Bioactive Principles in Two Polyherbal Traditional Anti-Diabetic Formulations

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Abstract: Bioactive principles in two polyherbal traditional anti diabetic formulations of different plants used in the treatment of diabetes mellitus mixed in different ratios were characterized using Infrared spectroscopy. Six medicinal plants with proven anti diabetic and related beneficial effects were selected for the preparation of two mixtures Acanthus montanus, Asystasia gangetica, Emilia coccinea, Hibiscus rosa-sinesis, Momordica charantia (Bitter melon), and Venonia amygdalina. Mixtures of the All-Six (AS) herbal leaves recorded these compounds 3-beta-acetoxy-5-ethenyl, acid dihydroxyacetone, acetobromo-alpha-D-galactose, dihydroxyacetone, ethylacetoxyacetate, 4-

Keywords: Bioactive compounds, characterization, diabetes, polyherbs.

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I. Introduction

Herbs are any Plants used for flavouring food, medicine and perfume. Herbal drugs have been used since the inception of human beings, and as a result are almost as old as life itself. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. In the world today, natural products and their derivatives represent more than 50% of the drugs clinically used (Ogbuji et al., 2017). Medicinal plants have attracted increased attention since they can serve as an excellently pool for the discovery of new drugs by the use of their bioactive compounds (Ogbuji et al., 2017). According to World Health Organization (WHO,1976), traditional medicine is defined as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance using natural products (especially plant materials).

Bioactive compounds are compounds synthesized by plants that have the potential to be used by human for a variety of applications. They are extra nutritional constituents that typically occur in small quantities in foods. The bioactive compounds vary widely in chemical structure and functions and are grouped accordingly. Bioactive compounds also have actions in the body that promote good health, and they have been studied in the prevention of cancer, heart disease, diabetes and other diseases. (National Cancer Institute, 2014).Examples of these bioactive compounds include Alkaloids, Flavonoids, Saponins, Tannins, Indoles, Lignans, Phenols, etc. Phenolic compounds including their subcategory flavonoids are present in all plants and have been studied extensively in cereals, legumes, nuts, olive oil, vegetables etc. Many of these phenolic compounds have been studied to have antioxidant properties. (Olabinri et al., 2010).When these bioactive compounds are introduced into the body, they bind to a particular biochemical targets, most notably to protein involved in signaling by hormones and neurotransmitters. This process is essentially, the basis for the effect of herbal medicine.

Herbal drugs are prescribed widely because of their effectiveness, less side effects, broad range of action and relatively low cost. However, the non trial drugs are usually not evaluated for purity and consistency of active compounds. They often contain contaminants and might show batch-to-batch variation. The exact mechanism of action, for example in lowering blood sugar is often not known. In addition, these herbs may not work well for everyone and their overall effects may vary in individuals, due to lack of standardization. Side

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effects can also be patient specific and a combination of these herbs may be required to obtain the desired effects which lead to development of pre-clinical trials for poly-herbal formulation.

At present there is extensive growth in the field of herbal mixture, and these mixtures are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines are derived from medicinal plants and minerals which are used for the treatment of different chronic diseases like asthma, diabetes, and so forth. There are about zero pure compounds from plant source reported to show anti-diabetic activity when assessed using presently available experimental techniques (Saifi, et al., 1999). Major hindrance in amalgamation of herbal medicine in modern medical practice is lack of scientific and clinical data proving their efficacy and safety. It is also important to establish the active component of these herbal extracts. (Aparajeya et al., 2013)

*Acanthus montanus* known as Bear’s Breech or Mountain Thistle or Alligator plant belongs to the family Acanthaceae. The plant is a striking small shrub with sparse branches and soft stem. It is also a branched perennial with basal clusters of oblong to leave-shaped glossy, dark green leaves reaching up to 12 inches (30cm) long. The leaves have silver marks and wavy margin. It reaches up to 6 ft (1.8m) tall and about 24 inches (61cm) inside. It prefers shady situations and occasional deep watering, but tolerates sunning, dry situation too. It is commonly found in South Eastern Nigeria and Congo DRC (Igoli, et al., 2004, Okoli, et al., 2008). It is also found growing in the wild field of grass lands, wood scrub and rocky hills of the Balkans, Romania, Greece and Eastern Mediterranean (Huxley, 1992).

*Asystasia gangetica* is a species of plant in the *Heanhaecae* family. It is commonly known as Chinese violet, coromandel. It is an ornamental plant and source of forage for cattle, goats, and sheep in South East Asia. The plant is a spreading herb or ground cover, reaching 600mm in height or up to 1m if supported. It is also used in the prevention of noxious weeds.

*Emilia coccinea* is an annual weed widely distributed in tropical Asia and some part of Africa (Nigeria and Ghana) (Chillendon, 1956). The plant is edible, commonly used as salad in South Asia and herbal medicine in Eastern part of Nigeria (Edeoga, et al., 2005; Jimoh, et al., 2010).

*Hibiscus rosa-sinensis* L. commonly referred to as Chinese hibiscus, China rose or shoe flower which belongs to the family Malvacaceae (Kirtikar and Basu, 1987). It is an ornamental plant widely grown in the tropical and subtropical region of East Asia. *Hibiscus rosa-sinensis* is a bushy evergreen shrub or small tree growing 2.5-5m (8-16fit) tall and 1.5-3m (5-10fit) inside, with glossy leaves and solitary, brilliant red flowers in summer and autumn. *Hibiscus rosa-sinensis* is considered to have a number of medical used in Chinese herbology. It can also be used as pH indicator, when used; the flower turns acidic solution to a dark pink or margenta colour and basic solution to green.

*Momordica charantia* is a member of the Cucurbitaceae family, also known as a bitter melon, bitter gourd, balsam pear, karela, and pare. It grows in tropical areas of the Amazon, East Africa, Asia, India, South America and the Caribbean and is used traditionally as both food and medicine. The plant is a climbing perennial with elongated fruit that resembles a warty gourd or cucumber. The seeds, fruits and roots of the plants have been used in traditional medicine for microbial infections, sluggish digestion and intestinal gas, menstrual stimulation, wound healing, inflammation, fever reduction, hypertension, and as a laxative and emetic. Extracts of *Momordica charantia* also enhances cellular uptake of glucose, promote insulin release and potentiate its effects (Yibchok, et al., 2006; Welihinda, et al., 1982), and increase the number of insulin producing beta cells in the pancreas of diabetic animals (Ahmed, et al., 1998). Although, various therapeutic qualities have been ascribed to it, it is most widely valued in traditional medicines as an anti-diabetic agent. As the name suggests, the fruit has an extremely bitter taste, which is due to the presence of non-toxic alkaloid.

*Vernonia amygdalina* is a member of the Asteraceae family, is a small shrub that grows in the tropical Africa. *Vernonia amygdalina* typically grows to a height of 2.5m. The leaves are elliptical and up to 20cm long. It barks is rough. It is commonly called bitter leaf in English. African common names include Onugbu (Igbo), Ewuro (Yoruba), Etidot (Ibibio), Oriwo (Edo), Itunu (Tiv).

Since ancient times, traditional medicines all over the world have advocated the use of plants to treat diabetes. A wide array of plant derived active principles representing numerous chemical compounds has demonstrated actively consistent with their possible use in the treatment if Non-Insulin Dependent Diabetes Mellitus (NIDDM) (Bailey and Day, 1989; Marles and Farnsworth, 1995). Also the ethnomedical information reports about 800 plants that may possess anti-diabetic potentials (Alarcon et al., 1998). Anti-diabetic plants are those plants that have blood sugar lowering properties, which are made useful for people with high risk of type II diabetes. Plants based therapies that have been shown in some studies to have anti-diabetic properties include *Aloe vera*, bitter melon (*Momordica charantia*), ginger etc. Also since ancient times, plants have been used as herbal medicines in the treatment of various human ailments as alternative medicines.

Infrared (IR) spectroscopy is the spectroscopy that deals with the infrared region of electromagnetic spectrum that is light with a longer wavelength than those of visible light and lower frequency and determines the chemical functional groups in the samples (Sherman, 2000).
In the present study, six plants have been selected for the preparation of two mixtures. The herbs of interest were *Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea*, *Hibiscus rosa-sinensis*, *Momordica charantia* (Bitter melon), and *Venonia amygdalina*.

### II. Materials And Method

#### Collection and preparation of herbal samples

Fresh leaf samples of *Acanthus montanus*, *Asystasia gangetica*, *Hibiscus rosa-sinensis*, *Momordica charantia* and *Venonia amygdalina* and *Emilia coccinea* were collected from Alaenyi Ogwa in Mbaiteoli L.G.A Imo state, Nigeria. The leaves were sorted, washed and dried in an oven at 60°C. Each of the sample was pulverized using a high speed grinder separately. A formulation of the pulverized samples comprising of *Acanthus montanus*, *Asystasia gangetica*, *Emilia coccinea*, *Hibiscus rosa-sinensis*, *Momordica charantia* and *Venonia amygdalina* in the ratio of 1:1 (4g each) (AS) and also a formulation of *Acanthus montanus*, *Hibiscus rosa-sinensis*, *Momordica charantia*, *Venonia amygdalina* in the ratio of 1:1 (4g each) (AF) were made and stored in an air tight containers separately.

#### Sample preparation

A 0.5g portion of the formulation (AS) and (AF) were grounded in an agate mortar with 1g of specially purified potassium bromide (KBr) to fine powder to remove scattering effect from large crystals. This powdered mixture was then pressed in a mechanical press to form a translucent pellet through which the beam of spectrometer can press through.

#### Scanning process

The pellet was placed on the center of the sample plate and the pressure arm was swung over the sample and adjusted until it touched the sample. The scanning process for the sample began as the pressure arm touched it to generate spectrum with different wave numbers for functional groups of different organic or biological compounds. These functional groups were correlated with different biological compounds present from the software library of the Pelkin Elmer spectrum BX 11 spectrometer.

#### Spectral data analysis and compound identification

The spectra of the three samples obtained from the Pelkin Elmer spectrum BX 11 spectrometer was analyzed and compounds were identified by searching and matching the data on the NIST (National Institute of Standards and Technology) data base, Sigma-Aldrich online catalog product list, and pubchem compound search database. The results obtained from these searches were then used to characterize the bioactive compounds present in the samples.

### III. Results

The spectra pattern and results of the spectral characterization of of the bioactive compounds in the All- Six (AS) herbal formulation are shown in fig. 1 and table 1 below.

![Fig.1: Showing the Spectra Pattern of Some of the Bioactive Compounds in the All- Six (AS) herbal formulation.](image-url)
<table>
<thead>
<tr>
<th>SPECTRA WAVENUMBER (CM⁻¹)</th>
<th>IDENTIFIABLE FUNCTIONAL GROUP</th>
<th>IDENTIFIED COMPOUND</th>
<th>STRUCTURE IDENTIFIED COMPOUNDS</th>
<th>MOLECULAR WEIGHT (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1043.76</td>
<td>Primary amine</td>
<td>4-Aminoacetophenone</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>135.1632</td>
</tr>
<tr>
<td>1043.76</td>
<td>Ether</td>
<td>3-Beta-Acetoxy-5-Etienic Acid</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>360.491</td>
</tr>
<tr>
<td>1043.76</td>
<td>Ether</td>
<td>Ethylacetohydroximate</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>103.12</td>
</tr>
<tr>
<td>1043.76</td>
<td>Ether</td>
<td>Ethyl-4-Chloro-2-Cyanoacetoacetate</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>189.59</td>
</tr>
<tr>
<td>1247.66</td>
<td>Ether</td>
<td>Dihydroxyacetone, Dimer</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>180.16</td>
</tr>
<tr>
<td>1324.61</td>
<td>Phenol or tertiary alcohol, OH bend</td>
<td>Ethylacetohydroximate</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>103.12</td>
</tr>
<tr>
<td>1324.61</td>
<td>Aromatic tertiary amine</td>
<td>P-Toloyacetonitrile</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>131.1745</td>
</tr>
<tr>
<td>Formula</td>
<td>CAS Number</td>
<td>Description</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------------</td>
<td>-------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1324.61</td>
<td>Nitro group (primary amine)</td>
<td>4-Aminoacetophenone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1643.36</td>
<td>Ether</td>
<td>Dihydroxyacetone, Dimer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1643.36</td>
<td>Ketone and Aldehyde</td>
<td>4-Aminoacetophenone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1643.36</td>
<td>Ether</td>
<td>3-Beta-Acetoxy-5-Etienic Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2927.0</td>
<td>Alcohol/phenol</td>
<td>Dihydroxyacetone, Dimer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1643.36</td>
<td>Ester</td>
<td>Acetobromo-alpha-D-galactose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: showing characterized bioactive compounds from the All-Six (AS) herbal formulation.
Fig. 2. The spectra pattern and results of the spectral characterization of some of the bioactive compounds in the All-Four (AF) herbal formulation.

**Table 2:** Showing characterized bioactive compounds from the All-Four (AF) herbal formulation.

<table>
<thead>
<tr>
<th>SPECTRA WAVE NUMBER (CM⁻¹)</th>
<th>IDENTIFIABLE FUNCTIONAL GROUPS</th>
<th>IDENTIFIED COMPOUND</th>
<th>STRUCTURE OF IDENTIFIED COMPOUND</th>
<th>MOLECULAR WEIGHT (g/mol)</th>
<th>BOND/VIBRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1051.0</td>
<td>Ethers</td>
<td>Ethylacetohydr oxamate</td>
<td><img src="image" alt="Structure of Ethylacetohydr oxamate" /></td>
<td>103.12</td>
<td>C – Br stretch C = O stretch C – O – C – O – C</td>
</tr>
<tr>
<td>1250.51</td>
<td>Ethers</td>
<td>Ethyl-4,4,4- Trichloroacetate</td>
<td><img src="image" alt="Structure of Ethyl-4,4,4- Trichloroacetate" /></td>
<td>233.48</td>
<td>C – O stretch C – N stretch</td>
</tr>
<tr>
<td>1250.51</td>
<td>Aromatic primary amine</td>
<td>4 – Amino – Acetophenone</td>
<td><img src="image" alt="Structure of 4 – Amino – Acetophenone" /></td>
<td>135.1632</td>
<td>C – H wag C – O stretch C = O stretch</td>
</tr>
<tr>
<td>1324.61</td>
<td>Phenol or tertiary alcohol, OH bend</td>
<td>Ethylacetohydr oxamate</td>
<td><img src="image" alt="Structure of Phenol or tertiary alcohol, OH bend" /></td>
<td>103.12</td>
<td>C – H stretch C – H bend C - O stretch</td>
</tr>
</tbody>
</table>
### IV. Discussion

Figures 1 and 2 show the infrared spectra of the two mixtures (AS and AF). Table 1 (AS) shows these compounds: 3-beta-acetoxy-5-etiene, acidi dihydroxacetone, acetybro-mo-alpha-D-galactose, dihydroxacetone, ethylacetohydroxamate, P-tolyacetonitrile, 4-aminoacetophenone, dihydroxyacetone, ethylacetoxyhydroxomat, ethyl-4-chloro-2-cyanoacetoacetate while Table 2 (AF) shows the following compounds ethylacetoxyhydroxamate, ethyl-4,4,4-trichloroacetate, 4 – amino – acetophenone, ethylacetohydroxamate, p – tolyacetonitrile, thiophene-2-acetonitrile, ethyl-4-chloro-2-cyanoacetoacetate, acetobromo-alpha-D-galactose, ethylacetoxyhydroxamate, 4-aminoacetophenone and thiophene-2-acetonitrile. The compound p-tolyacetonitrile is
an acetonitrile in which the enzyme arylacetonitrilase catalyses its hydrolysis to arylacetic acid ammonia without any formation of amide. This enzyme does not attack the nitrile groups attached to aromatic and hetero-aromatic rings (Nagasawa. et al., 1990). Arylacetic acids are useful intermediates in the synthesis of pharmaceuticals. P-tolyacetanilide is involved in the synthesis of phenylacetic acid which is useful as an antiseptic (ELF, 1998). P-tolyacetanilide has a boiling point of 242-243°C and melting point of 18°C and density of 0.992g/ml at 25°C. Acetobromo-alpha-D-galactose also known as Galactopyranosyl bromide is a versatile and important intermediate in carbohydrate chemistry (Gablie and Deshmukh, 2010). It has been utilized as a starting material in the synthesis of thiogalactosides. Acetobromo-alpha-D-galactose and other related compounds are known as acetyl derivatives of carbohydrates. However, acetyl derivatives of carbohydrates are interestingly becoming important in medicinal chemistry and industries (Dandale. et al., 2007). The compound was also identified in both mixtures.

Ethylacetohydroxamate is another compound which was identified in both mixtures, it belongs to the hydroxamic acid family. Hydroxamic acids are the family of compounds presenting the strong chelating properties towards various metal ions (Kurzak. et al., 1992). Compounds containing the hydroxamic group have been shown to inhibit the activities of various metallo proteinases such as urease (Stemmler. www.wikipedia.com 3 3 www.Chemicaland2.com re part of the protein containing the arginine. It has been part of the development of more potent antiinflammatory drugs. It has a boiling point and density of 226.4°C 75°C and 1.437g/cm 3 respectively.

2-Thiopheneacetanilide is another organo-sulphur heterocyclic compound having a nitrile and sulphur group. It is a nitrile as well as a thiophene as it possesses the N═C group and one thiophene ring. Thiophene derivatives are found in natural plant pigments. Biotin, a water-soluble β-complex vitamin, is a reduced thiophene derivative. Thiophene moiety is found in cephalothin antibiotics. Thiopheneacetanilide is used as an intermediate of antibiotics; cefoxitin, cephaloridine, and cephalothin (www.Chemicaland2.com). The compound is a clear colourless liquid after boiling.

The compound 3-beta-acetoxy-5-ethen-17β-carboxylic acid is an androstene (www.CTD.com, 2014). Androstanes are unsaturated derivatives of the steroid androstane containing at least one double bond at any site in any of the rings. 3-beta-androstane-3-one is a powerful methemoglobin former in vivo. The compound is a slightly yellow to brown crystalline powder with a melting point, boiling point and density of 103-107°C, 293°C and 0.777g/cm³ respectively.

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V. Conclusion

The characterization of the two mixtures of polyherbal antidiabetic formulation showed that there are sixteen bioactive compounds in both mixtures. Six of these secondary metabolites (bioactive compounds) are common in both formulations while four are not common in both formulations. Based on previous studies, these bioactive compounds characterized are of pharmacological and medicinal importance. A deeper insight into the isolation of the bioactive compounds characterized in these herbs may lead to the development of more potent anti-diabetic drugs.
References


