The FTIR and Heavy Metal Analysis of Some Anti-diabetic Herbal Drugs Sold in Ondo State, Nigeria

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Abstract: The use of herbal drugs for the treatment of various ailments especially diabetes mellitus has greatly increased recently due to the approval and recommendation from regulatory bodies. The safety level of these herbal drugs has not been clearly understood. This study was designed to determine the functional groups responsible for the anti-diabetic activity of these drugs and also to estimate their heavy metal concentrations. Three most popularly sold and readily available anti-diabetic herbal drugs were purchased from Okitipupa Market in Ondo State. The Fourier Transform Infrared Spectroscopy (FTIR) analysis was done by scanning the samples on Shimadzu FTIR machine at a wavelength of 4000 – 500 cm⁻¹. For the heavy metals analysis, the herbal drug samples were digested using standard method and analyzed on an Atomic Absorption Spectrophotometer (AAS). The FTIR spectral revealed absorption bands corresponding to -OH, C-H, C=O, C=C and S=O functional groups. The concentrations of the selected heavy metals are Copper (0.4328 to 0.2022 ppm), Cadmium (0.2763 to 0.022ppm), Nickel (0.8740 to 0.0551ppm), Lead (0.4333 to 0.3043 ppm), Manganese (1.5444 to 0.9405 ppm) and Zinc (0.2234 to 0.1224 ppm). The concentrations of these heavy metals were below the WHO maximum permissible limit for
herbal drugs. This study ascertained that these herbal drugs are not contaminated with heavy metals. However, prolong use of any of these herbal drugs should be avoided to prevent adverse effects of heavy metals bio-accumulation.

**Keywords:** Diabetes mellitus, heavy metals, herbal drugs and FTIR analysis.

1. Introduction

Diabetes Mellitus is a disease characterized by hyperglycemia that result from defects in insulin secretion and insulin action. The International Diabetes Federation, in the year 2015 estimated that about 415 million of the word populace are suffering from diabetes and predicted an increase to 642 million in the year 2040 [1]. Therefore, the need to develop effective treatment methods that are safe for diabetes treatment is a necessity.

Reports shows that medicinal plants play crucial role in the treatment of diabetes mellitus [2]. Recent advancement in pharmacological world has lead scientist and healthcare professionals to take advantages of herbal products in replacement of synthetic drugs [3, 22-25]. The general belief that herbal drugs are safer compared to synthetic drugs are not authenticated according to the recommended pharmaceutical guidelines and scientist discoveries because herbal drugs often contain some toxic substances in form heavy metals and other toxicants [4,26].

High concentration of heavy metals and polycyclic aromatic hydrocarbons in herbal based drugs have been documented [5]. Recently substantial amount of toxic metals such as cadmium, arsenic and mercury has been discovered in some herbal drugs [6]. Contaminations of herbal drugs with substantial amount of heavy metals may occur as a result of environmental pollution of farmlands where medicinal plants are harvested, poor processing methods and storage conditions [7].

The irreplaceable role of some essential metals such as copper and iron in critical enzyme systems of human are well pronounced. However, other metals such as lead and mercury are regarded as been xenobiotic because they exhibit no useful role in human physiology even at minute concentrations [8]. Essential metals have the prospective of been toxic at very high concentrations of exposure.

The United State Agency for Toxic Substances and Disease Registry (ATDSR) had listed lead, mercury and arsenic as the first three heavy metals base on their prevalence and toxicity. Inhalation of metal in form of tiny particulate matter or as dust, ingestion of metals through involuntary intake through food and water are the major exposure route to heavy metals [8]. Higher concentrations of toxic metals in herbal drugs can also be traced to contaminated water used for irrigation of farmland [3]. Heavy metals in soil are transported to medicinal plants via plant cell wall and membranes, these heavy metals are transferred to human cell via food web.
However, high concentration of heavy metals will affect both human and plants whenever their concentrations are beyond the maximum recommended limit [9]. Exposure to high concentration of heavy metals has been traced to the development of countless diseases [10]. Therefore, to resolve this impending health challenges, the concentration of heavy metals in herbal drugs must be evaluate before recommendation for sales and use.

2. Materials and Method

Three unregistered herbal drugs commonly used for the treatment of diabetes mellitus were purchased from Okitipupa market in Ondo State Nigeria. These anti-diabetic herbal drugs were selected based on the manufacturer’s reputation and popularity among the general public. The descriptions of the selected anti-diabetic herbal drugs are showed in Table 1.

Table. 1: Basic information on the herbal drug samples

<table>
<thead>
<tr>
<th>S/N</th>
<th>Product name</th>
<th>Same code</th>
<th>Claimed ingredients</th>
<th>Form</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Apase powder</td>
<td>A</td>
<td>Alstonia scholaris bark and Moringa oleifera stem</td>
<td>powder</td>
<td>2 sachets/day</td>
</tr>
<tr>
<td>2</td>
<td>Osaprapra powder</td>
<td>B</td>
<td>Amomum aromaticum root and Delonix regia stem</td>
<td>powder</td>
<td>2 sachets/day</td>
</tr>
<tr>
<td>3</td>
<td>Tajutaju herbal mixture</td>
<td>C</td>
<td>Ficus carica root, Lygodium flexuosum bark and Mangifera indica bark</td>
<td>liquid</td>
<td>5–10ml/twice daily</td>
</tr>
</tbody>
</table>

2.1. Preparation of the Selected Anti-diabetic Herbal Drugs for FTIR Analysis

The powdered anti-diabetic herbal drug samples where air dried and kept in an air tight container prior further analysis. The FTIR analysis of the herbal drugs were carried out at the Ladoke Akintola University of Technology Central Research Laboratory. 1.0g of each of the powdered anti-diabetic herbal drug samples were mixed with potassium bromide (KBr) while the liquid anti-diabetic herbal drug was run as neat and were scanned on a FTIR spectrophotometer Nicolet iS50 (Thermo Fisher Scientific, Waltham, MA, USA) at wavelength 4000 – 500 cm⁻¹.

2.2. Digestion of the Selected Anti-diabetic Herbal Drug Samples

For the powdered anti-diabetic herbal drug samples, about 1.0g was weighed and transferred into a clean beaker. 10 cm³ of analytically grade conc. nitric acid (HNO₃) was added. The mixture was kept in a fume cupboard overnight. The solution obtained was heated carefully with a heating mantle at 60 °C.
for 45 minutes until the emission of fume ceased. The container was cooled at room temperature and 5 cm$^3$ of 70 % analytical grade perchloric acid (HClO$_4$) was added and further heated at 60 °C till the sample almost dried. The residue obtained was cooled and transferred into 50 cm$^3$ volumetric flask and diluted with deionized water. The solution was filtered and kept in clean sample bottle for atomic absorption spectrophotometer analysis. This procedure was repeated for all the powered anti-diabetic herbal drug samples.

For the anti-diabetic herbal drug in liquid form, about 100 cm$^3$ was measured and transferred into a clean beaker. 5 cm$^3$ of concentrated analytical grade nitric acid was added and heated in a fume cupboard. HNO$_3$ was continually added until the appearance of a clear solution. The digested sample was quantitatively transferred to a 100 cm$^3$ volumetric flask and deionized water was added to make 100 cm$^3$. It was filtered and analyzed with an atomic absorption spectrophotometer (Shimadzu AA-6200, Japan).

3. Results and Discussion

3.1. FTIR Analysis of Selected Anti-diabetic Herbal Drugs

3.1.1. FTIR Analysis of Sample A

The FTIR spectrum of sample A showed in Figure 1 revealed an absorption band at wavelength 3421 cm$^{-1}$ which correspond to the stretching vibration of –OH of alcohol and the band at 2931 cm$^{-1}$ was attributed to the C-H stretching vibration of alkane groups [11]. The sharp band at 2360 cm$^{-1}$ was attributed to O=C=O stretching of carbon dioxide [12]. The strong absorption band at wavelength 1616 cm$^{-1}$ correspond to C=C stretching and the strong band at wavelength 1033 cm$^{-1}$ was attributed to S=O stretching of sulfoxide. This suggest that sample A contains -OH, C-H, and C=C functional groups.

![Figure 1: The FTIR Spectrum of Sample A](image-url)
3.1.2. FTIR Analysis of Sample B

The FTIR spectrum of sample B revealed broad absorption band at wavelength 3419 cm\(^{-1}\) correspond to -OH functional group [11]. The sharp band at 2910 cm\(^{-1}\) was attributed to the stretching vibrations of alkane groups [11]. The sharp band at 2362 cm\(^{-1}\) was attributed to O=C=O stretching of carbon dioxide [12]. The sharp band at 1735 cm\(^{-1}\) was attributed to C=O stretching of aldehyde, The strong absorption band at wavelength 1620 cm\(^{-1}\) correspond to C=C stretching of an alkene and the strong band at wavelength 1053 cm\(^{-1}\) was attributed to S=O stretching of sulfoxide. This suggest that -OH, C-H, C=O and C=C functional groups are present in sample B.

![Figure 2: The FTIR Spectrum of Sample B](image)

3.1.3. FTIR Analysis of Sample C

The FTIR spectrum of sample C displayed in Figure 3 revealed broad absorption band at wavelength 3416 cm\(^{-1}\) which correspond to stretching vibration of -OH functional group and the sharp band at 2926 cm\(^{-1}\) was attributed to the stretching vibrations of alkane groups [11]. The sharp band at 2360 cm\(^{-1}\) was attributed to O=C=O stretching of carbon dioxide [12]. The sharp short band at 1635 cm\(^{-1}\) was attributed to C=C stretching of alkene and the strong band at wavelength 1078 cm\(^{-1}\) was attributed to S=O stretching of sulfoxide. This suggest that -OH, C-H, C=O and C=C are the functional groups present in sample C. These functional groups might be responsible for the anti-diabetic activity of this herbal drug.
3.2. Concentrations of Heavy Metals in the Selected Anti-diabetic Herbal Drugs

3.2.1. Concentrations of Copper in the Selected Anti-diabetic Herbal Drugs

The concentrations of copper in the studied anti-diabetic herbal drugs ranges from (0.4328 to 0.2022 ppm). Sample B had the highest concentration of copper (0.4328 ppm) while sample A has least concentration of copper (0.2022 ppm) as showed in Table 2.

Table 2: Concentrations (ppm) of heavy metals in selected anti-diabetic herbal drugs

<table>
<thead>
<tr>
<th>Samples</th>
<th>Copper(Cu)</th>
<th>Cadmium(Cd)</th>
<th>Nickel(Ni)</th>
<th>Lead(Pb)</th>
<th>Manganese(Mn)</th>
<th>Zinc(Zn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.2022±0.02</td>
<td>0.2763±0.01</td>
<td>0.8740±0.01</td>
<td>0.4333±0.01</td>
<td>1.1455±0.01</td>
<td>0.2234±0.02</td>
</tr>
<tr>
<td>B</td>
<td>0.4328±0.01</td>
<td>0.0517±0.02</td>
<td>0.1111±0.01</td>
<td>0.3331±0.02</td>
<td>0.9405±0.01</td>
<td>0.1561±0.03</td>
</tr>
<tr>
<td>C</td>
<td>0.2449±0.02</td>
<td>0.022±0.01</td>
<td>0.0551±0.04</td>
<td>0.3043±0.01</td>
<td>1.5444±0.02</td>
<td>0.1224±0.01</td>
</tr>
<tr>
<td>FAO/WHO</td>
<td>0.14–0.48</td>
<td>0.30</td>
<td>1.63</td>
<td>10.00</td>
<td>44.6–339</td>
<td>50</td>
</tr>
</tbody>
</table>

(mean ± SD, n = 3)

The result obtained from the concentrations of copper in this study was higher than that obtained from the findings of [8] on the estimation of copper level in some unregistered powdered medicinal herb sold in Kano State, Nigeria (0.1369 to 0.2465 ppm). Copper in this study has its concentrations in the
range of standard set by WHO/FAO (0.14–0.48 ppm) for copper in herbal drugs [13]. Copper at lower concentration is regarded as essential component of many enzymes. However, high intake or exposure to copper may cause discoloration of hair or skin and damage the liver [14]. The increasing order of copper concentrations in herbal drugs was given in the sequence; sample B > C > A as showed in Figure 4.

![Figure 4: Concentrations of copper in selected anti-diabetic herbal drugs](image)

3.2.2. Concentrations of Cadmium in the Selected Anti-diabetic Herbal Drugs

Among the studied anti-diabetic herbal drugs presented in Figure 5. Sample A has the highest concentration of cadmium (0.2763 ppm) while sample C has the lowest concentration of 0.022 ppm. The concentrations of cadmium in this study were found lower than that obtained from the studies of [7] on the determination of cadmium concentrations in some valuable herbal drugs sold in Karachi city in Pakistan and on the determination of heavy metal concentrations in consumed herbal drugs obtained in Kano in Nigeria [8]. The concentrations of cadmium in this study were also lower than the maximum permissible limit set by WHO (0.30 ppm) for herbal drugs [13]. The concentrations of cadmium in this study suggested that the herbal drugs studied are safe for consumption. However, low concentration of this metal does not guarantee the safety for consumption as bio accumulative effect on prolong use might rendered the herbal drugs unsafe for use [15]. The increasing order of cadmium concentrations in herbal drugs was given as sample A > B > C
3.2.3. Concentrations of Nickel in the Selected Anti-diabetic Herbal Drugs

Sample A has the highest concentration of nickel (0.8740 ppm) and sample C had the lowest concentration of nickel (0.0551 ppm) as shown in Table 2. The highest concentration of nickel obtained from this study was found lower that the least concentration of nickel obtained from the findings of [16] on heavy metal estimation of some Pakistani branded herbal drugs used treating various ailments (1.2 – 56.3 ppm). The most prominent effect of exposure to high concentration of nickel are lung cancer and nasal cavities blockage. The FAO/WHO standard of Nickel in Herbal drug has reported by [13, 17] was 1.63 ppm. This suggest that the intake of these drugs might not result to ailment linked to nickel. The increasing order of nickel concentrations in the studied herbal drugs was A > B > C as shown in figure 6.
3.2.4. Concentrations of Lead in the Selected Anti-diabetic Herbal Drugs

From the concentrations of lead shown in (Table 2), sample A has the highest concentration of lead (0.4333 ppm) while sample C has the lowest concentration of 0.3040 ppm each. The concentrations of lead in this study were found higher than that obtained from the study of [8] on the analysis of heavy metal concentrations in some herbal drugs obtained from Kano state in Nigeria (0.0556 -0.0927 ppm). The WHO limit for lead in herbal drug was 10 ppm. Lead is one of the most toxic element capable of causing chronic poisoning and adverse effect on different body systems. Renal, digestive and brain damages has been documented as part of side effect of excessive exposure to lead [18]. Based on result obtained from this study, the concentration of lead in the anti-diabetic herbal drugs samples were below the permissible limit set by WHO. This suggest that the use of any of these herbal drugs might not result to any disorder associated with excessive intake of lead. The increasing order of lead in the studied anti-diabetic herbal drugs was given as A > B > C as shown in figure 7.

![Figure 7: Concentrations of Lead in Selected Anti-diabetic Herbal Drugs](image)

3.2.5. Concentrations of Manganese in the Selected Anti-diabetic Herbal Drugs

Manganese concentrations in this research work ranges from 1.5444 to 0.9405 ppm. Sample B has the minimum concentration of manganese while the maximum concentration of manganese was detected in sample C as showed in Table 2. The highest concentration of manganese detected in this study was found to be lower than the values reported in previous study on estimation of manganese in some medicinal plants [19]. Also manganese concentrations obtained from this study were below the WHO permissible limit range (44.6–339 ppm) set for manganese in herbs. Impaired growth and reproductive function has been reported as symptoms of manganese deficiency. The increasing order of manganese concentrations detected in this study was C > A > B as shown in figure 8.
3.2.6. Concentrations of Zinc in the Selected Anti-diabetic Herbal Drugs

The concentrations of zinc in the studied anti-diabetic herbal drugs range from (0.2234 to 0.1224 ppm). Sample A had the highest concentration of zinc (0.2234 ppm) while sample C has least concentration of zinc (0.1224 ppm) as shown in Table 2. The WHO/FAO permissible limit for zinc in herbal drug was 50 ppm. Notwithstanding the concentrations of zinc detected in this study were similar to the findings of [8]. Zinc has been regarded as an essential element needed for normal growth, proper thyroid performance and clotting of blood. There are minute evidence on zinc toxicity. Zinc has been reported to support the regulation of blood insulin levels and also help in improving the sensitivity of insulin in controlling of diabetes [20]. Aside the Anti-diabetic activities of these drugs, they could also be helpful in normal growth, proper thyroid performance and clotting of blood. However, exposure to high concentration of zinc beyond permissible limit may lead to toxic effect that could hamper the proper functioning of the immune system in the body [21]. The increasing order of zinc evaluated from this study was A > B > C as shown in figure 9.
Figure 9: Concentrations of Zinc in the selected anti-diabetic Herbal Drugs

4. Conclusion

The FTIR analysis revealed that -OH, C-H, C=O and C=C functional groups found in these anti-diabetic herbal drugs might be responsible for their anti-diabetic activities. The heavy metal analysis of all the anti-diabetic herbal drug samples confirmed the presence of Copper, Cadmium, Nickel, Lead, Manganese and Zinc at concentrations below the permissible limits set by WHO. This study therefore ascertained that the studied anti-diabetic herbal drugs are safe for human consumption if consumed at low dosage. The increase in circulation of unregistered herbal drugs in Nigeria market should be discouraged and continuous monitoring of herbal drugs should be embarked on to prevent toxicity effect which may later endanger the users.

References


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