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UV-VISIBLE AND FTIR SPECTROSCOPIC ANALYSIS OF THE CRUDE ETHANOLIC EXTRACT OF *PEURARIA PHASEOLOIDE* LEAF (ROXB) BENTH. (FABACEAE)

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ABSTRACT

The present study was carried out to characterize the bioactive constituents present in the Ethanolic extract of *Peuraria phaseoloides* leaves using UV-Vis and FTIR spectroscopic techniques. UV-VIS method was performed using a Perkin-Elmer Lambda 19 spectrometer while the FTIR method was performed using Buck scientific M530 system which was used to detect the characteristic peak values and their functional groups. UV-Visible spectrum revealed varying peaks in the range of 220-900nm recording the absorptions at its respective wavelengths. It showed a λmax peak at 500nm, with peaks at 420, 440 and 460nm indicating the presence of flavonoids. The FTIR analysis revealed the highest peak at 3291.098cm-1, which signifies the presence of R2NH 20 amine functional group. Other functional groups such as Halogen, ether, ethane, carboxylic acid, carbonyl, nitrile and alcohol were equally present. The presence of various functional groups and phytocompounds in *Peuraria phaseoloides* confirm that it act as a most important source of drugs against various ailments.

KEYWORDS: *Peuraria phaseoloides*, FTIR, UV-Vis absorption, functional groups.

INTRODUCTION

The important use of natural products from different kinds of plant species and their derivatives in management of human health, prevention and treatment of pathological conditions (such as bacterial infections etc) both in the classical and modern medicine cannot be over emphasized (Bruce et al., 2016). Modern medicines make use of plant derived compounds as the basis for evidence based pharmacological agents (Onyegbule et al., 2019). There abounds scientific evidence which have given credence that natural products exhibit various medicinal properties and act as major sources of therapeutic agents (bruce and Onyegbule, 2021). However, some of these plants contain complex chemical structure as a result of diversities of inherent compounds in them (bruce and Onyegbule, 2021; Ihekwereme et al., 2020). Thus, to ascertain these efficacies and phytochemical diversities, crude plant samples are subjected to laboratory tests using various techniques such as FTIR, GC-MS, UV-Vis spectrum, NMR, etc. Considering these, Okoye et al., (2020) carried out a phytochemical test on the ethanolic leave extract of *Peuraria phaseoloides*, which showed the presence of alkaloids, tannins, cardiac glycosides, saponins, carbohydrates, reducing sugars, flavonoids and terpenoids. Asha et al., (2017) studied GC-MS analysis on the whole plant *Drosera indica* and revealed some biological properties such as phytol, 4 methyl-4-nonadecene, 4',5,7-Trihydroxyisoflavone,1,2-Benzene dicarboxylic acid, bis [2-methyl propyl] ester, Tetradecanoic acid, Eicosane, 2,6,10,14,18–pentamethyl, Tetracosane, Lochnerine and methyl–n-hexadecylketone. The result shows that these plants contain some of these bioactive components and thus, would require further test such as Ultraviolet- Vis/ Infrared Spectroscopy for further characterization. UV-Vis and FTIR spectroscopy are amongst the effectively used methods in phytoconstituent characterization and have been used as primary methods to identify medicines for pharmacopeia in several countries over the years. The UV-visible spectra help in identification and quantification of organic and inorganic compounds while the FTIR spectroscopy which is a non-destructive technique is based on the vibrations of atoms of a molecule, providing information on the energy levels of the molecules in wave numbers (cm-1) in the region of electromagnetic spectrum which are useful source of information for qualitative and quantitative applications. Therefore, this present study is structured as follows: Section one contains the introduction and literature review and section two contains the methods while section three is result and findings, section four is discussion.

Spectroscopy by definition is the study that investigates the interaction between electromagnetic radiation and matter. During this interaction, energy is either absorbed or emitted by the matter individually and this is called quanta. Spectroscopy can be of two types viz: Atomic and Molecular spectroscopy, with the latter explaining the techniques of interest in our present study.

Ultra violet visible spectrophotometry (UV-Vis) study helps to ascertain the spectral features of photons in the UV-visible region. The color of the chemicals is responsible for the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic Spectrum (Gunasekaran, 2003). Also, is useful in identifying varying physicochemical properties of compounds, hence are capable of providing data as to the identity of a particular compound. UV-visible spectra are generally known for the production of few broad absorbance bands which provides scanty qualitative information while FTIR on the other hand produces many narrow bands.

Fourier Transform Infrared Spectroscopy (FTIR) is a vibrational spectroscopic technique that makes use infrared radiation to vibrate molecular bonds in a given sample. It is one of the most widely used method to categorize the chemical constituents and has been used as a necessary method to identify medicines for pharmacopeia in several countries (Subashini et al., 2015). A non-destructive, simple and cost-effective method, helps to characterize and identify functional groups. FTIR can be described as a highly legalized technique in the evaluation of compositional and structural information in plant samples. During analysis, the wavelength of light evoked serves to represent the chemical bond and might be visible in the spectrum.

The GC-MS analysis of Peuraria phasoeloides ethanolic leaf extract revealed the presence of Glycerin, 4H-pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, Phytol, 9,12,15-Octadecatrienoic acid, Octadecanoic acid, ethyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxyl-methyl) ethyl ester, Squalene, Vitamin E, Stigmasterol, D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.) as well as their corresponding biological effects described in Bruce et al., 2021.

Therefore, this present study reveals the UV-Vis and FTIR spectroscopic techniques of the ethanolic extract of *Peuraria phaseoloides* leaves.

MATERIALS AND METHODS

Plant materials

Fresh *Pueraria phaseoloides* leaves were collected from Nando in Oyi Local Government Area of Anambra State, Eastern Nigeria in July 2019. The plant material was identified, confirmed and authenticated by a taxonomist in the Pharmacognosy and Traditional Medicine Department of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Nigeria. Herbarium specimen deposited with herbarium number PCG 474/A/030.

Equipments

Electronic digital weighing balance (Ohaus Corp, USA), water bath (Serological, England), beakers (Pyrex;10, 50, 100 and 1000 ml), measuring cylinders, mechanical grinding machine (GX160 Delmar 5.5HP), rotary evaporator (RE300 Model, United Kingdom), refrigerator (Thermocool, England), cotton wool (Pyrex. Nig), Glass funnel (Pyrex), Muslin cloth, Filter paper (Number 1 Whatman), UV-visible spectrophotometer, Buck scientific M530 USA FTIR.

Reagents and chemicals

Ethanol (JHD), Distilled water (NBC, Nig), Deuterated triglycine sulphate, Potassium bromide.

Preparation of plant material

The leaves of *Pueraria phaseoloides* were carefully washed and shade dried at room temperature for 14days. The dried leaves were pulverized with a mechanical grinding machine and sieved to control the particle size, then stored in an airtight container for further analysis (Bruce *et al*., 2016).

Extraction

Peuraria phaseoloides leave powder (600 g) was extracted using ethanol (2500 ml) with occasional stirring for 72hrs by cold maceration. The mixture was sieved with Muslin cloth and filtered using a filter paper. Finally, the filtrate was concentrated to dryness in *vacuo* using rotary evaporator (RE300 Model, United Kingdom) at 40°C and stored in a refrigerator for use.

Ultra Violet-Visible Spectroscopy

A Perkin-Elmer Lambda 19 spectrometer was used in recording the absorption spectra of the study sample. Peuraria phaseoloides leaves extract was centrifuged at 3000 rpm for 10mins and filtered through Whatmann No. 1 filter paper using high pressure vacuum pump. The sample was diluted to a concentration of 1:10 with the extraction solvent. The study compound was measured in the UV-visible spectrum at a range of 220-900nm with a spectral band width of 2nm and scan speed of 240nm min- 1. Prominent characteristic peaks were detected and their absorbance noted (Dhivya and Kalaichelvi, 2017).

Fourier Transform Infrared Spectroscopic (FTIR) Analysis

Buck scientific M530 USA FTIR was used for the analysis. This instrument was equipped with a detector of deuterated triglycine sulphate and beam splitter of potassium bromide. The software of the Gram A1 was used to obtain the spectra and to manipulate them. An approximately of 1.0g of samples, 0.5mi of nujol was added, they were mixed properly and placed on the salt pallet. During measurement, FTIR spectra was obtained

at frequency regions of 4,000-600cm^1 and co-added at 32 scans and at 4cm^1 resolution. FTIR spectra were displayed as transmitter values (Van der et al., 2004).

RESULTS

Table 1: UV-VIS Spectrum Peak Values of Ethanolic Extract of *Peuraria phaseoloides* Leaves.

S/No.	Wavelength (nm)	Abs.
1	240	0.076
2	260	0.067
3	280	0.045
4	300	0.056
5	325	0.078
6	340	0.089
7	365	0.098
8	380	0.099
9	395	0.102
10	420	0.111
11	440	2.344
12	460	2.566
13	480	2.788
14	500	2.898
15	520	2.455
16	540	2.234
17	560	2.31
18	580	1.822
19	600	1.533
20	620	1.874
21	640	0.927
22	660	0.989
23	680	0.899
24	700	0.677
25	720	0.271
26	740	0.215
27	760	0.142
28	780	0.116
29	800	0.112
30	820	0.11
31	840	0.089
32	860	0.089
33	880	0.08
34	900	0.092

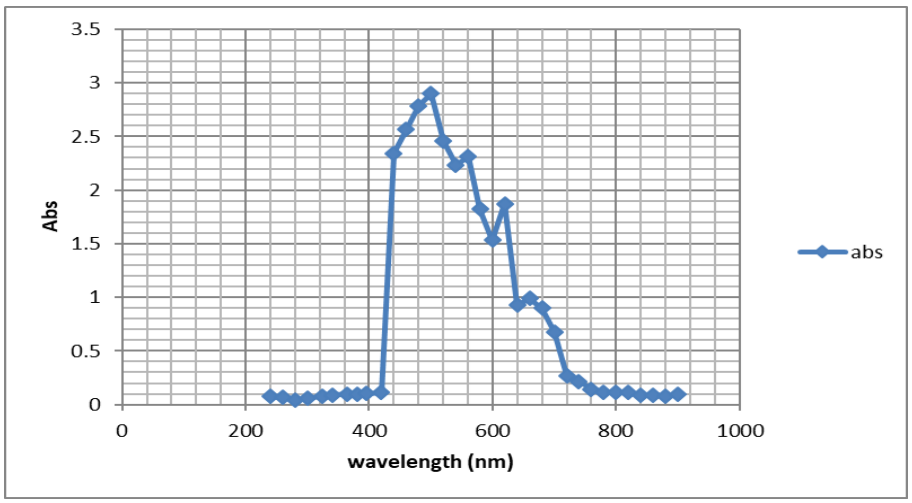

Fig. 1: UV-VIS Spectrum of Ethanolic Extract of *Peuraria phaseoloides* Leaves.

Interpretation of FTIR Spectra

Table 2: FTIR Interpretation of compounds of ethanolic extract of *P. phaseoloides* leaves.

S/N	Frequency	Functional group	Compounds
1	840.4413	C-CI	Chloro Cl symmetric stretch
2	1295.274	R-0-R	Ether C0 symmetric stretch
3	1417.323	H_2C=CH	Ethene CH symmetric stretch
4	1632.317	RNH_3	1^0 amine NH stretch
5	2041.271	RC00H	Carboxylic acid C0 stretch
6	2217.593	RC=0	Carbonyl C0 antisymmetric stretch
7	2455.486	R-C≡N	Nitriles CN antisymmetric stretch
8	2511.817	R-C≡N	Nitriles CN antisymmetric stretch
9	2717.020	R-C≡N	Nitriles CN antisymmetric stretch
10	2891.118	R-C≡N	Nitriles CN antisymmetric stretch
11	3039.616	RCH0H	1^0 alcohol 0H stretch
12	3291.098	R_2NH	2^0 amine NH stretch

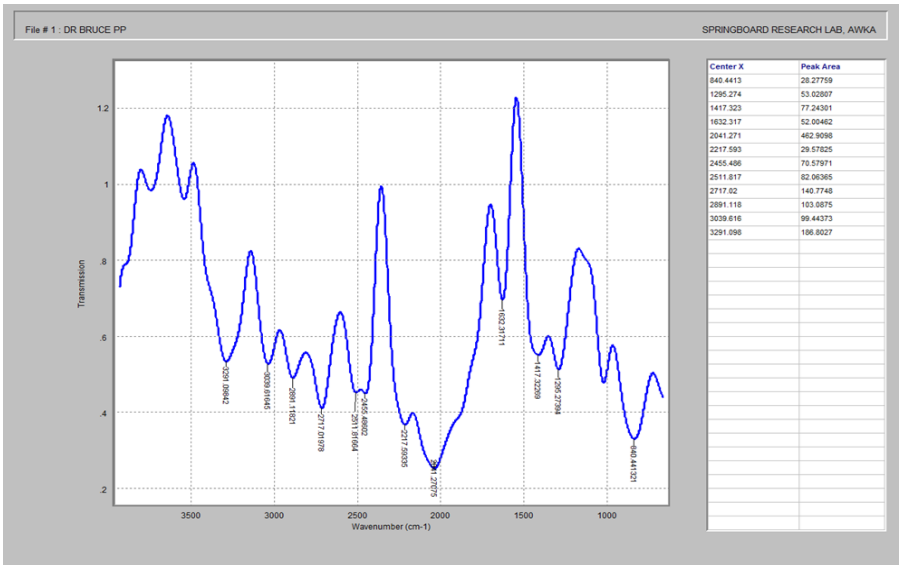

Figure 2: FTIR Spectrum analysis of ethanolic extract of *P. phaseoloides* leaves.

DISCUSSION

Spectrophotometry techniques are those techniques utilized in qualitative and quantitative analysis of pharmaceutical and biological materials (Dhivya and Kalaichelvi, 2017). Amongst all spectroscopic techniques, UV-Vis spectroscopy has been considered of great advantage because of its exactness and reproducibility in measuring absorbance against wavelength. It uses light in the visible ranges and the colours of the chemicals present have a direct impact on its absorption.

The UV-Vis spectrum of ethanolic extract of *Peuraria phaseoloides* displayed absorption bands in the wave length of 220-900nm, considering the sharpness of the peaks and proper baseline. Varying peaks observed during analysis were indicative of many type of bonds within the molecule and the electronic transitions which in turn signifies various chemical constituents responsible for the biological activities.

Peuraria phaseoloides extracts upon analysis showed peaks at 240nm, 560nm and 900nm with absorption of 0.076, 2.31 and 0.092 respectively. Peaks at 420, 440 and 460 shows the presence of flavonoids and at 700nm it designates chlorophyll. A bathochromic (Red) shift was observed in the violet region as the wavelength increased while the on red region, a Hypsochromic (Blue) shift was equally observed, from a decreased wavelength. Kalsi (2004) investigations revealed that for the classification of multiple bonds and aromatic conjugations of organic compounds, UV-Vis spectroscopy might be utilized.

Fourier transform infrared (FTIR) spectroscopy is a vibration spectroscopic method that makes use of infrared radiation to vibrate molecular bonds inherent in the sample that absorbs it. FTIR technique is important in identifying the characteristic functional groups, which are instrumental in determination of functional groups and organic compounds present in any given sample based on the peak values in the region of IR radiation. On the point of sample introduction, the components are isolated which gives a clear view of its highest proportion. The results of FT-IR spectroscopy confirmed

the presence of various chemical constituents such as halogen, ether, ethane, amine, carboxylic acid and carbonyl, nitrile, methylene, alcohol in the ethanolic of *Peuraria phaseoloides* leaves. Table 2 and Figure 2 shows the presence of 9 functional groups from the ethanolic extract of *Peuraria phaseoliodes* leaves. The broad band around 3039.62 and 3291.10 were designated to OH stretching vibration of 1 and 2 alcoholic compound. The bands; 2455.49, 2511.82, 2717.02 and 2891.11 which are designated to Nitriles CN antisymmetric stretch shows the presence of Nitriles in the ethanolic leave extract. The band around 1417.32cm was designated to C=C stretching vibration of ethene and some other groups such as Halogen, Ether, Amine, Carboxylic acid and Carbonyl at 840.44, 1295.27, 1632.31, 2041.27 and 2217.59 respectively.

Starlin et al., (2012) carried out an FTIR analysis on the ethanolic extracts of Ichnocarpus frutescens and this resulted in functional groups like: Amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens. Nithyadevi and Sivakumar (2015) on the same vain studied the FTIR analysis using the methanolic leave extract of Solanum torvumto which revealed alcohol, alkanes, aromatic carboxylic acid, halogen and alkyl halide. In the present study, FTIR analysis revealed the presence of Halogen, ether, ethene, amine, carboxylic acid, carbonyl, nitrile, methylene and alcohol and this is in agreement with a previous study of FTIR analysis on the methanolic extract of Faidherbia albida stem bark, revealing Methylene, amine, alcohol, carbonyl, halogens with only the alkene group none present in the present study (Maitera and Chukkol, 2016). FTIR analysis of methanolic extract of different parts of Caralluma fimbriyata revealed functional groups like Lactose, aromatic compounds, halogen, ethers, alcohol, alkenes, aldehydes, esters, acid halides, carboxylic acids, amides, showing varying peaks at different ranges with that of Halogen at 840.96cm (Packialakshmi and Naziya, 2014) which is similar to the peak value of halogen, 840.44cm in the present study.

Implication to Research and Practice

Spectroscopic techniques provide specific information, opens hidden areas of interest that will in turn enable further analysis/screening on the compounds by researchers and scholars as the data will be documented on journals, reference books (monographs, codex, compendia etc). Also these techniques when used for analysis, equally reveals information on the nutritional and pharmacological relevance of the compounds which promotes their use by industrial chemist in food and pharmaceutical industries.

CONCLUSION

From the just concluded study on UV-Visible and FTIR analysis of *Peuraria phaseoloides* leaves, it can be established that the above techniques proved effective in characterization and identification of the chemical/biological components present in the leaf extract as a good number of them were recorded. Hence, confirming the aim and objectives of the present study.

Future Research

These compounds can be isolated further, screened for different kind of biological activities, depending on their therapeutic uses. Further research will be needed to find out the structural analysis of *Peuraria phaseoloides* by use of different analytical methods such as NMR and GC-MS.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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