Bioactive composition, antimicrobial activities and the influence of Agrocybe aegerita *(Brig.) Sing on certain quorum-sensing-regulated functions and biofilm formation by* Pseudomonas aeruginosa

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Abstract

Agrocybe aegerita (Brig.) Sing is known as a white rot fungus. Antioxidant, antimicrobial activity and antiqourum effect on *Pseudomonas aeruginosa* of *A. aegerita* methanolic extract was investigated. The extract showed good antimicrobial activity. Effects of *A. aegerita* methanolic extract regulated virulence factors in quorum sensing (QS) test, as well as biofilm formation on *P. aeruginosa* and demonstrated reduction of pyocyanin production, twitching and swimming motility. Methanolic extract exhibited high antioxidant effect. The studied mushroom is a rich source of carbohydrates and proteins. Trehalose was the main free sugar while malic acid was the most abundant organic acid. α-, β-, γ- and δ-Tocopherols were also found, and oleic acid was the most prevalent fatty acid. *A. aegerita* can be a good source of nutritional and bioactive substances. This research is of great importance due to the prevalence of drug-resistant microorganisms and health-beneficial effects of antioxidants.

Key words: *Agrocybe aegerita*, antimicrobial activity, antiquorum effect, *Pseudomonas aeruginosa,* antioxidant potential, chemistry

1. Introduction

An emerging problem associated with misuse of antibiotic therapy is the worldwide emergence of higher level tolerance of target organisms against available broad spectrum antibiotics.[1] As a result, and considering the rapid spread of multidrug resistance, the development of new antimicrobial or antipathogenic agents that act upon newly adapted microbial targets has become a very pressing priority.[2]

Quorum sensing (QS) plays an important role in biofilm formation. It is an intercellular signaling system in which bacteria communicate and regulate gene expression by releasing small compounds called autoinducers in environment.[3] Due to its role in various regulatory processes it can serve as an important target. Knowledge about the biofilm formation and quorum sensing are resulting in identification of new targets for therapeutics against *Pseudomonas aeruginosa* infection.[4]

Despite the huge diversity of compounds with antimicrobial properties, resistance of microorganisms to them is increasing dramatically. This fact certainly demands our attention and immediate response. Therefore, the search for new sources of compounds with antimicrobial properties in natural matrices has been intense in the last years.[5,6]

Mushrooms emerged as a good alternative source of new antimicrobials. Some common edible mushrooms like *Ganoderma lucidum* and *Lentinus edodes* have been studied for that purposes,[7,8] but isolated substances or crude extracts derived from lesser-known edible mushrooms are also interesting. Those species are potential sources of diverse biomolecules with nutritional and/or medicinal properties, and could be used for the development of medicines, nutraceuticals and food supplements.[9,10]

Furthermore, wild mushrooms have also emerged as a source of antioxidant compounds, very important to eliminate free radicals and other reactive species produced as a part of the normal process of aerobic metabolism, and that can cause structural damage to cells, being implicated

in several chronic diseases such as various types of cancer, cardiovascular diseases or diabetes.[11,12]

It is estimated that there are about 140000 species of mushrooms on earth and of these only 22000 are known and only a small percentage (5%) has been investigated.[6] Therefore, it is necessary to expand the knowledge and methods available to identify the bioactive components in mushrooms that act on specific target microorganisms or responsible for antioxidant properties.

Edible mushroom *Agrocybe aegerita* (a synonym of *Agrocybe cylindracea*; black poplar mushroom) is an agaric fungus that colonizes deciduous wood and bark mulch, preferably stumps of poplar trees. This mushroom is found in North America, Europe and Asia, and it seems to prefer warm or mild climates. It is a popular edible mushroom in southern Europe, especially in Italy (so called Pioppino mushroom) where it is also commercially cultured.[13] *A. aegerita* is a popular and highly nutritional edible mushroom, which has been used as a traditional Chinese herbal medicine. It has an abundant amount of proteins, which accounts for 25–30% of dry fruiting bodies.[14]

In the present study, a methanolic extract obtained from a wild sample of *A. aegerita,* collected in Serbia, was explored for its antimicrobial activity against plant, animal and human pathogens, as well as food spoilage agents, possible inhibition of quorum sensing activity on *P. aeruginosa,* and antioxidant potential. Furthermore, being an edible species, the mushroom was fully characterized regarding nutritional properties, hydrophilic and lipophilic compounds.

2. Material and methods

2.1. Standards and reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers and standards of tocopherols, sugars, organic acids and phenolic compounds, and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Mueller-Hinton agar (MH) and malt agar (MA) were obtained from the Institute of Immunology and Virology, Torlak (Belgrade, Serbia). Dimethylsulfoxide (DMSO), (Merck KGaA, Germany) was used as a solvent. Methanol and all other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.2. Mushroom species

Agrocybe aegerita was collected from the wood logs of poplar trees at Jabučki rit (Northern Serbia) during April 2012 and authenticated by Dr Jasmina Glamočlija (Institute for Biological Research "Siniša Stanković"). Voucher specimen has been deposited at the Fungal Collection Unit of the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia, under number Aa-001-2012. Fresh fruiting bodies were randomly divided to smaller samples and immediately dried by lyophilization (LH Leybold, Lyovac GT2, Frenkendorf). When reaching constant mass, specimens were milled to a fine powder, mixed to obtain an homogenate sample, and kept at 4ºC untill further analysis.

2.3. Preparation of the extract

The methanolic extract was obtained by stirring the dry fruiting body of *A. aegerita* (8 g) with 100 mL of methanol for 1 h and subsequently filtered through Whatman No. 4 paper. The residue was then re-extracted with 20 mL of methanol for 1 h. The combined methanolic extracts were evaporated at 40ºC (rotary evaporator Büchi R-210) to dryness. The extracts were re-dissolved in *a)* methanol for the antioxidant activity assays (20 mg/mL), *b)* 5% solution of DMSO in distilled water for the antimicrobial activity and antiqourum assays (100 mg/mL).

2.4. Evaluation of the antimicrobial activity

2.4.1. Antibacterial activity

The Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240) and *Listeria monocytogenes* (NCTC 7973), and the Gram-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210), and *Enterobacter cloacae* (human isolate), were used. The antibacterial assay was carried out by a microdilution method.[15,16] The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. Mushroom methanolic extract was dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (10 mg/mL) and added in Tryptic Soy broth (TSB) medium (100 µL) with bacterial inoculum (1.0×10^4 CFU per well). The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MICs obtained from the susceptibility testing of various bacteria to tested extracts were determined also by a colorimetric microbial viability assay based on reduction of an INT ((p-iodonitrotetrazolium violet) [2-(4-iodophenyl)-3-(4-nitrphenyl)-5-phenyltetrazolium chloride; Sigma]) color and compared with positive control for each bacterial strains. The MBCs were determined by serial sub-cultivation of 2 µL into

microtitre plates containing 100 μL of broth per well and further incubation for 24 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate manager 4.0 (Bio-Rad Laboratories) and compared with a blank (broth medium plus diluted extracts) and the positive control. Streptomycin (Sigma P 7794) and Ampicillin (Panfarma, Belgrade, Serbia) were used as positive controls (1 mg/mL in sterile physiological saline). Five percent DMSO was used as a negative control.

2.4.2. Antifungal activity

Aspergillus fumigatus (human isolate), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Penicillium verrucosum* var. *cyclopium* (food isolate) were used for this test. In order to investigate the antifungal activity of mushroom extract, a modified microdilution technique was used.[17,18] The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (*v/v*) and spore suspension was adjusted with sterile saline to a concentration of 1.0×10^5l. Extract was dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) (10 mg/mL) and added in broth Malt medium with inoculum (0.005-3 mg/mL for extracts). The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of a 2 μL of tested compounds dissolved in medium and incubated for 72 h at 28°C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, and commercial fungicides, bifonazole (Srbolek, Belgrade, Serbia) and ketoconazole

(Zorkapharma, Šabac, Serbia), were used as positive controls (1-3000 µg/mL). Five percent DMSO was used as a negative control.

2.5. Antiqourum (AQ) sensing activity

2.5.1. Bacterial strains, growth media and culture conditions

P. aeruginosa PA01 (ATCC 27853) used in this study is from the lab collection. Bacteria were routinely grown in Luria-Bertani (LB) medium (1% w/v NaCl, 1% w/v Tryptone, 0.5% w/v yeast extract) with shaking (220 rpm) and cultured at 37 °C.

2.5.2. Biofilm formation

Biofilm was grown as described previously.[19] The effect of different concentrations of extract (ranging from 0.5 to 0.125 of MIC) on biofilm forming ability was tested on polystyrene flat-bottomed microtitre 96 well plates as described by Spoering and Lewis,[20] with some modifications. Briefly, 100 µL of overnight culture of *P. aerugonosa* was added to each well of the plates in the presence of 100 µL subinhibitory concentrations (subMIC) of extract (0.5, 0.25 and 0.125 MIC; 0.30 mg/mL, 0.15 mg/mL, 0.07 mg/mL) or 100 mL medium (control). After incubation for 24 h at 37° C, each well was washed twice with sterile PBS (pH 7.4), dried, stained for 10 min with 0.1 % crystal violet in order to determine the biofilm mass. After drying, 200 µL of 95% ethanol (v/v) was added to solubilize the dye that had stained the biofilm cells. The excess stain was washed off with dH_2O. After 10 min, the content of the wells was homogenized and the absorbance at λ= 625 nm was read on a Sunrise™ - Tecan ELISA reader.

2.5.3. Discs-diffusion method for determination of AQ activity against P. aeruginosa

Filter paper discs were impregnated with tested extract solutions (subMIC; 0.30 mg/disc, 0.15 mg/disc, 0.07 mg/disc), streptomycin and ampicillin (subMIC; 0.5 MIC, 0.25 MIC and 0.125

MIC; 0.1, 0.05, 0.025 mg/disc for streptomycin and 0.4, 0.2 and 0.1 mg/disc for ampicilin) to determine whether they have antiqourum activity against bacteria and impair bacterial growth. To do this, filter paper (filter paper 4 mm; Whatman) were used. Discs were soaked in the indicated solutions, then dried at room temperature (3 h, protected from light), and aseptically placed onto the plates prior to bacterial inoculation. After incubation, it was recorded whether the inhibition or antiqourum zones were obtained.[21]

2.5.4. Twitching and flagella motility

After growth in the presence or absence of extract, the cells of *P. aeruginosa* PA01 were washed twice with sterile PBS and resuspended in PBS at 1 x 10^8 cfu/mL (OD of 0.1 at 660 nm). Briefly, cells were stabbed into a nutrient agar plate with a sterile toothpick and incubated overnight at 37° C. Plates were then removed from the incubator and incubated at room temperature for two more days. Colony edges and the zone of motility were measured with a light microscope.[22] Fifty microlitres of tested extract (sub MIC; 0.5 MIC, 0.25 MIC and 0.125 MIC) was mixed into 10 mL of molten MH medium and poured immediately over the surface of a solidified LBA plate as an overlay. The plate was point inoculated with an overnight culture of PAO1 once the overlaid agar had solidified and incubated at 37° C for 3 days. The extent of swimming was determined by measuring the area of the colony.[23]

2.5.5. Anti-QS against P. aeruginosa PA01 Pyocyanin

Overnight culture of *P. aeruginosa* PA01 was diluted to OD_{600} nm 0.2. Then, tested extract (250 µL disolved as 0.5 MIC, MIC and 2 MIC) was added to *P. aeruginosa* (4.75 mL) and incubated at 37 °C for 24 h. The treated culture was extracted with chloroform (3 mL), followed by mixing the chloroform layer with 0.2 M HCl (1 mL). Absorbance of the extracted

organic layer was measured using the UV-visible spectrophotometer (UV1601, Shidmazu, Kyoto, Japan) at 520 nm.[24]

2.6. Evaluation of the antioxidant potential

2.6.1. General

Successive dilutions were made from the stock solution and submitted to different *in vitro* assays to evaluate the antioxidant activity of the samples.[25] The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (ferricyanide/Prussian blue assay) against sample concentrations. Trolox was used as standard.

2.6.2. Folin-Ciocalteu assay

The extract solution (1 mL) was mixed with *Folin-Ciocalteu* reagent (5 mL, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40°C for colour development. Absorbance was then measured at 765 nm (Analytikjena spectrophotometer; Jena, Germany). Gallic acid was used to obtain the standard curve and the reduction of *Folin-Ciocalteu* reagent by the samples was expressed as mg of gallic acid equivalents (GAE) per g of extract.

2.6.3. Reducing power or ferricyanide/Prussian blue assay

The extract solutions with different concentrations (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50°C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48 wells plate, the same with

deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm in ELX800 Microplate Reader (Bio-Tek Instruments, Inc; Winooski, USA).

2.6.4. DPPH radical-scavenging activity assay

This methodology was performed using the Microplate Reader mentioned above. The reaction mixture was made in a 96 wells plate and consisted of 30 µL of a concentration range of the extract and 270 µL methanol containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 30 min in the dark, and the absorption was measured at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample and A_{DPPH} is the absorbance of the DPPH solution.

2.6.5. Inhibition of β-carotene bleaching or β-carotene/linoleate assay

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two millilitres of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40ºC under vacuum and linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into test tubes containing 0.2 mL of a concentration range of the extract. The tubes were shaken and incubated at 50ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: Absorbance after 2h of assay/initial absorbance) × 100.

2.6.6. Thiobarbituric acid reactive substances (TBARS) assay

Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000*g* for10 min. An aliquot (100 µL) of the supernatant was incubated with 200 µL samples of a concentration range of the extract in the presence of $FeSO_4$ (10 mM; 100 µL) and ascorbic acid (0.1 mM; 100 µL) at 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 µL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 µL), and the mixture was then heated at 80ºC for 20 min. After centrifugation at 3000 *g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A - B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively.

2.7. Chemical characterization

2.7.1. Nutritional value

The samples were analysed for their chemical composition (moisture, proteins, fat, carbohydrates and ash) through AOAC procedures.[26] The crude protein content (N ×4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15ºC. Total carbohydrates were calculated by difference. The energy contribution was calculated according to the following equation: Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

2.7.2. Sugars

Following the extraction procedure described by Reis et al.,[25] free sugars were determined by a High Performance Liquid Chromatography (HPLC) system consisting of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000) and auto-sampler (AS-2057 Jasco), coupled to a refraction index detector (RIdetector Knauer Smartline 2300). Sugars identification was made by comparing the relative retention times of sample peaks with standards. Data were analyzed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the internal standard (IS, raffinose) method and by using calibration curves obtained from the commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

2.7.3. Organic acids

Following the extraction procedure described by Barros et al.,[27] organic acids were determined by ultra fast liquid chromatography (UFLC, Shimadzu 20A series) coupled with a photodiode array detector (PDA). The organic acids were quantified by the comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight.

2.7.4. Fatty acids

Following the extraction transesterification procedures described by Reis et al.,[25] fatty acids were determined using a gas chromatographer (DANI 1000) equipped with a split/splitless injector and a flame ionization detector (GC-FID). Fatty acids identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.7.5. Tocopherols

Following the extraction procedure described by Heleno et al.,[28] tocopherols were determined by HPLC (equipment described above, for sugars composition), and a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by chromatographic comparison with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in µg per 100 g of dry weight.

3. Results and Discussion

3.1. Antimicrobial activity

It was determined that the yield of the methanolic extraction from *A. aegerita* fruiting body is very good, and is 1.40 g out of 5.00 g of fruiting body.

The methanolic extract of *A. aegerita* was tested against four species of Gram-negative bacteria, four species of Gram-positive bacteria, and eight species of fungi. Extract exhibited excellent activity at tested concentrations on all tested microorganisms. Among the tested bacteria, *S. aureus* and *P. aeruginosa* were the most susceptible to the activity of the extract, with MIC (0.59 mg/mL) and MBC (1.18 mg/mL). Commercial antibiotics, streptomycin and ampicillin, showed inhibitory activity in the range of 0.05-0.30 mg/mL, and bactericidal activity in the range of 0.10-0.50 mg/mL. *L. monocytogenes* was the most resistant bacteria with MIC (4.74 mg/mL) and MBC (9.49 mg/mL) (**Table 1**).

Regarding micromycetes, *A. versicolor* was strongly inhibited by the extract, with MIC value of only 0.025 mg/mL, and MBC 0.95 mg/mL. The most resistant microfungi was *A. fumigatus*

with inhibitory concentration at 2.38 mg/mL and fungicidal concentration 18.96 mg/mL (**Table 1**). Antifungal drugs ketoconazole and bifonazole exhibited high activity in the range of 0.10-3.50 mg/mL (**Table 1**). It is important to notice that the extract showed inhibitory activity against all the tested bacteria and micromycetes.

Numerous mushroom extracts have been reported to have antimicrobial activity against pathogenic microorganisms, including extracts from *Agaricus bisporus* (the most cultivated mushroom in the world), *Armillaria mellea*, *Boletus edulis*, *Cantharellus cibarius*, just to mention a few.[9] It should be highlighted that as far as our literature survey could ascertain, scarce information is available on the *in vitro* antimicrobial activity of *A. aegerita* extracts. There are literature data (from 1992) concerning the antifungal activity of the extract from the submerged culture, and since then methodology has significantly changed concerning the isolation of the extracts and assays for antimicrobial activity evaluation.[29] Also, data concerning the compound agrocybolacton, derived from the culture representatives of the genus *Agrocybe*, showed moderate antibacterial activity against Gram-positive bacteria *B. subtilis* and *M. smegmatis*.[30] Agrocybin, a peptide isolated from *A. dura* and *A. cylindracea* exhibited activity against Gram-negative bacteria[31] and also antifungal activity.[32] Since there are no literature data providing information about antimicrobial activity of crude extracts of *A. aegerita*, these results are of great importance.

3.2. Biofilm formation

The effect of *A. aegerita* extract on biofilm formation of *P. aeruginosa* was tested with 0.5, 0.25 and 0.125 of determined MIC. **Table 2** shows that the extract tested reduced biofilm formation better than streptomycin and ampicillin, especially at 0.5 MIC. The extract reduced biofilm formation in 84.24%, while streptomycin and ampicillin reduced biofilm in 50.60% and 30.84%, respectively.

The quorum-sensing inhibition zones were determined by disc diffusion method. It can be seen that extract showed antiquorum sensing (AQ) activity at all concentrations in range of 7.70-10.30 mm. Ampicillin possessed AQ activity at higher concentration (7.60 mm), while streptomycin showed the best AQ activity presenting the zones in range of 15.50-22.06 mm (**Table 2).**

The flask incubation assay was used to quantify quorum sensing inhibitory activity of the extract that produced quorum-sensing inhibition zones. The tested extract of *A. aegerita* demonstrated concentration-dependent pyocyanin inhibitory activity and showed reduction of pigment in all tested concentration, sub MIC, MIC and 2 MIC. The best reduction pyocyainin ability was noticed at 2 MIC. In general, all the tested concentrations (sub MIC, MIC and 2 MIC) showed higher reduction ability of pigment. A decrease level in green pigment content was demonstrated in the extract at all the tested concentrations. The extract showed higher reduction of pigment than ampicillin and streptomycin (**Figure 1**). Promising anti-quorum sensing compounds have been demonstrated to disrupt bacterial biofilms and make the bacteria more susceptible to antibiotics, and these compounds also provide the ability to reduce bacterial virulence factors as well as promote clearance of bacteria in infectious animal models. Many mechanisms of actions have been proposed to interfere with the quorum sensing system such as inhibition of biosynthesis of autoinducer molecules, inactivation or degradation of the autoinducer, interference with the signal receptor, and inhibition of the genetic regulation system.[33]

In addition to QS, the initiation of biofilm formation by *P. aeruginosa* depends on two cell-associated structures; the flagellum and type IV pili.[22,34] The flagellum is responsible for swimming motility while the type IV pili are responsible for twitching motility.[35] Both types of motility are important in the initial stages of biofilm formation by *P. aeruginosa*.[22,34] Therefore, we tried to determine if our extract influence on either one or both motilities. On

swimming plates, the motile strain PAO1 was used as the 100% standard (control) for motility while the Petri dishes with the same strain plus *A. aegerita* extract were compared with control. Extract reduced the twitching motility of *P. aeruginosa*. The normal colonies of *P. aeruginosa,* i.e. in the absence of the extract, were flat with a rough appearance displaying irregular colony edges (**Fig. 2B**) and a hazy zone surrounding the colony. The cells were in a very thin layer. After 2 days of incubation at ambient temperature, colony expansion occurred very rapidly due to twitching motility, the control *P. aeruginosa* isolates produced swimming zones to 100% (**Table 2)** and it was 14.0 mm. Bacteria that were grown with the *A. aegerita* extract solution were incapable of producing such a twitching zone and had almost round, smooth, regular colony edges, the flagella were reduced both in size and in numbers, and the colony diameter swimming zones was also reduced (15.33 mm) (**Fig. 2A**, **Table 2**). Streptomycin reduced the flagellas completely (**Fig. 2C**), while Ampicillin did not affect the formation of flagella at all (**Fig. 2D**).

In summary, our study indicated that *A. aegerita* extract possessed antimicrobial, antibiofilm and anti-quorum sensing activity. Inhibition of bacterial quorum sensing offers new strategy for the treatment of bacterial infections. Anti-quorum sensing property of this plant species may play an important role in antibacterial activity and offers an additional strategy for fighting bacterial infection.

3.3. Aantioxidant activity

Antioxidant activity of the methanolic extract was measured by four different methods (**Table 3**). These assays measured free radical scavenging activity, reducing power and lipid peroxidation inhibition. The extract gave 17.36 mg GAE/g extract in the Folin-Ciocalteu assay, and revealed high DPPH radical-scavenging activity assay (EC_{50}= 7.23 mg/mL). Slightly higher effect was observed in the β-carotene/linoleate assay (EC_{50} = 6.11 mg/mL),

while Ferricyanide/Prussian blue assay and TBARS assays showed even higher effects (EC_{50} = 2.66 mg/ml; EC_{50} = 0.39 mg/mL, respectively). The same behavior was previously reported for other mushroom species.[36] The observed antioxidant activity may be the consequence of the presence of different antioxidant compounds described in the chemistry section, mainly phenolic acids. Lo and Cheung[13] reported antioxidant activity of the methanol crude extract of *A. aegerita* and its fractions, isolated by liquid–liquid partition, using scavenging activity of 2,20-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS) and inhibition of lipid peroxidation of rat brain homogenate. The ethyl acetate (EA) fraction, which showed the most potent antioxidant activity in the mentioned two assays, was further fractionated by a Sephadex LH-20 column into four subfractions (EA1–EA4). EA3 exhibited the strongest radical-scavenging activity in the ABTS and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and showed a similar extent of *in vitro* inhibition of human LDL oxidation to caffeic acid. Significant correlation was found between the total phenolic content and the antioxidant activity ($p < 0:01$) in the EA fraction and its subfractions.

3.4. Chemical composition

Results regarding the nutritional value of *A. aegerita* are presented in **Table 4**. Carbohydrates and proteins are the most abundant compounds (84.51 g/100 g dw and 6.68 g/100 g dw, respectively). Mushrooms are generally considered to be a good source of digestable proteins, and are reported to contain all the essential amino acids needed in the human diet.[37] Ash content is low (6.69 g/100 g dw); *A. aegerita* is also poor in fat (2.13 g/100 g dw) and energetic value (383.91 kcal/100 g dw), which makes this mushroom a good candidate for low-caloric diets (**Table 4**). Trehalose was the dominant sugar (12.49 g/100g dw), while mannitol was presented at 0.93 g/100g dw (**Table 4**). This is in concordance with previous reports on mushroom sugars composition that also mentioned these molecules as the most

predominant.[38] Malic acid was the most abundant organic acid (1.82 g/100 g dw), followed by citric acid (0.88 g/100 g), then fumaric acid (0.26 g/100 g) and oxalic acid (0.09 g/100 g) (**Table 4)**.

A. aegerita polyunsaturated fatty acids- PUFA (78.60%) levels were higher than saturated and monounsaturated fatty acids (17.93% and 3.47%, respectively) (**Table 5**). As already mentioned, mushrooms are known for their low fat content, but also for predominance in PUFA over SFA.[39] The main fatty acids found by Shuai et al.[40] in *A. aegerita* were linoleic acid (C18:2n6c) > palmitic acid (C16:0) > oleic acid (18:1n9c). A total FA value of 33.13 mg/10 g was reported in literature for *A. aegerita*, while ratio between unsaturated fatty acids and saturated fatty acids was 3.80.

Tocopherols were also determined, since these compounds have the important ability to scavenge free radicals, and are considered to protect our organisms against degenerative diseases. For a long time, α-tocopherol was considered to be the most active form of vitamin E and was reported to have the highest biological activity. However, recent studies have shown that the other forms are also active.[29] For the studied mushroom, γ-tocopherol was the most abundant isoform (86.08 µg/100 g dw), followed by β-tocopherol (8.80 µg/100 g dw), δ-tocopherol (3.40 µg/100 g dw) and α-tocopherol (2.10 µg/100 g dw; **Table 5).** Our literature survey showed that there are only few avalialble studies concerning *A. aegerita* chemical composition. Diyabalanage et al.,[41] described the presence of ceramide, methyl- β-D-glucopyranoside and α-D-glucopyranoside, along with linoleic acid and its methyl ester. Our studies are in accordance with previous studies reporting the presence of palmitic acid, linoleic acid, mannitol and trehalose in the fruiting body of *A. aegerita*.[41] A novel lectin was isolated from water extract *A. aegerita* from China by affinity chromatography.[42] *A. aegerita* was also reported to contain several bioactive metabolites, such as indole derivatives with free radical-scavenging ability,[42] polysaccharides with hypoglycemic activity[43] and agrocybin, a

peptide with anti-fungal activity.[32] Previously, Gao et al.,[44] reported that the protein components from *A. aegerita* showed tumor rejection activity. Also two antitumor lectins, AAL and AAL-2, were identified from the protein components of *A. aegerita*.[45,46]

Nowadays, mushroom research is intense and hundreds of species have demonstrated a broad spectrum of activities, including antimicrobial and antioxidant.[47] It is evident that there are very good effects of extracts, but there is a gap in the identification of the individual compounds responsible for antimicrobial properties. Only a few low-molecular weight compounds and some peptides and proteins have been described. After elucidation of the mechanism of mushroom extracts activity or pure compounds activity, mushroom metabolites or other related compounds could be used to develop nutraceuticals or microorganisms effective drugs.

The reason that some of the mushroom extracts/preparations are not available yet as medicines may be the difficulty relating to massive production. Since *A. aegerita* is part of the mushroom cultivation, and the fungal material is primarily available, in the light of new data of the antimicrobial activity and mechanisms of action concerning antiqourum activity of the extract, attention should definitely be dedicated to it.

4. Conclusion

Our results concerning chemical profile and bioactive characteristics of *A. aegerita* extract and gaining knowledge about the mechanisms of action of the different mushroom compounds might lead to the usage of new active principles from this mushroom as a source of functional food.

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Conflict of interest

There is no conflict of interest associated with the authors of this paper.

References:

1. H. Harbottle, S. Thakur, Z. D. G. White, Genetics of antimicrobial resistance. *Anim. Biotechnol.* 2006, **17**, 111-124.

2. A. Coates, Y. Hu, R. Bax, C. Page, The future challenges facing the development of a new antimicrobial drugs. *Nat. Rev. Drug Discov.* 2002, **1**, 895-910.

3. S. Favre-Bonté, E. Chamot, T. Köhler, J. A. Romand, C. Van Delden, Autoinducer production and quorum-sensing dependent phenotypes of *Pseudomonas aeruginosa* vary according to isolation site during colonization of incubated patients. *BMC Microbiol.* 2007, **7**, 33.

4. G. Sharma, S. Rao A. Bansal, S. Dang, S. Gupta, R. Gabrani, *Pseudomonas aeruginosa* biofilm: Potential therapeutic targets. *Biologicals*, 2014, **42**, 1-7.

5. L. Barros, R. C. Calhelha, J. A. Vaz, I. C. F. R. Ferreira, P. Baptista, L. Estevinho, Antimicrobial activity and bioactive compounds of Portugese wild edible mushrooms methanolic extracts. *Eur. Food Res. Technol.* 2007, **225**, 151-156.

6. U. Lindequist, J. H. Timo, W. D. Julich, The pharmacological potential of mushrooms. *Evid-Based Compl. Alt.* 2005, **2**, 285-299.

7. B. Boh, M. Berovic, J. Zhang, L. Zhi-Bin, *Ganoderma lucidum* and its pharmaceutically active compounds. *Biotechnol. Annu. Rev.* 2007, **13**, 265-301.

8. E. Altobelli, Lignicolous fungi with medicinal properties. *Scientifica Acta*, 2011, **5**, 10-19.

9. M. J. Alves, I. C. F. R. Ferreira, J. Dias, V. Teixeira, A. Martins, M. Pintado, A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta Med.* 2012, 78, 1707-1718.

10. V. Popović, J. Živković, S. Davidović, M. Stevanović, D. Stojković, Mycotherapy of cancer: an update on cytotoxic and antitumor activities of mushrooms, bioactive principles and molecular mechanisms of their action. *Curr. Top. Med. Chem.* 2013, **13**, 2791-2806.

11. M. Carocho, I .C. F. R. Ferreira, A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem. Toxicol.* 2013, **51**, 15-25.

12. I. C. F. R. Ferreira, L. Barros, R. M. V. Abreu, Antioxidants in wild mushrooms. *Curr. Med. Chem.* 2009, **16**, 1543-1560.

13. K. M. Lo, C. K. P. Cheung, Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. *alba*. *Food Chem.* 2005, **89**, 533–539.

14. Y. Chen, J. Shuai, J. Yanxia, Y. Yalin, Y. Guojun, L. Xianqing, C. Mingyao,

15. CLSI Clinical and Laboratory Standards Institute, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 8th ed. CLSI publication M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA, 2009.

16. T. Tsukatani, H. Suenaga, M. Shiga, K. Noguchi, M. Ishiyama, T. Ezoe, K. Matsumoto, Comparison of the WST-8 colorimetric method and the CLSI broth microdilution method for susceptibility testing against drug-resistant bacteria. *J. Microbiol. Meth.* 2012, **90**, 160-166.

17. A. Espinel-Ingroff, Comparation of the E-test with the NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi. *J. Clin. Microbiol.* 2001, **39**, 1360–1367.

18. H. Hanel, W. Raether, A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example, *Mycoses* 1998, **31**, 148-154.

19. E. Drenkard, F. M. Ausubel, *Pseudomonas* biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature* 2002, **416**, 740-743.

20. A. L. Spoering, K. Lewis, Biofilms and planktonic cells of *Pseudomonas aeruginosa* have similar resistance to killing of antimicrobials. *J. Bacteriol.* 2001, **183**, 6746-6751.

21. C. O'May, N. Tufenkji, The Swarming Motility of *Pseudomonas aeruginosa* Is Blocked by Cranberry Proanthocyanidins and Other Tannin-Containing Materials. *Appl Environ. Microb.* 2011, **77**, 3061-3067.

22. G. A. O'Toole, R. Kolter, Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Mol. Microbiol.* 1998, **28**, 449-461.

23. S. Y. Siew-Mian, T. Foong-Yee, Anti-quorum sensing and antimicrobial activities of some traditional Chinese medicinal plants commonly used in South-East Asia. *Malaysian J. Microbiol.* 2012, **8**, 11-20.

24. D. W. Essar, L. Eberly, A. Hadero, Crawford I. Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: Interchangeability of the two anthranilate synthases and evolutionary implications. *J. Bacteriol.* 1990, **172**, 884–900.

25. F. S. Reis, A. Martins, L. Barros, I. C. F. R. Ferreira, Antioxidant properties and phenolics profile of the most widely appreciated cultivated mushrooms: a comparative study between *in vivo* and *in vitro* samples. *Food Chem. Toxicol.* 2012, **50**, 1201-1207.

26. AOAC, Official methods of analysis, 16th ed. Arlington VA, USA. Association of Official Analytical Chemists, 1995.

27. L. Barros, E. Pereira, R. C. Calhelha, M. Duenas, A. M. Carvalho, C. Santos-Buelga, I. C. F. R. Ferreira, Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of *Chenopodium ambrosioides* L. *J. Funct. Foods* 2013, **5**, 1732-1740.

28. S. A. Heleno, L. Barros, M. J. Sousa, A. Martins, I. C. F. R. Ferreira, Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chem.* 2010, **119,** 1443-1450.

29. K. Stransky, M. Semerdzieva, M. Otmar, Ž. Procházka, M. Buděšínský, K. Ubik, J. Kohoutová, L. Streinz, An extract from a submerged culture of the mushroom *Agrocybe aegerita* contains antifungal antibiotic. *Collection of Czechoslovak Chemical Communications*, 1992, *57*, 590-603.

30. L. H. Rosa, K. M. G. Machado, C. C. Jacob, M. Capelari, C. A. Rosa, C. L. Zani, Screening of Brazilian Basidiomycetes for Antimicrobial Activity. *Memórias do Instituto Oswaldo Cruz* 2003, **98**, 967-974.

31. F. Kavanagh, A. Hervey, W. J. Robbins, Antibiotic substances from Basidiomycetes. VI. Agrocybe dura. *Proceedings of the National Academy of Sciences of the United States of America*, 1950, **36**, 102-106.

32. P. H. Ngai, Z. Zhao, T. R. Ng, Agrocybin an antifungal peptide from edible mushroom *A. cylindraceae*. *Peptides* 2005, **26**, 191–196.

33. T. B. Rasmussen, M. Givskov, Quorum sensing inhibitors: a bargain of effects. *Microbiology* 2006, **152**, 895-904.

34. G. A. O'Toole, R. Kolter, Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol.* 1998, **30**, 95-304.

35. J. Henrichsen, Bacterial surface translocation: a survey and a classification. *Bacteriol. Rev.* 1972, **36**, 478–503.

36. V. Vieira, A. Marques, L. Barros, J. C. M. Barreira, I. C. F. R. Ferreira, Insights in the antioxidant synergistic effects of combined edible mushrooms: phenolic and polysaccharidic extracts of *Boletus edulis* and *Marasmius oreades*. *J. Food Nutr. Res.* 2012, **51**, 109-116.

37. A. M. Mshandete, J. Cuff, Proximate and Nutrient composition of three types of indigenous edible wild mushrooms grown in Tanzania and their utilization prospects, *African Journal of Food, Agriculture, Nutrition and Development* 2007, **7***,* 1-6.

38. Bernas, E., Jaworska, G., & Lisiewska, Z. (2006). Edible mushrooms as a source of valuable nutritive constituents. *ACTA Scientiarum Polonorum,* 5, 5-20.

39. Yilmaz, N., Solmaz, M., Ibrahim, T., & Elmastas, M. (2006). Fatty acid composition in some wild edible mushrooms growing in the middle Black Sea region of Turkey. *Food Chemistry,* 99*,* 168–174.

40. J. Shuai, C. Yijie, W. Man, Y. Yin, Y. Pan, B. Gu, G. Yu, Y. Li, B. H. C. Wong, Y. Liang, H. Sun, A novel lectin from *Agrocybe aegerita* shows high binding selectivity for terminal N-acetyl glucosamine. *Biochem. J.* 2012, **443**, 369-378.

41. Y. Zang, G. L. Mills, M. G. Nair, Cycloxygenase inhibitory and antioxidant compounds form edible mushroom *Agrocybe aegerita*. *Phytomed.* 2003, **10**, 386–390.

42. W. G. Kim, , L. I. Kee, J. P. Kim, I. J. Ryoo, H. Koshino, I. D. Yoo, New indole derivatives with free radical scavenging activity from *A. cylindraceae. J. Nat. Prod.* 1997, **60**, 721–723.

43. K. Tadashi, S. Sobue, S. Ukai, Polysaccharides in fungi XXXI, structural features of and hypoglycemic activity of two polysachcharides from hot water extracts of *Agrocybe cylindrical*. *Carbohyd. Res.* 1994, **251**, 81-87.

44. T. Gao, H. Bi, S. Ma, J. Lu, The antitumor and immunostimulating activities of water soluble polysaccharides from Radix aconiti, Radix aconiti Lateralis and Radix aconiti Kusnezoffii. *Nat. Prod. Commun.* 2010, **5**, 447-455.

45. C. Zhao, H. Sun, X. Tong, Y. Qi, An antitumour lectin from the edible mushroom *Agrocybe aegerita*. *Biochem. J.* 2003, **374**, 321-327.

46. L. Feng, H. Sun, Y. Zhang, D. F. Li, W. Da-Cheng, Structural insights into the recognition mechanism between an antitumor galectin AAL and the Thomsen-Friedenreich antigen. *FASEB Journal,* 2010, **24**, 3861-3868.

47. J. Petrović, D. Stojković, F. S. Reis, L. Barros, J. Glamočlija, A. Ćirić, I. C. F. R. Ferreira, M. Soković, Study on chemical, bioactive and food preserving properties of *Laetiporus sulphureus* (Bull.: Fr.) Murr. *Food Funct.*, DOI:10.1039/C4FO00113C.

Table 1. Antibacterial and antifungal activities of *Agrocybe aegerita* methanolic extract (mg/mL) (mean ± SD).

	Bacteria	*S. aureus*	*B. cereus*	*L.monocytogenes*	*M.flavus*	*P.aeruginosa*	*E.coli*	*S.typhimurium*	*En. cloacae*
A. aegerita	MIC	0.59±0.01	1.18±0.01	4.74±0.01	2.38±0.02	0.59±0.02	2.38±0.02	2.38±0.01	1.18±0.03
	MBC	1.18±0.02	2.38±0.03	9.49±0.03	4.74±0.03	1.18±0.03	4.74±0.03	4.74±0.01	2.38±0.01
Streptomycin	MIC	0.25±0.03	0.05±0.02	0.15±0.03	0.125±0.02	0.05±0.03	0.05±0.01	0.05±0.02	0.05±0.03
	MBC	0.5±0.01	0.10±0.03	0.30±0.01	0.25±0.01	0.10±0.03	0.10±0.01	0.10±0.02	0.10±0.02
Ampicillin	MIC	0.10±0.03	0.10±0.01	0.15±0.03	0.10±0.03	0.10±0.01	0.30±0.02	0.15±0.01	0.15±0.01
	MBC	0.15±0.01	0.15±0.02	0.30±0.03	0.15±0.02	0.20±0.03	0.50±0.03	0.20±0.03	0.20±0.01
	Fungi	*A.fumigatus*	*A.versicolor*	*A.ochraceus*	*A.niger*	*T.viride*	*P.funiculosum*	*P.ochrochloron*	*P.cyclopium*
A. aegerita	MIC	2.38±0.01	0.025±0.01	1.19±0.01	2.38±0.02	2.38±0.02	2.38±0.02	1.19±0.01	2.38±0.00
	MFC	18.96±0.02	0.95±0.03	4.74±0.03	9.49±0.03	2.38±0.03	4.74±0.03	4.74±0.01	4.47±0.01
Ketoconazole	MIC	0.20±0.03	0.20±0.02	0.15±0.03	0.20±0.02	0.20±0.03	2.50±0.01	0.20±0.02	0.25±0.03
	MFC	0.50±0.01	0.50±0.03	0.20±0.01	0.50±0.01	0.30±0.03	3.50±0.01	0.50±0.02	0.50±0.02
Bifonazole	MIC	0.15±0.03	0.10±0.01	0.15±0.03	0.15±0.03	0.10±0.01	0.20±0.02	0.20±0.01	0.25±0.00
	MFC	0.20±0.01	0.20±0.02	0.20±0.03	0.20±0.02	0.20±0.03	0.25±0.03	0.25±0.03	0.50±0.03

Table 2. Effects of *Agrocybe aegerita* methanolic extract on biofilm formation of *P. aeruginosa* (PAO1), disc-diffusion method for detection of antiquorum (AQ) concentrations and twitching activity.

Biofilm formation* (%)				AQ** (mm)		
Agents	0.5MIC	0.25MIC	0.125MIC	0.125 MIC	0.25 MIC	0.5 MIC
A. aegerita	15.76±0.03	44.97±0.30	80.63±0.46	7.70±0.58	8.00±0.00	10.30±0.58
Ampicillin	69.16±0.65	56.46±0.46	92.16±0.37	-	-	7.6±0.6
Streptomycin	49.40±0.46	70.97±0.36	88.36±0.42	-	15.0±2.1	22.6±2.3

Twitching activity			
Agents	Colony diameter (mm ± SD)	Colony color	Colony edge
A. aegerita	15.33±4.51	green with white edges	reduced flagella
Streptomycin	5.00±0.06	white	flat
Ampicillin	12.00±1.00	white	regular flagella
Control P.a. 10^9	14.00±1.00	green	regular flagella

*Biofilm formation values were calculated as: (mean A_{620} treated well)/(mean A_{620} control well)x100.
Values are expressed as means ± SD. In each column, different letters mean significant differences between samples ($p<0.05$).
** - No effect of AQ

Table 3. Antioxidant properties of the studied edible mushroom (mean ± SD).

	Reducing power		Scavenging activity	Lipid peroxidation inhibition	
Species	Folin-Ciocalteu (mgGAE/g extract)	Ferricyanide/Prussian blue (EC_{50};mg/ml)	DPPH radical-scavenging activity (EC_{50}; mg/ml)	β-carotene/linoleate (EC_{50}; mg/ml)	TBARS (EC_{50}; mg/ml)
A. aegerita	17.36 ± 0.88	2.66 ± 0.10	7.23 ± 0.18	6.11 ± 1.60	0.39 ± 0.06

In each row different letters mean significant differences between species ($p<0.05$). Concerning the *Folin-Ciocalteu* assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential. EC_{50}: Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the Ferricyanide/Prussian blue assay.

Table 4. Nutritional value and hydrophilic compounds of *Agrocybe aegerita* (mean ± SD).

Ash	Proteins	Fat	Carbohydrates	Energy
(g/100 g dw)	(g/100 g dw)	(g/100 g dw)	(g/100 g dw)	(kcal/100 g dw)
6.69 ± 0.33	6.68 ± 0.26	2.13 ± 0.02	84.51 ± 0.24	383.91 ± 1.02
Mannitol	Trehalose	Total		
(g/100 g dw)	(g/100 g dw)	(g/100 g dw)		
0.93 ± 0.01	12.49 ± 0.09	13.42 ± 0.08		
Oxalic acid	Malic Acid	Citric Acid	Fumaric Acid	Total
(g/100 g dw)	(g/100 g dw)	(g/100 g dw)	(g/100 g dw)	(g/100 g dw)
0.09 ± 0.01	1.82 ± 2.21	0.88 ± 0.01	0.26±0.01	3.06±2.23

Table 5. Lipophilic compounds of *Agrocybe aegerita* (mean ± SD).

C6:0	0.10 ± 0.01
C8:0	0.13 ± 0.01
C10:0	0.08 ± 0.00
C12:0	0.06 ± 0.00
C14:0	0.23 ± 0.00
C14:1	0.01 ± 0.00
C15:0	0.43 ± 0.00
C16:0	13.07 ± 0.11
C16:1	0.29 ± 0.00
C17:0	0.25 ± 0.00
C18:0	2.13 ± 0.02
C18:1n9	3.03 ± 0.00
C18:2n6	78.40 ± 0.10
C18:3n3	0.07 ± 0.00
C20:0	0.49 ± 0.00
C20:1	0.05 ± 0.00
C20:2	0.03 ± 0.00
C20:3n3+C21:0	0.08 ± 0.00
C20:5n3	0.02 ± 0.00
C22:0	0.40 ± 0.01
C22:1n9	0.02 ± 0.01
C23:0	0.11 ± 0.01
C24:0	0.45 ± 0.01
C24:1	0.07 ± 0.02
Total SFA (% of total FA)	17.93 ± 0.07
Total MUFA (% of total FA)	3.47 ± 0.03
Total PUFA (% of total FA)	78.60 ± 0.10
α-Tocopherol	2.10 ± 1.10
β-Tocopherol	8.80 ± 2.20
γ-Tocopherol	86.08 ± 12.90
δ-Tocopherol	3.40 ± 0.20
Total (μg/100 g dw)	100.38 ± 13.81

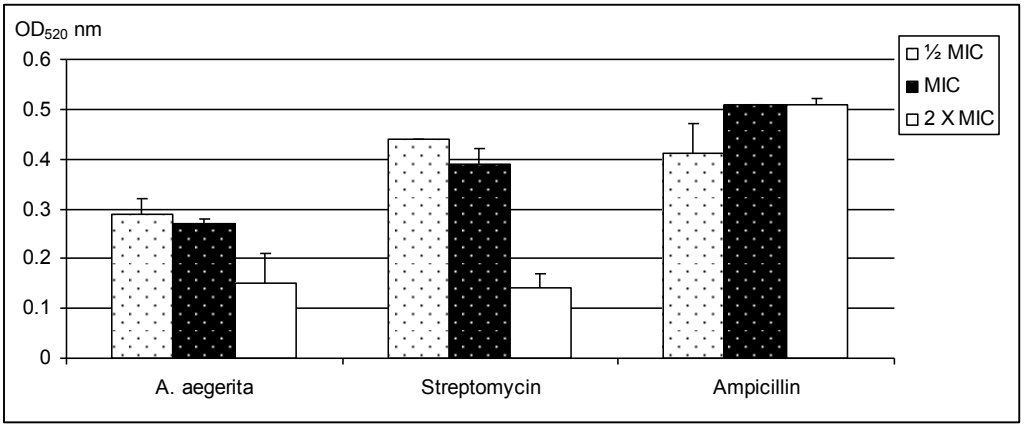

Figure 1. Reduction of pyocyanin pigment by *Agrocybe aegerita* extract.

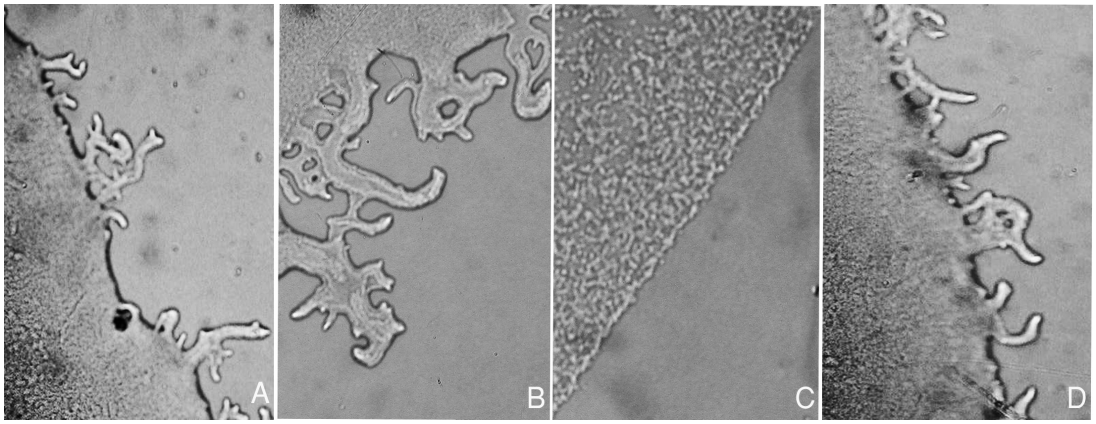

Figure 2. Light microscopy of colony edges of *P. aeruginosa* in twitching motility plates, grown in the presence or absence of *A. aegerita* extract.

The colonies from the bacteria grown with *A. aegerita* extract (**A**) were with small changes in flagella shape and numbers. *P. aeruginosa* produced a flat, widely spread, irregularly shaped colony in the absence of extracts (**B**). *P. aeruginosa* colony with presence of Streptomycin without flagella (**C**) and Ampicillin with almost regularly formed flagella (**D**). Magnification: (**A-D**) x100.