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FOURIER-TRANSFORM INFRARED SPECTROSCOPIC CHARACTERISATION OF THE DIFFERENT FRACTIONS FROM *Anthocleista vogelii* PLANCH LEAF EXTRACTS

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ABSTRACT

Medicinal plants are used to treat and prevent ailments largely due to the presence of phytochemicals in them. This study was aimed at identifying the phytochemicals present in the leaf of *Anthoclestia vogelii* using established methods. The air-dried leaves were pulverized and One kilogram of the sample was extracted in absolute methanol for seventy two hours. The concentrated extract weighs 271.25 g. The crude extract (7.0 g) was loaded on a silica gel packed column using gradient elution of n-hexane (100%), ethyl acetate-methanol (50:50) and methanol (100%). Characterisation of the fractions was done using Fourier-transform infrared spectroscopy. Phytochemical screening of the lyophilized brown viscous methanol extract of *A. vogelii* shows the presence of tannins, saponins, flavonoids, alkaloids, and phenols. The fractionated and purified crude extract of *A. vogelii* with n-hexane, ethyl acetate-methanol and methanol yielded 1.75, 0.32 and 0.84 g labeled as X, Y and Z respectively. The FT-IR spectra revealed prominent absorption bands at 2855 – 2955, 1714, 1461 and 1379-1006 cm^{-1} in Fraction X, while 3321, 2885 – 2970, 1647, 1326 – 1449 and 1043 cm^{-1} in Fraction Y, and 3321, 2829 – 2981, 1416 – 1446, 1110 cm^{-1} in Fraction Z. The absorption bands suggested the presence of C=C bearing phytochemicals in Y, –OH bearing phytochemicals in Y and Z while C=O bearing phytochemicals in Fractions X.

Keywords: *Anthocleista vogelii*, Fractionation, Chromatograhy, FTIR, Phytochemical screening and characterisation.

INTRODUCTION

Phytochemicals are secondary metabolites of low-molecular-weight that occur naturally in plants. The usage of medicinal plants to treat and prevent ailments is primarily due to phytochemicals present in those plants. A renewal of interest in secondary plant metabolites has developed due to the growing epidemiological evidence showing a protective effect of vegetable and fruit consumption against several diseases(Ahmed, 2018) [1]. Some other phytoconstituents that occur in plants are; glycosides, flavonoids, alkaloids, saponins, coumarins, phenol, carboxylic acids and terpenes. These phytoconstituents convene specific characteristics and properties on plants. Hence, the study of these constituents can help in determining various biological activities of plants.

Anthocleista vogelii Planch is a tree (of the Loganiaceae family) which is found growing commonly in marshy areas of the tropical humid

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forest of West Africa [2]. It can be found from Sierra Leone, Kenya, Zambia, Angola, Cameroon and Gabon [3,4].

Anthocleista vogelii has been reported for its antiulcerogenic activities, antidiabetes, anti-diuretic, and laxative in rats [5-7]. The seed, stem bark and root of *A. vogelii* are used as traditional medicines for the management of stomach troubles, wounds, inflammations and venereal diseases, while its leaves and roots have been used for the treatment of typhoid and throat problems [8-11]. Some studies have corroborated the antibacterial and the antifungal properties of crude extracts of the leaves and stem bark of *A. vogelii* [7,11,12].

In Zambia, the trunks are cut for hollows in canoes. In Congo, the leaves are sandwiched between tobacco leaves during dehydrating to strengthen the tobacco, and a decoction of the leaves has been reported to avert malaria and lessen symptoms of malaria fever. The leaves of *A. vogelii* is also used as haemostatic and to treat jaundice. In Cameroon, the stem bark has been reportedly used in treating abdominal pains [13]. In Ghana, the wood-ash is used as a mordant to fix colours, while the potash of the wood is used in making soap [14]. A decoction of the stem bark in Libreville, Gabon, is used in the treatment of cardiovascular diseases [17].

The stem bark and seed in Nigeria are used as a strong purgative agent and as a diuretic. It is also used in management of irregular menstrual cycle, constipation, scrotal elephantiasis, leprosy, stomach-ache, and oedema [16,17].

Secologanic acid, vogeloside, and sweroside, fagaramide has been reported from the plant [18]. The focal objective of this study is to identify the phytoconstituents in the different fractions from the leaf of *A. vogellii* using FTIR profile.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical grade. n-Hexane and acetone were from Surechem (Suffolk, England),while ethylacetate, magnesium ribbon, acetic acid and methanol were obtained from Lobachemie PVT (Mumbai, India).

Sample collection and preparation

Plant material collection and identification

Anthocleista vogelii leaves were collected from the botanical garden of Olabisi Onabanjo University (OOU), Ago-Iwoye, Nigeria. It was identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Nigeria with herbarium number FHI 10906 and a voucher specimen deposited.

Extraction of crude plant content

The leave was washed, air-dried and pulverized. One kilogram of the pulverized leaf sample was extracted in absolute methanol at room temperature for seventy two hours, stirring intermittently. The mixture was then filtered with muslin cloth, and filter papers to obtain a filtrate that was concentrated with a vacuum rotary

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evaporator (Eyela N-1300) at 40 ºC. The solvent was driven-off on Memmert W270 waterbath (Schwabach, Germany) and freeze dried on Dynavac freeze drier (Dynavac Engineering, Australia).

Phytochemicals screening

Phytochemical constituents of *A. vogelii* was determined using methods of Ayoola *et al.* (2008) [19] and Samejo *et al.* (2013) [20]. The extracts were screened for phenols, flavonoids, glycosides, saponin, phenols, terpenoids, alkaloids, steroids and anthraquinones.

Extraction and Purification of bioactive compounds from plant samples

Seven grams of crude extract was fractionated using gravity column Z163988-1IA (70 cm ×24 mm ×28 mm, Zigma-Aldrich, St. Louis, MO, USA) as described Bajpai *et al.* (2016) [21] and eluted under gravity with solvents of differing polarities. The extracts obtained were concentrated on a vacuum rotary evaporator (Eyela N-1300) and freeze dried (DYNAVAC, Dynavac Engineering, Australia).

Characterisation of bioactive compounds

The fractionated extract was subjected to FT-IR analysis. 1 mg of the dried powder was encapsulated in 10 mg of KBr pellet and loaded on the FTIR spectroscope (Agilent Cary 630 FTIR), with a scan range of 600-4000cm^{-1} with a resolution of 4 cm^{-1}.

RESULTS AND DISCUSSIONS

The methanol extract obtained after drying was a brown viscous solid with percentage yield (27.1%). A total of 15 fractions of 100 mL each were collected and analyzed using Thin layer Chromatography (TLC). Retention factor (Rf) values of the TLC spots were determined and the fractions were combined on that basis.

Table 1: Solvent system used in the column-chromatography for the purification of bioactive molecules from *A. vogelli*

Solvent	Ratio	Volume (mL)	Fraction
n-Hexane	100%	500	X
Ethyl acetate : Methanol	1 : 1	500	Y
Methanol	100%	500	Z

The results of the phytochemical screening of *A. vogelli* leaves showed the presence of tannins, saponins, flavonoids, alkaloids, and phenols that are consistent with previous studies [22, 23, 24]. The results of the fractionated and purified crude extract (7.0 g) of *A. vogelli* showed the yields of

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1.75, 0.32 and 0.84 g labeled as fractions X, Y and Z for n-hexane, ethyl acetate-methanol and methanol respectively.

Fraction X was a yellowish, viscous liquid, Y was a brown, viscous liquid and Z was a colourless crystal. The Thin Layer Chromatography (TLC) analysis of the fraction revealed single spots on the plates.

The FTIR shows different absorption bands with different intensities indicating different functional groups. Prominent absorption bands observed were at 2855 – 2955, 1714, 1461, 1379-1006 cm-1 for Fraction X, 3321, 2885 – 2970, 1647, 1326 – 1449, 1043 cm^{-1} for Fraction Y, and 3321, 2829 – 2981, 1416 – 1446, 1110 cm^{-1} for Fraction Z.

The absorption bands 3321 cm^{-1}, in Fractions Y and Z, are indicative of the presence of strong, broad OH functionality in both fractions which suggests the presence of OH-bearing phytochemicals. The presence of C=O str. and C=C str. absorption were evident in Fractions X and Y with absorption bands at 1714 and 1647 cm^{-1}, respectively. The absorption band of Fraction Y indicated an unsaturated compounds while that of Fraction X was ketonic and of low intensity. All the fractions showed the presence of alkane absorption (C–H, 2829 – 2981 cm^{-1}). Fraction Y showed absorption of C-H bending of alkane at (1449 cm^{-1}; 1379 cm^{-1}) and of 1, 2, 4-trisubsustituted (879 cm^{-1}). It also showed the presence of a primary alcohol absorption band at 1084 cm^{-1} while fractions X and Z showed absorption bands of secondary alcohol at 1092 cm^{-1} and 1110 cm^{-1}, respectively. Fraction Y showed absorption of sulfonamide at 1326 cm^{-1}, sulfoxide at 1043 cm^{-1}, while fraction Z showed absorption of sulfoxide at 1021 cm^{-1} and sulfate at 1416 cm^{-1}.

Table 2: FTIR ABSORPTION PEAKS OF FRACTIONS X, Y AND Z.

Fractions	IR Absorption peaks (cm^{-1})	Some major functional groups present
X	2955, 2922, 2855, 1714, 1461, 1379, 1289, 1218, 1133, 1092, 1062, 1006, 887, 760, 723, 663	C–H str., C=O str., C–H bend and C–O
Y	3321, 2970, 2885, 1647, 1449, 1416, 1379, 1326, 1084, 1043, 879	O-H str., C–H str., C=C str., C–H bend and C–O
Z	3321, 2981, 2944, 2881, 2821, 1446, 1416, 1110, 1021	O-H str., C–H str., C–H bend and C–O

Similarly, hydroxyl group in conspicuously absent in fraction X unlike Y and Z. The presence of these characteristic functional groups could be that of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, organic

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halogens and carbohydrate. They may be responsible for the various medicinal properties of *A. vogelii.*

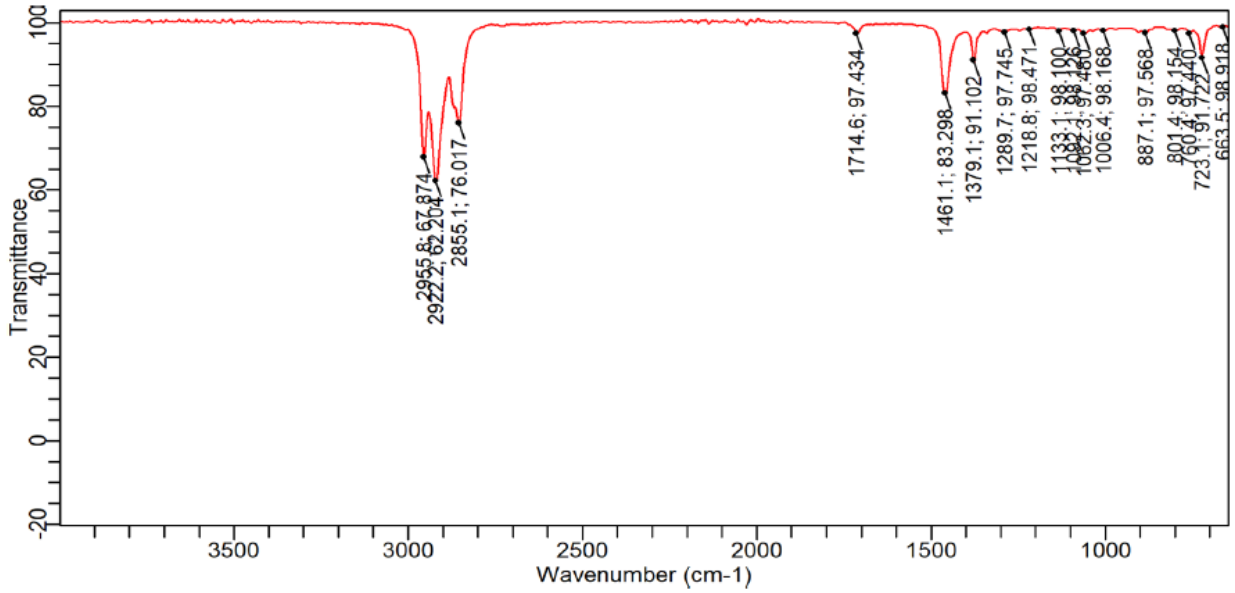

Figure 1: FTIR spectrum of n-hexane fraction of *A. vogelii* leave extract

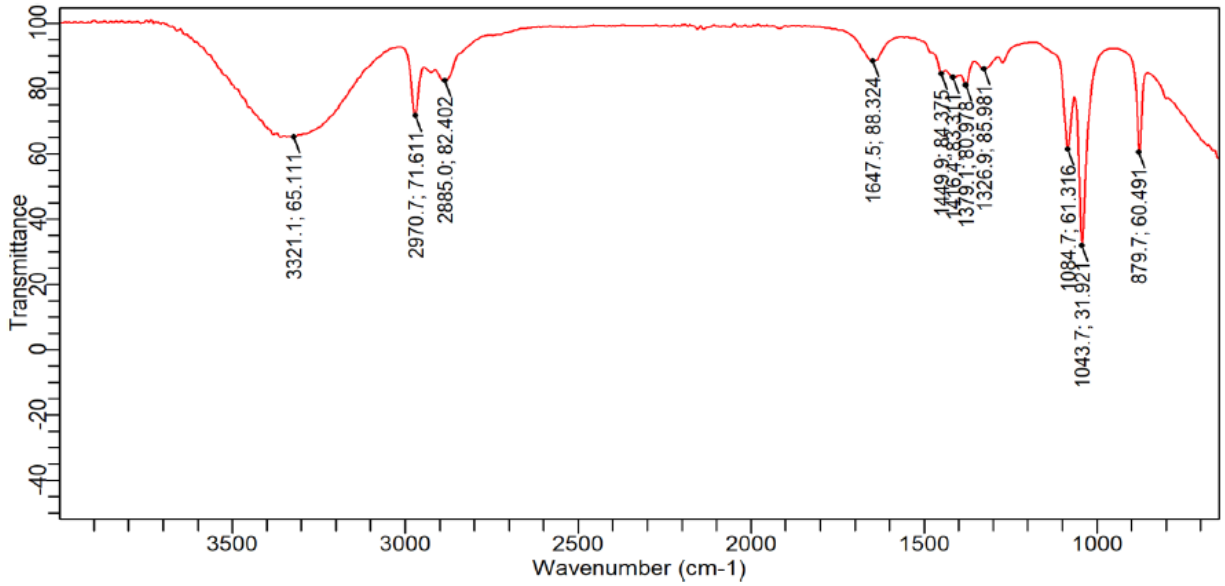

Figure 2: FTIR spectrum of ethyl acetate – methanol fraction of *A. vogelii* leave extract

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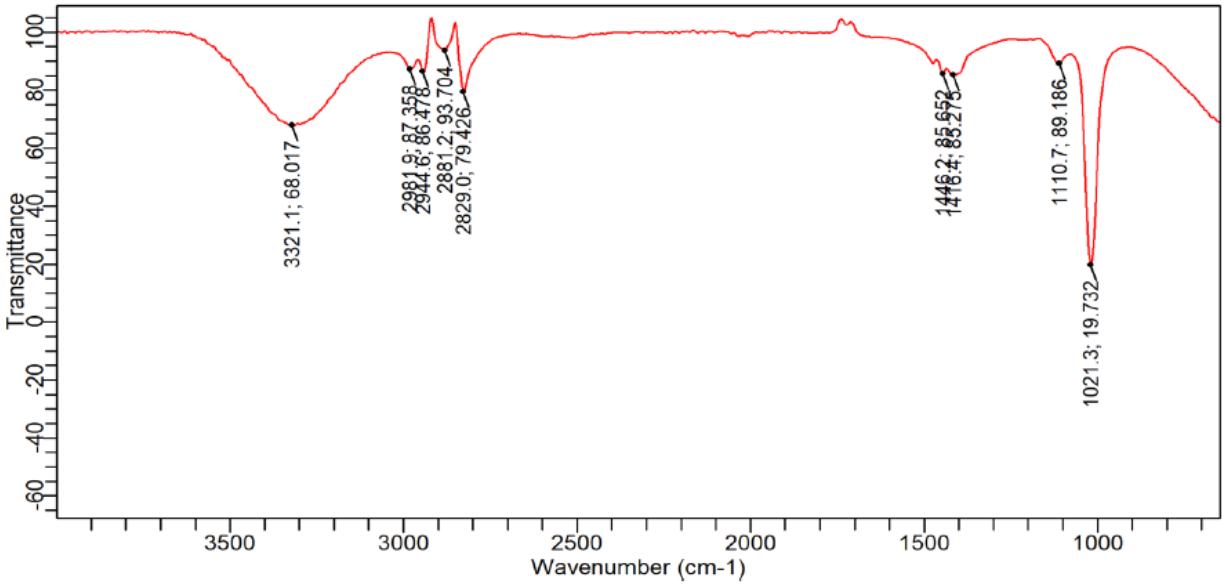

Figure 3: FTIR spectrum of methanol fraction of *A. vogelii* leave extract

CONCLUSION

In the present study qualitative phytochemical screening of the methanol extract of *A. vogelli* revealed the presence of alkaloids, flavonoids, saponins, tannins and phenols. Fractionation of the methanol extract followed by FTIR spectroscopy showed the presence of compounds with hydroxyl, unsaturated and carbonyl functional groups which can be said to be responsible for various medicinal properties of plant. These fractions could be further purified to isolated, characterize and carry out the bioassay of the isolates for different kind of biological activities depending on their therapeutic uses.

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