ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Solid State Fermentation and Characterization of Natural Pigments from *Aspergillus niger* Isolated from Corncob.

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ABSTRACT

Aspergillus niger is saprophyte fungi that live in organic material such as corncob. *A. niger* was then isolated from corncob to obtained natural pigments from *A. niger*. The solid state fermentation process was done using corncob as medium fermentation (solid medium). Results showed that the optimum condition of fermentation of *A. niger* was of 6 weeks after cultivation with temperature was of 30^0C. Characterization of natural pigments produced by using Thin Layer Chromatography (TLC) technique found that there are three compounds with Rf 0.64; 0.56 and 0.52. Characterization of UV-Vis spectrophotometry for first and second isolate of *A. niger* isolated from corncob obtained maximum wavelength is 203.40 nm and 201.80 nm. Further characterization of the infrared spectroscopy indicates the first and second isolate of *A. niger* isolated from corncob in the form of functional groups were OH stretch, bend aliphatic CH, CH stretch, stretch CC (alkyne), stretch C = O, strain C = C (alkenes), bond O - H, strain C - O and C - H aromatic.

Keywords: *A. niger, solid state fermentation, characterization, pigment*

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ISSN: 0975-8585

INTRODUCTION

Colour is a vital constituent and is probably one of the first characteristics perceived by the senses [1]. Synthetic colourant has been used widely in industrial process. Synthetic colourant is known as stable colourant that obtained from synthetic compound. However, synthetic colourant has negative effect for health. The use of synthetic colourant can be replaced by natural colourant or natural pigment. Natural pigment has less sideeffect than synthetic colourant. Moreover, it can be used as colourant as food colourant, cosmetic colourant and as colourant in pharmaceutical formulation.

Natural pigment are non-toxic, non-polluting, and less hazardous. Moreover, their antioxidant and antimicrobial nature further adds to their positive effect [2]. Natural pigment can be obtained from various sources like plants, animals and microbes. Production of natural pigment from microbes can be alternative issues to obtained natural pigment. Fungi is found to be an interesting ecological source of natural pigment as several fungal species are rich in stable colourant such as anthraquinone, carbixcylic acids, pre-anthraquinone, etc.[3]. *Aspergillus niger* is one of fungal species that can be used to obtained natural pigment. The used of *A. niger* as source to obtained natural pigment has potentially develop. *A. niger* is saprophyte fungi that live in organic material such as corncob. Corncob is commonly regarded as a waste product in agricultural processing. Fermentation technology that used agricultural waste as medium of fermentation has been done by many researches. The fermentation process of *A. niger* to obtained natural pigment was conducted using corncob as medium of fermentation. The use of corncob as medium of fermentation is cost effective and environmental friendly. Moreover, corncob can be us directly as medium of fermentation without having to add any other minerals [4, 5].

A study about fermentation of *A. niger* using solid and liquid medium to obtained chitosan has been reported. The result was fermentation of *A. niger* in solid medium has yield 15 – 20 times larger than fermentation of *A. niger* in liquid medium [6]. A study about the use of corncob and kernel of corn as medium fermentation of *Neurospora sp* to obtained pigment has been done. The result was the growth of *Neurospora sp* in corcob medium wa higher than kernels of corn [4]. Moreover, a study about the use of corncob as medium fermentation of *Neurospora sitophila* to obtained lacasse has been report [7].

The present study aims to know the optimum condition of *A. niger* fermentation to obtained natural pigment using corncob as medium fermentation and to know the characteristic of natural pigment obtained from *A. niger.*

EXPERIMENTAL SECTION

Collecting of fungal sample

Fungal sample obtained from corncob as agricultural waste in Padang City, West Sumatra, Indonesia.

Isolation and purification of *A. niger*

Fungal was isolated from corncob as agricultural waste. Fungal was then isolated into PDA medium in a Petri dish using sterile needle. Fungus incubation at a temperature of 240 - 280C for 5-7 days. Make observations after two days of incubation, in the event of contamination by other fungi, do insulation back to obtain pure cultures of fungi.

Identification of *A. niger*

Identification of A. *niger* in the Central Regional Veterinary II, Baso West Sumatra.

Fermentation of *A. niger*

Fermentation of *A. niger* was done using corn cob as solid medium**.** Fermentation on solid medium was done using corn cob that has been powdered and add sterile distilled water in the ratio of 90: 10% (w / w)

[4]. The powder of 50 grams of corn cobs put into 500 ml of sterile infusion bottles, inoculation of *A. niger* to solid media, then incubation at room temperature. Observed on 2, 3, 4, 5, 6, 7 and 8 weeks after cultivation.

Extraction of natural pigment from *A. niger*

Extraction performed on weeks 2, 3, 4, 5, 6, 7 and 8 after cultivation using maceration method with methanol. Separate between sample and methanol extract using filter paper, then extract of methanol was collected. Methanol was evaporate using rotary evaporator to obtained thick ecxtract. This was done 2 – 3 times.

Fractination of natural pigment from *A. niger*

Extract that waas obtained on weeks 2, 3, 4, 5, 6, 7 and 8 after cultivation was dried *in vacuo*. The dry extract was dissolved in 25 mL distilled water and then fractionation using 25 mL ethyl acetate. Separate the ethyl acetate fraction of the fermentation medium using a funnel. This was done 7 – 8 times. Ethyl acetate was evaporate using rotary evaporator to obtained thick fraction.

Determination of natural pigment yield

Fraction that was obtained on weeks 2, 3, 4, 5, 6, 7 and 8 after cultivation was then evaporated to obtain a dry fraction. Measure the weight of dry fraction (g) compared with volume of fermentation medium (ml) multiplied by 100%. Compare the yield obtained from each day of fermentation.

Characterization of isolate compound

Thin Layer Chromatography (TLC) pattern

Fraction obtained from *A.niger* was then characterization using Thin Layer Chromatography (TLC). Sample was spotted on TLC plate at stance of 0.5 cm. Enter TLC plate into a chamber that has been saturated by mobile phase, let the mobile phase elution rise to the limit. Observe the nodes under UV light with wavelength of 254 nm.

Examination of UV-Vis spectrum

A total of 1 mg of sample was dissolved in 1 mL of methanol pa then measuring the absorption at a wavelength of 200-400 nm. Determine the wavelength of maximum absorbance.

Examination of the infrared spectrum

Ribbon separation results determined by TLC Preparatif infrared spectrum using the FTIR spectrophotometer (PerkinElmer®).

RESULTS AND DISCUSSION

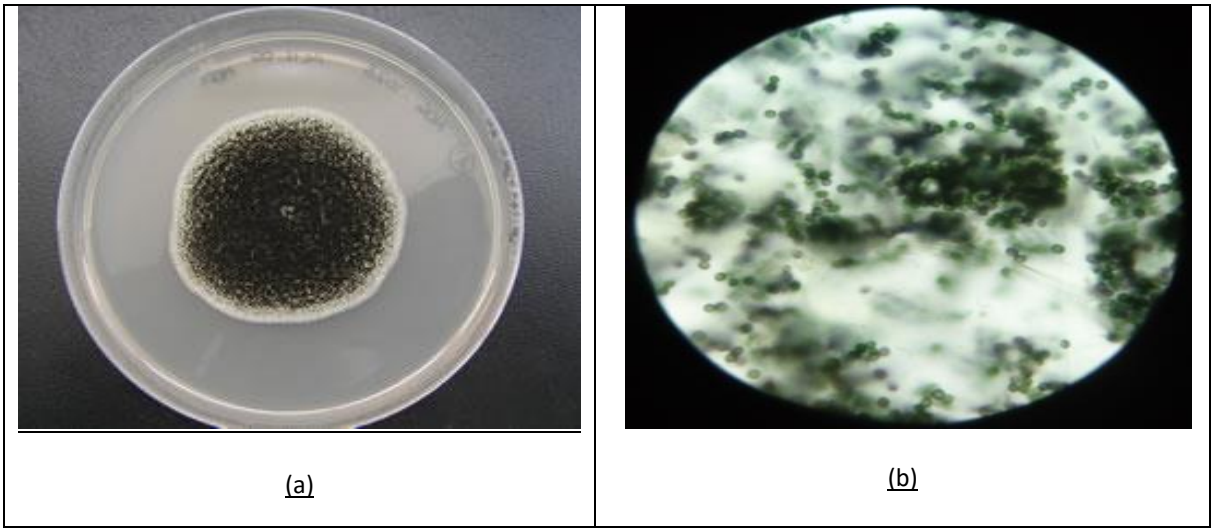
Isolation and identification of *A. niger*

Samples of *A. niger* was isolated from corn cobs as agricultural waste as shown in Figure 1 and Table 1. Isolate were then identified macroscopically and microscopically. Based on identification by Balai Veteriner Regional II, Bukittinggi, West Sumatra, Indonesia showed the result was *Aspergillus niger.*

Table 1: Characterization of Isolated of *A. niger*

Observation	Explanation
Macroscopic	A. niger is composed of a collection of fine threads, called hyphae, have a basic white colored fur with black colored conidia [8].
Microscopic	Microscopic observation using methylene blue Lacto phenol shows conidiophores of *A. niger* are round with black colored conidia is split by age fungal cells [8].

ISSN: 0975-8585



(a) (b)

Figure 1: Macroscopic (a) and microscopic (b) profile of isolated *A. niger* from

Fermentation of *A. niger*

The optimum time for fermentation of *A. niger* in solid medium to obtained natural pigment was of 6 weeks after cultivation at temperature was 30^{0}C. The yield of natural pigment was 4,03% (w/v).

Fermentation profile of solid state medium using corn cob that was done using powdered corn cob with addition of distilled water was shown in Figure 2. The aims of powdered corn cob was to enlarge the surface area of the fermentation medium so that the number of microbes grow became more. The addition of distilled water on fermentation media with ratio of 90: 10% (w / w). According Chutrtong (2014) ratio of 90: 10% (w / w) between corncobs and distilled water gives the highest mold growth results in producing dyestuffs. It is based on the results of the optimization of fermentation media with different composition ratio of corn cobs and distilled water.

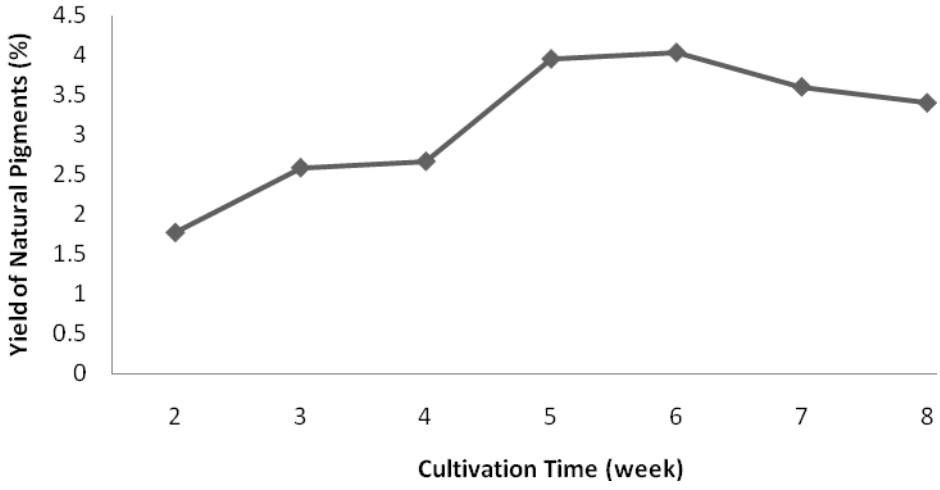


Figure 2: Relationship between cultivation time and yield of natural pigments

A decrease in the yield of natural pigments on 6 – 8 weeks after cultivation showed the decrease in the production of natural pigment of *A. niger*. This decrease may be due to the reduced number of fungal cells to grow due to the reduced of nutrient for the growth of yeast cells so that many fungal cells die.

ISSN: 0975-8585

Characterization of natural pigment

Characterization of natural pigment obtained from *A. niger* was conducted using Thin Layer Chromatography (TLC), UV-Vis Spectrophotometry and Infra-Red Spectrophotometry. The natural pigment isolated from *A. niger* has three nodes in TLC chromatogram as shown in Figure 3 and Table 2.

Table 2: Retention factor (Rf) natural pigments obtained from *A. niger*

Compound	Rf
a	0.64
b	0.56
c	0.52

a: First isolated compound produced by *A. niger*
b: Second isolated compound produced by *A. niger*
c: Third isolated compound produced by *A. niger*

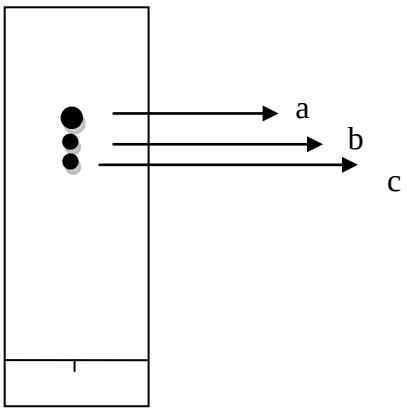


Figure 3: Thin Layer Chromatography (TLC) patterns of natural pigment from *A. niger* after 6 weeks cultivation

Three nodes were seen on TLC pattern then separated using TLC preparative. The purpose of using TLC preparative is to separate the compounds from the test sample to obtained single isolate in the form of a ribbon on a preparative TLC. This ribbon that contained of single isolate is use for further analysis such as UV-Vis spectrophotometer and infrared spectrometer. The results of separation using preparative TLC obtained two ribbons for ethyl acetate fraction of *A. niger.* The optimum condition for fermentation *A. niger* to obtained natural pigments was of 6 weeks after fermentation with temperature was 30^0C. The yield of natural pigment was 4.03% (w/v). Characterization of TLC pattern has three nodes with Rf values are 0.64 for first node, 0,56 for second node and 0.52 for third node.

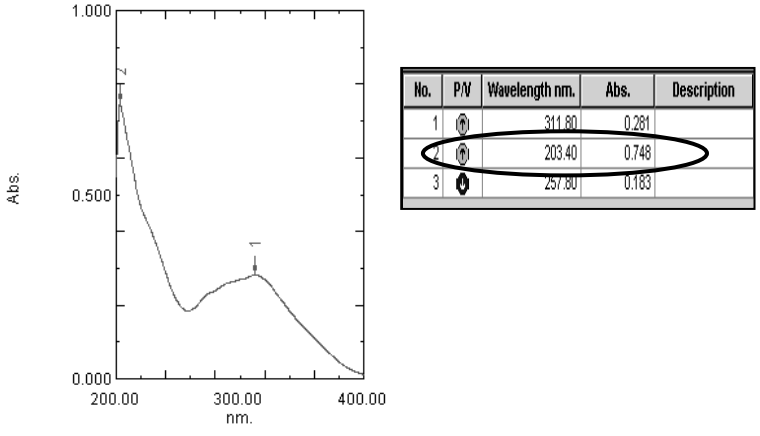
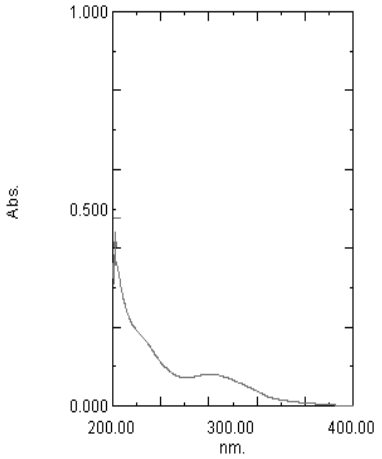


Figure 4: UV-Vis spectrum of first isolate of natural pigment from *A. niger* isolated from corncob.

ISSN: 0975-8585



No.	P/V	Wavelength nm.	Abs.	Description
1		201.80	0.391	
2		331.40	0.021	
3		261.80	0.072	

Figure 5: UV-Vis spectrum of second isolate of natural pigment from *A. niger* isolated from corncob.

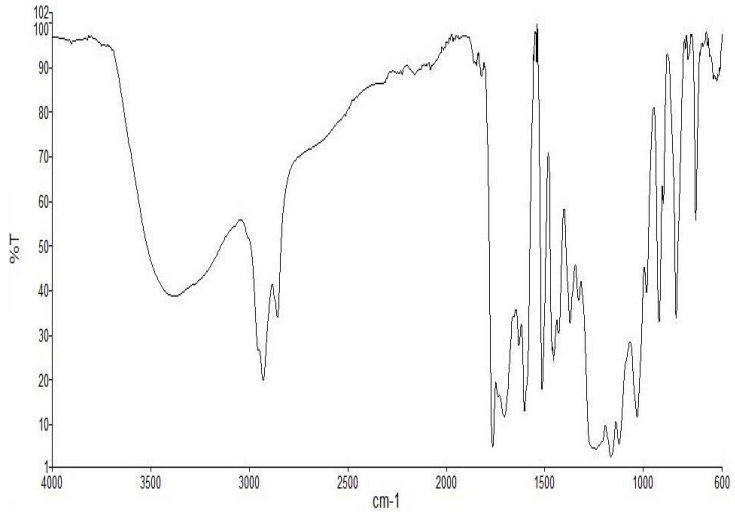


Figure 6: Infrared spectrum of first isolate of natural pigment from *A. niger* isolated from corncob.

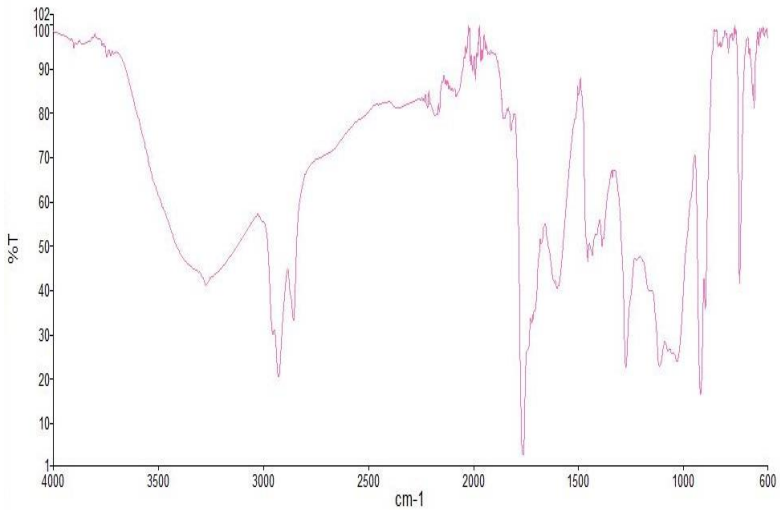


Figure 7: Infrared spectrum of second isolate of natural pigment from *A. niger* isolated from corncob.

Other characterization that uses is infrared spectrophotometer. The goal is to determine the type of bond structure and the functional group of the test sample through the data obtained wave number. Characterization of UV-Vis spectrum of test samples intended to determine the maximum wavelength of the test sample [9].

Characterization of the infrared spectrum of the first isolate of *A. niger* isolated from corncob was obtained absorption at various wave number. The 3374.17 cm^{-1} is probably derived f strain OH; 2926.22 cm^{-1} is probably derived from aliphatic CH bending; 2852.57 $cromm^{-1}$ is probably derived from C-H stretch; 2172.02 cm^{-1} probably derived from the strain C C; 2014.82 cm^{-1} is probably derived from the stretch C = O; 1656.00 cm^{-1} is probably derived from C = C (alkenes); 1328.21 cm^{-1} is probably derived from bond O - H of alcohol or phenol; 744.18 cm^{-1} is probably derived from C - H aromatic [10-11]. While the second isolate of *A. niger* isolated from corncob was also obtained absorption at various wave number. The 3384.53 cm^{-1} is probably derived from buckling OH; 2927, 23 cm^{-1} is probably derived from aliphatic CH bending; 2852.84 cm^{-1} is from the strain CH (metin (-CH); 2147.26 cm^{-1} is probably derived from C – C; 1930.06 cm^{-1} is probably derived from C = O; 1656.58 cm^{-1} is probably derived from strain C = C (alkenes); 671.94 cm^{-1} is probably derived from C - H aromatic [12].

Table 3: Functional group of infrared spectrum

Wave number (cm^{-1})	Functional Group
3570-3200 (broad)	- OH stretch
2935-2915	- CH stertch
2260 – 2100	Alkynes stretch
2100 – 1800	- C = O stretch
1680 – 1620	Alkenes stretch
1420 – 1330	- OH bond
900 – 670	- CH aromatic

CONCLUSION

The optimum condition for fermentation *A. niger* to obtained natural pigments was of 6 weeks after fermentation with temperature was 30^0C. The yield of natural pigment was 4,03% (w/v). Characterization of TLC pattern has three nodes with Rf values are 0.64 for first node, 0.56 for second node and 0.52 for third node. Characterization of UV – Vis spectrophotometer for first and second isolate of *A. niger* isolated from corncob obtained maximum wavelength is 203.40 nm and 201.80 nm. Characterization of the infrared spectroscopy indicates the first and second isolate of *A. niger* isolated from corncob in the form of functional groups were OH stretch, bend aliphatic CH, CH stretch, stretch CC (alkyne), stretch C = O, strain C= C (alkenes), bond O - H, strain C - O and C - H aromatic.

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