Journal of Agricultural Science; Vol. 7, No. 11; 2015
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Antibacterial Activity of Spent Substrate of Mushroom *Pleurotus ostreatus* Enriched with Herbs

Maricela Ayala Martínez[1], Deyanira Ojeda Ramírez[1], Sergio Soto Simental[1], Nallely Rivero Perez[1], Marcos Meneses Mayo[2] & Armando Zepeda-Bastida[1]

[1] Área Académica de Medicina Veterinaria y Zootecnia, Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, México

[2] Facultad de Ciencias de la Salud (Nutrición), Universidad Anáhuac México-Norte, México

Correspondence: Armando Zepeda-Bastida, Área Académica de Medicina Veterinaria y Zootecnia, Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Avenida Universidad s/n km 1, Tulancingo, Hidalgo, C.P. 43600, México. Tel: 52-771-717-2000 ext. 2449. E-mail: azepeda@uaeh.edu.mx

Received: August 10, 2015 Accepted: September 11, 2015 Online Published: October 15, 2015

doi:10.5539/jas.v7n11p225 URL: http://dx.doi.org/10.5539/jas.v7n11p225

Abstract

The recurrent use of antibiotics has given the guideline so that bacteria will develop resistance to drugs used in medicine, which is why recent investigations have been directed to evaluate natural sources such as plants or fungi, which can fight the bacteria. Here the antibacterial activity of spent substrate of *Pleurotus ostreatus* combined with medicinal plants was evaluated. We designed six mixtures (barley straw, barley straw/*Chenopodium ambrosioides* L., barley straw/*Mentha piperita* L., barley straw/*Rosmarinus officinalis* L., barley straw/*Litsea glaucescens Kunth* and barley straw/*Tagetes lucid Cav*) to be used as a substrate of cultivation of mushroom. These were recovered after the harvest. We obtained aqueous extracts from spent substrates and resuspended them to different concentrations (25, 50 and 100 mg mL^{-1}). These were tested for antibacterial activity against *Staphylococcus epidermidis*, *Bacillus subtilis* and *Escherichia coli*; as a positive control we used azithromycin, cephalexin and dicloxacillin. The protocol was a completely randomized assay with a factorial arrangement design. The data were analyzed with PROC GLM, SAS. The spent substrate from *Pleurotus ostreatus* that contained *Mentha piperita* L. presented the largest zone of inhibition against *Staphylococcus epidermidis* (40.00 mm) which was similar to control antibiotics (40.00 mm). Second in toxicity was the spent substrate from barley straw extract (33.33 mm). In conclusion, the results suggest that it is possible to use the spent substrate of *Pleurotus ostreatus* as source of extracts with antibacterial activity, being the best option the combination of barley straw with *Mentha piperita* L.

Keywords: mushroom, *Escherichia coli*, *Staphylococcus epidermidis*, *Bacillus subtilis*, medicinal plant

1. Introduction

The indiscriminate use of antimicrobials has generated serious problems on global health, since the bacteria have developed resistance to them, both in veterinary medicine as well as in human medicine, so the World Health Organization is considering infections caused by drug-resistant bacteria as an emergent global disease and a major public health problem. The development of novel therapeutic strategies, however, seems to have reached a dead end. Despite the urgent need to find new antibacterial products, many pharmaceutical companies have abandoned antibiotic drug discovery programs. For this reason, pharmacological alternatives have been sought, such as the use of fungi and medicinal plants (Roca et al., 2015).

Fungi have an important role in the degradation of organic matter (Chang & Miles, 1984). In addition these are a source of bioactive substances to produce antibiotics or pharmaceutical drugs, such as functional food and additives in feeding stuffs (Santoyo, Ramírez-Anguiano, Reglero, & Soler-Rivas, 2009). Cultured mushrooms (*Pleurotus ostreatus*), are a source of agricultural by products. Their organic waste can be used as a source of food with high protein content and as an alternative pharmaceutical treatment; *Pleurotus ostreatus* is a fungus that grows on agricultural wastes, accelerates biodegradation and recycling, and thus prevents trash burning and the subsequent environmental pollution (Varnero, Quiroz, & Álvarez, 2010).

Pleurotus ostreatus grows on a large number of substrates, amply used is straw, used as a source of carbon to

increase the nutritional value and palatability of the fruiting body; lately, production has been combined with substrates of aromatic or medicinal plants such as *Chenopodium ambrosioides* L., *Mentha piperita* L., *Rosmarinus officinalis* L., *Litsea glaucescens* Kunth and *Tagetes lucida* Cav, that also have antibacterial activity (Sánchez, 2010). The rapid worldwide growth in mushroom production has resulted in large quantities of spent substrate (about 13.6 million t year^{-1}). The massive amount of waste is an environmental problem and research to develop technologies for its treatment or use is underway (Lin, Ge, & Li, 2014).

Recently, there has been a need to study phytochemicals with antimicrobial potential to generate new pharmacological options. For this reason, it was decided to determine the antibacterial activity of the spent substrate from *Pleurotus ostreatus* combined or not with herbs (*Chenopodium ambrosioides* L., *Mentha piperita* L., *Rosmarinus officinalis* L., *Litsea glaucescens* Kunth and *Tagetes lucida* Cav). These spent substrates were tested against *Staphylococcus epidermidis*, *Escherichia coli* and *Bacillus subtilis*.

2. Method

2.1 Strain, Substrates and Cultivation Method

Substrate blocks (1 Kg) were obtained from Centro de Investigaciones Biológicas of the Universidad Autónoma del Estado de Hidalgo, México. The blocks were formed by a mixture of barley straw and medicinal plants (*Chenopodium ambrosioides* L., *Mentha piperita* L., *Rosmarinus officinalis* L., *Litsea glaucescens* Kunth and *Tagetes lucida* Cav); which were purchased in Central Abastos in Pachuca Hidalgo, México. The taxonomic identification was performed by Dr. Miguel Angel Villavicencio-Nieto; the specimens are deposited at the Herbarium of the Centro de Investigación de Ciencias Biológicas, of the Universidad Autónoma del Estado de Hidalgo, México. To form the substrate, the plants were dehydrated at room temperature in an area protected from dust, and mixed with barley straw (20:80), to be later colonized with mycelium of *Pleurotus ostreatus* UAEH-003 in solid substrate fermentation. Mushrooms were harvested at 40 d and spent substrate from each treatment was obtained.

2.2 Preparation of Organic Extracts

The extracts were obtained by mixing 300 g of spent substrate mushroom from each treatments and 150 mL distilled water. Then mix was macerated for 24 h using agitation. Then, it was filtered using gauze to separate solid and liquid parts, which were filtered through paper Whatman® #41. The extract was placed into a ball flask and water was evaporated (55 °C, 75 rpm) using a rotatory evaporator (Büchi R-215, Company Labor Technik, Flawil, Switzerland) adapted with a vacuum pump (V-700) and vacuum controller (V-855).

2.3 Test Organisms

The test organisms were *Staphylococcus epidermidis* ATCC12228, *Escherichia coli* ATCC25922 and *Bacillus subtilis* ATCC10718.

2.4 Antimicrobial Assay

In vitro antimicrobial activity was carried out by agar disc diffusion method. To perform the test the extracts were dissolved in water for obtain different concentrations. Then a 100 µL inoculum (*Escherichia coli*, 298×10^5 CFU mL^{-1}, *Staphylococcus epidermidis* 128×10^5 CFU mL^{-1} or *Bacillus subtilis* 350×10^5 CFU mL^{-1}), was spread on the surface of the agar plate prepared for the growth of bacteria, incubated at 37 °C (Forma Series II Water Jacket CO_2, Incubator, Model 3100, Thermo Scientific, USA) during 24 h. Disc (6 mm diameter) contain different concentrations (25, 50 or 100 mg mL^{-1}) was placed on the surface of the inoculated media. Negative controls were prepared with distilled water; positive controls were azithromycin, cephalexin and dicloxacillin (Sigma, Aldrich) with the same concentrations that extracts. Antimicrobial activity was then determined by measuring the diameter of inhibition generated around each disc, and express in millimeter.

2.5 Statistical Analysis

Data were analyzed (factorial design 3 × 3 and blocked by extract type and extract concentration and bacterial strain as factors; a PROC GLM procedure and LSMEANS option) using SAS statistical software (2002).

3. Results

Antibacterial activity of spent substrate of *Pleurotus ostreatus* extracts combined or not with herbs against *Escherichia coli*, *Staphylococcus epidermidis* and *Bacillus subtilis* is show in Table 1; the tested antibacterial activity was quantitatively assessed measuring the diameter of inhibition generated for each sample. The results showed that the spent substrate extracts combined with *Menta piperita* L., presented greater inhibitory effect (23.37 mm) followed by the spent substrate without herbs (16.22 mm). In the case of spent substrate extracts combined with *Litsea glauescens* Kunth, *Chenopodium ambroioides* L., *Tagetes lucida* Cav. and *Rosmarinus*

officinalis L. inhibition was lower (12.22, 12.69, 10.74 and 6.85 mm, respectively) than the treatment, which only contained barley straw (16.22 mm). Positive controls azithromycin, cephalexin and Dicloxacillin were larger diameter of inhibition (39.35, 42.80 and 35.31 mm, respectively), than all spent substrate extracts combined or not with herbs.

Antibacterial activity of spent substrate *Pleurotus ostreatus* extracts combined or not with herbs against different bacterial strains is show in Table 2; the tested bacterial was quantitatively assessed by measuring the diameter of inhibition generated for each sample. The results showed that the spent substrate extracts combined with *Menta piperita* L., presented highest inhibitory effect against *Staphylococcus epidermidis* and *Escherichia coli* (32.78 and 22.33 mm, respectively). The spent substrate extracts combined with *Chenopodium ambroioides* L. and *Litsea glauescens* Kunth showed the greater inhibition against *Escherichia coli* (16.67 and 16.94 mm, respectively) and *Chenopodium ambroioides* L., *Litsea glauescens* Kunth and *Tagetes lucida* Cav showed the highest inhibition against *Bacillus subtilis* (13.61, 15.83 and 14.72 mm, respectively); the barley straw extract showed greater inhibition against *Staphylococcus epidermidis* (25.27 mm). Positive controls azithromycin, cephalexin and dicloxacillin were larger diameter of inhibition for three bacterial strains, than all spent substrate extracts combined or not with herbs.

Antibacterial activity of spent substrate extracts from *Pleurotus ostreatus* cultures combined or not with herbs at different concentrations is shown in Table 3; the tested antibacterial activity was quantitatively assessed by measuring the diameter of growth inhibition generated for each sample. The results showed that the spent substrate extracts barley straw and barley straw combined with *M. piperita* L. presented highest inhibitory effect against *Staphylococcus epidermidis* (33.33, 40.00 mm, respectively) a 50 mg mL^{-1}, similar to the positive controls to the same concentration (dicloxacillin 46.67 mm and cephalexin 40.00 mm). For *E. coli* greater inhibition spent substrate extracts combined with *M. piperita* L. were at 50 mg mL^{-1} (29.17 mm) and spent substrate extracts combined with *Chenopodium ambroioides* L. and *Litsea glauescens* Kunth were at concentration of 100 mg mL^{-1} (18.33 and 20.00 mm, respectively). For *Bacillus subtilis* greater inhibition spent substrate extracts combined with *M. piperita* L. were at 25 mg mL^{-1} (25.00 mm) and spent substrate extracts barley straw or combined with *Chenopodium ambroioides* L. and *Tagetes lucida* Cav were at concentration of 100 mg mL^{-1} (18.50, 15.83 and 17.50 mm, respectively).

Table 1. *In vitro* antibacterial activity of spent substrate of *Pleurotus ostreatus* extracts combined or not with herbs against *Escherichia coli*, *Staphylococcus epidermidis* and *Bacillus subtilis*

Treatment	Diameter of inhibition (mm)
Barley straw	16.22^{e}±0.62
Barley straw/*C. ambroioides* L.	12.69^{f}±0.62
Barley straw/*M. piperita* L.	23.37^{d}±0.62
Barley straw/*L. glauescens* Kunth	12.22^{f}±0.62
Barley straw/*T. lucida* Cav	10.74^{f}±0.62
Barley straw/*R. officinalis* L.	6.85^{g}±0.62
Azithromycin	39.35^{b}±0.62
Cephalexin	42.80^{a}±0.62
Dicloxacillin	35.31^{c}±0.62

Note. ^{abcdefg} Literal different ranks indicate significant differences ($P < 0.0001$) with the Tukey test.

Table 2. *In vitro* antibacterial activity of spent substrate *Pleurotus ostreatus* extracts combined or not with herbs against different bacterial strains

Treatment	Diameter of inhibition (mm)		
	B. subtilis	*E. coli*	*S. epidermidis*
Barley straw	10.61^{b} ±1.08	12.78^{b} ±1.08	25.27^{a} ±1.08
Barley straw/*C. ambroioides* L.	13.61^{a} ±1.08	16.67^{a} ±1.08	7.78^{b} ±1.08
Barley straw/*M. piperita* L.	15.00^{c} ±1.08	22.33^{b} ±1.08	32.78^{a} ±1.08
Barley straw/*L. glauescens* Kunth	15.83^{a} ±1.08	16.94^{a} ±1.08	3.88^{b} ±1.08
Barley Straw/*T. lucida* Cav	14.72^{a} ±1.08	9.17^{b} ±1.08	8.33^{b} ±1.08
Barley straw/*R. officinalis* L.	11.39^{a} ±1.08	9.17^{a} ±1.08	0.00^{b} ±1.08
Azithromycin	36.11^{b} ±1.08	31.94^{c} ±1.08	50.00^{a} ±1.08
Cephalexin	43.33^{a} ±1.08	39.72^{b} ±1.08	45.55^{a} ±1.08
Dicloxacillin	23.33^{c} ±1.08	34.83^{b} ±1.08	47.78^{a} ±1.08

Note. ^{abcdefg} Literal different between columns indicate significant differences ($P < 0.0001$) with the Tukey test.

Table 3. *In vitro* antibacterial activity of spent substrate of *Pleurotus ostreatus* extracts combined or not with herbs at different concentrations

Treatment	Concentration (mg mL^{-1})	Diameter of inhibition (mm)		
		B. subtilis	*E. coli*	*S. epidermidis*
Barley straw	25	0.00^{b}±1.87	13.33±1.87	27.50^{a}±1.87
	50	13.33^{a}±1.87	13.33±1.87	33.33^{a}±1.87
	100	18.50^{a}±1.87	11.67±1.87	15.00^{b}±1.87
Barley straw/*C. ambroioides* L.	25	10.00±1.87	15.00±1.87	9.17±1.87
	50	15.00±1.87	16.67±1.87	9.17±1.87
	100	15.83±1.87	18.33±1.87	5.00±1.87
Barley straw/*M. piperita* L.	25	25.00^{a}±1.87	20.00^{b}±1.87	18.33^{b}±1.87
	50	10.00^{b}±1.87	29.17^{a}±1.87	40.00^{a}±1.87
	100	10.00^{b}±1.87	17.83^{b}±1.87	40.00^{a}±1.87
Barley straw/*L.glauescens* Kunth	25	15.83±1.87	15.83±1.87	0.00^{b}±1.87
	50	15.00±1.87	15.00±1.87	0.00^{b}±1.87
	100	16.66±1.87	20.00±1.87	11.66^{a}±1.87
Barley straw/*T. lucida* Cav	25	13.33±1.87	0.00^{b}±1.87	5.83^{b}±1.87
	50	13.33±1.87	13.33^{a}±1.87	5.00^{b}±1.87
	100	17.50±1.87	14.17^{a}±1.87	14.17^{a}±1.87
Barley straw/*R. officinalis* L.	25	10.00^{b}±1.87	0.00^{c}±1.87	0.00±1.87
	50	10.00^{b}±1.87	12.50^{b}±1.87	0.00±1.87
	100	14.17^{a}±1.87	15.00^{a}±1.87	0.00±1.87
Azithromycin	25	36.66 ±1.87	30.00±1.87	50.00±1.87
	50	31.66±1.87	32.50±1.87	50.00±1.87
	100	40.00±1.87	33.33±1.87	50.00±1.87
Cephalexin	25	40.00^{b}±1.87	40.00±1.87	50.00^{a}±1.87
	50	40.00^{b}±1.87	39.17±1.87	40.00^{b}±1.87
	100	50.00^{a}±1.87	40.00±1.87	46.67^{a}±1.87
Dicloxacillin	25	15.83^{b}±1.87	34.17±1.87	46.67±1.87
	50	17.50^{b}±1.87	36.67±1.87	46.67±1.87
	100	36.67^{a}±1.87	33.67±1.87	50.00±1.87

Note. ^{abcdefg} Literal different ranks indicate significant difference between the concentration of treatments ($P < 0.0001$) with the Tukey test.

4. Discussion

In recent decades, bacteria have become multi-resistant to antibiotics (Nehra, Meenakshi, & Yadav, 2012). This has led to the search for alternative treatments, such as medicinal plants and fungi to counteract this resistance. The oils obtained from medicinal plants have shown to be a viable alternative since they exhibit bacteriostatic activity against *Staphylococcus* sp; likewise, hydroalcoholic extracts from leaves and stems of *Rosmarinus officinalis* L., *Chenopodium ambrosioides* L., *Laurus nobilis* and *Tagetes minuta* are bactericidal activity against Gram-positive (*S. epidermidis*, *B. subtilis*) and Gram-negative (*E. coli*) bacteria (Wang, Li, Luo, Zu, & Efferth, 2012; Petrolini et al., 2013; Chmit et al., 2014; Shirazi, Gholami, Kavoosi, Rowshan, & Tafsiry, 2014; Ye et al., 2015). Edible fungi such as *Pleurotus ostreatus* have shown anti-inflammatory and antibacterial activity, and a high nutritional value as food; however, most of the research has been based on the study of the fruiting body and not in extracts (Krishnamoorthy & Sankaran, 2014). We show that aqueous extract of spent substrate from *Pleurotus ostreatus* cultures combined with barley straw has antibacterial activity against *S. epidermidis*, showing a greater inhibition at a concentration of 50 mg mL^{-1} (33.33 mm) and intermediate antibacterial activity at a concentration of 50 mg mL^{-1} against *E. coli* and *B. subtilis* (13.33 mm), similar to that obtained using extracts of mushroom *Pleurotus ostreatus* obtained with different organic solvents (24.56 and 14 mm) for Gram positive and Gram negative bacteria (Nehra et al., 2012); It has a concentration of 25 mg mL^{-1} not be found antibacterial activity against *B. subtilis*.

In the case of spent substrate *Pleurotus ostreatus* extracts combined with *Chenopodium ambrosioides* L., the antibacterial activity was lower compared to extracts combined with barley straw; none of the three concentrations exhibited antibacterial activity, even though this plant has antiparasitic properties (Avila-Blanco, Rodríguez, Moreno-Duque, Muñoz-Ortega, & Ventura-Juárez, 2014; Ye et al., 2015) and anti-inflammatory activity (Trivellato Grassi et al., 2013). Extracts combined with *Litsea glaucescens* Kunth and *Tagetes lucida* Cav showed the same antibacterial activity against *B. subtilis* and *E. coli*, that combined with barley straw, but were less active against *S. epidermidis*, which contrasts with the results reported in experiments using oils obtained from such plants, which showed a higher zone of inhibition (25.4 mm) for Gram-positive and Gram-negative bacteria (Dadalioglu & Evrendilek, 2004; Shirazi et al., 2014; Evrendilek, 2015). Spent substrate *Pleurotus ostreatus* extracts did not inhibit *S. epidermidis* growth; but a concentration of 100 mg mL^{-1}, showed antibacterial activity against *E. coli* and *B. subtilis*, with a zone of inhibition of 14.17 mm and 15.00 mm, respectively, which contrasts with previous reports where the extract of the plant, which showed a zone of inhibition of 8 mm for *E. coli* and no effect against *B. subtilis* (Mathlouthi et al., 2012).

Substrate spent extracts from *Pleurotus ostreatus* cultures that contained *Mentha piperita* L. showed the highest antibacterial activity at a concentration of 50 and 100 mg mL^{-1} with inhibition zone of 40.00 mm against *S. epidermidis*, at a concentration of 50 mg mL^{-1} with a zone of inhibition of 29.17 mm against *E. coli* and 25 mg mL^{-1} with a zone of inhibition of 25.00 mm against *B. subtilis*, similar to what reported when using oil or methanolic extracts, with a zone of inhibition of 17.20 mm for *S. aureus* and 5.1 mm for *E. coli* (McKay & Blumberg, 2006; Singh, Shushni, & Belkheir, 2015), the highest inhibition displayed on our results may suggest a process of synergy between *Mentha piperita* L. and barley straw.

The combination of medicinal plants with barley straw as a substrate for the cultivation of *Pleurotus ostreatus*, led to the production of spent substrates containing antibacterial activity. These findings suggest that it is feasible to use these substrates to obtain antibacterial compounds and at the same time reduce the pollution produced by spent substrate accumulation. In addition, including these medicinal plants in the substrate for *Pleurotus ostreatus* opens the possibility of improving the palatability and nutraceutical properties of edible fungi.

References

Ávila-Blanco, M. E., Rodríguez, M. G., Moreno, J. L. D., Muñoz-Ortega, M., & Ventura-Juárez, J. (2014). Amoebicidal activity of essential oil of *Dysphania ambrosioides* (L.) Mosyakin & Clemants in an Amoebic Liver Abscess Hamster Model. *Evidence-Based Complementary and Alternative Medicine*, *2014*, 930208. http://dx.doi.org/10.1155/2014/930208

Chang, S. T., & Miles, P. G. (1984). A new look at cultivated mushrooms. *BioScience, 34*, 358-362. http://dx.doi.org/10.2307/1309726

Chmit, M., Kanaan, H., Habib, J., Abbass, M., Mcheik, A., & Chokr, A. (2014). Antibacterial and antibiofilm activities of polysaccharides, essential oil, and fatty oil extracted from Laurus nobilis growing in Lebanon. *Asian Pacific Journal of Tropical Medicine, 7S1*, S546-552. http://dx.doi.org/10.1016/S1995-7645(14)60288-1

Dadalioglu, I., & Evrendilek, G. A. (2004). Chemical compositions and antibacterial effects of essential oils or Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas* L.) and fennel (*Foeniculum vulgare*) on common foodborne pathogens. *Journal of Agricultural and Food Chemistry, 52*, 8255-8260. http://dx.doi.org/10.1021/jf049033e

Deepalakshmi, K., & Mirunalini, S. (2014). *Pleurotus ostreatus*: An oyster mushroom with nutritional and medicinal properties. *Journal of Biochemical Technology, 5*, 718-726.

Evrendilek, G. A. (2015). Empirical prediction and validation of antibacterial inhibitory effects of varios plant essential oils on common pathogenic bacteria. *International Journal of Food Microbiology, 202*, 35-41. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.02.030

Lin, Y., Ge, X., & Li, Y. (2014). Solid state anaerobic co-digestion of spent mushroom substrate with yard trimmings and wheat straw for biogas production. *Bioresource Technology, 169*, 468-474. http://dx.doi.org/10.1016/j.biortech.2014.07.020

Mathlouthi, N., Bouzaienne, T., Oueslati, I., Recoquillay, F., Hamdi, M., Urdaci, M., & Bergaoui, R. (2012). Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: *in vitro* antimicrobial activities and effects on growth performance. *Journal of Animal Science, 90*, 813-823. http://dx.doi.org/10.2527/jas.2010-3646

McKay, D. L., & Blumberg, J. B. (2006). A Review of the Bioactivity and potential health benefits of Peppermint Tea (*Mentha piperita* L.). *Phytotherapy Research, 20*, 619-633. http://dx.doi.org/10.1002/ptr.1936

Nehra, K., Meenakshi, M. K., & Yadav, A. (2012). Evaluation of antimicrobial potential of fruiting body extracts of Pleurotus ostreatus (oyster mushroom). *International Journal of Microbial Resource Technology, 1*, 391-400. Retrieved from http://ijmrt.inpressco.com

Petrolini, F. V., Lucarini, R., de Souza, M. G., Pires, R. H., Cunha, W. R., & Martins, C. H. (2013). Evaluation of the antibacterial potential of Petroselinum crispum and Rosmarinus officinalis against bacteria that cause urinary tract infections. *Brazilian Journal of Microbiology, 44*, 829-834. http://dx.doi.org/10.1590%2FS1517-83822013005000061

Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., ... Vila, J. (2015). The global threat of antimicrobial resistance: Science for intervention. *New Microbes and New Infections, 6*, 22-29. http://dx.doi.org/10.1016/j.nmni.2015.02.007

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbiology and Biotechnology, 85*, 1321-1337. http://dx.doi.org/10.1007/s00253-009-2343-7

Santoyo, S., Ramírez-Anguiano, A., Reglero, G., & Soler-Rivas, C. (2009). Improvement of the antimicrobial activity of edible mushroom extract by inhibition of oxidative enzymes. *International Journal of Food Science & Technology, 44*, 1057-1064. http://dx.doi.org/10.1111/j.1365-2621.2008.01896.x

SAS Institute Inc. (2002). *SAS User's guide* (Stat, version 6.03). SAS Institute Inc., Cary, NC, USA.

Shirazi, M. T., Gholami, H., Kavoosi, G., Rowshan, V., & Tafsiry, A. (2014). Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetes minuta* and *Ocimum basilicum* essential oils. *Food Science & Nutrition, 2*, 146-155. http://dx.doi.org/10.1002%2Ffsn3.85

Singh, R., Shushni, M. A. M., & Belkheir, A. (2015). Antibacterial and antioxidant activities of *Mentha piperita* L. *Arabian Journal of Chemistry, 8*, 322-328. http://dx.doi.org/10.1016/j.arabjc.2011.01.019

Trivellato Grassi, L., Malheiros, A., Meyre-Silva, C., da Silva, Z. B., Dalmarco, E. M., Fröde, T. S., ... de Souza, M. M. (2013). From popular use to pharmacological validation: A study of the anti-inflammatory, anti-conceptive and healing effects of *Chenopodium ambrosioides* extract. *Journal of Ethnopharmacology, 145*, 127-138. http://dx.doi.org/10.1016/j.jep.2012.10.040

Varnero, T. M., Quiroz, M. S., & Álvarez, C. H. (2010). Utilización de residuos forestales lignocelulósicos para producción del hongo ostra (*Pleurotus ostreatus*). *Información Tecnológica, 21*, 13-20. http://dx.doi.org/10.4067/S0718-07642010000200003

Wang, W., Li, N., Luo, M., Zu, Y., & Efferth, T. (2012). Antibacterial Activity and Anticancer Activity of *Rosmarinus officinalis* L. Essential Oil Compared to That of Its Main Components. *Molecules, 17*, 2704-13. http://dx.doi.org/10.3390/molecules17032704

Ye, H., Liu, Y., Li, N., Yu, J., Cheng, H., Li, J., & Zhang, X. Z. (2015). Anti-Helicobacter pylori activities of

Chenopodium ambrosioides L. *in vitro* and *in vivo*. *World Journal of Gastroenterology, 21*, 4178-4183. http://dx.doi.org/10.3748/wjg.v21.i14.4178