Introduction

Common cockscob (Celosia cristata) is a herbaceous plant that has been classified as a member of the family Amaranthaceae. This annual plant has a distinctive characteristic inflorescence, which looks like the crest of a rooster or convoluted brain after developing (Bojian et al., 2003). Celosia cristata was found to be able to produce purplish or reddish pigment in tissue culture system and when analysed was found to contain cyanidin, a kind of anthocyanin (Taha and Wafa, 2012).

The colored pigments obtained both in vivo and in vitro have potential to be used as paints or natural coating materials. The production of paint from plant sources is desirable due to environmental friendly, economical and safer compared to synthetic compounds. For instance, anthocyanin extracts obtained from Gerbera jamesonii Bolus ex. Hook F. was shown to be successful in natural paint production when combined with polyurethane (Taha...
et al., 2011). In addition, Abidin et al. (2006) also reported that natural paint was successfully produced from curcumin with acrylic resin and dammar as binders.

In recent advances of plant biotechnology techniques, there have been a number of reports on the production of secondary metabolites such as alkaloids, quinones, terpenes, and flavonoids in plant tissue culture (Mori et al., 1994). Anthocyanin pigments and derivatives are flavonoid and unique to the plant kingdom. Interest in these pigments is not only due to their potential as natural colorants but also because of their therapeutic properties. They are expected to be beneficial to human health as potential anti-cancer, cardio protective, anti-inflammatory and antioxidant properties (Castonguay et al., 1997; Liu et al., 2002).

Many plants have been investigated in the search for novel antioxidants. There has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing tissue injuries caused by free radicals (Jimoh et al., 2009). The use of plants and their products for medicinal benefits have played a significant role in nearly every culture on earth. Plants have also been the source of well-known anticancer drugs such as camptothecin, podophyllotoxin and paclitaxel (Stahlhut et al., 2009; Lee, 2004).

Objectives of the current study were; to induce the colored callus to identify the anthocyanin produced; to investigate the cytotoxic activity and antioxidant study of Celosia cristata.

Methods and Materials

Plant materials and Seeds of Celosia cristata:

collected from natural resources of Malaysia were surface sterilized. The seeds were cultured on MS media without hormones, supplemented with 30 g/l sucrose, pH 5.6±0.1, solidified with 2 g/l gelrite and maintained in the culture room at 25±1 ºC with 16 hours light and 8 hours dark for 4 weeks.

Callus induction

Different explants from stem, leaf, petal and root of aseptic seedlings were inoculated in Murashige and Skoog medium fortified with different concentrations of BAP, NAA and IBA. The cultures were maintained at 25 ºC with 16 h light and 8 h darkness. The colored callus obtained was then dissolved in absolute alcohol and spectroscopic analysis was then carried out (Rusli et al., 2009).

Antioxidant activity

DPPH assay:

DPPH reagent prepared in absolute ethanol (90 µM; 950 µl) was added to 50 µl of the plant extract (100, 200, 300, 400, 500 & 1000 µg/ml) and the volume was adjusted to 4 ml using 95% ethanol before incubation at room temperature in the dark for 120 min. Scavenging of DPPH reduced the color of the and was measured using a spectrophotometer at 515 nm. Comparison of the reduction of color in the examined samples with the blank (solution without plant extract) was used to measure the potential of scavenging capacity of our plant extracts using the following equation Rafat et al. (2010);
Radical scavenging capacity (%) = \[(\text{Blank} – \text{Sample A}) / \text{Blank}] \times 100

**Superoxide dismutase (SOD) assay:**

Superoxide dismutase (SOD) assay kit (Sigma Chemical Co., St. Louis, MO) was used to determine the SOD activity of prepared extract. Plant extract (20 µl) of different concentrations (200, 400, 600, 800, 1000 µg/ml) was added to 200 µl of the kit working solution. After a gentle shaking, the mixture was incubated at 37°C for 20 min after adding 20 µl of the kit enzyme working solution. The absorbance of the mixtures was measured at 450 nm using a microplate reader (Varioscan Flash, Thermo, Finland) and the SOD activity was calculated using the following equation (Xing et al., 2010):

Percentage of inhibition (SOD activity) = \[\frac{[(\text{blank 1} – \text{blank 2}) – (\text{sample A} – \text{blank 3})]}{\text{blank 1} – \text{blank 3}}\] × 100

Where; blank 1 was a mixture of the working solution (200 µl) and enzyme working solution (20 µl) containing 20 µl DMSO. Blank 2 contained the plant extract (20 µl) with working solution (200 µl) and dilution buffer (20 µl), while DMSO (20 µl) was added to the working solution (200 µl) and dilution buffer (20 µl) in the blank 3. Ascorbic acid was employed as the positive control in this study.

**Cytotoxic assay:**

*Celosia cristata* extracts were treated against HCT 116 (Human colorectal carcinoma) cell lines using MTT Cell Proliferation Assay. Extracts were dissolved in DMSO with the concentration of 40 mg/ml. Cells were harvested by centrifugation following trypsin treatment. Cells were seeded in 96-well plates at a density of 10^4 cells per well in 100µl culture medium and then in order to have cell attachments, the plate went under incubation for 24 hours. Cells were treated with different concentrations of plant extracts (1.56 - 100 µg/ml) for 72 hours. MTT (10 µl, 12 mM) was added to each well. A row of negative control well was selected including 10 µl of the MTT stock solution added to 100 µl of medium alone with no extracts. Plate was incubated at 37 °C for 4 hours. DMSO solution (100 µl) was added to each well and mix thoroughly using the pipette and absorbance (OD) was measured at 570 nm using a micro-plate reader (Varioscan Flash, Thermo, Finland).

Percentage of residual cell viability was determined as \[1 – (\text{OD of treated cells}/\text{OD of control cells})\]×100

**Results and Discussion**

Apart from propagation and cloning purposes, tissue culture can also be used for producing valuable chemicals for industrial and medicinal uses. *Celosia cristata* is very responsive in culture. Successful plant regeneration, in vitro flowering and cellular behavior studies have been already reported (Taha and Wafa, 2012). Callus growth was observed after 7-10 days depending on the media and type of organs used. Stem and leaf explants could produce the highest reddish purple callus on MS medium containing 0.5 mg/l BAP, 0.5 mg/l NAA, 1.0 mg/l BAP and 1.0 mg/l NAA (Fig. 1). Moreover, cultured petals explants on MS medium supplemented with 2.0 mg/l NAA also induced reddish colored callus after 20 days.

The results showed that callus from stem explants had the peaks at 532 nm, 472 nm and 212 nm, whilst callus from leaf explants had the peaks at the following wavelength, 536.50 nm,
482.50 nm and 210.50 nm, respectively. Callus from root explants had a peak at 214 nm (Fig. 2). It can be deduced that 532 nm and 536.5 are the actual peaks for the pigments compared with spectroscopic reading of 70 % alcohol. The pigment was suggested to be cyaniding, an anthocyanin, which is useful in coloring and dye industries (Fig. 3a). The pigment in the colored callus from petals was a flavanon, which has the peaks at 290 nm and 320 nm. Flavanon is a common pigment in the flowers of many species (Fig. 3b).

Based on the DPPH scavenging activity assay results, the IC50 value (the concentration that scavenges 50% of the DPPH radical) for *Celosia cristata* extract was found in 670.46 µg/ml (Fig. 4). The examined plant extract showed significantly lower DPPH scavenging activities as compared to ascorbic acid (control). The antiradical activity usually depends on the reaction kinetic behavior of different antioxidants at the same time and concentration. Different antioxidants had varied reaction rates for DPPH that produced different reaction kinetic behaviors (Xing *et al.*, 2010). Ascorbic acid was the only example of rapid kinetic behavior in this study, reacting very rapidly with DPPH and reaching the steady state in less than 2 min. Based on the result of SOD assay (Fig. 5), the extract showed significantly lower inhibition rates compared to ascorbic acid (positive control).

According to the United States NCI plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity, if the IC50 value is indicated less than 20 µg/mL (Boik, 2001). It was observed about 18% of inhibition with the concentration of 20 µg/mL of extract solution in *Celosia cristata* (Fig. 6). Therefore, this species was not active against HCT116 cell lines. However, it is still an open way to carry out a higher concentration treatment for this extract.

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Reference


Figure 1: Red callus of *Celosia cristata* in different concentrations of NAA and BAP

![Figure 1](image)

Figure 2: The UV-vis absorption spectra of callus from stem, leaf, root and petal

![Figure 2](image)
Figure 3: Structure of a. cyanidin-3-sophoroside and b. flavanon

Figure 4: Antioxidant activity of *Celosia cristata* extract using DPPH scavenging activity assay. Ascorbic acid was used as the positive control.
Figure 5: Antioxidant activity of *Celosia cristata* extract using superoxide dismutase (SOD) assay. Ascorbic acid was used as the positive control.

Figure 6: Antitumor activity of *Celosia cristata* extract against HCT116 cell line.