Antioxidant in natural blue colour of *Gynochthodes sublanceolata* (pitang leaves)

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Abstract

Gynochthodes sublanceolata express several color due to present of various pigments. Extraction of the leaves produce a dark-purplish color and analysis of the extract shows higher antioxidant properties with percentage of DPPH inhibition (75.7 ± 0.05), in comparison to *Salacca edulis* (salak fruit), *Hylocerous polyrhizus* (red pitaya), and *Ipomea batatas* L. (purple sweet potato). *Gynochthodes sublanceolata* seem to have high potential as food colorant which provide beneficial values such as antioxidant which not provided by synthetic coloring.

Keywords *Gynochthodes sublanceolata, Antioxidant, Anthocyanin.*

Introduction

Pitang leaves *(Gynochthodes sublanceolata)* are from the family of Rubiaceae [1]. It is a glabrous woody climber with stems several meters long, branches terete, and shiny dark blackish-brown when dry. Pitang leaves are considered as a rare plant and hard to find. It mostly can be found in Thailand including Songkhla, Pattani, and Narathiwat [2]. Since it able to turn the color of the whitish rice to dark purple, it is now widely used as a food colorant for *'nasi kerabu'*[1] in Kelantan.

The main factor which determined the color of plant tissues is plant pigments such as chlorophyll, xantophyll, carotene, flavone, flavonol, and anthocyanin. Chlorophyll pigments possessed by G. *sublanceolata* allow photosynthesis process to occur at the cellular level, allowing plants to appear green in color [3]. Chlorophyll is not a very stable compound, thus it can be destroyed due to temperature and pH changes [4]. However, as chlorophyll is destroyed, the other pigments such as carotenoid and anthocyanin are expressed [3].

In other cases, the color of the anthocyanin can be influenced by pH [5]. The anthocyanin turns into bright pink in acidic solution, reddish-purple in neutral solutions and green in alkaline or basic solutions [6]. Anthocyanins are antioxidant flavonoids which improved human health condition. Besides, the antioxidant supplementation can block NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation as well as inhibit NF-kB activity. Since NF-kB is responsible to cancer and inflammatory, thus it indirectly plays important role to inhibit cancer and inflammatory through mechanisms distinct from redox regulation [7]

Materials and Method

Sample Collection and Extraction

G. sublanceolata samples were collected from Tumpat, Kelantan. The Pitang leaves samples were blended in mixture of methanol: water (50% v/v + 0.1 % HCl). Then, the solvent was removed from samples using rotary evaporator/ rotavap (Büchi, Switzerland). Freeze dryer was used to transform the samples into powder form.

Analysis byDPPH free radical scavenging

Radical scavenging activity of the plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate, sigma-Aldirich Chemie, Steinheim, Germany) was determined by spectrophotometer. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (deep-violet to light-yellow) were measured at 517 nm. The extract stock solutions were prepared in 100 mg/ml in ethanol. Methanol extract was not soluble in ethanol (even after treating solutions for 5 min in an ultrasonic bath), therefore it is dissolved in dimethylsulphoxide.

[1] *Nasi kerabu* is a type of rice which is blue in colour, which originated in Kelantan state.

The working solution was prepared using methanol in a concentration of 500 μg/ml. the solution of DPPH in methanol 2.5 mg/ml was freshly prepared. 5 μl of this solution was mixed with 100 μl extract solution in 96 wells plate. The samples were kept in the dark for 30 min at ambient temperature and then the decrease in absorption was measured by using microtitre plate reader (Labsystems iEMS Reader MF). Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The experiments were carried out in triplicate.

Results and Discussion

The extracts of *G. sublanceolata* give rise to blue-indigo liquid solution which can be used as natural food colorant. The use of the pitang leaves as natural food colorants have more advantages compared to synthetic/ artificial coloring such as tartrazine and amaranth. Natural food coloring can provide beneficial values such as antioxidant that synthetic coloring do not provide. Furthermore, FDA (Food and Drug Administration) also has strictly prohibited the use of synthetic coloring for food coloring agent.

Analysis of the extract shows higher antioxidant activities with percentage of DPPH inhibition (75.7 ± 0.05) compared to *Salacca edulis* (salak fruit), *Hylocerous polyrhizus* (red pitaya), and *Ipomea batatas* L. (purple sweet potato). The high level of activities of antioxidant in pitang leaves contribute a major advantage for medicinal purposes. NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF-kB plays a key role in regulating the immune response to infection. Conversely, incorrect regulation of NF-kB has been linked to cancer. The nuclear factor (NF-kB) was proposed to be a pivotal protein in the link between inflammation and cancer [8]. NF-kB characterizes all inflammatory responses and is also a major hallmark of tumors [9]. Antioxidant supplementation can block NF-kB activation and inhibit NF-kB activity through mechanisms distinct from redox regulation [7]

Conclusion

Gynochthodes subalanceolata seem to have high potential for food industries and also medicinal value. As food colorant, *G. sublanceolata* can provide a beneficial value as antioxidant which is not available from synthetic coloring. Further studies on antioxidant activities of *G. sublanceolata* for its medicinal role either as anti-inflammatory or anti-cancer compound is much needed.

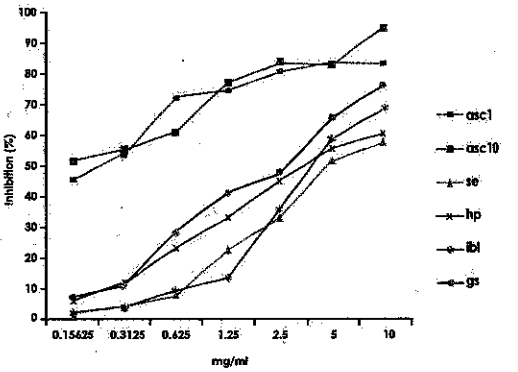

Figure 1 : *Antioxidant activities of Gynochthodes sublanceolata (gs) compared to Salacca edulis (salak fruit) (se), Hylocerous polyrhizus (red pitaya) (hp), and Ipomea batatas L. (purple sweet potato) (lbl) determined by DPPH.*

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