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Prospect of Button Mushroom (*Aga Ricus Bisporus* (J.E. Lange) Imbach) and Shitake (*Lentinula Edodes* (Berk) Pegler) as an Antibacterial and Colorectal Anti-Cancer

Nia Rossiana*, Kartiawati Alipin, Keukeu Kaniawati Rosada, Mia Miranti Rosada, Annasa Rizki Kamalina

Biology Departement, Faculty of Mathematics and Natural Science, Padjadjaran University, Raya Bandung-Sumedang St. KM 21 Jatinangor 45363

Corresponding author:
Nia Rossiana

niarossiana@yahoo.com

Tel: 022-84288889

Biology Departement, Faculty of Mathematics and Natural Science, Padjadjaran University, Raya Bandung-Sumedang St. KM 21 Jatinangor 45363

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Abstract

The aim of this study is obtaining sensitive extract to *Enterococcus faecalis* ATCC 29212, *Eschericia coli* ATCC 25922, and *Salmonella typhimurium* ATCC 49416, obtaining IC50 values and getting effective extract as colon anticancer with the HCT-116 cell line. This study use experimental and study literature. The measured parameter was the Inhibition Zone using disk diffusion, percentage of inhibition of HCT-116 cell lines using the MTT-assay method the data were analyzed profit to determine the IC50 value. The result study found that the largest inhibition zone of indicated bacterial test *E. faecalis* administered mushroom extract of the concentration studs 100mg/ml by 32.53 mm. IC_{50} value for *Agaricus bisporus* is 29.20 ppm and for *Lentinula edodes* is 6.11 ppm. According to U.S National Cancer Institute, the lower the IC_{50} value, the higher the cytotoxic activity. In summary ethyl acetate extract of fruit body sensitive to test bacterial, in anticancer test show that *Lentinula edodes* have more effectiveness to inhibit proliferation HCT-116.

Keywords: *Agaricus bisporus; Enterococcus faecalis; Eschericia coli*; HCT-116; IC_{50}; *Lentinula edode*; MTT-based cytotoxicity assay; *Salmonella typhimurium*

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Introduction

Cancer is formed from uncontrolled growth of abnormal cells in the body. Cancer develops when the body's normal control mechanisms stop working [1]. Colorectal cancer is cancer of the colon tissue, consisting of the colon (the longest part of the large intestine) and / or rectum (the last small part of the large intestine before the rectum) [2]. Cancer can be affected by 5 behavioral risks, that is excess body weight, low fruit and vegetable intake, lack of physical activity, tobacco and alcohol use. Several studies have shown that many bacterial species appear to be associated with the pathogenesis of colorectal cancer. Bacterial species such as *Clostridium septicum, Enterococcus faecalis, Streptococcus bovis, Bacteroides fragilis, Helicobacter pylori, Escherichia coli* and *Fusobacterium* spp. has been detected and has a role as a colorectal pathogenesis [3].

With drug resistance currently occurring, it is necessary to seek new drugs from natural sources so that they do not cause complications. This new drug discovery effort is required to have high sensitivity and the potential to be developed into new drugs. Various types of mushrooms have been developed into herbal medicines such as Lingzhi mushroom from the genus Ganoderma which is believed to be anti-diabetic, anti-hypertensive, and anti-tumor [4]. Other types of fungi that have the potential to become new drugs are also being developed.

In this study, the ethyl acetate extract of button mushroom (*Agaricus bisporus*) fruit body was used and shitake (*Lentinula edodes)* to test for antibacterial and anticancer potential. The antibacterial activity test was carried out using the disk diffusion assay and the literature against colorectal pathogenic bacteria, namely, *Enterococcus faecalis* ATCC 29212, *Eschericia coli* ATCC 25922, and *Salmonella typhimurium* ATCC 49416. Cytotoxic test was performed using MTT-based cytotoxicity assay with HCT-116 (human colonic epithelial carcinoma*)* cell line.

Material and Methods

Disk diffusion assay

Antibacterial test using disc paper method using ethyl acetate extract of button and shitake mushroom fruit body. The three tested bacteria consisted of two gram-positive bacteria *Enterococcus faecalis* ATCC 29212 and *Eschericia coli* ATCC 25922, and one gram-negative bacterium, *Salmonella typhimurium* ATCC 49416. Bacteria were grown on MHA medium (Medlab) for 24 hours at 37°C. Bacteria were diluted to McFarland 0.5 (9 x 108 cells / ml sample). Mushroom extract was made into a concentration series then the disc discs were immersed in the concentration series for 2 hours. The antibiotics that were used as controls were Amoxicillin and Ciprofloxacin in the form of disc paper.

The MHA medium that had been inoculated with the test bacteria was added with disc paper with a series of extract concentrations. The clear zone can be seen after incubation for 24 hours. The MIC and MBC values were tested in the literature due to the conditions that were not possible.

Mtt-based cytotoxicity assay

Button mushroom and shitake extracts were made into a concentration series based on logs, 0,1,10,100, and 1000 ppm. The HCT-116 cell line was prepared using complete RPMI medium warmed in a water bath (37 ° C) for ± 30 minutes. A total of ± 5 ml of RPMI medium was then put into a 15 ml centrifugation tube. The cell suspension in liquid nitrogen was taken and put in a water bath at 37 ° C for ± 5 minutes until the cells were homogeneous. After thawing, the cell suspension was immediately transferred to a 15 ml centrifugation tube containing complete RPMI medium and then centrifuged for 5 minutes at a speed of 1500 rpm. The centrifuged supernatant was discarded, while the cell pellets at the bottom of the centrifugation tube were resuspended with 5 ml of the new RPMI medium. The cell suspension was then implanted in a sterile flask measuring 25 cm^2 and incubated in a 5% CO_2 incubator at 37 ° C [5].

This test uses 96 well plates. Each well was inoculated as many as 10^4 cells, and 200 µL of RPMI medium was added, then incubated in a 5% CO_2 incubator for 24 hours. After incubation, 200 µL of new RPMI medium was added, dissolved with ethyl acetate extract of button mushrooms and white shitake mushrooms with the final concentration series, namely 0, 1, 10, 100, and 1000 ppm. Cell culture was incubated in a 5% CO_2 incubator for 48 hours. After incubation, 100 µL of medium was taken from each well, then 10 µL of MTT kit stock solution was added and incubated for 2 - 4 hours. After incubation, the MTT kit and medium were discarded and 100 µl of DMSO was added as a stop reaction. The absorbance value of the cell suspension was read at a wavelength of 450 nm with a Multiwell plate reader which was then analyzed using probit to obtain the IC_{50} value.

Probit analysis

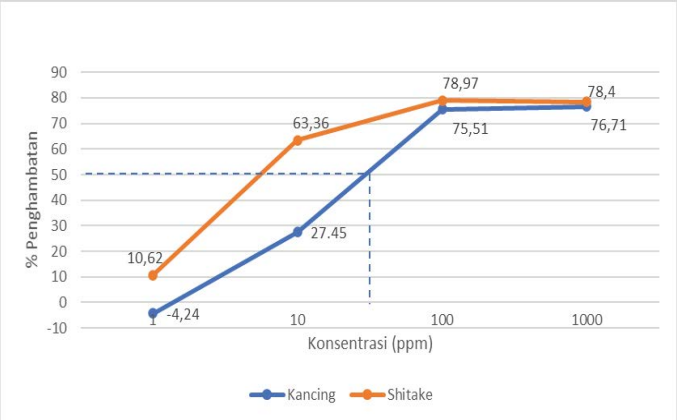
The data obtained were then converted into percentage inhibition and analyzed probit. Probit analysis was used to obtain the IC_{50} value of each mushroom using Microsoft Excel 365 proplus and using Fisher and Yates (1948) probit table. The results are presented in (**Table 1**). The concentration of 0 ppm was used as a control with various concentrations of 1,10,100 and 1000 ppm.

Result and Discussion

Antibacterial test

The clear zone results using a disk diffusion assay with the test bacteria, *Enterococcus faecalis* ATCC 29212, *Eschericia coli* ATCC 25922, and *Salmonella typhimurium* ATCC 49416 can be seen in (**Figures 1, 2 and 3**) and in (**Table 2**). All extracts show sensitivity

Table 1. Correlation curve of percentage inhibition of HCT-116 cell line proliferation with extract concentration.



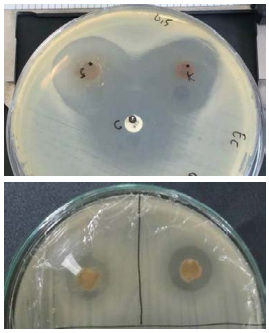


Figure 1 The clear zone of the extract against *E. coli* 100% concentration (top) 50% concentration (bottom).

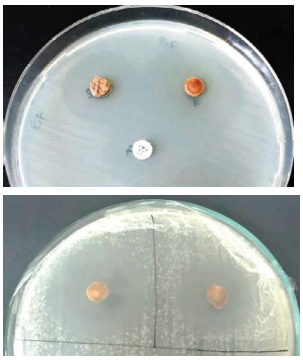


Figure 2 The clear zone of the extract against E. faecalis 100% concentration (top) 50% concentration (bottom).

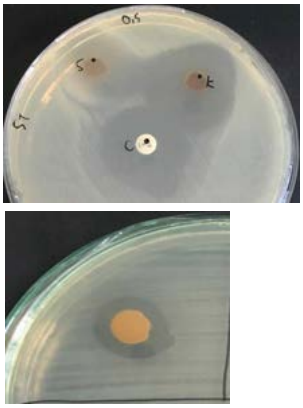


Figure 3 The clear zone of the extract against Salmonella typhimurium 100% concentration (top) 50% concentration (bottom).

Table 2. Inhibition zone.

Jenis Jamur	Bakteri Uji	Konsentrasi	zona hambat (mm)	Sumber
Agaricus bisporus	*Enterococcus faecalis*	100	32,53	*
		50	22,71	*
		30	13	Waithaka *et al*, 2017
		20	10	Waithaka *et al*, 2017
		10	0,9	Waithaka *et al*, 2017
		Kontrol (+)	23,7	*
	Eschericia coli	100	26,72	*
		50	14,85	*
		30	18	Waithaka *et al*, 2017
		20	13	Waithaka *et al*, 2017
		10	10	Waithaka *et al*, 2017
		Kontrol (+)	30,37	*
	Salmonella thypimurium	100	25,235	*
		50	16,33	*
		Kontrol (+)	29,465	*
Lentinula edodes	*Enterococcus faecalis*	100	27,22	*
		50	17,52	*
		Kontrol (+)	23,7	*
	Eschericia coli	100	29,075	*
		50	13,65	*
		25	8,5	Bastida et al 2016
		12,5	9,2	Bastida et al 2016
		6	8,83	Bastida et al 2016
		Kontrol (+)	30,37	*
	Salmonella thypimurium	100	26,28	*
		50	15,42	*
		25	29,4	Bastida et al 2016
		12,5	29,6	Bastida et al 2016
		6	30,3	Bastida et al 2016
		Kontrol (+)	29,465	*

* Keterangan : Hasil penelitian saat ini

to the tested bacteria. The largest clear zone was formed by the ethyl acetate extract of the fruit body of button mushrooms (*Agaricus bisporus*) at a concentration of 100% with the test bacteria *Enterococcus faecalis* ATCC 29212 with a diameter 32.53mm. These results indicate that the formed inhibition zone is included in the very sensitive group at 100% concentration because the inhibition zone is formed >20mm and is sensitive at 50% concentration because the inhibition zone is formed 10-20mm.

The results of the antibacterial activity test were indicated by the formation of an inhibition zone around the disc paper. The concentration given for testing indicates the presence of this antibacterial activity. In this test, a positive control was used in the form of amoxicillin antibiotics for *E. coli* (inhibition zone 30.37mm) and *E. faecalis* (23.7mm inhibition zone) and ciproflolaxacin for *S. thypi* test bacteria with an inhibition zone of 29.46mm.

The diameter of the resulting inhibition zone indicates the sensitivity of bacteria to antimicrobial substances, the larger the diameter, the more sensitive it is and the less likely the resistance [6]. Antimicrobial substances in shitake have been identified as compounds that act as antibiotics including lentin (a protein), lenthionine (a sulfur-containing exobiopolymer), lentysine (a purine compound), lentinamycin A and B (polyacetylene derivatives) (Mizuno in 1995) [7].

The difference in sensitivity to the extracts used is thought to be due to differences in the sensitivity of the tested bacteria to the compounds contained in the extracts used. Research conducted [8] by comparing the test bacteria *E. coli* (gram negative) with *Staphylococcus aureus* (gram positive) states that gram-positive bacteria are more susceptible than Gram-negative bacteria because there is a difference in fatty acid sensitivity between gram-positive and gram-negative bacteria. The impermeability of the outer membrane of gram-negative bacteria which is an effective barrier against hydrophobic substances makes gram-negative bacteria more resistant to inactivation by medium and long-chain fatty acids than gram-positive bacteria. As in *E. coli*, it has a three-layer (multilayer) cell wall with a high fat content (11-22%) and has a peptidoglycan layer in a rigid layer of about 10% dry weight so that antibacterial compounds are difficult to absorb [9-15].

The MIC and MBC values obtained were not based on the inhibition zone test carried out in the laboratory by the researcher. Based on the results of literature studies, the MIC value of button mushrooms is shown in (**Table 3**). Ethyl acetate extract of shitake mushroom fruit body is proven to have better bacteriostatic and bactericidal properties than button mushroom extract, as according to [16] that the Polysaccharides contained in this species strengthen the immune system and are strong antibacterials. Apart from polysaccharides, several antimicrobial agents have been identified, many of which have been patented. Shitake is therefore a great source of antibacterial and antifungal compounds. The isolated antibiotics include: lentins (protein), lenthionine (a sulfur-containing exobiopolymer), lentysine (purine compound), lentinamycin A and B (polyacetylene derivative).

Cytotoxic assay

The cytotoxic activity of button and shitake mushrooms was determined by MTT-based cytotoxicity assay. The IC_{50} values of

Table 3. MIC and MBC value.

Mushrooms	Bacteria Species	MIC (mg/ml)	MBC (mg/ml)
Agaricus bisporus	*Enterococcus faecalis*	1,25[1]	4,5[1]
	Eschericia coli	8[2]	4,7[2]
	Salmonella typhimurium	1,15[2]	0,6[2]
Lentinula edodes	*Enterococcus faecalis*	0,2[3]	0,2[5]
	Eschericia coli	3[4]	1[6]
	Salmonella typhimurium	2[4]	1[6]

the ethyl acetate extract of button and shitake mushroom fruit bodies against the HCT-116 cell line were 29.20ppm and 6.11ppm (**Table 4**). Based on the results of the study, both extracts have potential as anticancer compounds because they have an IC_{50} value which is classified as very toxic.

Table 4. Average percentage and IC_{50} value.

Ekstrak Etil Asetat	Konsentrasi (ppm)	Parameter				
		Rata-rata Pengambatan Proliferasi	% Penghambatan Proliferasi	Probit	Nilai IC_{50} (ppm)	Kategori
Agaricus bisporus	0	0,471			29.20	Cukup toksik
	1	0,491	.-4.24 ± 0,04	-		
	10	0,342	27.45 ± 0,01	43.992		
	100	0,115	75.51 ± 0,007	56.903		
	1000	0,11	76.71 ± 0,008	57.290		
Lentinula edodes	0	0,468			6.11	Sangat toksik
	1	0,418	10.62 ± 0,009	37.519		
	10	0,171	63.36 ± 0,04	53.398		
	100	0,098	78.97 ± 0,08	58.030		
	1000	0,101	78.4 ± 0,01	57.858		

The anti-proliferation activity indicated by the IC_{50} value of each type of edible mushroom extract in each type of cell line has a different value depending on the composition of the bioactive compounds contained in the fungus and the defense system of the cell line itself. The IC_{50} value of this study is 29.20ppm for button mushrooms and 6.11ppm shitake mushrooms, which are classified as strong cytotoxic against HCT-116 cell lines compared to other types of mushrooms. The button mushroom and shitake extract likely have the same cytotoxic properties when tested on other cell line types such as HeLa and HepG2 because they have a range of IC_{50} values 1-30 ppm.

The difference in IC_{50} value is possible in many ways such as the type of solvent used, fungal habitat, the method used and the compounds that play a role in the mechanism of inhibiting cell proliferation. Various types of mushroom from the Basidiomycetes class have antitumor activity with a direct or indirect mechanism where inhibition of proliferation occurs due to the presence of polysaccharides or polysaccharide protein complexes isolated from fungi showing cytotoxicity to tumor cells.

The percentage of inhibition corresponds to the IC_{50} value. Approximate IC_{50} values can be seen on the curve. At a concentration of 10-100ppm the button mushroom extract had an inhibitory percentage of 27.45-75.51%, which means that the 50% inhibition value existed in that concentration range. The peak of cell line inhibition was at a concentration of 100 ppm. Inhibition tended not to differ much in the concentration range of 100-1000 ppm. The curve above shows that the higher the concentration used the greater the percentage inhibition of HCT-116 cell line.

Inhibition occurs due to the presence of influential compounds such as lentinan and arginine which increase the body's immune system thereby activating the proliferation of T cells and B cells. Compounds such as flavonoids according to research results [17] has the potential to inhibit cancer cell growth with mechanisms of action such as inactivation of carcinogens, anti-proliferation, stopping the cell cycle, inducing apoptosis, promotion of differentiation, inhibiting angiogenesis, antioxidants and modulating multidrug resistance.

Conclusion

The ethyl acetate extract of button and shitake mushroom fruit bodies is sensitive to all tested bacteria and is effective in inhibiting the proliferation of HCT-116 cell lines with IC_{50} values for button mushrooms 29.20ppm and shitake 6.11ppm.

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