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Use of Genipin as a Natural Amino Acid Based Fingerprint Enhancing Reagent and Comparison with Frequently Used Ninhydrin

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Abstract: ***Genipin is a natural product obtained directly from Genipa americana and after the hydrolysis of iridoid glycoside called Geniposide, found in the fruit of Gardenia jasminoides Ellis (Rubiaceae). It is colourless but generates dark blue pigment spontaneously on reacting with amino acids. It has recently been investigated as a fingerprint enhancement reagent on absorptive surfaces. Genipin also exhibits high luminescence characteristics at 590 nm excitation and 635 nm emission wavelength without further treatment. Studies have indicated that it provides better quality prints than the most frequently used fingerprint reagents, ninhydrin on papers of high luminescent background, and moreover it is eco-friendly. Because ninhydrin has various toxic effects on mammals, we suggest Genipin can act as a potential substitute of ninhydrin in near future. The scope of this article covers the above short discussion in detailed manner while reviewing the recent research works done in past.***

Keywords: Fingerprint enhancement reagent, Genipin, Gardenia jasminoides, Ninhydrin

1. Introduction

Fingerprints have often been and still are considered as the gold standard for personal identification in forensic science. Since the19th century, Forensic fingerprinting analysis has been used to help investigators link one crime scene to another involving the same individual. Fingerprints are the most frequently used as physical evidence used to establish the identity of the individual. Since no two individual's fingerprints are exactly alike, Forensic fingerprint analyst visually compares the deposited or latent fingerprint from the crime scene to the suspect [1]. When the finger touches any surface, the sweat from the pores on friction ridge skin of the fingerprints deposit in the form of contours which are exact mirror or negative image of the ridge pattern [2,3,4].

The human body possesses three types of sweat glands: Eccrine, Apocrine and Sebaceous. The secretion of Eccrine gland mainly contributes to the deposition of a fingerprint. The Eccrine sweat gland is distributed throughout the complete body surface except on the lips and glans penis [5]. Composition of Eccrine gland can differ according to hydration, fitness level and region of the body [6,7,8]. Composition of Eccrine gland is mostly water but also consist traced amount inorganic salts and organic derivatives like urea, creatinine, choline, sugar, amino acids and protein [9]. Since sweat is colourless in nature, the deposited patterns are also not visible and hence are called "latent fingerprints". Amino acids are, however, of primary importance for the visualization of the latent fingerprint impression details.

There are wide ranges of chemical methods for visualizing latent prints. Ninhydrin is one of the promising amino acid-based methods. In 1954, Ninhydrin was found to be an important reagent to develop fingerprints on porous surfaces [10] where Ninhydrin react with amino acids to the fingerprint content to produce the nonfluorescent purple colour known as Ruhemann's purple [11] after the name of the scientist Siegfried Ruhemann, who first synthesized it in 1910. Since that time, ninhydrin and its analogues have been synthesized and used for the purpose of fingerprint development or enhancement. Although, this method has been successfully used for years in the forensic field, Scientists continue to identify and use alternate natural products for fingerprint detection and examination to decease print development safety hazards as it is also important that fingerprint enhancement techniques are safe to carry out. One such natural product is Genipin, which have recently been identified as a naturally sourced plant product to chemically visualize latent prints by Almog and coworkers [3].

2. Source of Genipin

2.1. Gardenia jasminoides

Gardenia jasminoides is a Chinese medicinal plant belonging to the Gardenia genus and Rubiaceae family. It is also referred to as Cape jasmine and mainly grows and cultivated in most Asian continents like China, Vietnam, Taiwan, Japan and India. Typically, it can grow up to 6-8 ft high. It has been reported that fruit of *Gardenia jasminoides* can be used to treat headache, hypertension, inflammation, hepatic disorder and edema [12]. The extract of gardenia fruit is the natural source of geniposide and genipin. Geniposide is the main iridoid glycoside in ripe Gardenia Fruit [13] and when geniposide got hydrolysed by b-glucosidase, it gives genipin [14].

2.2. *Genipa americana L.*

Genipa americana L. is species of trees that belongs to Rubiaceae family. The tree can grow upto110 ft with a tall, slender trunk and spreading branches. It occurs throughout Brazil especially in lowland areas. It is cultivated as ornamental tree and for its fruits. The fruit of Genipa americana L. is also known as Genipa, Genipap or Jagu [15] and has been traditionally used as colorant in many industrial purposes. The main compounds of Genipa are genipin, and genipin 1-β-gentiobioside [16].

3. Structure of Genipin

Structure of Genipin was discovered in1960's by Djerassi and his colleagues using NMR spectroscopic data and experiments on chemical degradation. The empirical formula of Genipin is $C_{11}H_{14}O_5$ and possesses a dihydropyran ring in its structure [17]. The blue pigment form when Genipin in the presence of oxygen react with primary amine sources such as amino acids and proteins [18]. The structure of Genipin is shown in Fig 1(a)

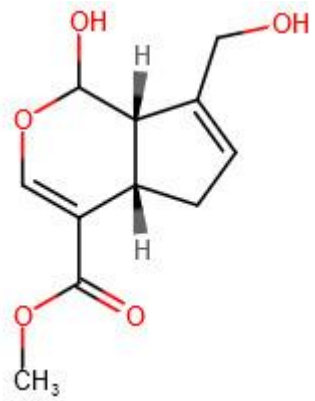


Figure 1(a): Structure of Genipin [17]

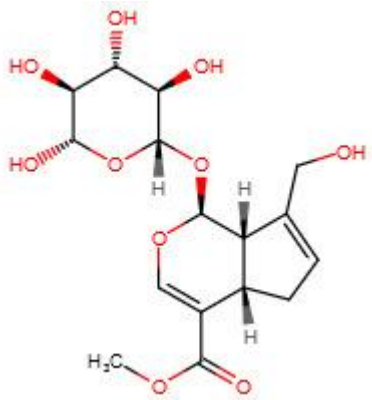
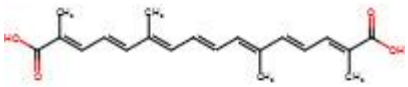


Figure 1(b): Structure of Geniposide [18]

4. Phytochemistry

When we talk about the major constituents of *Gardenia jasminoides*, it has geniposide and geniposidic acid, crocin and crocetin apart from the Genipin plays a major role in pigment formation [23]. The figures {Figure-1(a), Figure-1(b), Figure-3, Figure-4 and Figure-5) described the chemical structure of different components. Figure-1(a), Figure-1(b), Figure-2, Figure-3 and Figure-4 represents Structure of Genipin, Geniposide, Crocetine, Geniposidic acid and Crocin respectively [23].



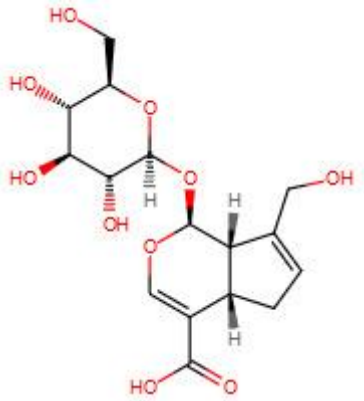


Figure 2: Crocetine [23]Figure-3 Geniposidic acid [23]

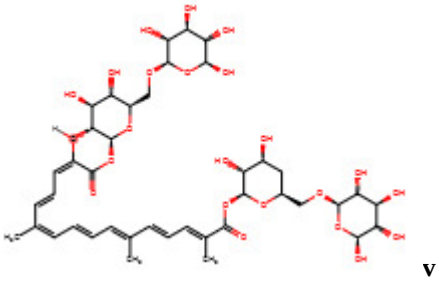


Figure 3: Crocin [23]

5. Isolation of Geniposide and Genipin

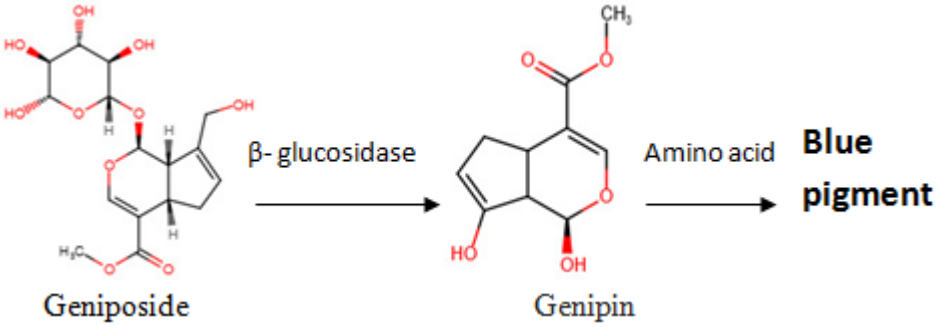
Extraction of Geniposide and Genipin

In 1973, Endo and Taguchideveloped method of extraction of geniposide and genipin from Gardenia Jasminoides which is described below [24]. The same method is still in use, but various modifications in this method have also been introduced now.

- The Gardenia Jasminoides fruit is first pulverized.
- This Pulverized fruit is extracted with Chloroformto remove oil and fat and then with methanol for three times at room temperature.
- This combined methanol extract is then to *a small volume* under reduced pressure result *in* brown mass.
- The charcoal filtered extract is then washed withwater, then with 10% aqueous ethanol which will separate sugar. This is then washed with methanol to separate glycosides.

- The glycosidic fraction is then applied to a silica gel column using a mixture of ethanol and methanol (7:3 (v: v)) and crystallized from acetone to colorless needles of genopiside.
- The isolated genopiside and β-glucosidase is added to acetate buffer at pH-5.
- This mixture is then incubated at 37°C for three hours and three times extracted with diethyl ether.
- The extract is then dried, filtered and concentrated.
- The concentrated mixture is then crystallized from diethyl ether to yield colorlessgenipin.

6. Mechanism of Blue Pigment Formation in of Gardenia Fruit



7. Comparison between Ninhydrin and Genipin for Latent Fingerprint Development

As earlier we have discussed about the formation of colour upon reaction of amino acid (can be encountered in fingerprints as a major component) with Genipin or Ninhydrin, now we can also consider Genipin as a natural fingerprint developing substance. But as an organic substance we must consider the advantages or disadvantages while using genipin. So, the comparison of Genipin is done below with Ninhydrin (commonly used chemical for latent fingerprint development).

In a study the amino acids taken in aqueous medium were plotted on a TLC plate and the separation was done in phenol and water in a ratio of 3:1. Both the considered colouring agents (Ninhydrin and Genipin) were sprayed onto the plate and further steps were carried out as per the standard procedure [20]. The results obtained from the study indicated that majority of amino acids reacted with the natural substance Genipin achieved more molar absorptivity with respect to the Ninhydrin. Further it also shows the stability of genipin is more than ninhydrin. The strength of genipin is well described considering pH, temperature, light etc; in a different section of this article. Major drawback was noted when metal ions were taken into consideration. The ninhydrin solution developed colour, wherein genipin fails to generate colour upon reaction with metal ions [20]. In a study latent fingerprints were developed using both genipin and ninhydrin. The results obtained from genipin was having better resolution as compare to the ninhydin [28].

8. Factors Influencing Physical Stability of Pigment

The *Gardenia jasminoide*'s fruit can be commonly encountered in southern parts of Korea. Apart from its application considering forensic aspects, these can also be implicated in medical cases such as medicine for antiphlogistic. It is also used as a laxative, choleretic and diuretic etc, in similar manner. Moreover, for food and fabric colourant or dye (Yellow), this is being used traditionally in various part of Asian countries. Carotenoids and some other related components usually form the yellow pigment. We can also get blue colour pigment just by modifying enzymatic reactions and amine treatment.

In a study by Young-Sook Paik and his colleagues in 2001 they monitored the physical stability of the pigment (Blue) derived from Geniposide [21]. They considered various factors such as pH, temperature and light etc to conduct this experimental analysis [21]. Upon hydrolysis with alpha-glucosidase the *Gardenia* got transformed into blue pigments, which involves reaction with amino acid at the terminal step [22]. Study indicates that the suitable pH for formation of blue pigment is 7.0. Amino acids which are giving the best results are- Glycine, Lysine, Phenylalanine. Further UV-Vis spectroscopy suggested the rapid disappearance of genipin upon reaction with amino acids at 240nm, while keeping the scanning interval at 5 minutes. Also stated that, at 290 nm the intermediate peak initiated to be visible and around 570-600 nm gives the pigment (Blue). Now if we are considering the amino acids individually, the pigment formation can be achieved as per the table below (TABLE-1).

Table 1

Amino Acid	Absorption Maxima formed at	Reference
Glycine	Lowest Absorption Maxima (580nm)	
Lysine	Absorption Maxima more than Glycine (583nm)	[21]
Phenylalanine	Highest Absorption Maxima (589nm)	

The stability can be greatly influenced by heat, Light and pH. Study suggests after Thermal degradation rection while treatment under variable pH and temperature condition shows a higher stability in alkaline condition rather than acidic condition. The absorption fluctuation suggested the extent of degradation of pigment. The experiment pin-pointed the percentage degradation at various stages. At time interval of 10hrs under 60degree maximal temperature and variable pH of 5.0, 7.0, 9.0 suggested 97%, 99% and 105% of degradation fluctuation rate. It clearly indicates the additional synthesis of pigments in the alkaline environment. At the same experiment, it also suggested the lysine can

dramatically increase this synthesis process. When the genipin was treated with lysine to form the blue pigment and was treated under 60degree maximal temperature and variable pH of 5.0, 7.0, 9.0 (time interval- 10hrs) gives the degradation percentage 104%, 102% and 110% respectively. The experimental values clearly determined the high thermal stability of the blue pigments formed by genipin.

Table-2 below represents the degradation in percentage when Glycine was treated with Genipin at pH 5:

Table 2

Time Interval (in hour)	At Temperature 60° C	At Temperature 70° C	At Temperature 80° C	At Temperature 90° C	Reference
0	100%	100%	100%	100%	
02	99%	94%	90%	89%	
04	98%	92%	87%	85%	[21]
06	97%	92%	84%	82%	
08	97%	92%	83%	80%	
10	97%	90%	81%	75%	

Table-3 below represents the degradation in percentage when Glycine was treated with Genipin at pH 7.0:

Table 3

Time Interval (in hour)	At Temperature 60° C	At Temperature 70° C	At Temperature 80° C	At Temperature 90° C	Reference
0	100%	100%	100%	100%	
02	101%	99%	98%	96%	
04	101%	98%	97%	93%	
06	101%	97%	94%	90%	[21]
08	101%	96%	94%	88%	
10	99%	94%	94%	85%	

Table-4 below represents the degradation in percentage when Glycine was treated with Genipin at pH 9.0:

Table 4

Time Interval (in hour)	At Temperature 60° C	At Temperature 70° C	At Temperature 80° C	At Temperature 90° C	Reference
0	100%	100%	100%	100%	
02	102%	102%	102%	101%	
04	103%	103%	103%	101%	
06	104%	104%	102%	98%	[21]
08	104%	103%	99%	94%	
10	105%	103%	99%	93%	

Table-5 below represents the degradation in percentage when Lysine was treated with Genipin at pH 5:

Table 1

Time Interval (in hour)	At Temperature 60° C	At Temperature 70° C	At Temperature 80° C	At Temperature 90° C	Reference
0	100%	100%	100%	100%	
02	101%	99%	100%	87%	[21]
04	102%	99%	98%	83%	
06	102%	99%	98%	81%	
08	103%	100%	95%	79%	
10	104%	100%	95%	79%	

Table-6 below represents the degradation in percentage when Lysine was treated with Genipin at pH 7:

Table 6

Time Interval (in hour)	At Temperature 60° C	At Temperature 70° C	At Temperature 80° C	At Temperature 90° C	Reference
0	100%	100%	100%	100%	
02	102%	101%	99%	98%	
04	101%	101%	98%	94%	
06	101%	102%	97%	91%	[21]
08	101%	102%	95%	89%	
10	102%	101%	94%	85%	

Table-7 below represents the degradation in percentage when Lysine was treated with Genipin at pH 9:

Table 7

Time Interval (in hour)	At Temperature 60° C	At Temperature 70° C	At Temperature 80° C	At Temperature 90° C	Reference
0	100%	100%	100%	100%	
02	103%	103%	105%	105%	
04	105%	106%	105%	105%	
06	107%	108%	105%	103%	
08	109%	109%	103%	101%	[21]
10	110%	108%	102%	102%	

9. Conclusion

Detection and visualization of fingerprints with the use of natural *reagent* (Genipin) represent a positive alternative to Ninhydrin which has toxic effects such as skin allergy, somnolescence and arterial or venous dilation. Moreover, *the durability* of the blue spots from genipin and amino acid reaction is high and remains longer than *conventional methods*, ninhydrin on TLC plates. Thus, Genipin has a huge potential of becoming a very important tool in forensic science.

10. Acknowledgement

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