Egypt. J. Soil Sci. **Vol. 54**, No. 3, pp. 229 -235 (2014)

Anti-microbial and Anti-oxidant Agents in Mushroom Fruit Bodies

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POTENTIAL drugs from mushrooms are useful for mankind. The scientific basis for their traditional medicinal uses, the anti-microbial activities of the crude polysaccharide extract from the mushroom *Pleurotus ostreatus* were investigated. In this study, ethanol and methanol were tested for extraction of mushroom fruit bodies. Ethanolic and methanolic extracts of fungal fruit bodies showed somewhat low antagonism towards *Staphylococcus aureus* and *Salmonella typhi*. No anti-biosis due to either ethanolic or methanolic extracts was detected against *Enterobacter faecalis* or *Lactobacillus bulgaricus.* The fungal members *Aspergillus flavus* and *Candida albicans*, as well, did successfully withstand the existence of three extracts with no apparent inhibition. Total phenols and ascorbic acid contents as antioxidant components in the mushrooms fruit bodies were also detected.

Keywords: Mushroom, Anti-biosis, Anti-oxidants.

The anti- microbial activity of mushrooms have been known since 1946, when Bose isolated pollporin, a compound active against Gram-positive and Gram-negative bacteria with no toxicity to experimental animals. Recently studies of Smania *et al*. (1995, 1997) showed that the basidomycete *Pycnoporus sanguineus* produces cinnabarine, an orange pigments active against wide species of pathogenic bacteria. As mentioned by Chang (1999), edible and medicinal mushroom fungi not only convert huge cellulose biomass waste into human food, but also most remarkably can produce notable pharmaceutical substances. Some of these substances contain several anti-bacterial and anti-tumor polysaccharides (Mizuno, 1999). The workers (Azoro, 2002, Stowe, 2003 and Shcherba & Babitskaya, 2004) recorded high concentration of the active principle polysaccharides, terpenoids, alkaloids, tannins and phenols in the fruit body of *Pleurotus tuberregium*. Mushrooms also contains appreciable amounts of niacin, pantothenic acid and biotin (Subramanian, 1986). The recent study of Scherba and Babitskaya (2004) on carbohydrates of submerged mycelium showed that the qualitative composition of Xylographie basidomycetes were mainly structural polysaccharides which were hetero- and homo-glycans. Phenolics are among the major groups of non-essential dietary components that have been associated with the inhibition of cancer (Williams and Iatropoulos, 1997). The bio-activity of phenolics might be related to their ability to chelate metals, inhibit lipoxgenase

and scavenge free radicals (Decker, 1997). Ascorbic acid is also among the components responsible for the antioxidants properties

The aim of this work was to identify the anti-microbial and anti-oxidant properties of mushroom fruit bodies.

Material and Methods

Growth media

Garlic (*Allium sativum* L.), jasmine (*Jasminum officinale* L.) and basil (*Ocimum basilicum* L.) wastes as well as rice straw were chopped into small pieces of 2-3 cm long, soaked in water up to 75% moisture content. Polyethylene bags (25 x 35 cm) were filled with 100g dry weight substrate, tied up and autoclaved at 120 ^{o}C for one hour. The different mixture of rice straw : waste were prepared (w/w), 100:0, 75:25, 50:50 and 25 75%, as media for growing *Pleutrotus ostreatus* mushroom.

Preparation of mushroom fruit bodies extracts

At maturity stage, the fruit bodies of *Pleurotus ostreatus* from the various experimental treatments were prepared as powder samples, the prepared samples of each treatment were extracted with both methanol and ethanol alcohols for assessment the anti-oxidant activity using a modified procedure of Yang *et al*. (2002). Dried samples (10g) were stirred with 10 ml of alcohol at 150 rpm for 24 hr and filtered through Whatman No. 4 filter paper. The residue was re-extracted with two additional 100 ml alcohol as previously mentioned. The combined extracts were then rotary-evaporated at 40^{o}C to dryness. The dried product was used as such for anti-oxidant component analysis and/or re-dissolved in alcohol to a concentration of 50 ml and stored at 4^{o}C. Samples of water extracts were also prepared as control.

Anti-oxidant and anti-microbial effects of Mushroom extracts

Ascorbic acid and total phenols as anti-oxidants were determined according to Klein and Perry (1982) and Taga *et al*. (1984), respectively. The anti-microbial activity of mushroom frut bodies extracts were monitored against a number of microbes selected on the basis of their pathogenicity, in general. The *Enterococcus faecalis*, *Lactobacillus bulgaricus, Salmonella typhi* and *Staphilococcus aureus,* in addition to same fungal strains *Aspergillus niger, Aspergillus flavus* and *Candian albicans*. The bacterial strain *E. faecalis* and *L. bulgaricus* were grown and maintained on Eliker medium (Dave and Shah, 1996) while nutrient agar medium was used for *Salmonella typhi* and *Staphilococcus aureus.* The potato dextrose-agar medium was adopted for either *A. niger, A. flavus* and/or *Candian albicans.*

The crude ethanolic, methanolic and aqueous polysaccharide extracts were obtained from the mushroom powdered samples using the method described by Mizuno (1999). Anti-microbial assay was done adopting the agar-disc diffusion

technique (Rasoanaiva and Ratsimamanga-Urveg, 1993). After 18 hr incubation at 37 ^{o}C, the inhibition zone diameters were measured.

Results and Discussion

Mushrooms have long been appreciated for their flavor and texture. Nowadays, they are recognized as a nutritious food as well as an important source of biologically active compounds of medicinal value (Breene, 1990). These microorganisms accumulate a variety of secondary metabolites including phenolic, terpenes and steroids compounds. Therefore, the ant-oxidative activity of the mushroom *Pleurotus ostreatus* in relation to its phenolic and ascorbic acid contents was measured. The results showed that the fungal candidate did possess an anti-oxidant activity that protects against oxidative stress which provide a pharmaceutical explanation for uses of *Pleurotus ostreatus* in folk medicine in Egypt. In addition, bacterial and three fungal strains were examined for their susceptibility to ethanolic, methanolic and aqueous polysaccharide extracts of the mushroom fruit bodies. Also the obtained results showed no antibiosis to either ethanolic or methanolic extracts against *Enterobacter faecalis* or *Lactobacillus bulgaricus.* The fungal members *Aspergillus flavus* and *Candian albicans*, as well, did successfully withstand the presence of the extracts with no apparent inhibition. *Staphilcoccus aureus* seemed somewhat sensitive in *comparison* with Salmonella *typhi* to the alcohols extracts (Table 1). Aqueous extracts of the mushroom fruit bodies showed no antibiosis against either tested oraganisms. This in genral, indicates that pleurotus ostreatus contained active principles which justify its use to treat some diseases associated with bacterial infections as reported by many researches (Fasidi and Ekuere, 1993 and Fasidi & Olorunmaiye, 1994). Ezeronye *et al*. (2005) investigated the anti- bacterial effect of crude polysaccharide extracts of *Pleurotus tuberregium* on some bacterial pathogens. They reported that ethanolic polysaccharide extracts of the mushroom fruit bodies exhibited inhibition zone of 19, 21, 23 and 7 mm on *Enterobacter faecalis*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi.* The aqueous extracts did not produce any observable inhibition zone. The inability of ethanolic and methanolic extracts to inhibit some other tested microorganisms using the agar-disc diffusion technique may be due to easy evaporation of the components on paper discs or that the quantity of extracts absorbed by the paper was too small. Even in the agar-cup technique (Ezeronye *et al*., 2005) the concentration is an important factor, since the higher concentration gives wider zones of inhibition. They added that the spectrum of anti-bacterial activity of the crude polysaccharide extracts of the *P. tuberregium* fruit body is comparable to that of gentamycin.

Total phenols and ascorbic acid as the major naturally occurring anti-oxidant component in the mushroom fruit body were found in the methanolic extract in concentration varying from 15.5 to 208.2 ug ml^{-1} and from 183.3-342.2 ug ml^{-1}, respectively (Table 2). The findings of Lo and Cheung (2004) exhibited a strong positive correlation between the total phonolic content and anti-oxidant activity in the methanol crude extract of the mushroom *Agrocybe aegerita* var.*alba.*

TABLE 1. Sensitivity of *Staphilococcus aureus* and *Salmonella typhi* strains to ethanolic and methanolic extracts of the mushroom fruit bodies developed on different growth media.

Substrates	Ethanol		Methanol	
	St.aureus	***S.typhi***	***St.aureus***	***S.typhi***
Mono-substrate				
Rice straw (R	3*	-	4	6
Basil(B)	3	-	4	-
R:W mixtures %ً				
R:B (75:25)	-	-	-	-
R:B (50:50)	2	-	3	-
R:B (25:75)	-	-	2	-
R:J (75:25)	3	-	-	-
R:J (50:50)	-	-	2	-
R:J (25:75)	-	-	2	-
R:G (75:25)	-	-	-	-
R:G (50:50)	-	-	2	3
R:G (25:75)	1	4	2	3

B: Basil, G: Garlic, J: Jasmine, W: waste.
*Inhibition zone diameter in mm
-No effect.

TABLE 2. Contents of total phenols and ascorbic acid in methanolic exrract of *Pleurotus ostreatus* fruit bodies developed on different growth media (ug ml^{-1}).

Substances	Total phenols	Ascorbic acid
Mono-substrate		
Rice straw (R	15.5	183.3
Basil(B)	134.5	230.9
R:W mixtures %ً		
R:B (75:25)	23.4	203.2
R:B (50:50)	32.5	213.7
R:B (25:75)	208.2	342.2
R:J (75:25)	50.6	253.5
R:J (50:50)	49.5	279.9
R:J (25:75)	nd	nd
R:G (75:25)	82.4	302.4
R:G (50:50)	71.0	232.3
R:G (25:75)	nd	nd

nd: not determined.

In conclusion, based on our data, *Pleurotus ostreatus* could be an appropriate organism to cope with the difficult task of remediating a number of medicinal and aromatic plant wastes. This is possible not only by elevating some toxic load and facilitating further biological treatment and exploitation of these pollutants, by yielding other products of important value. The findings of this preliminary work emphasize the need for further research to evaluate other species that have potential for bio-converting plant wastes into most appropriate and bio-available pruducts

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(*Received* 4/12/2013;
accepted 17/ 8/2014

العوامل المضادة للميكروبات والمضادة للاكسدة فى فطر المشروم

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يحتوى فطر عيش الغراب على خواص طبية غاية فى الاهمية لبنى الانسان حيث تستخدم هذه المركبات كعلاج وكذلك تأثيرها كمواد مضادة للميكروبات قد تم دراستها .وتم استخلاص هذه المركبات بواسطة كحول الايثانول وكذلك كحول الميثانول من مستخلص عديدى السكريات لفطر *Pleurotus otreatus* . وهذه الكحولات تعرف كمذيبات عضويه لها قدرة عاليه على استخلاص المكونات العضويه مقارنة بالماء كمستخلص . وهذه المستخلصات الكحوليه بنوعيها كان لها تأثير مضاد محدود على ميكروبى *Staphylococcus aureus* , *Salmonella typhi* ولكنها غير ذات تأثير على ميكروبى *Enterobacter faecalis* and *Lactobacillus bulgaricus* ايضا لم تظهر المستخلصات الكحولية او المستخلص المائى اى تأثير مضاد على فطريات *Candida albicans* and *Aspergillus flavus* و *Aspergillus niger*. محتوى المستخلصات من الفينول الكلى وحمض الاسكوربك كمركبات مضادة للاكسدة قد تم ايضا دراستها فى مستخلص كحول الميثايل وقد اظهرت النتائج احتوائها على هذه المركبات بكميات محسوسه اختلفت باختلاف نسبة المكونات فى بيئة نمو الفطر.

مما سبق يتضح ان فطر المشروم *Pleurotus otreatus* يحتوى على العديد من المركبات ذات الفائدةالعلاجية والدوائية ومن ثم كان الموضوع يحتاج لمزيد من الدراسة.