EXTRACTION AND CHARACTERIZATION OF PURPLE PIGMENT FROM *Chromobacterium violaceum* GROWN IN AGRICULTURAL WASTES

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ABSTRACT

There has been an increasing trend towards replacement of synthetic colorants with natural pigments in last decades because of the strong consumer demand for more natural products. Among three groups of main natural pigments, bacterial pigments are considered as an alternative to synthesized dye. Production and extraction of violet pigment of *Chromobacterium violaceum* grown on agricultural waste such as solid pineapple waste (SPW) and brown sugar (BS) was studied. From the study, the optimum growth temperature of *C. violaceum* and pigment production is at 25°C and optimum pH is 7. The pigment was extracted from the growth media using two solvents which were methanol and ethyl acetate. Characterization of the purple pigment was carried out using UV-VIS spectrophotometer, FTIR and ^{1}H and ^{13}C-NMR. UV-VIS analysis of the purple pigment samples from nutrient broth (NB), BS and SPW media shows λ_{max} at 566.50, 567.50 and 571.94 nm respectively. FTIR spectrum of purple pigment pellet from BS growth medium showed a broad peak at 3430.10 cm^{-1} assigned to OH stretching, overlapping of N-H bond with O-H stretching observed at 3330.1 cm^{-1}, two stretching bonds at 1640 cm^{-1} and 1723.5 cm^{-1} assigned to the C=O amide groups and C=C peak at 1615.92 cm^{-1}. ^{1}H-CNMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 and 20 carbon peaks and also 13 proton peaks appeared in the result to confirm the present of violacein in the samples. Lastly, stability of the produced pigment towards changes of the pH was examined. The pigment shows different colors at different pH.

ABSTRAK

Terdapat peningkatan hala tuju dalam beberapa dekad ini terhadap penggantian pewarna sintetik dengan pigmen asli disebabkan peningkatan permintaan pengguna terhadap produk-produk asli. Di antara tiga kumpulan utama pigmen asli, pigmen daripada bakteria dianggap sebagai alternatif kepada pewarna sintetik. Penghasilan dan pengekstrakan pigmen ungu oleh *Chromobacterium violaceum* yang dikulturkan di atas sisa pertanian seperti sisa pepejal nenas (SPW) dan gula perang telah (BS) telah di kaji. Daripada kajian ini, suhu optimum untuk pertumbuhan dan penghasilan pigmen oleh *C. violaceum* ialah pada 25°C dan pH optimum ialah 7. Pigmen tersebut diekstrak daripada media pertumbuhan menggunakan dua pelarut iaitu metanol dan etil asetat. Pencirian pigmen ungu ini dilakukan dengan menggunakan spektrofotometer UV-VIS, FTIR dan ^{1}H dan ^{13}C-NMR. Analisis UV-VIS ke atas sampel pigmen ungu yang diperolehi daripada kaldu nutrien (NB), BS dan SPW masing-masing memberikan λ_{max} pada 566.50, 567.50 dan 571.94 nm. Spektrum FTIR untuk pelet pigmen ungu daripada media pertumbuhan gula perang menunjukkan jalur yang lebar pada 3430.10 cm^{-1} mewakili regangan O-H, pertindihan jalur regangan ikatan N-H dan O-H pada 3330.1 cm^{-1}, dua jalur regangan pada 1640 cm^{-1} dan 1723.5 cm^{-1} mewakili kumpulan amida C=O dan jalur ikatan C=C pada 1615.92 cm^{-1}. Spektra ^{1}H -NMR dan ^{13}C-NMR telah direkodkan menggunakan pelarut DMSO-d_6 dan didapati 20 puncak karbon dan 13 puncak proton muncul, mengesahkan kehadiran violacein tulen di dalam sampel. Akhir sekali, kestabilan pigmen yang dihasilkan terhadap perubahan pH turut dikaji. Pigmen ungu memberikan warna yang berbeza dalam pH yang berbeza.

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LIST OF ABREVIATIONS

IR	Infrared
ml	Milliliter
NMR	Nuclear Magnetic Resonance
ppm	part per million
s	singlet
d	doublet
TLC	Thin Layer Chromatography

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

For decades, both natural pigments and synthetic dyes have been extensively used in various fields of everyday life such as food production, textile industries, paper production, agricultural practices and researches, water science and technology (Tibor, 2007).

According to green technology curriculum, less toxic products and more natural starting material is favorable for today's production lines. In case of dyes, it is well known that some synthesized dye's manufacturing is prohibited due to the carcinogenicity of the precursor or product and also because of the effects of disposal of their industrial wastes on the ecosystem. The wastewater generated from dye and dye intermediate industries mainly have intense color having various shades of red, blue green, brown and black through the production of different color containing dyes and usually have high level of COD, BOD, acidity, chlorides, sulphates, phenolic compounds and various heavy metals like copper, cadmium and chromium (Yogendra, 2008).

Dyes, as they are intensively colored, cause special problems in effluent discharge (even small amount is noticeable). The effect is aesthetically more displeasing rather than hazardous, and can prevent sunlight penetration decreasing photosynthetic activity in aquatic environment. Although, some azo dyes that causes the effluent color have been implicated as being mutagenic/carcinogenic as well as toxic to aquatic life (Yogendra, 2008).

Thus, extensive research has been conducted to find alternative dyes whose production and use would meet high environmental and safety requirements (Georgeta et al, 2004).

Increasingly, with the improvements in fermentation and other biotechnological techniques, bacteria, single-celled fungi and protozoa offer considerable scope for the commercial production of many pigments. There are many source of natural pigments which are derived from plants, animal, fungi and bacteria. Several intensely colored compounds have been isolated from certain bacteria which have resemblance to pigments in other biological systems (Britton, 1983).

Indigoidine or bacterial indigo, a dimeric pyridine structurally unrelated to the indigo of plants, is found in *Pseudomonas indigofera.* The highly pigmented *Chromobacterium* has also yielded the dark antibiotic prodigiosin with almost uncommon structure, a trimeric pyrrole (Hendry and Houghton, 1996).

The same genus also produces dimeric indoles such as the purple violacein pigment, although this one has, at least, some resemblance to the indole derivatives of higher plants (Hendry and Houghton, 1996).

Natural pigments not only have the capacity to increase the marketability of products, they also display advantageous biological activities as antioxidants and anticancer agents. Synthetic pigments, on the other hand, cause considerably environmental pollution and adverse toxicological side effects. Both classes of pigment exhibit considerable structural diversity (Tibor, 2007).

1.2 Statement of Problem

The use of synthetic dye has several disadvantages amongst them are carcinogenicity, ambient pollution possibility and increase of the cutaneous allergies for the user of the product.

Green technology is leading all producers to go towards ecological and less polluted products with fewer by-products; in the case of synthesized dye, natural pigments can be considered as an ideal alternative.

The most important issue regarding natural pigment is the price of final product which is more expensive than cheap synthesized dye. In this research possibility of using cheap growth media (agricultural wastes) such as Solid Pineapple Waste (SPW) and Brown Sugar (BS) which leads to inexpensive and competitive product, have been studied.

1.2 Objectives and Scope of Study

The objective of this study is to extract the purple pigment, violacein, from *Chromobacterium violaceum* which was grown on SPW and BS. The characterization of the pigment was made using UV-VIS spectrophotometer, IR, and NMR.

1.3 Significance of Study

This study aims at introducing bacterial pigments as an alternative to synthetic dye. In this study cheap medium were employed for bacterial growth and the simplest method for bacterial pigment extraction was developed to overcome the higher price of natural pigments compare to synthesized dye.

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