Determination of Indigo in Indigo wood root tea

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Abstract

As a common medicine, people usually drink indigo wood root tea to prevent and cure cold in china. The indigo (indole group) was found as a useful component, not only could cure cold, but also have good effect for other diseases. Moreover, it does not have negative effect. The separation and extraction technology are used to mensurate indigo from the indigo wood root tea. We determined the absorption of indigo in the tea solution at wavelength of 290nm with HPLC and found the tea has some indigo (or components with indole group) in it. As a tea with good effect for healthy we suggest to drink it when feel catch cold or have a fever.

Keywords: Indigo (Indigotin), Indigowoad root (Indigo wood root), HPLC, UV-Spectrum, absorption.
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Background:

Indigo wood root is a kind of traditional medicine in China. Indigo and indirubin are the available components in the indigo wood root. We can easily find the indigo wood root tea are wildly used in China. According to research of the past, indigo is good for protecting liver. Indirubin can not only cure the tumor, but also be used for curing some kinds of leucocythemia. The research of Liu Yi et al. has studied the extraction and determination of mass ration of indigo in indigo wood root. They found the indigo was scanned from 240nm to 700nm, and at 285nm the indigo have a largest peak.

With the improve of science the Liquid chromatography (LC) combined with ultraviolet-visible(UV-VIS) and mass spectrometric(MS) detection was utilized to study the chemical components present in extracts of natural plants.

Indigowoad [B][Y]

a. source:

The indigowoad is Baphicacanthus cusia bremek, isatis indigotica fort, indigofera suffruticosa mill, polygonum tinctorium ait.

b. shape:

Growth period is two year. The stalk is vertical. There are a lot of branches at the top of indigowoad. Leaves at the bottom and shape oblong. The length is 15~30 cm, the width is 3~7 cm.
Group 1

The leaves in the stalk shape acicula and circle. The length is 3–5 cm, the width is 0.5–3.5 cm. The plant is raceme. The flower is yellow. There are 4 sepals, 4 petals and 6 androeciums. The florescence is 4–5 months, fruit period is 6 months.

c. collection:

at the early of winter, it should be dug with mud. Then through solarization, get rid of mud, make it dry in order to save it.

d. efficacy:

Clear heat, detoxification, and cool blood

e. function cure:

it can cure cold, epidemic spinal cord brain fever, epidemic second cephalitis, pneumonia, erysipelas, fever toxin, pharynx turgescence, tumefaction.

f. taste:

it taste bitter and cold.

g. chemical component:

Indoxyl-B-glucoside \( \left( \text{C}_{14} \text{H}_{17} \text{NO}_6 \right) \), isatin, \( \text{C}_5 \text{H}_7 \text{NO}_3 \), \( \text{C}_{26} \text{H}_{48} \text{O}_{16} \), \( \text{C}_{18} \text{H}_{30} \text{O}_{10} \), vegetable albumen, sugar and so on.

**Indigowoad root**[Y][Z]

It is the root of indigowoad. It shapes slight column. The length is 10–20–30 cm. the diameter is 3–8 mm. the surface is tough and light lark. The basic is a little big. Its quality is massy and
crisp. Moreover, it tastes sweet.

In China, people usually make some tea of indigo wood root product. Then take it to prevent and cure cold. It is very convenient, impact and good taste. In addition, another important thing is that it is made from plants. Then it does not have bad effect of health. In China, Indigowoad Root is popular for preventing cold, cure flu, hepatitis and parotitis. Indigo is one of the most important compounds in Indigowoad Root and it is a kind of dyeing for chemical and eatable industry. The blue dye indigo was used to create beautiful patterned fabrics, paintings, and color paper for letters, medicine wrappings and religious writings.

Historically, indigo was used by European herbalists to treat ulcers and several types of infections, including those affecting the mouth and gums, lymph nodes, and throat. [Hoffmann D. The New Holistic Herbal. Shaftbury, Dorset, UK and Rockport, MA: Element, 1990, 241.]

For the research of indigo has a long experience. People was used HPLC for determined the indigo from the leaves and roots of plants (Baphicacanthus cusia Bremek, Isaatis indigotica Fort, Indigofera suffruticosa Mill, polygrown tinctorum Ait). They found the wavelength of indigo is 288nm to 292nm with a UV-Spectrogram test. [Y]

In 2003 there also have some people did the experiment about extraction and determination of mass ratio of indigo in indigowoad root. They found the wavelength of indigo is 285 to 601nm with UV-Spectrogram test. [Journal of China Agricultural University]

In our experiment we use indigo and Indigowoad Root (from China). We want to find the determination of indigo in indigo wood root tea.

**Problem Formulation**

Can we determine indigo in indigo wood root tea?
Theory

1. HPLC

HPLC (High Performance Liquid Chromatography) is used for testing the absorption of the indigo wood root products. It is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. [H]

The sample comes from the injector, and then comes into the detector. A detector measures response changes between the solvent itself, and the solvent & sample when passing through it. The electrical response is digitized and sent to a data system. [H1]

A chromatographic column is shown in the picture up. A length of it, about 15 cm would peacked with stationary phase. By change the concentration of the solvent(methanol and water) will change the separate time of the sample in the column ,and the result will be showed as a curve.[C]
In the experiments of us we use two column on one HPLC (result part b) for more separated. And this can make the main column more obturation.

2. UV spectrum (ultraviolet spectrum)

Spectrophotometry is the quantitative study of electromagnetic spectra. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near ultraviolet, and near infrared. In addition, the term does not cover time-resolved spectroscopic techniques.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer (a device for measuring light intensity) that can measure intensity as a function of the color, or more specifically, the wavelength of light. There are many kinds of spectrophotometers. Among the most important distinctions used to classify them are the wavelengths they work with, the measurement techniques they use, how they acquire a spectrum, and the sources of intensity variation they are designed to measure. Other important features of spectrophotometers include the spectral bandwidth and linear range.
The main theory of UV-Spectrum formed of the complementary of the color. The light that can be seen by human eyes is from blue to red. The color from blue moves to red, then the wavelength becomes long. It has been used on the large astronomical telescope to test the distance of the star and the main compounds of the star. Because the different element have a different wavelength when the light cross it. For example, the blue solution will absorb the yellow light. And the result will shows a large peak at the wavelength of 600nm. The UV light (ultraviolet) can not be seen by human eyes, but only test by the equipment.

3. GCMS
GCMS is an abbreviation for Gas chromatography-Mass spectrometry. It is an advanced piece of chemical analytical equipment typically used to characterize complex organic mixtures.
Mass spectrometry makes the samples evaporation and ionization, then form the ions columns. Through the pressure make the ions columns speedup and focalize. And go through the magnetic field and electric field. So different ion will focalize different places forced by the magnetic field and electric field. Finally, we can get the mass chart of the researched matters.

4. Distribution and extraction
Extraction is used for the isolation of enrichment of certain compounds or groups of compounds in chemical mixtures. The tea includes water, indigo, sugar, dextrin and any other compounds. Because of the HPLC is very sensitive, so the better way is use an organic compound as a carriers to extract the indigo. As the indigo wood root tea is a liquid phase, normally choose the continuous liquid – liquid extraction. Because of the
process is more easily to be done with the same state of the different compounds. The extraction can be performed readily in a separate funnel.

The liquid are allowed to separate into two layers, and the denser liquid is with drawn through the stopcock.[C]

For the indigo wood tea solution, when it mixed with dichloromethane and be separated, the aqueous phase can contains sugar, dextrin and indigo will story in the dichloromethane. Because of sugar and dextrin are polar matters like water. And the polar compounds are easy to stay in the polar solution. The indigo is a completed organic compound. Then it will much more stay in the dichloromethane.

**Results and discussions**

1. The wavelength of indigo of UV spectrum

The curve on the down page shows the wavelength of indigo. When we used HPLC then choose the wavelength as 290 and 600. (melting point: 390~392°C) The indigo solution does not like the other blun solution, and do not absorb the yellow light(at 400nm) but at 290nm in UV area. We do not find indigo when work with HPLC at the wavelength this as curve shows the large peak at 740nm.
2. indigo determination

Indigo can be obtained from indigofera. The glucoside and indoxyl with water can becomes D-glucose with indole. And oxidization it then get indigo.[L]

The test results from three different HPLC are different.

Part A

Figure 1 is the peak of pure indigo with DMF solution in 290nm wavelength

Figure 2 show the mass spectrum of indigo. The mass of indigo is 262(C16H10N2O2)
Group 1

Figure 3 shows the tea solution (with DMF) and indigo. At the wavelength of 290, indigo comes out at the ratio time at 10.42 and 10.72, it is different with the indigo standard. Figure 4 is the pure tea solution (with DMF). It seems like almost no indigo inside. The reason may become the pollution of the column or the indigo in indigo wood root tea is very little, and the concentration of the sample is too low, so the indigo cannot be checked out.

Part B

The result of part B is controlled by “chrome leon” version 6.40 [cn]

Figure 5 shows the indigo sample in dichloromethane at the wavelength of 290nm shows the peak at the ratio time of 6.36, and the peak of UV spectrum is 267.5. (Figure be marked on the paper)
Figure 5 (a)

Overlay of Samples and Spectra from Integration View

REPORT DEF/Overlay UV Spectra Print

Chromeleon (c) Dionex 1996-2001
Version 6.40 SP1a Build 719
Figure 5 (b)

Overlay of Samples and Spectra from Integration View
Figure 6 run with another column at wavelength of 290nm. The solution mixture with tea and dichloromethane, at the ratio time 6.061, is a strong peak with almost the same ratio time as part B in indigo sample.

The GCMS was used for test the mass spectrum. As a melting of 390 – 392, the system cannot separate it well and to find out it.

To find a good mass spectrum is necessary for more good analysis of HPLC. But it is hard to find it because the column is not the same state in the different day. For a not so stable system (may include pollution by the different chemical compounds), the result will have a big different. Also the different column will give a different result at the different ratio time. The result can not explain the peak at the different wavelength (260nm and 290nm), may because the size of column and the pollution of the solvent that used with HPLC.
Figure 6 (a with 100 micro litter)
Conclusion:

This report has discussed the determination of indigo in indigo wood root tea. Include UV-spectrum, HPLC and extraction from the mixture of different state. For a completed organic compound like indigo, it is very hard to discover all the properties by doing several experiments. Because when a question is worked out and then comes more for the science. Each layer unraveled reviews new secrets, but also new mysteries. That is one rational reason of we keep on working harder on the way of science. Otherwise, these mysteries always keep our curiosity, and help us to discover ourselves, and the world we are living on.

The determination we have discussed can be used in any other traditional Chinese medicine or product with the similar properties like indigo. As today, it seems like we should use some time to look back what we have had in history. When the synthetically medicine do not as well used as before. Although the unknown part of the traditional medicine may be as many as the new things, but the advantage is that we have a long history to use it and that is the priceless experience.

Experiment process

This experiment takes two months. We have tried a lot of methods and use different equipments to research on the indigo and the product. In our university, three HPLC equipments can analyze compounds. Moreover, our group has tried them all in order that make sure we can get the scientific results. Therefore, we divided three steps to describe our experiment.

Step 1 Use UV spectrum to test the wavelength

Sample: indigo (Indigo, use the compound from sigma-aldrich[S]) mixed with dichloromethane.
Concentration: put 15 mg indigo in a 50ml flask and filled up with dichloromethane to 50ml.

1. First, use electronic balance to weigh 15mg indigo.
2. Use graduated flask to measure 35ml of dichloromethane. Mix them together to make solution as sample.
3. Take the sample into the vial for UV spectrum and test it with the wavelength from 900nm to 270nm.

**Step 2** Use HPLC to test the absorption of Indigo and the products of Indigo wood root

Sample: indigo (Indigo, use the compound from sigma-aldrich[S]) mixed with dichloromethane

Concentration: put 15 mg indigo in a 50ml flask and filled up with dichloromethane to 50ml (may changed with methanol)

1. First, make sample as above.
2. Make a HPLC solution of which concentration is 50% methanol + 50% miniq water (may changed with 80% methanol + 20% miniq water) then inject 150ul of sample to the hole like the picture below. After that close the hole, circumgyrate the button to start to analyze.

Injector the sample [H]

Use a syringe to take 100-150ul the mixture of the sample and inject into the HPLC.
[H]Wait for the separation. The curve can be drawn automatically by the separation.

Another equipment.

This equipment is the most advanced of the three equipments that we have told you. In this machine, we can get the result of HPLC and UV spectrum in one time just through computer that we can control the experiment using the program. It is very convenient and exact. At last, we can get a piece of paper in which we can see the result clearly and easily. However, in this step, we have a problem, it is that the equipment is too advanced to use.

**Step3 Use dicholomathane to separate the tea**

1. Add 5 gram tea in to 100 ml hot water (at around 100 degree), and dissolved it well. The solution shows color in brown.
2. Use ice to cool it around 0 degree.
3. Take 20 ml tea into the separate funnel, and add 20 ml dicholomathane inside. Shake it well.
4. Wait for about 3 to 5 minutes, and the solution will be separated in two phases. The first phase is water phase with sugar and the below one contains dicholomathane with the other things. The color of the water face is very light yellow and the dicholomathane is almost brown.
5. Open the stopcock to get the solution of dicholomathane in a beaker.
6. Add bitter salt (about 10mg or more) into the solution to make it saturation. Moreover, put the beaker on a hot plate, use magnetic stirrer to mix it well. In addition, waiting about one hour for gets rid of the water in the solution.
7. Use the filter paper and funnel to get rid of the deposit in the solution, until the solution becomes the color like clear brown.
8. Move the solution into a pear-shaped flask and use a Rota vapor to evaporate the liquid.
9. Dissolve the crystal matter with 5ml DMF (Dimethylformamide), and mixture well.
10. Use a syringe to make the sample. One is 25 micro litter of the solution and 975 micro litter of DMF, and the other one is 50 and 950. All samples contains in auto sampler vials.

**Perspective:**

Now we find that the indigo wood root tea contains indigo and some other similar compounds. Of course, that is why it can prevent and cure cold. It is not the same as normal medicine. It is much healthier and better taste than medicine. Everyone knows that if you take too much bills, which will be dangerous for your health. Nevertheless, it is very different of the indigo wood root tea. Because it is made from crude plants. Therefore, it cannot be bad for your health. In addition, when you are free or you are afraid of that, you maybe catch cold; you can make a tea of this product. Then you will be safe that you need not to worry. Although it is a common medicine in china. Every Chinese people know such a good product. No one who is from other countries knows it. Therefore, we want to introduce this good product to everyone. Moreover, we want to
introduce all Chinese medicines to everyone. As you know, western medicine can get quick effects, but it may have side effect because of their sources ---chemical compounds. On the other side, Chinese medicines usually taste bitter, and the impact comes slowly. Its advantage is that they are made from plants. Then they will not bring in other problem.

Let us think. If we can join them, two type of medicine. Then we can assume that our products can be the most popular products. From now on, we will try to find this kind of products to prove assume of us. What we will do can not only improve economic, but also be useful for medicine. Imagine if we can develop a new product that is a good taste drink. Then you can take care of your body while you are having the drink.

**Acknowledgement**

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**Reference List**


[H]. High Performance Liquid Chromatography (HPLC): A Users Guide, the University of Kentucky.


[S]Sigma-Aldrich: the company of chemical products.


Appendix:

Indigo

Name: Indigo, synthetic [M]
Catalog Numbers: 21213-0000, 21213-0250, 21213-1000
Synonyms: C.I. 73000, Vat Blue 1
Molecular Formula: C\textsubscript{16}H\textsubscript{10}N\textsubscript{2}O\textsubscript{2}\text{(C 73.28\%, H 3.64\%, N 10.68\%, O 12.20\%)}
Molecular Weight: 262.26

Physical and chemical properties [M]

Physical State: Powder
Color: dark blue-violet
pH: 9.5(indole group)
Freezing/Melting Point: 390 deg C
Flash Point: 390 deg C (734.00 deg F)
Decomposition Temperature: 390 deg C