Ministry of Education
Department of Higher Education (Lower Myanmar)
Dawei University

Dawei University Research Journal

Vol. 4, No.1 December, 2012

CONTENTS

Extraction, Preparation and Modification of Organic Pigments for Pharmaceutical, Food, Cosmetic and Allied Industries

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Abstract

The recent research related to an efficient method for the extraction, modification and preparation natural dyes (organic pigments) from fruits of *Gardenia jasminoides* Ellis. The process comprising steps of mixing fruits powder of *Gardenia jasminoides* Ellis. with food-grade solvent, macerating and agitating, filtering the macerated mixture to remove undesirable fibrous plant material, freezing the filtrate immediately to remove fat and oil content. Then, concentrating the cooled filtrate under reduced pressure to obtain crude material reddish brown color mass. Those reddish brown mass was hydrolyzed by β-glucosidase in buffer solution, resulting colorless genipin and then simply reacted with amino acid (taurine) and to obtain stable brilliant blue color. Moreover, purple and green colors were also modified during process.

Introduction

The market for natural food colours has been steadily growing over recent years. However, the expectation that a large proportion of synthetic colours would gradually be replaced by natural colours has proved too optimistic. Even though many consumers state a preference for naturally coloured food the attractiveness of synthetically coloured products does appeal too many. In comparison natural colours are usually less bright. Still, while consumers look for foodstuffs with a fresh and appealing colour, a decreasing number of people are willing to eat or drink foodstuffs, which are coloured synthetically. Because of this situation, there is a clear tendency towards more genuine natural food colours. Manufacturers of genuine natural food colours only use edible fruits, vegetables and plants as basic raw materials and they try to utilize manufacturing processes that are as close to nature as technically possible.

As the natural source, *Gardenia jasminoides* Ellis is from the plant family Rubiaceae and its native to China. It is an evergreen shrub. That grows 4-8 feet tall and wide. It is a fragrant flowering evergreen tropical plant which is commonly grown in Vietnam, Southern China, Taiwan and Japan. The fruits are oval in shape about one-half inch to one inch long and orange in color (Figure 1 to 6). The major component of the fruit of *Gardenia*

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jasminoides Ellis is the iridoid glycoside geniposide.

Genipin is hydrolytic product of geniposide, which is found in the fruits of *Gardenia jasminoides* Ellis. The components of the fruit have been used in traditional Chinese medicine and as a blue colorant by food industries. The blue pigments are edible and are currently being used as a blue food colorant in East Asia. Djerassi and his colleagues discovered the structure of genipin in the 1961. It possesses the molecular formula $C_{11}H_{14}O_5$ and contains a dihydropyran ring. Genipin itself is colorless but it reacts spontaneously with amino acids to form the blue pigments (Djerassi *et al.*, 1960).

The fruit of *Gardenia jasminoides* Ellis has been used as traditional Chinese medicine to treat irritability in febrile diseases, jaundice, acute conjunctivitis, epitasis, hematemesis, pyrogenic infections and ulcer of skin, and externally on sprains and painful swelling due to blood stasis (Tsai *et al.*, 2002). Gardenias are widely used as exotic ornamental flowers in corsages, as houseplants, and in some regions, as outdoor plants. A yellow silk dye has been made for centuries from the chemical compound crocetin extracted from the gardenia berry.

Moreover, Sung and his co-workers (1999) conducted an in vivo experiment to test the ability of gelatin crosslink with genipin glue as a bioadhesive for closing skin wounds. Bioadhesives are used in surgery for tissue adhesion and also for stopping the flow of blood. The current bioadhesive being used is gelatin crosslinked with glutaraldehyde. However, as stated earlier the toxicity of glutaraldehyde has led to the investigation of the use of other crosslink reagents. Furthermore, the gelatin crosslink with genipin glue was found to be more flexible than the gelatin crosslink with glutaraldehyde glue. In addition to these activities, genipin crosslinked gelatin has also been conducted as a conduit for peripheral nerve regeneration (Chen *et al.*, 2005) and genipin crosslink chitosan for protein drug delivery (Chen *et al.*, 2004).

Therefore, genipin will become an important crosslink reagent for biomaterials in the future. So production of genipin might also be essential. In recent investigation, reliable methods for produce genipin glue and food colorant Gardenia blue and purple were described.

Aims and Objectives

-To develop and efficient method for the production of high stability, superior quality, value added, standardized, ready to use genipin and blue, green, purple pigment.

-To develop a concentrate used in pharmaceutical, food, cosmetic and allied industries.

-To develop a water soluble concentrate.

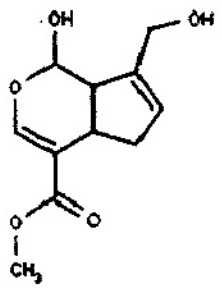
-To growing concern over the safety of synthetic dyes.

-To product organic certified natural colorant from Myanmar source.

Materials and Methods

The fruits of *Gardenia jasminoides* Ellis were collected from Site Khung village, Sei Sai Township, Southern Shan State. They are dried at room temperature about one month. After that, continue to dry in temperature controlled oven at 40°C for 6 hours. Then, they were grind to get powder and store in an air tight container to prevent moisture changes and contamination. As the chemical materials, Ethyl alcohol, acetone, β-glucosidase and taurine were used in recent investigation.

The resent invention relates to an efficient method for the extraction of high stability, superior quality, value added, standardized, ready to use genipin and blue, purple, green pigments; said process comprising steps of mixing Gardenia fruit powder with food graded-solvent, macerating and agitating the mixture with continuance protection from light condition, filtering the macerated mixture to remove undesirable fibrous plant material, freezing the filtrate immediately for remove fat and oil components. After 24 hours, the yellow precipitate was seen in solution (Figure 7, 8). Filtered them then, the remnant portion is hydrolyzed by β-glucosidase.



Genipin

100 gram powder of *Gardenia jasminoides* fruits and 500ml 50% ethanol were mixed and macerated at 80°C efficiently for 2 hours and filtered to afford an aqueous material, which was store in deep freezer. After 24 hours filtered gain the yellow precipitate (16 g) from the solution. The rest aqueous fraction 350 ml was reddish brown in color. The remnant was continuing extracted two times with 500ml distilled water. To obtain genipin, that reddish

brown color solution and water extracted fraction were combined and hydrolyzed by β-glucosidase at 60°C with continuous shaking for 4 hours. Then, that solution was crystallized with acetone and form colorless amorphous crystals, genipin. Then, to obtain blue color (Gardenia blue), reacted with amino acid in pH 3-9 at 40-90°C for 2-10 hours and to be stable brilliant blue color, kept at 80°C for 4 hours. According to recent result, taurine was the best amino acid to obtain stable color.

The combine ratios of Genipin and taurine were 1:1 at pH8 weakly alkaline buffer solution for getting purple color (Figur 11); and 2:1 for pH 6 weakly acidic buffer solutions to get brilliant blue (Figure 12). Furthermore, green color could be simply modified by combining of yellowish brown and brilliant blue color in 1:9 ratios.

Result and Discussion

As a result, recent research produced reliable methods for high stability, superior quality, value added, standardized, ready to use genipin and blue, purple pigment for pharmaceutical and food industries (Figure 11, 12).

Natural colors have historically been an essential component of many food products. Practically all consumers judge the palatability of foods not only on flavor, texture, and aroma, but also on appearance. A large number of these consumers would find foods that did not meet their expectations for vibrant yet reliable colors to be unappealing and perhaps would suspect that they are not sufficiently nutritious or even, in some cases, safe to eat. Consumer acceptance of these foods is therefore based in large part upon the ability of processed food manufacturers to utilize food colors to maintain expected and desirable appearances for their products (UNCTAD, 2005)

Organic consumers expect no less from their foods. Organic foods are chosen by consumers because they know that they are healthy and reliable, but also because they look good to consumers. For many foods, a large part of this positive appearance is due to the use of natural colors. In all cases, the consumer is guaranteed that in addition to the great flavor and health benefits of eating organic foods, they also have the expected appearance and a highly desirable palatability. The use of natural colors in organic and traditional foods is critical due to the processes involved in food production (Hendry and Houghton, 1992; Lauro and Francis, 2000). In many processes there is at least one and occasionally several heating

steps involved in the conversion of raw ingredients to final food products. In other cases the blending of ingredients changes the pH or increases the rate of oxidation. An object, therefore, of the present invention is to produce colors from the Gardenia fruits derived genipin, which is stable over a broad pH range and in an ethanol containing aqueous solution.

According to United States Patent 4878921, when analyzed by high-pressure chromatography, produces a chromatogram containing the following six principal components, A to F in blue dye, which respectively have approximate retention time of 1.6 min., 2.3 min., 2.7 min., 2.9 min., 3.6 min., 4.4 min., with components A, B and C each having an absorption maximum (λ_{max}) of about 592 nm, component D with λ_{max} of about 602 nm, component E with λ_{max} of about 614 nm, component F with λ_{max} of about 627 nm, as measure in 40% ethanol.

Conclusion

The information on analysis and chemical composition of Gardenia nuts are of academic interest. No concrete information is available in the literature for the quantitative isolation and modification of pigments. Hence, recent information might be able to used for production of natural colorant from *Gardenia jasminoides* Ellis, especially Myanmar lacks organic certified natural colorants for National health. Because there is no current supply of organic certified natural colorants from international sources, and because there is no current supply of organic certified natural colorant from Myanmar source, and because natural colorant of levels below 5% greatly improve the visual appearance of organic foods and beverages, this research seeks the addition of organic pigments (natural colorants) to the National List.

Acknowledgement

The authors acknowledge the Ruby Dragon Companies for the supplement of the plant sample and matured fruits samples for recent research. I would like to express my thanks to Professor and Head of Department of Botany, Dawei University, Dr. Daw San Wai Aung for the permission of this research to do well.

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Fig.1. Habit of *Gardenia jasminoides* Ellis

Fig. 2. Flower of *Gardenia jasminoides* Ellis

Fig.3. Fruits of *Gardenia jasminoides* Ellis

Fig.4. Dried fruits of *Gardenia jasminoides* Ellis with

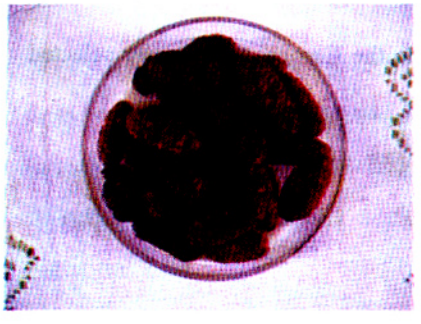

Fig.5. Dried fruits of *Gardenia jasminoides* Ellis without

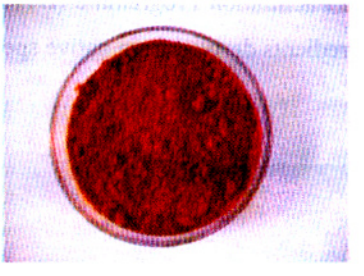

Fig.6. Dried fruits Powder of *Gardenia jasminoides* Ellis

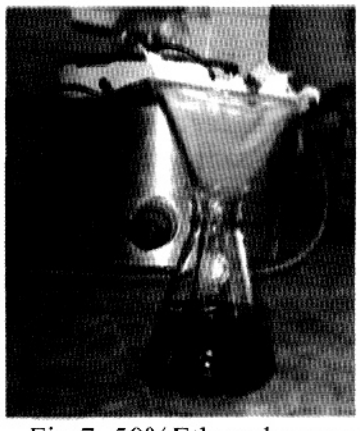

Fig.7. 50%Ethanol extract of dried fruits Powder of *Gardenia jasminoides* Ellis

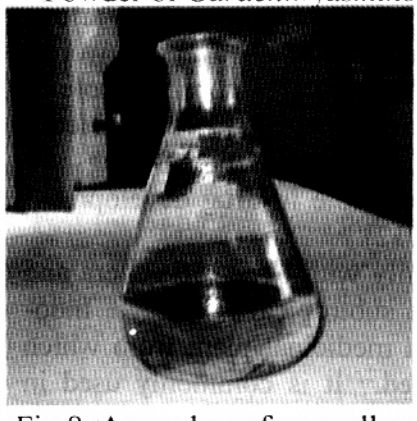

Fig.8. Amorphous form yellow ppt saffron with solvent

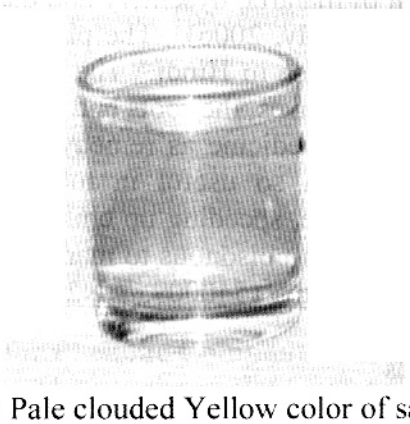

Fig. 9. Pale clouded Yellow color of saffron when dissolves in water

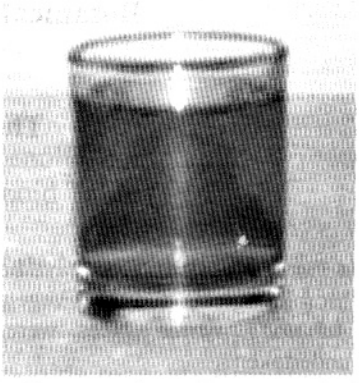

Fig. 10. Orange clear (honey color) when dissolves in water

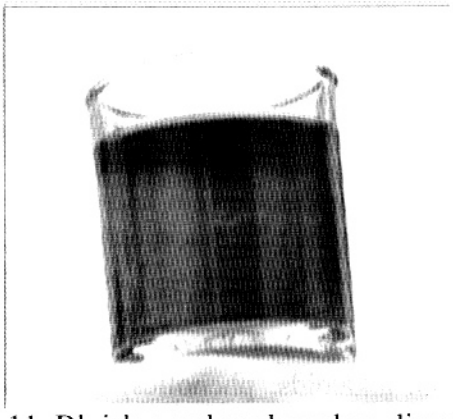

Fig. 11. Bluish purple color when dissolves in water

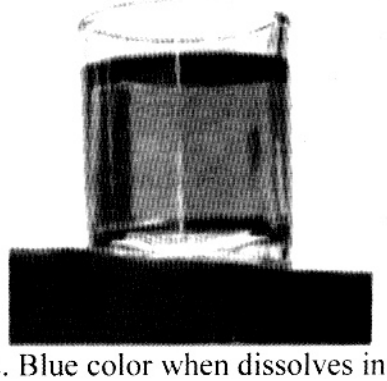

Fig. 12. Blue color when dissolves in water