010).

[22] Bouallagui Z. and Sayadi S., Production of high hydroxytyrosol yields via tyrosol conversion by *Pseudomonas aeruginosa* immobilized resting cells. Journal of agricultural and food chemistry, 54 (26) 9906-9911(2006).

[23] Masi M., Maddau L., Linaldeddu B.T., Scanu B., Evidente A. and Cimmino A., Bioactive metabolites from pathogenic and endophytic fungi of forest trees. Current medicinal chemistry, 25(2), 208-252(2018).

[24] Koutrotsios G., Kalogeropoulos N., Stathopoulos P., Kaliora A. C. and Zervakis G.I., Bioactive compounds and antioxidant activity exhibit high intraspecific variability in *Pleurotus ostreatus* mushrooms and correlate well with cultivation performance parameters. World Journal of Microbiology and Biotechnology, 33(5), 98(2017).

[25] Di Benedetto R., Varì R., Scazzocchio B., Filesi C., Santangelo C., Giovannini C. and Masella R., Tyrosol, the major extra virgin olive oil compound, restored intracellular antioxidant defences in spite of its weak antioxidative effectiveness. Nutrition, Metabolism and Cardiovascular Diseases, 17(7), 535-545(2007).

[26] Evidente A., Cimmino A. and Masi M., Phytotoxins produced by pathogenic fungi of agrarian plants. Phytochemistry Reviews, 18(3), 843-870(2019).

[27] Reveglia P., Pacetti A., Masi M., Cimmino A., Carella G., Marchi G. and Evidente A., Phytotoxic metabolites produced by *Diaporthe eres* involved in cane blight of grapevine in Italy. Natural product research, 1-9(2019).

[28] Gamboa-Angulo M.M., García-Sosa K., Alejos-González F., Escalante-Erosa F., Delgado-Lamas G. and Peña-Rodríguez, L.M., Tagetolone and tagetenolone: two phytotoxic polyketides from *Alternaria tagetica*. Journal of agricultural and food chemistry, 49 (3), 1228-1232(2001).

[29] Venkatasubbaiah P., Baudoin A.B.A.M., Chilton W.S., Leaf spot of hemp dogbane caused by *Stagonospora apocyni*, and its phytotoxins, Journal of Phytopathology, 135 (4) 309-316(1992).

[30] Albuquerque P., Casadevall A., Quorum sensing in fungi-a review. Medical Mycolgy, 50(4),337-345(2012).