Analysis of Fake Honey and Sildenafil Adulterated Honey

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GRAPHICAL ABSTRACT

(a) Example of honey sample and
(b) Viagra tablet

ABSTRACT

Recently, there has been a report in local newspaper for fake and adulterated honey. Fake honey was prepared using sucrose while adulterated with sildenafil for the pleasure that it brings. In this study, local honey was used and analyzed for sucrose while for comparison laboratory prepared sucrose was also analyzed. Since adulterated honey with sildenafil was not obtained, laboratory simulation or doped was prepared. Characterization of fake honey and adulterated was carried using Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) Spectroscopy and Ultra Violet-Visible (UV-Vis) Spectroscopy respectively. Qualitative analysis of honey, sildenafil and sucrose using FTIR-ATR showed a huge difference between honey and sildenafil, however there is no difference between honey and sucrose. So, the honey sample was suspected to contain sucrose and this is also supported by the analysis using UV-Vis that showed the peak at 280 nm to represent sucrose. The analysis of sildenafil using UV-Vis spectroscopy at 306 nm illustrated a good result with calibration curve linear up to 0.9975. The percentage of error and percentage of recovery are 1.7 % and 94.5 % respectively. The analysis of sucrose at 280 nm gave the regression line up to 0.9320 and the percentage of error and percentage of recovery are 2.7 % and 94.5 % respectively. From the UV analysis, the honey sample was identified positive to contain 23 ± 1 µg/mL of sucrose meanwhile negative for sildenafil.

Keywords: Fake honey, prepared sucrose, FTIR-ATR spectroscopy, honey sample, adulterated honey, sildenafil, UV-Vis spectroscopy.

1. INTRODUCTION

Honey is one of the precious natural food commodities from ancient times and there is high market demand for natural honey. Original and pure honey has been reported to contain about 200 substances. Honey is composed primarily of fructose and glucose but also contains fructo-oligosaccharides and many amino acids, vitamins, minerals and enzymes. Most of those compound works together to provide a synergistic antioxidant effect. [1]

Honey consumption has grown significantly during the last few decades due to its high nutritional value and unique flavor. The price of natural bee honey is much higher than other sweeteners making it susceptible to prepare the fake honey using sucrose. [2] However, according to the international regulations, any commercially available "pure"-labeled honey products that are found to have in excess of 5% by weight of sucrose or maltose are considered to be adulterated. [2] A number of studies done proved that the honey products were adulterated by other active pharmaceutical ingredients and synthetic drugs such as anti-obesity and sex stimulant agent such as sildenafil. [3]

Viagra is the brand name for a medicine known as Sildenafil (Figure 1) was being proved that would induce marked penile erection. However, over dosage of this drug will give hazardous effects to the users. Unfortunately, there were still many people who abuse this drug for recreational and unaware of the hazardous effect of sildenafil. Manufacturers on the other hand take advantage by selling these products. In Malaysia, it was reported that sildenafil was adulterated in honey bee to enhance the sexual pleasure.
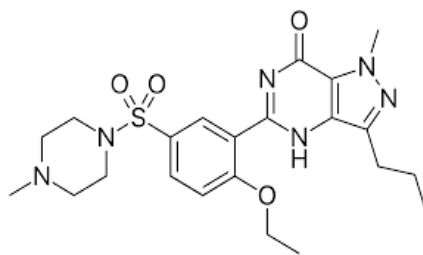

Figure 1 Chemical Structure of Sildenafil

This research will emphasize on the development a simple, easy in handling and cost effective method to identify the fake honey, to detect the presence of sildenafil and sucrose in local honey products and to analyse the presence of sildenafil and sucrose in local honey product qualitatively and quantitatively.

2.   EXPERIMENTAL

In this chapter, there are two major parts of the research which are the analysis of local honey, sildenafil and sucrose by using FTIR-ATR. Then, the research proceeds with an analysis on the presence of sildenafil and sucrose in local honey qualitative and quantitatively by using UV-Vis Spectroscopy.

Firstly, the analysis of local honey and sildenafil will be starting by buying a local honey from supermarket and sildenafil from the pharmacy. Meanwhile, the sucrose obtained by heating the sugar on a hot plate until its melts. Then, the honey sample and sucrose directly injected to the instrument while the sildenafil need to crush first before analyse by using FTIR-ATR.

Next, several series of concentration of sildenafil was prepared after mixed with a honey in order to analyse it using UV-Vis Spectroscopy. Standard solutions of 100, 200, 300, 400 and 500 µg/mL of sildenafil were used to plot the calibration curve.

One of the concentrations of sildenafil was taken for further analysis. The solution was analysed repeatedly for ten times in order to determine the percentage of error. The percentage of error can be calculated from the result obtained based on formula as belows:

$$Percentage\ error = \frac{\frac{\Sigma X - \bar{\mathrm{x}}}{10}}{\bar{\mathrm{x}}} \times 100\%$$

The research proceeded with an analysis of sucrose in honey by using same methods as sildenafil.

3.   RESULTS AND DISCUSSION

3.1.   Qualitative analysis of local honey, sildenafil and sucrose using FTIR-ATR

Currently, infrared spectroscopy is one of the most common spectroscopic techniques used in the food industry. With the rapid development of infrared spectroscopic instrumentation software and hardware, the application of this technique has expanded into many areas of food research. It has become a powerful, fast and nondestructive tool for food quality analysis and control. FTIR-ATR technique used to identify the functional group present in the local honey sample, sildenafil and sucrose respectively. The IR spectrums are as in Figure 1, Figure 2 and Figure 3 respectively.
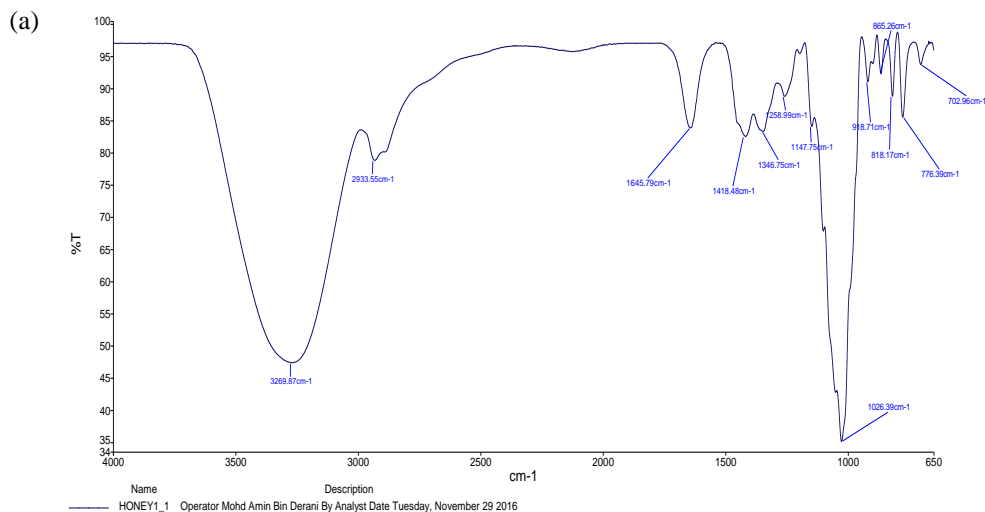

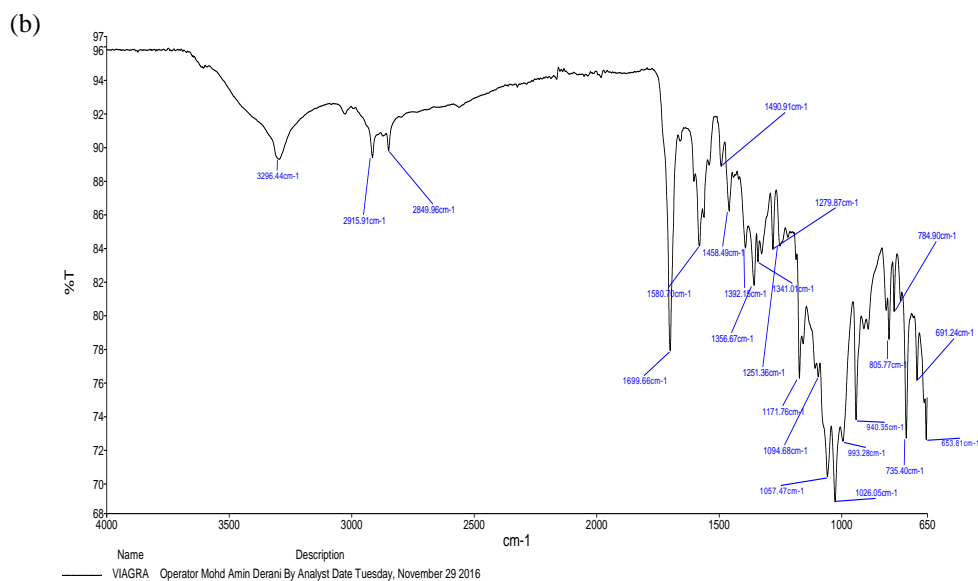**Figure 1** Infrared Spectrum of Honey

(b)

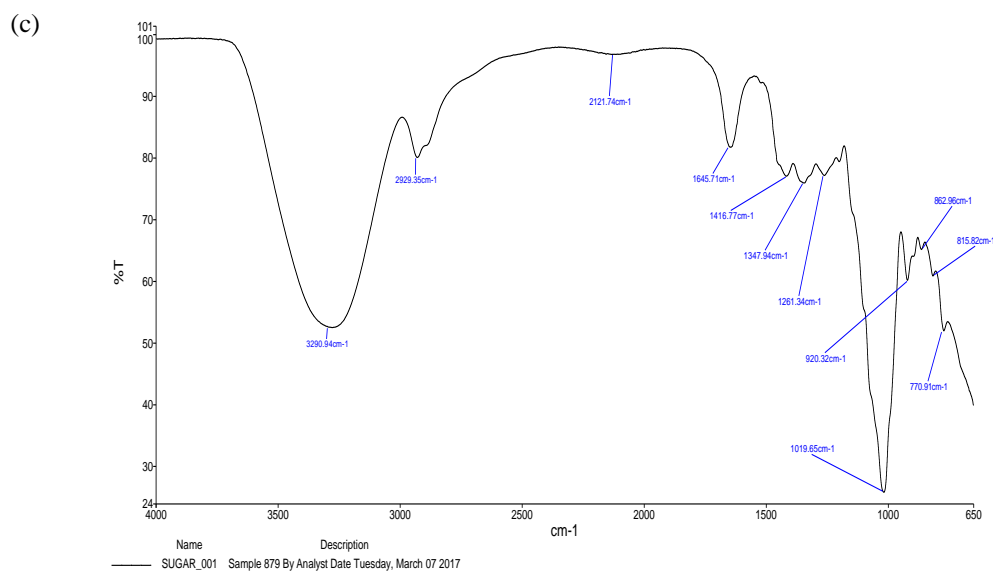**Figure 2** Infrared Spectrum of Sildenafil

(c)

Figure 3 Infrared Spectrum of Sucrose

From the results of FTIR-ATR, it was observed that the difference between infrared spectrum of sildenafil and infrared spectrum of honey. In the spectrum of honey, there is a broad and intense peak around 3268.35 cm^{-1} suspected of the presence of hydroxyl group (OH$^-$) while a moderate peak around 3296.44 cm^{-1} presence in the spectrum of sildenafil. Besides, there is one peak around 2933.09 cm^{-1} presences in the spectrum of honey while there are two peak about 2915.91 cm^{-1} and 2849.96 cm^{-1} in the spectrum of sildenafil. Furthermore, we can clearly see that the intense peak presence in the spectrum of sildenafil about 1699.66 cm^{-1} suspected for the presence of carbonyl group (C=O) while the medium peak presence in the spectrum of honey. Then, there is no aromatic peak presence in the spectrum of honey but there are peaks around 1580.70 cm^{-1} and 1458.49 cm^{-1} indicates the aromatic exists in the sildenafil.

By comparing the infrared spectrum of honey and sucrose, it was clearly show that there is no difference between these two results. So, it can be conclude that the honey sample may be sucrose and not a pure honey. The peaks of sildenafil also do not appears in the infrared spectrum of honey indicates that the honey sample does not contain sildenafil. This statement can be strengthen from the result of an analysis of local honey using UV-Vis Spectroscopy that showed at peak at 280 nm (wavelength for sucrose) and there is no peak at 306 nm (wavelength for sildenafil).

3.2   Quantitative analysis of sildenafil in adulterated local honey using UV-Vis Spectroscopy

Analysis of sildenafil in local honey using UV-Vis spectroscopy gives the absorbance of sildenafil at 306 nm wavelength. The absorbances are different based on the concentration of sildenafil in the solutions. The absorbance detected for respective solution was as shown in the Table 1. Tables 4.1 show the concentration sildenafil citrate using UV-Vis method at wavelength 306 nm due to the high correspondent of sildenafil. From the table, this method has successfully determined the presence of sildenafil at different concentration related with the intensity peak of the spectrum.

Table 1 Absorbance of Sildenafil at 306 nm wavelength

Concentration of Sildenafil (µg/mL)	Absorbance
0	0.000
100	0.105
200	0.412
300	0.760
400	1.139
500	1.536

Figure 4 was demonstrated the calibration curve of absorbance and sildenafil concentration. It shows the correlation value of sildenafil concentration to the respective absorbance. This calibration curve was very useful to determine quantitatively the concentration of sildenafil in the samples based on the absorbance recorded.
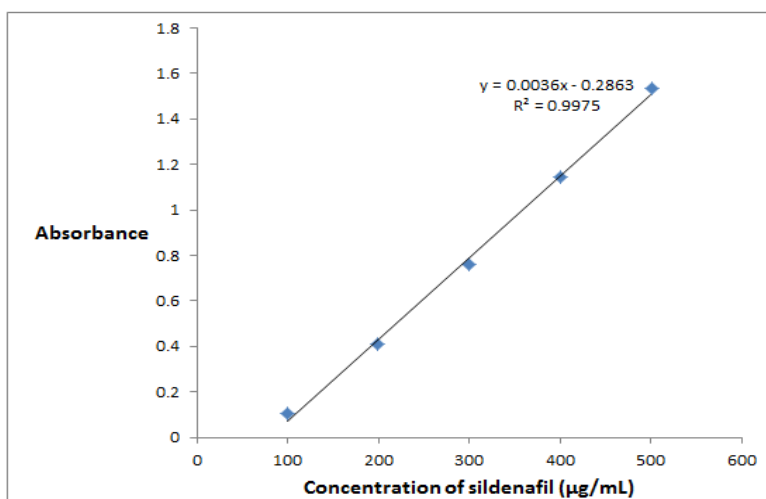

Figure 4 Calibration curve of absorbance against sildenafil concentration

Table 2 Results of Ten Replicates 100 µg/mL Standard Solution of Sildenafil

Number of replicates	Absorbance
1	0.164
2	0.168
3	0.170
4	0.166
5	0.166
6	0.169
7	0.186
8	0.184
9	0.183
10	0.182

In addition to the qualitative and quantitative analysis to the concentration of sildenafil, some analytical calculations were done for a method validation. The percentage of error was evaluated by testing ten replicates of 100 µg/mL standard solution of sildenafil within the day. The result was being demonstrated by the Table 2 as below. From the calculation, the percentage of error for sildenafil was 1.7%.

3.3   Quantitative analysis of sucrose in adulterated local honey using UV-Vis Spectroscopy

Analysis of sucrose in local honey using UV-Vis spectroscopy gives the absorbance of sildenafil at 280 nm wavelength. The absorbance is different based on the concentration of sucrose in the solutions. The absorbance detected for respective solution was as shown in the Table 3.

Table 3 Absorbance of Sucrose at 280 nm wavelength

Concentration of Sucrose (µg/mL)	Absorbance
0	0.000
100	0.216
200	0.295
300	0.368
400	0.624
500	0.850

Figure 6 was demonstrated the calibration curve of absorbance and sucrose concentration. It shows the correlation value of sucrose concentration to the respective absorbance.
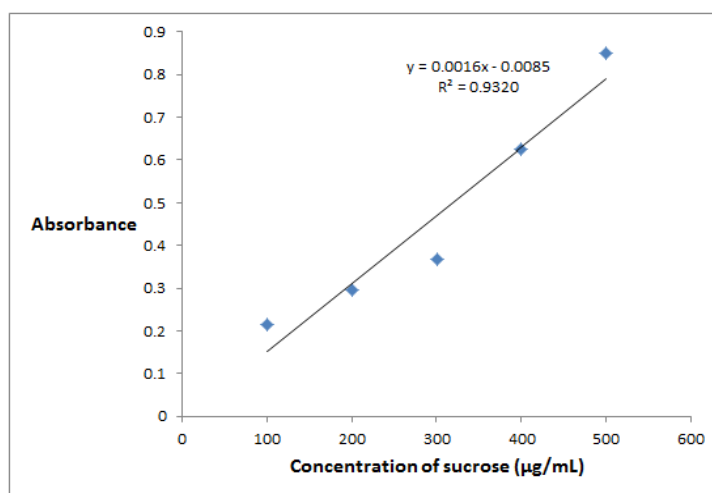

Figure 6: Calibration curve of absorbance against sucrose concentration

Table 4: Results of Ten Replicates 300 µg/mL Standard Solution of Sucrose

Number of replicates	Absorbance
1	0.368
2	0.368
3	0.368
4	0.395
5	0.368
6	0.382
7	0.395
8	0.368
9	0.368
10	0.355

In addition to the qualitative and quantitative analysis to the concentration of sucrose, some analytical calculations were done for a method validation. The percentage error was evaluated by testing ten replicates of 300 µg/mL standard solution of sucrose within the day. The result was being demonstrated by Table 4 below. The percentage of error for sucrose was 5.3% obtained from calculation.

3.4   Determination of Sildenafil and Sucrose in Local Honey Sample

To determine the amount of sildenafil and sucrose in the samples, the sample was analyzed using UV-Vis Spectroscopy. From the spectra obtained, the absorbance of the sample was recorded. Quantitation analysis of concentration of sucrose in the sample was done using calibration curve equation $y = 0.0016x - 0.00085$. Calculated amount of concentration of sucrose was 23 ± 1 µg/mL while for the sildenafil, there is no peak presence in the spectrum indicates that the sildenafil absence in the sample.

4.   **CONCLUSION**

Through this study, two methods had successfully developed in identifying the fake honey and detecting the presence of sildenafil and sucrose in local honey products. The qualitative and quantitative analysis was done by FTIR-ATR and UV-Vis spectroscopy and these two proposed method are simple, easy in handling and cost effective method to analyse the local honey for sucrose and sildenafil adulteration. The development of the effective UV-Vis spectroscopy in screening, detection and confirmatory of sildenafil and sucrose hopefully can help the enforcement agencies to do inspection regularly on the product available in the market, and thus can reduce illegal business of sildenafil-sucrose contained products. Thus, hopefully it could increase the awareness to the society in Malaysia and all over the world about doped and hazards of sildenafil. As the conclusion, the combination of all these analysis method including FTIR-ATR and UV-Vis spectroscopy proved that it's able to identify the fake honey and quantitate the presence of sildenafil and sucrose in the honey samples. The resulted spectrums gave major information in determination and quantitation the presence of sildenafil and sucrose.

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