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Evaluation of Spectrometric Characterization of Bioactive Compounds in *Hibiscus Rosa-Sinesis*

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***Abstract**: Infrared Spectrophotometric technique was employed to characterize some bioactive compounds present in the leaf extracts of Hibiscus rosa-sinensis. The compounds characterized were 2-thenoyltrifluoroacetophenone, p-tolyacetonitrile, 1,2,4- trichloroacetophenone, 4-chloroacetophenone, ethyl-4-chloro-2-cyanoacetoacetate,2,4,6-trihydroxyacetophenon,ethyl-4,4,4- trichloroacetoacetate, 1-acetonaphthane, and 3-indoleacetontrile were characterized in Hibiscus rosa-sinensis. The presence of these bioactive compounds in these herbs may be the key to their suggestive efficacy and application in ethno-medicine.*

***Keywords:** Bioactive compounds, infrared spectroscopy, Spectrometric, Hibiscus rosa-sinensis.*

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I. Introduction

Bioactive compounds in plants are compounds produced by plants having pharmacological or toxicological effects in man and animals (Aksel, 2010). Although, nutrients show pharmacological or toxicological effects when ingested at high dosages (e.g. vitamins and minerals), nutrients in plants are generally not included in the term bioactive plant compound. The typical bioactive compounds in plants are produced as secondary metabolites. Hence, bioactive compounds in plants are also identified as metabolites eliciting pharmacological or toxicological effects in man and animals (Aksel, 2010; Gablie and Deshmukh, 2010).These secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes of compounds aimed at plant growth and development, such as carbohydrates, amino acids, proteins and lipids. A 2010 release by the Norwegian Academy of Science classified bioactive compounds in plants according to different criteria. These classifications were based on pharmacological and toxicological effects, biological effects and botanical categorization based on families and genera of plants producing the bioactive compounds. However, bioactive compounds in plants include glycosides, saponins, diterpenoids, resins, lignans, alkaloids, tropane alkaloids, furocoumarines and naphthodianthrones, proteins and peptides, flavonoids, tannin, nicotine and terpenoids (Anil and Ashatha, 2012).

In the past years, different traditional formulations termed anti-diabetic traditional formulations have been developed for the cure of diabetes. This is based on the different herbs being combined to achieve the formulation. However, bioactive compounds present in the various plants used in formulations have been identified as the major constituents responsible for the anti-diabetic property. In order to characterize the bioactive compounds present in such formulation, the isolation of the bioactive compounds for proper identification is required. This cannot be achieved without first carrying out extraction with appropriate solvents and extraction techniques. Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical component from the plant materials for further separation and characterization. The basic operation include steps such as prewashing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytical extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to ensure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples (Sasidharan *et al*., 2011). Due to the fact that plants extract usually occur as a combination of various types of bioactive compounds with different polarities, their separation still remains a big challenge for

the process of identification and characterization. However, it is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, Column Chromatography and HPLC, should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity using spectroscopic (Sasidharan *et al*., 2011). Spectroscopic techniques such as ultraviolet-visible spectroscopy, infrared spectroscopy and mass spectrometry are used in the characterization of bioactive compounds in plant materials or extracts. Most times, these instruments are coupled together to obtain a more complex system for more efficient analysis. Infrared spectroscopy is one of the most common spectroscopic techniques used by organic and inorganic chemists. It is the absorption measurement of different infrared frequencies by a sample positioned in the path of an infrared beam. The main goal of infrared spectroscopic analysis is to determine the chemical functional groups in the sample. Different functional groups absorb characteristic frequencies of infrared radiation. Using various sampling accessories, infrared spectrometers can accept a wide range of sample types such as gases, liquids and solids. Thus, infrared spectroscopy is an important and popular tool for structural elucidation and compound identification (Sherman, 2000).

Hibiscus rosa-sinensis, a well known member of the family *malvaceae* grows as an ever green herbaceous plant. A native to tropical and sub-tropical regions, this plant is extensively cultivated as an ornamental plant. It bears large flowers on the bushy hedges and these flowers are usually dark-red in colour (Anil and Ashatha, 2012). The roots of the plant are cylindrical, 5-15cm in length and 2cm in diameter, off-white and with light brown transverse lenticels. The leaves are simple ovate or ovatelancolate, and are entire at the base and coarsely toothed at the apex (Imafidon and Okunrobo, 2010).

The flowers are pedicillate, actinomorphic, pentamerous and complete. The corolla consists of five petals, red-coloured and about 8cm in diameter. Traditionally *Hibiscus rosa-sinensis* is used for the control of dysfunctional uterine bleeding and as an oral contraceptive. It has been reported that the leaves of *Hibiscus rosa-sinensis* as well as the flowers have various pharmacological properties (Imafidon and Okunrobo, 2010). This study is aimed at using spectroscopic technique in the characterization of bioactive compounds in the leaves *Hibiscus rosa-sinensis.*

II. Materials And Method

Sample Collection

The leaves of *Hibiscus rosasinensis,* were collected from Abia State Polytechnic Botanical Garden (Science Laboratory Technology Department). The leaves were then taken to the Biology Department of Abia State Polytechhnic, Aba and were properly identified.

Preparation of Leaf Samples

The leaf samples collected were washed very well under running tap water and air-dried at room temperature for 7days. After proper drying, the leaves were grinded using an electric blender into a powdered form and the moisture content of the grinded samples was determined. The grinded sample (50g) was stored in an air tight container in a cool dry place till further analysis.

Analysis using Pelkin Elmer infrared spectrum bx11 spectrometer

Sample Preparation

A 0.5g portion of sample was ground in an agate mortar with 1g of specially purified potassium bromide (KBr) to a fine powder to remove scattering effects from large crystals. This powdered mixture was then pressed into a mechanical press to form a translucent pellet through which the beam of the spectrometer passed.

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 spectrum of the sample 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 sample.

III. Results And Discussion

The spectrum pattern and results of the spectral characterization of the bioactive compounds in *Hibiscus rosa-sinensis* are shown in fig. 1 and table 1

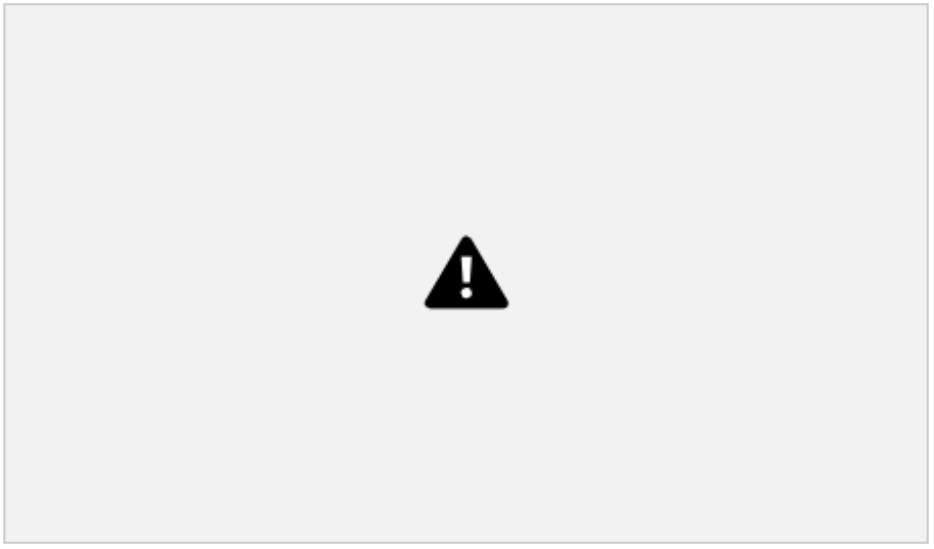
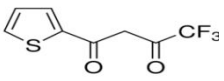
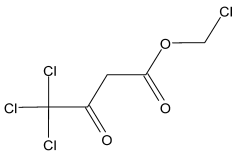
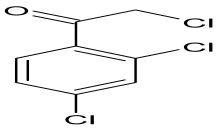
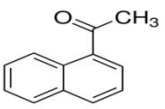
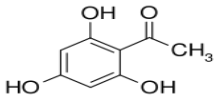
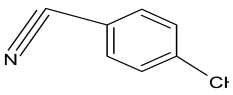
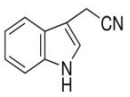


Fig. 1: Showing the Spectrum Pattern of the Bioactive Compounds in *Hibiscus rosa-sinensis.*

Table 1: Showing the Results of the Spectrum characterization of the Bioactive compounds in *Hibiscus rosa-sinensis.*

SPECTRA WAVENUMBER (CM^{-1})	IDENTIFIABLE FUNCTIONAL GROUP	IDENTIFIED COMPOUND	STRUCTURE OF IDENTIFIED COMPOUNDS	MOLECULAR WEIGHT (g/mol)	BOND/VIBRATION
544.00	Acetals, substituted Ketones	Ethyl-4-Chloro-2-Cyanoacetoacetate		189.60	O=C-N deformation C-O-C-O deformation C-Halide Stretch
1042.00	Aliphatic Fluoro-compounds, Ethers, Esters, Alkoxy	2-Thenonyl Trifluoro-Acetone		222.18	C-F Stretch C-N Stretch C-O Stretch
1247.66	Alkyl Halides, Alkoxy, Esters, Ethers.	Ethyl-4,4,4-Trichloroacetate		233.48	C-H Wag C-O Stretch
1324.61	Aromatics, Ethers, Acetals, Chloro compounds	1-2,4-Trichloro-Acetophenone		233.48	C-O Stretch C-H Halides C-C Stretch
1401.55	Aromatics, Aryl substituted Ketones, Phenyl group	1-Acetonaphthane		170.21	C-C Stretch C-C=O Stretch
1636.00	Substituted Ketones and Aldehydes, Phenyl group	2,4,6-Trihydroxy-Acetophenone		168.15	C=O Stretch C=C Stretch
2371.42	Nitriles, Alkanyls	P-Tolyacetonitrile		131.1745	CΞN Stretch H-C-H Asym Stretch
2931.42	Nitriles, Azo compounds, Phenyls	3-Indoleacetonitrile		156.1839	CΞN Stretch N-H Bend C-C Stretch

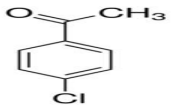
3437.00	Phenyl group, Aryl substituted Ketones	4-Chloro-acetophenone		154.594	O-H Stretch C=O Stretch

Figure.1 shows the infrared spectrum of *Hibiscus rosa-sinensis,* The characteristic of this spectrum is that it contains various organic functional groups in the functional group region (1300-4000cm^{-1}) and in the finger print region (400-1300cm^{-1}). Functional groups such as those of the nitriles, benzene, acetals, cyano-compounds, amines, amides, substituted and conjugated ketones and aldehydes, alkaloids, phenyl groups, chlorocompounds, bromo sugar, glycosides, thiophene derivatives, amino substituted compounds and indoles, were identified. The table contains information including the wave numbers, bonds and vibrations that gave rise to the peaks, identifiable functional groups, identified compounds, structure of the compounds identified and molecular weight of the compounds. The bioactive compounds identified in *Hibiscus rosa-sinensis*, were 2-thenolytrifluoroacetone, p-tolyacetonitrile, 1-2,4-trichloroacetophenone, 4-chloroacetophenone, ethyl-4-chloro-2-cyanoacetoacetate,2,4,6-trihydroxyacetophenone, ethyl-4,4,4-trichloroacetoacetate, 1-acetonaphthane and 3-indoleacetonitrile. The compound 1,2,4-trichloroacetophenone characterized is an alpha-haloketone. Alpha-haloketones are very useful alkylating agents for coupling to sulphuhydril-containing biomecules (Weihua *et al*., 2002). Alpha-haloketones have been reported to be highly reactive compounds which are known to undergo enzymatic reduction to methyl ketones. Brundin *et al*., 1982) reported that 1,2,4-trichloroacetophenone is reduced to 2,4-dichloroacetophenone by gluthathione-dependent cytosolic enzymes present in the liver, kidney and brain. It has been discovered that 4-chloroacetophenone can be used as tear gas. Among other side effects, chloroacetophenone can evoke toxic and allergic contact dermatitis, and more rarely exanthematous or anaphylactic reactions (U.S National Library of Medicine Toxicological data, 2013). The biochemical changes in blood samples of rats at different intervals after exposure to chloroacetophenone have been studied and it was reported that the compound induced hyperglycemia and the level of plasma urea was unaltered. 1-Acetonaphthane also called 1-acetonaphthone and 1-acetonaphthalene is an acetyl- substituted naphthalene. It is used as a bactericide and as an antitubercular agent. Its end application include soap, detergent, beauty care products, household products, some acetonaphthanes are also used as mosquito repellent (WWW.metabolomicscentre.ca/metaboloured for 3-indoleacetonitrile. Ethyl-4,4,4-trichloroacetoacetate belongs to the family of acetoacetates and is an ethyl ester of acetoacetic acid. It is mainly used as a chemical intermediate in the production of a wide variety of compounds, such as amino acids, analgesics, antimalarial agents, antipyrine and aminopyrine, and vitamin B, (WWW.Chemicalland2.com/1-acetonaphthane).The compound 3-indoleacetonitrile also seen belongs to the class of compounds called indoles. 3-indoleacetonitrile is a phytoalexin. Phytoalexins are antibiotics produced by plants that are under attack. Phytoalexins tend to fall into several classes including terpenoids, glycosteroids and alkaloids; however, researchers often find it convenient to extend the definition to include all phytochemicals that are part of the plants defensive arsenal (WWW.NIST.org), 3-indoleacetonitrile is common in cruciferous vegetables such as cabbage, cauliflower, broccoli and brussel sprouts (Stemmler *et al*,. 1995). Dietary indoles in cruciferous vegetables include cytochrome P450 enzymes and have prevented tumors in various animal models. Consumption of brassica vegetables is associated with a reduced risk of cancer of the alimentary tract in animal models and human population (Ciska and Pathak, 2004). Dihydroxyacetone dimer is a simple carbohydrate which is the normal form of Dihydroxyacetone (DHA) also known as glycerone. Its phosphate form dihydroxyacetone phosphate (DHAP) takes part in glycolysis, and it is an intermediate product of fructose metabolism. DHAP is also the product of the dehydrogenation of L-glycerol-3-phosphate, which is part of the entry of glycerol into the glycolytic pathway (Weihua *et al*., 2002). Also studies have reported the use of DHA as an oral drug for assisting children with glycogen storage diseases. Other studies have also shown that DHA reacts chemically with the amino acids in the skin, which are part of the protein containing keratin layer on the skin surface. Various amino acids react differently to DHA, producing different tones of colouration (Faurschou and Wulf, 2004).

IV. Conclusion

This work focused on the use of infrared spectroscopy (a spectrometric technique) in the characterization of bioactive compounds in *Hibiscus rosa-sinensis.* The infrared spectrum of the plant shows the presence of several functional groups, hence the presence of several compounds which have different physiological activities in humans, animals and plants. Some of the compounds characterized were polysulpuric compounds, chloro compounds, substituted sugar compounds and haloketones. Some of these compounds have been reported by recent studies to have anti-cancer, anti-tubercular, antimalarial, antimicrobial, antifungal,

therapeutic and inhibitory activities; and also have cholesterol lowering effects. Some also have been reported to be useful intermediates in physiological reactions, in the synthesis of pharmaceuticals and other products.

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