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EFFECT OF ULTRAVIOLET RADIATION ON THE FORMATION OF ERGOCALCIFEROL (VITAMIN D2) IN *Pleurotus ostreatus*

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ABSTRACT

Naturally the content of ergocalciferol (vitamin D_2) in mushrooms is small, but mushrooms are rich in ergosterol which is known as a precursor of ergocalciferal. Ergosterol is converted to ergocalciferal by UV light irradiation following a heat reaction. The aim of the study is to observe formation of ergocalciferol from its precursor in the *Pleurotus ostreatus* using UV radiation at 254 and 366 nm. Crude samples were irradiated for 15, 30, 60, 90, and 120 minutes. The samples were then extracted using a reflux for one hour. The ergocalciferol content was determined using spectrophotodensitometer. The results showed that the contents of ergocalciferol after 15, 30, 60, 90, 120 mins radiation under UV 254 nm were 24.12 ± 3.46; 28.15 ± 1.43; 38.22 ± 2.11; 48.19 ± 3.68; and 14.81 ± 3.41 µg/g respectively, whereas under UV 366 nm in similar duration the content were 9.59 ± 5.35; 20.6 ± 5.58; 27.67 ± 4.5; 10.62 ± 2.81; 8.76 ± 0.32 µg/g, respectively. No ergocalciferol was found in the control. It is concluded that the ergocalciferol was formed in mushroom after irradiating with ultraviolet light. The highest ergocalciferol content was found in the sample irradiated under UV light 254 nm for 90 minutes.

Key words: Ergocalciferol, ergosterol, *Pleurotus ostreatus*, spectrophotodensitometer, ultraviolet light

PENGARUH DURASI PENYINARAN SINAR ULTRAVIOLET TERHADAP PEMBENTUKAN ERGOKALSIFEROL (VITAMIN D_2) PADA JAMUR TIRAM PUTIH (*Pleurotus ostreatus)*

ABSTRAK

Kandungan ergokalsiferol (vitamin D2) pada jamur umumnya sedikit, namun kaya akan ergosterol yang merupakan prekursornya. Ergosterol dapat dimanfaatkan untuk produksi ergokalsiferol melalui metode radiasi sinar ultraviolet pada panjang gelombang tertentu yang dilanjutkan dengan reaksi termal (pemanasan). Penelitian ditujukan untuk mempelajari proses pembentukan ergokalsiferol dari prekursornya (ergosterol) pada simplisia jamur tiram setelah diradiasi dengan sinar ultraviolet pada panjang gelombang 254 dan 366 nm. Simplisia diradiasi dalam rentang waktu 15, 30, 60, 90, dan 120 menit, kemudian diekstraksi dengan metode refluks selama 1 jam. Kadar ergokalsiferol setelah perlakuan ditentukan menggunakan spektrofotodensitometer. Kadar ergokalsiferol pada simplisia yang telah diradiasi sinar UV 254 nm selama durasi tersebut adalah masing- masing 24,12 ± 3,46; 28,15 ± 1,43; 38,22 ± 2,11; 48,19 ± 3,68; dan 14,81 ± 3,41 µg/g. Sedangkan untuk simplisia yang diradiasi oleh UV 366 nm adalah masing-masing 9,59 ± 5,35; 20,6 ± 5,58; 27,67 ± 4,5; 10,62 ±2,81; 8,76 ± 0,32 µg/g simplisia. Keduanya dibandingkan terhadap simplisia yang tidak diradiasi (sebagai kontrol). Hasil menunjukkan terjadinya pembentukan senyawa ergokalsiferol pada jamur tiram yang telah diradiasi sinar ultraviolet. Kadar tertinggi ergokalsiferol yang terbentuk berdasarkan durasi penyinaran pada percobaan diperoleh pada jamur yang diradiasi pada panjang gelombang 254 nm dengan lama penyinaran 90 menit.

Kata kunci: Ergocalciferol, ergosterol, lama penyinaran, *Pleurotus ostreatus*, spectrophotodensitometer, sinar ultraviolet

INTRODUCTION

Pleurotus ostreatus or oyster mushroom is commonly consumed in Indonesia. This type of mushroom can be processed into various foods. The nutrient content is complete and it is known for treating anemia and diabetic. The values of protein, fat, phosphorus, thiamin and riboflavin are higher than any other fungus (Jaelani, 2008, Djarijah, 2001, Agustin, 2005, Parjimo & Agus Andoko, 2007, Pasaribu, 2002, Suriawira, 2002).

Mushroom membrane sterol contains ergosterol. Previous research showed that the product of photochemical and thermal reactions from ergosterol yield several compounds that had been characterized, which were ergocalciferol (vitamin D2), complex-lumisterol ergocalciferol 1:1 (vitamin D1). Vitamin D3 and D4 were found in fish oil and animal or human skin which are exposed to sunlight (Mann, 1994). Therefore, oyster mushrooms can be used as natural source for the production of vitamin D2. Vitamin D2 is important because it increases calcium absorption which acts as one of the essential mineral for maintaining bone health. Vitamin D2 can be found either in active (calciferol) or passive (ergocalciferol) forms in the body. In the inactive form, vitamin D2 acts as a hormone because it sends the signal to increase the absorption of calcium and phosphorus in the intestine. UV radiation can be used to increase the levels of ergocalciferol in oyster mushrooms. Jasinghe and Perera (2006) proved that ultraviolet B (290-315 nm) radiation in one hour (35 ^{o}C, 80% moisture) shows the highest concentration of ergocalciferol. The formation of ergocalciferol was not influenced by postharvest time, no significant degradation was found on ergocalciferol formation 1-4 days after harvest (Roberts *et al.* 2008). These two researches used HPLC to quantify the ergocalciferol content in mushrooms. Koyyalamudi *et al.* (2009) develop a quantification method of ergocalciferol using HPLC MS/MS while no publication was found that mention ergocalciferol quantification using spectrophotodensitometer.

The aim of the study was to explore optimum conditions that can increase the production of vitamin D2 in mushroom oyster under ultraviolet radiation within a certain time frame and to develop an analytical method for analyzing ergocalciferol using spectrophotodensitometer.

MATERIAL AND METHODS

Materials

White oyster mushroom (*Pleurotus ostreatus*) used as powder crude drug (Ditjen POM, 1985; 1986, 1995; 2000) was obtained from Cisarua West Java. Reagents are distilled water, glycerin, a solution of chloral hydrate 70%, 25% ammonia, chloroform, concentrated hydrochloric acid, Dragendorff reagent, magnesium powder, amyl alcohol, sulfuric acid, sodium hydroxide, Steasny reagent, sodium acetate, ether, a solution of iron (III) chloride, Mayer reagent, Liebermann-Burchard reagent, methanol, ethanol, ethyl acetate, n-hexane, acetic acid, benzene, aluminum (III) chloride, ergosterol (Sigma-Aldrich), ergocalciferol (Supelco), glassware, UV spots DESAGA, syringe, thin layer chromatography (TLC) GF_{254}.

Material characterization

Water content determination (WHO, 1998), water dissolved extractable matter, ethanol dissolved extractable matter, total ash, loss and drying (DITJEN POM, 1978).

Phytochemical Screening

Identification of flavonoids, alkaloids, saponins, quinone, tannins, steroids/triterpenoid (Farnsworth, 1966, Gritter, 1991, Harborne, 1987).

Radiation process

The material used was powder crude drug with water content of 6.25%. Radiation was carried out using a UV lamp at 254 nm and 366 nm that existed at spotting detector DESAGA for 15, 30, 60, 90, and 120 minutes. Crude drug was placed on a flat container and the irradiation was carried out at a distance of 10 cm from the source of radiation at the laboratory temperature.

Extraction

Crude drug (4gr) which has been irradiated by UV light was extracted using 50 ml n-hexane using reflux for 1 hour at 60 °C. The extract was then filtered with filter paper and then concentrated using a hair dryer until the solvent evaporated completely. The extract was dissolved in 5 ml n-hexane and then put into a vial for further analysis.

Extract Monitoring

Monitoring extracts were performed by thin layer chromatography (TLC) using silica gel GF_{254} as a stationary phase. The mobile phase was n-hexane-ethyl acetate (3:1v/v), and chloroform-benzene (1:1 v/v). UV light at 254 and 366 nm and sulfuric acid in 10% methanol were used as visualization agents.

Determination of Ergocalciferol Level

Preparation of calibration curve was performed using a standard solution in the concentration of 500 ppm. These standards were spotted on TLC plates in a number of specific volumes 1, 2, 4, 8, 12, and 16 mL. The responses of the instrument were plotted to the ergocalciferol mass.

Determination of Ergocalciferol Level in Samples

Samples were prepared for analysis and then spotted on TLC plates which were developed with the systems previously mentioned. The development was done in a closed chamber. Determination of ergocalciferol levels was done by comparing the Rf of samples and standard. Spectrophotodensitometry was used in this process (λ_{max} 265 nm).

Accuracy and Precision Test Accuracy

These assay testings were performed by the standard addition method. 1 ml of Ergocalciferol standard solution (100 ppm) equivalent to 100 µg ergocalciferol was added to the control samples. Furthermore, these standards were treated similarly with the samples preparation procedure.

RESULT AND DISCUSSION

Materials examination was aimed to determine the quality of materials used in this study and the of the results examination were shown in Table 1. Phytochemical screening showed that the oyster mushrooms contain only a steroid group. No Alkaloids, flavonoids, saponin, quinine, were present in this test. Ultraviolet irradiation was done to form heterocyclic ring opening reaction in ergosterol (Figure. 1).

Table 1. Result of the quality characteristics oyster mushrooms

Characteristic	Result
Water content	6.25% (v/b)
Water soluble compounds	1.55% (b/b)
Ethanol soluble compounds	5.10 % (b/b)
Ash total	5.46% (b/b)
Loss and drying	11.33% (v/b)

The reaction was affected by light. In this reaction intermediate product will be formed before pre-vitamin D. The next stage was heating of the system, double bonds at position 1 and 7 were shifted. The product of this reaction was called ergocalciferol (Figure. 2). Heating process was carried out by reflux which was functioned as an extraction process. Reflux was chosen because the process involved heat that was needed for ergocalciferol (vitamin D2) formation. N-hexane was used as an extracting solvent because of its polarity.

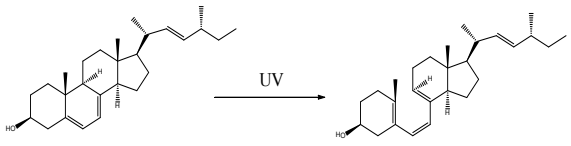

Figure 1. Opening of diene ring system in ergosterol (Mann *et al,* 1994)

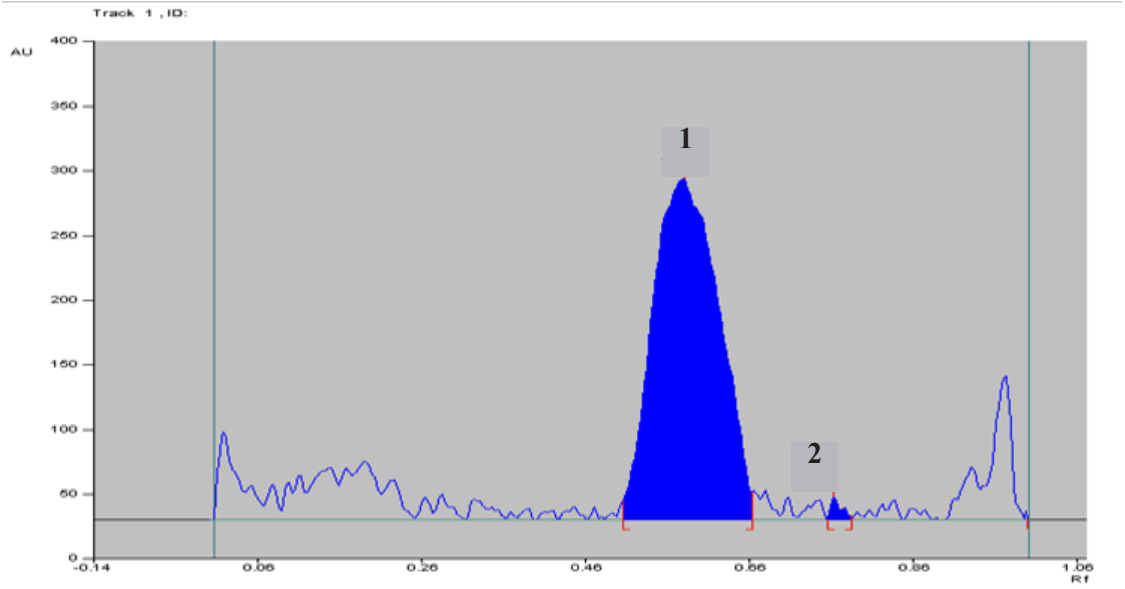

Figure 3. Densitometer Chromatogram of control (without radiation). Description: (1) ergosterol, (2) other compounds that have fluorescence at 366 nm.

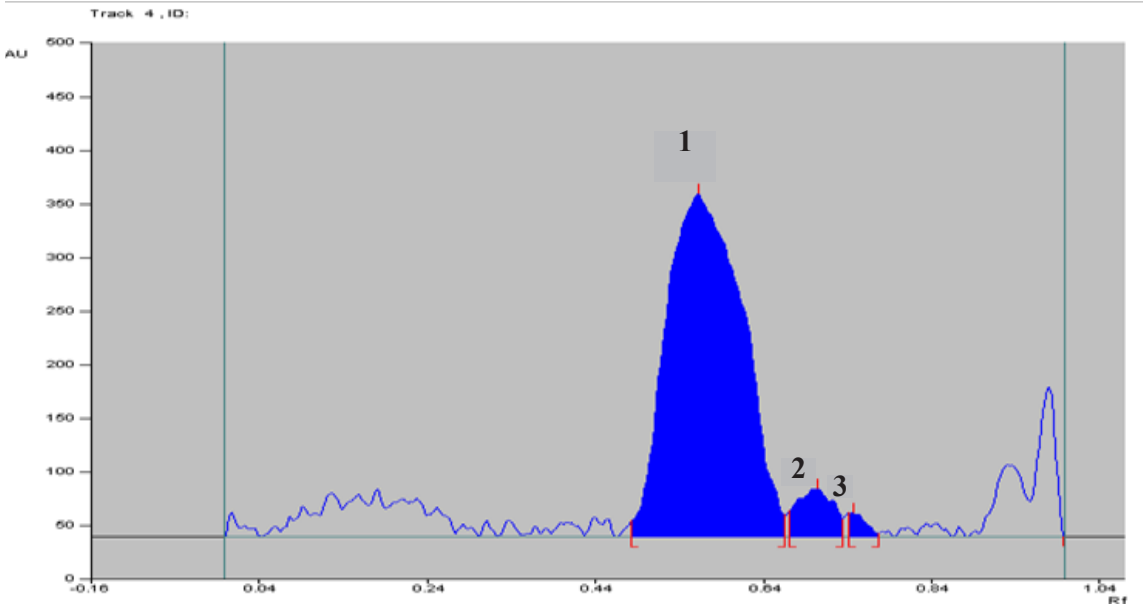

Figure 4. Densitometer Chromatogram of irradiated extract. Description: (1) rgosterol, (2) ergocalciferol and (3) other compounds that have fluorescence at 366 nm

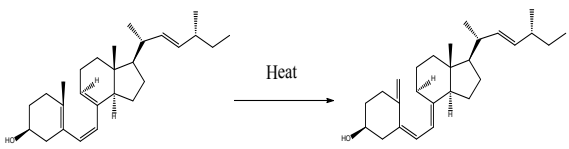

Figure 2. Sigmatropic shift on the carbon position 1.7 (Mann *et al,*1994)

The extract was monitored by thin layer chromatography (tlc) using silica gel GF_{254} as stationary phase while n-hexane-ethyl acetate (3:1 v/v) and chloroform-benzene (1:1 v/v) were used as mobile phases. Different kinds of mobile phases were used to enhance the resolution between two solutes that were difficult to separate. Spots were detected under UV light 254 nm, 366 nm, and 10% concentrated sulfuric acid in methanol.

Ergocalciferol was found in the crude drug irradiated by UV light and no ergocalciferol was found in control group. Extracts monitoring can be seen on the densitometer chromatogram (Figure. 3 and 4).

The aim of the study is to observe elevation levels of ergocalciferol in oyster mushrooms after being irradiated with ultraviolet light at 254 and 366 nm. Calculation of ergocalciferol levels in the extracts was made using a linear regression equation. The ergocalciferol calibration curve was made to show the relationship between ergocalciferol mass to the AUC. Stock solutions of ergocalciferol were spotted on TLC plates in specific volume (1, 2, 4, 8, 12, 16 mL). The plates were developed using n-hexane: ethyl acetate (3:1 v/v). Absorbance of each volume was determined using spectrophotodensitometry. Measurements were made at 265 nm (Figure. 5).

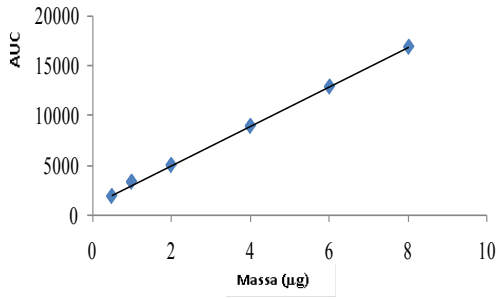

Figure 5. Calibration curve of ergocalciferol in different concentrations (λ=265 nm)

Area under curve (AUC) was plotted against the equivalence of mass ergocalciferol. The linear regression equation is y = 1973.7 x + 1060.9 and the correlation coefficient (r) is 0.9997, while y indicates the AUC and x indicates ergocalciferol mass (g). The ergocalciferol level in the samples calculated as ergocalciferol in µg/g crude drug. Table 2 shows the effect of UV light duration on ergocalciferol formation. Based on the results, the maximum level of ergocalciferol was occurred after 90 minutes exposure at 254 nm, while under UV 366 nm, the maximum ergocalciferol level was obtained after 60 minutes radiation. Longer duration of irradiation did not effect ergocalciferol level.

The formation of ergocalciferol in crude drug by UV radiation at 254 nm was higher than at 366 nm. This was because the energy produced at 254 nm was higher than the energy produced at 366 nm. Sufficient energy was required on the diene bond in ergosterol. If the amount of energy was sufficient, the formation of previtamin D2 will be optimum. Further, formation of previtamin D2 will be obtained by optimum temperature and heating duration.

Intermediate reaction affected by light (previtamin D) absorbs UV radiation that will cause formation of new compounds such as tachysterol and lumisterol. Longer irradiation process will produce irreversible products due to dimerization and ring cleavage (Braun, 1991).

Previtamin D production increases with the length of ultraviolet irradiation. But after certain time, previtamin D will react with ultraviolet light through pericyclic reaction

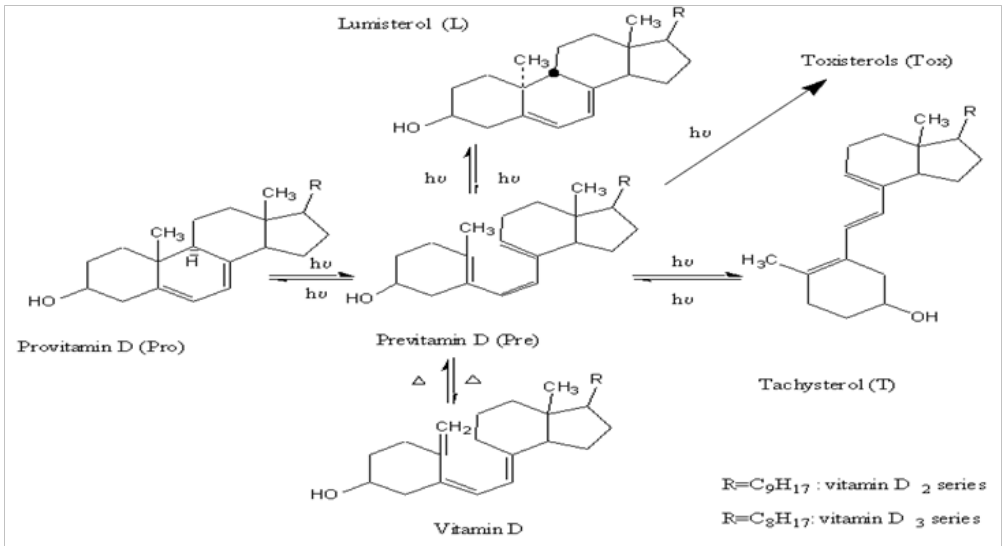

Figure 6. Scheme of ergosterol photolysis reaction and its products (Dmitrenko and Reischl)

mechanism. Consequently, production of previtamin D will be reduced because of lumisterol convertion into ergosterol isomer. This explains why the level of ergocalciferol was decreased at 254 nm after 120 mins exposure. This happened also in crude drug irradiated with UV 366 nm after 90 and 120 mins exposure. Complete photolysis reaction of ergosterol into its products can be explained in Figure. 6.

Table 2. Levels of Ergocalciferol (µ/g) after irradiation in various duration

Treatment (minute)	Ergocalciferol (µg/g)	
	UV- 254 nm	UV 366 nm
0 (control)	0	0
15	24.12 ± 3.46	9.59 ± 5.35
30	28.15 ± 1.43	20.6 ± 5.58
60	38.22 ± 2.11	27.67 ± 4.5
90	48.19 ± 3.68	10.62 ± 2.81
120	14.81 ± 3.41	8.76 ± 0.32

Accuracy testing was performed by the standard addition method. One ml of ergocalciferol standard solution (100 ppm) equivalent to 100 µg ergocalciferol was added to the control. Furthermore, these standards were treated similarly with the samples preparation procedures. The calculation of accuracy parameters was obtained by calculating the average percent recovery of added analyte whereas the precise value was determined from the relative standard deviation of percent recovery (Table 3).

Table 3. Test Method of Accuracy and Precision

Amount added (µg)	Measured (µg)	Recovery (%)	SD	RSD (%)
100	107,98	107,98	6,98	6,34
	106,04	106,04		
	115,92	115,92		
		109,98		

The results showed that the accuracy of the test was 109.98%. According to AOAC, range of acceptability by the number of recoveries of added analyte was 90-107%. Value of relative standard deviation (precision) was 6%, higher than the requirements (5.3%). Precision values were high due to high deviation in the measurement of analytes, therefore the RSD obtained was also higher.

CONCLUSION

The highest ergocalciferol formation was found in crude drug irradiated with UV light 254 nm for 90 mins. Longer time radiation did not increase ergocalciferol level. Sphectrophotodensitometer could be used for analyzing ergocalciferol content in oyster mushrooms.

REFERENCES

Agustin W. 2005. Usaha Pembibitan Jamur, Jakarta: Penebar Swadaya.

Ditjen POM Depkes R.I. 1985. Cara Pembuatan Simplisia. Depkes RI. Jakarta. Hlm. 1, 4, 7, 8, 10-18.

Ditjen POM Depkes R.I. 1986. Sediaan Galenik, Depkes R.I. Jakarta. Hlm. 1-2, 8, 10-13, 16-17, 25-26.

Ditjen POM Depkes R.I. 1995. Farmakope Indonesia. Edisi IV. Depkes RI. Jakarta. hlm. 7.

Ditjen POM Depkes R.I. 2000. Parameter Standar Umum Ekstrak Tumbuhan Obat, Depkes R.I. Jakarta. Hlm. 7-11.

Dmitrenko & Reischl. Previtamin D Photo-synthesis in Vitro. Institute of Surface Chemistry. Kiev. Ukraine.

Farnsworth, N. R. 1966. Biological and Phytochemical Screening of Plant. J., Pharm., Sci. 55(33),225-268.

Gritter, R. J. J. M, Bobbit, & A. E. Schwarting. 1991. Pengantar Kromatografi. Terjemahan K. Padmawinata. Ed 2. Bandung: Penerbit ITB. Hlm. 107, 116-129, 157-159, 160-165.

Harborne, J. B. 1987. Metode Fitokimia: Penuntun Cara Modern Menganalisa Tumbuhan. Terjemahan K. Padmawinata dan I. Soediro. Bandung: Penerbit ITB. 8-9, 21, 24-26, 27-30, 70-71, 155, 234-235.

Jaelani. 2008. Jamur: Berkhasiat Obat. Jakarta: Pustaka Obor Populer, 39.

Jasinghe, V. J., & C. O. Ferera. 2006. Ultraviolet irradiation: The generator of Vitamin D_2 in edible mushrooms. Food chemistry, 95:638-645.

Koyyalamudi, S. R., S. C. Jeong, C. H. Song, K. Y. Cho, & G. Pang. 2009. Vitamin D2 Formation and bioavailability from *Agaricus bisporus* button mushrooms treated with ultraviolet radiation. J. Agric. Food Chem, 57:3351-3355.

Mann, J. R. S & Davidson. 1994. Natural Products. England: Longman Scientific & Technical. Hlm. 341.

Nunung Marlina Djarijah. 2001. Budi Daya Jamur Tiram. Yogyakarta: Kanisius.

Parjimo & Agus Andoko. 2007. Budi Daya Jamur. Jakarta: Agromedia Pustaka.

Pasaribu, T. 2002. Aneka Jamur Unggulan yang Menembus Pasar. Jakarta: PT,Gramedia.

Roberts, J. S., A. Techert, & T. H. McHugh. 2008. Vitamin D_2 Formation from Post-Harvest UV-B treatment of mushrooms (*Agaricus bisporus*) and retention during storage. J. Agric. Food Chem, 56:4541-4546.

Suriawira, H. & Unus. 2002. Budi Daya Jamur Tiram, Yogyakarta: Kanisius. Hlm. 11, 12, 14.

World Health Organization. 1998. Quality Control Methods for Medical Plant Materials, World health Organizaton. Geneva. p. 31.