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Antimicrobial and Antioxidant Potentials of *Agaricus bisporus*

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Abstract: *Agaricus bisporus* is an edible macrofungus, the methanolic extract of *A. bisporus* exhibited antimicrobial properties against some tested pathogenic bacteria of public health importance. Inhibition zone ranges from 4.33 ± 0.58 to 9.00 ± 1.00mm at 100mg/ml as Minimum Inhibitory Concentration (MIC) for the entire tested organism except *S. dysenterae 006*. The antioxidant activity was analyzed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (OH^-) radical scavenging assays. The extract showed more scavenging activity on DPPH (IC_{50} = 0.139) more than OH^- (IC_{50} = 0.149). The phenolics composition of *A. bisporus* methanolic extracts was analyzed by High Performance Liquid Chromatography (HPLC) and found to contain rutin, gallic acid, caffeic acid and catechin which contributed to the antimicrobial and antioxidant activity. Total phenol (280-480mg/l) and ascorbic acid (2.0±1.0mg/g) contents also contributed to its antioxidant activity. *A. bisporus* is a natural source of antioxidant and antimicrobial agent against the tested organisms and had a potential as anticancer.

Key words: Antimicrobial · Antioxidant · *Agaricus bisporus*

INTRODUCTION

Free radicals, including the superoxide radicals ($O2^-$), hydroxyl radical (OH), hydrogen peroxide(H2O2) and lipid peroxide radicals have been implicated in a number of diseases processes, including asthma, cancer, cardiovascular disease, cataracts, diabetics, gastrointestinal inflammatory diseases, liver disease, macular degeneration, periodontal disease and other inflammatory processes. These reactive oxygen species (ROS) are produced as a normal consequence of biochemical processes in a body and as a result of increased exposure to environmental and/or dietary xenobiotics [1-4]. Therapy using free radical scavengers (antioxidants) has potential to prevent, delay or ameliorate many of these disorders [5]. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to non-radical forms and functions as natural anti-oxidant in human body. Due to depletion of immune system natural anti-oxidant in different maladies, consuming anti-oxidant as free radical scavengers may be necessary [6-8]. Currently available synthetic antioxidant like Butylated Hydroxyl Anisone (BHA), Butylated Hydroxyl Toluene (BHT), tertiary butylated hydroquinone and gallic acid esters, has been suspected to prompt or cause negative health effects. Hence, strong restriction has been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, this synthetic antioxidant also shows low solubility and moderate antioxidant activity [9, 10]. Antimicrobial activities of several mushroom has been documented [11, 12]. Metabolite such as phenolics haboured by most macrofungus has been responsible for their antimicrobial and antioxidant activity of mushrooms. The antioxidant capacity of phenolic compounds as compared to gallic acid standard has been documented [13]. Phenolic compound could inhibit low density lipoprotein oxidation [14]. Total phenol inhibits occurrence of atherosclerosis and cancer [15]. Enzymes like superoxide dismutase and catalase and compounds such as tocopherol and ascorbic acid can protect organisms against free radical damage [16]. The extractable products from medicinal mushrooms, designed to supplement the human diet not as regular food, but as

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the enhancement of health and fitness, can be classified into the category of dietary supplements/mushroom nutriceuticals [17]. This study therefore investigates the *in vitro* antimicrobial activity of methanolic extract of *A. bisporus* using the agar well diffusion method against some food borne and clinical pathogens isolates. Its phenolic compositions and other antioxidant compound along with the *in vitro* antioxidant activity were also determined.

MATERIALS AND METHODS

Chemicals, Reagents and Media: All chemicals listed were of analytical grade and freshly prepared before use. Ethylenediaminetetraacetic acid (EDTA), folin-ciocalteu's (analytical grade-baker analyzed) phenol reagent, 2,2-diphenyl-1-picrylhydrazyl, dimethylsufoxide (DMSO) (analytical grade-kermel), gallic acid, butylated hydroxyl anisone (BHA), 2,6-di-tert-butyl-4-methyphenol (BHT), α-tocopherol, methanol (Analytical grade-BDH), ethanol (Merck), ferric chloride (Sigma), sodiumcarbonate, Tris-HCl buffer, Thiobabituric acid (TBA) (TCA-analytical grade Riedel-de-Haen). Mueller Hinton agar (MHA) (oxoid), Tripticase Soy Broth (TSB), Nutrient broth and Sauboraud dextrose agar.

Source of *A. bisporus:* *A. bisporus* was obtained from a cabbage dump beside a flowing stream in Ogugu, Kogi State, Nigeria and identified to be *A. bisporus* by group of Mycologist.

Preparation of Methanolic Extract of *A. bisporus:* Fresh macrofungus was collected and dried at room temperature; it was then grounded into powder with the aid of a blender after reducing it to blendable size by a mortar and pestle. The materials were then loaded into soxhlet apparatus and extracted in methanol at 60°C for 4 hours. The methanolic extract was then filtered and concentrated using a rotary evaporator (RE 300) at 50°C for 2 hours. Extracts recovered from rotary evaporator was then lyophilized in vacuo and stored at 4°C before use.

Test Organisms: Test organisms include, some selected foodborne and clinical pathogens of public health concern such as; *Pseudomonas aeruginosa, Escherichia coli* ATCC 25922, *Shigella flexneri* 004, *Shigella dysenterae* 006, *Bacillus cereus, Salmonella typhi, Candida albicans* and *Listeria monocytogenes. L. monocytogenens* was grown in Tripticase Soy Broth (TSB) while the rest organisms were grown in nutrient broth and were standardized using 0.5 McFarland standards. All organisms were obtained as pure isolates from Nigerian Institute of Medical Research (NIMR).

Agar Well Diffusion Method: Petridishes with 10ml 0f MHA was prepared and inoculated with 100µl of respective standardized culture suspension in broth. The well were made with the use of cork borer and the extracts which were dissolved in DMSO in varying concentrations of 100, 200 and 300mg/ml were added to the inoculated plate. Tetracycline (250mg) Nystatin (200mg) dissolved in 1ml of DMSO was used as a reference value and pure DMSO was used as a control. The entire preparation was carried out under PCR8 Recirculating Laminar Flow Preparation Station before incubating in an incubator (Electro-thermal incubator model-DNP) for 24 hours. After incubation, inhibition zones were measured from the edge of the well to the edge of the zone in millimeter with a venier caliper.

HPLC Analysis Condition: The HPLC system consist of a shimadzu LC-10ADVP pump, a 20µl sample loop, SCL-10AVP. UV-DAD detector and analysis were carried out using Agilent Zorbax Eclipse XDB-C18 column (4.6 × 250 mm, 5 ìm). The mobile phase was 3% acetic acid/methanol at a flow rate of 0.8 ml/min and UV detection was at 278 nm.

Antioxidant Activity

DPPH Scavenging Activity: The ability of the extracts to scavenge 2, 2-diphenyl-1-picrylhydrazyl radicals was spectrophotometrically assessed by the method of Gyamfi *et al*. [18], with slight modification. A 50µl aliquot of each extract in 80% aqueous methanol at 0.05, 0.1 and 0.15, was mixed with 450 µl Tris–HCl buffer (50 mM, pH 7.4) and 1.0 ml 2,2-diphenyl-1-picrylhydrazyl (0.1 mM, in methanol). After a 30 min reaction period, the resultant absorbance was recorded at 517 nm. Test was carried out in triplicate. BHT was used as positive control. The percentage inhibition and the IC_{50} value estimated.

Hydroxyl Radical Scavenging Assay: Scavenging hydroxyl radical was spectrophometrically measured by the method described by Halliwell and Gutterdge [19] with slight modification. All solution was freshly prepared. 200µl of 2.8mM 2-deoxy-2-ribose, 5 µl of extracts (0.05, 0.1

and 0.15g/ml), 400 µl of 200µM $FeCl_3$, 1.04mM EDTA (1:1 V/V), 200 µl of H_2O_2 (1.0mM) and 200 µl ascorbic acid (1mM) was mixed to form a reaction mixture. After an incubation period of one hour at 37°C the extent of deoxyribose degradation was measured by the thiobarbituric acid (TBA) reaction: 1.5ml of 2.8% TCA was added in the reaction mixture and kept for 20minutes at 100°C taking Vitamin E as positive control. Absorbance was measured at 532nm and the Percentage inhibition was calculated. The IC_{50} values were estimated from the graph of percentage inhibition against concencentration.

Total Phenol Content: Total phenols were estimated as gallic acid equivalents [20]. To a 6.0 ml ultra-pure water, a 100 µl sample in 80% aqueous methanol at 0.05, 0.1 and 0.15g/ml were transferred to a 10.0 ml volumetric flask, to which was subsequently added 500 µl undiluted Folin-Ciocalteu reagent. After 1 min, 1.5 ml 20% (w/v) Na2CO3 was added and the volume was made up to 10.0 ml with ultra-pure water. After 30 min incubation at 25°C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve based on the formula: Absorbance = Concentration × 0.0011. The total phenol concentration was then recorded in mg/l.

Determination of Ascorbic Acid: Ascorbic acid were spectrophotometrically determined according to the method described by Klein and Perry [21], dried methanolic extract 50mg) was extracted with 10ml of 5mg/ml metaphosphoric acid for 45 minutes at room temperature and filtered through a disposable filtrate (0.45 millipore) 1ml of the filtrate was then mixed with 9ml of 2, 6-Dichloroindole phenol and the absorbance was measured at 515nm against blank. Content of ascorbic acid was calibrated on the basis of calibration curve of authentic L-ascorbic acid. The assay was done in triplicate.

RESULTS

The activity of the methanolic extract measured as zones of inhibition in millimeter is shown as mean ± SD (Table 1). The extract had activity at 100mg/ml (MIC) except for *S. dysenterae.* The zones of inhibition increase with increase in concentration. The zones of inhibition at 100mg/ml range from 4-9mm, 5-10mm and 6-11mm at 200mg/ml and 300mg/ml respectively. All the organisms exhibited better activity than tetracycline used as a positive control or standard for bacteria and nystatin the fungus. *P. aeruginosa* was most susceptible to the extract at all concentration. *L. monocytogenes* exhibited the lowest susceptibility to the extract. However, all the tested organisms were found to be susceptible to the extract. HPLC analysis of phenolics composition detected the presence of catechin, gallic acid, caffeic acid and rutin in 4.99mg/l, 0.06mg/l, 2.11mg/l and 0.51mg/l respectively (Table 2). DPPH free radical scavenging of the extract increases with concentrations The IC_{50} (concentration of the extract required in inhibiting 50% of the free radicals) = 0.139 (Figure 1).

The IC_{50} value of BHT used as a positive control is 0.0628 (Figure 2). Hydroxyl radical scavenging of the extract yielded IC_{50} = 0.149 (Figure 3) and IC_{50} = 0.1233 for vitamin E used as a positive control. (Figure 4). Total phenols Concentration also increase with increase in the concentration of the extract (Figure 5). Ascorbic acid content was found to be 2.0 ± 1.0 mg/g.

Table 1: Zones of Inhibitions in millimeter expressed in Mean ± SD

Organisms	100mg/ml	200mg/ml	300mg/ml	T (250mg/ml)	N (200mg/ml)
P. aeruginosa	9.00 ± 1.00	10.67 ± 1.53	11.67 ±1.53	5.33 ± 1.53	
E.coli.*	7.30 ± 1.53	8.33 ± 5.77	8.67 ± 5.77	5.33 ± 1.53	
S. flex.004	5.67 ± 5.77	7.00 ± 1.00	7.67 ± 1.53	7.33 ± 0.58	
S.dysen.006.	0.00 ± 0.00	7.00 ± 0.00	9.33 ± 0.58	7.33 ± 0.58	
B. cereus	6.33 ± 0.58	7.67 ± 1.15	9.67 ± 1.15	7.33 ± 2.0	
S. typhi	8.67 ± 0.58	9.67 ± 2.08	9.67 ± 1.53	5.00 ± 1.00	
C. albicans	8.33 ± 0.58	9.67 ± 0.58	10.67 ± 0.58	NT	8.00 ± 1.00
L. monoc.	4.33 ± 0.58	5.67 ± 0.58	6.67 ± 0.58	6.33 ± 0.58	

NT = Not tested, T = Tetracycline, N = Nystatin, dysen.=dysenterae, monoc.=monocytogenes, flex. = flexneri, * = ATCC 25922

Table 2: Amount of Phenolic Compositions

Phenolic components	Amount (Mg/L)
Catechin	4.99
Gallic acid	0.06
Rutin	0.51
Caffeic acid	2.11

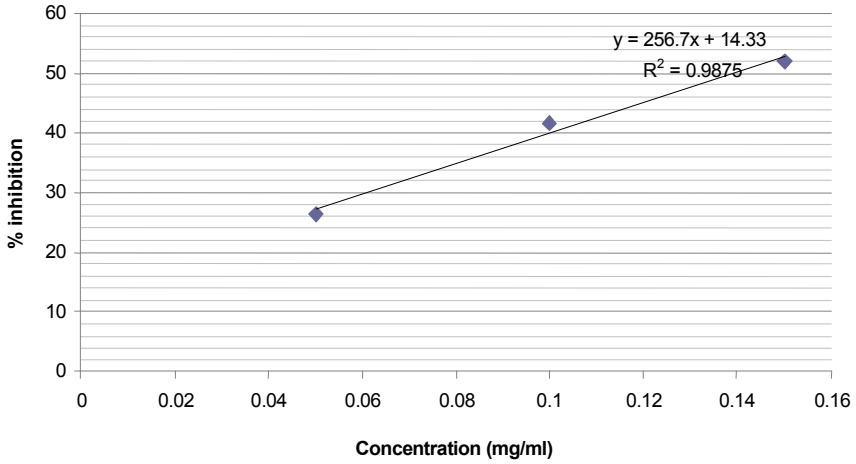

Fig. 1: Percentage Inhibition against Concentrations of Extract

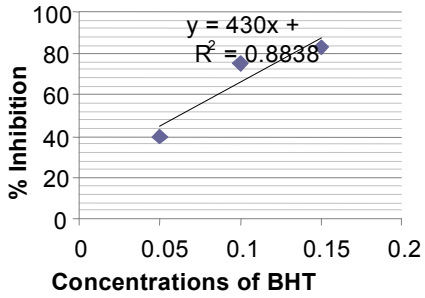

Fig. 2:% Inhibition Against Concentrations of BHT in g/ml

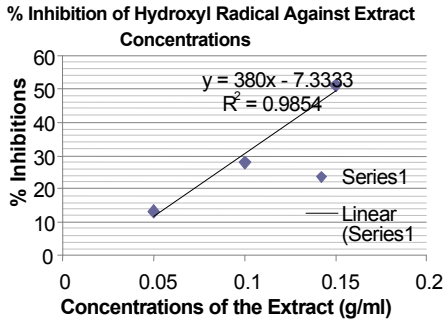

Fig. 3:

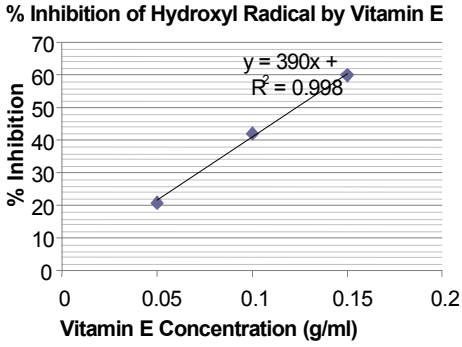

Fig. 4:

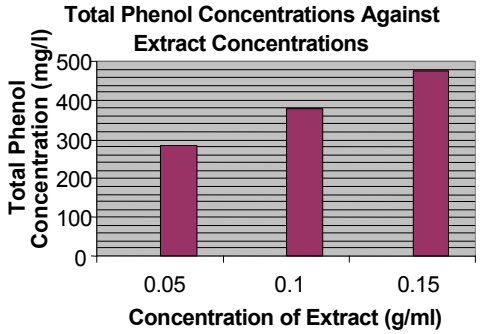

Fig. 5:

DISCUSSION

The susceptibility of these organisms to *A. bisporus* methanolic extract denotes the use of these macrofungus in the treatment of infections with these organisms as aetiologic agents. Addition of these macrofungus as additives to food could reduce occurrences of infections due to foodborne pathogens such as *Bacillus cereus.* It has been documented that certain extractable product from macrofungus (neutricicals) can be used as supplements in human diet to enhance health and fitness because of their medicinal properties [17]. It had been proven that mushroom exhibit antimicrobial effect [22, 23]. Susceptibility of *P. aeruginosa, C. albicans* and *B. subtilis* among other organism had been reported [24]. Antimicrobial activity of *A. bisporus* must have been due to the presence of essential bioactive components. Catechin which is one of phenolic components has been found to exhibit antimicrobial, antioxidant, anticancer and antiallergy properties [25, 26]. Caffeic acid and rutin has been shown to exhibit antimicrobial activity [25].

Gallic acid is a bioactive compound which is widely present in plants [27]. It is a strong natural antioxidant; it has also been shown to have anti-inflamatory, antitumor, antibacterial and antifungal activity [28-31]. The antioxidant activity of *A. bisporus* methanolic extract was also due to these bioactive compounds as most of them exhibited both antimicrobial and antioxidant activity. Flavonoid and phenolic compounds are potent water soluble and free radical scavenger which prevent oxidative cell damage [33-35]. Presence of ascorbic acid and phenolic compound in *A. bisporus* comfirms its antioxidant activity [16] A number of studies have been focused on biological activities of phenolic compound as a potential antioxidant and free radical scavengers [36, 37]. Presence of phenols in *A.bisporus* made this macrofungus a good candidate for formulating antioxidant products. The methanolic extracts of *A. bisporus* scavenge more DPPH radical than hydroxyl radical. The scavenging potentials increase with decrease in IC_{50} value. The result of this study has revealed *A. bisporus* as a natural source of antimicrobial against the tested organisms and a validation of its antioxidant activity. Some of the bioactive components as revealed in this research, like catechin had been labeled as anticancer, thus the possibility of *A. bisporus* as anticancer.

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