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Spectroscopic characterization of the antidiabetic properties of partially purified ethanolic extract of *Hibiscus Cannabinus*, *Vernonia Amygdalina*, *Murraya Koenigii* and *Telfairia Occidentalis*

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Abstract
Spectroscopy plays a major role in phytochemical characterization of medicinal plants. In this research, we report the characterization of antidiabetic fractions of partially purified ethanolic leaf extract of *Murayya koenigii, Telfairia occidentalis, Vernonia amygdalina* and *Hibiscus cannabinus* using UV-Visible spectroscopy, Infrared spectroscopy (IR) and Scanning Electron Microscopy (SEM). Various fractions each were obtained from *plants extract* by column chromatography. Spectroscopy studies on the various fractions of *Telfairia occidentalis* absorbed maximally at 385nm, while 1st fraction of *Murayya koenigii* absorbed maximally at 340nm. Also, *Hibiscus cannabinus* absorbed maximally at 420nm, while the 2nd fraction of *Vernonia amygdalina* absorbed maximally at 350nm. The Infrared spectrum of *Murayya koenigii* transmit maximally at 3899.06cm^{-1}, 3446.59 cm^{-1}, 2342.57 cm^{-1}, 1732.73 cm^{-1}, 1652.03 cm^{-1}, 1507.02 cm^{-1}, and 1489.39 cm^{-1} which indicates the presence of functional groups such as O-H stretch of Alcohols, C-H stretch of Alkanes, C≡C stretch of Alkynes, C=O stretch of Ketones, C=O stretch of Aldehyde, ring C=C stretch of Aromatic compound and C=C stretch of Aromatic compound respectively. While the infrared spectrum of *Telfairia occidentalis* transmit maximally at 3711.40 cm^{-1}, 3445.08 cm^{-1}, 2921.93 cm^{-1}, 1506.37 cm^{-1} and 1077.75 cm^{-1}. These indicate the presence of functional group such as O-H stretch of Alcohol, C=C stretch of Aromatic compound, N-H stretch of secondary amine and N-H stretch of primary amines respectively. The Infrared spectrum of *Hibiscus cannabinus* transmit maximally at 3445.21cm^{-1}, 2923.88 cm^{-1}, 2359.13 cm^{-1}, 1738.76 cm^{-1}, 1682.68 cm^{-1}, 1557.73 cm^{-1}, and 1455.79 cm^{-1} which indicates the presence of functional groups such as O-H stretch of Alcohols, C-H stretch of Alkanes, C≡C stretch of Alkynes, C=O stretch of Ketones, C=O stretch of Aldehyde, ring C=C stretch of aromatic compound and C=C stretch of Aromatic compound respectively. While the infrared spectrum of *Vernonia amygdalina* transmit maximally at 3646.20 cm^{-1}, 3445.23 cm^{-1}, 2341.38 cm^{-1}, 1557.75 cm^{-1} and 1456.09 cm^{-1}; indicating the presence of functional group such as O-H stretch of Alcohol, C=C stretch of Aromatic compound, N-H stretch of secondary amine and N-H stretch of primary amines respectively. Scanning electron microscope (SEM) was carried out for all fractions to view the structural morphology of the plant's micrograph; were oval, cubic and spherical shapes where commonly associated with the extracts. The implication of the various findings were highlighted and discussed.

Keywords: spectroscopic characterization; antidiabetic properties; ethanolic leaf extract; *Murayya koenigii, Telfairia occidentalis, Vernonia amygdalina*; *Hibiscus cannabinus*

Introduction
Medicinal plants are widely used against diabetes throughout the world but few of them have been validated by scientific criteria. Great diversity of animal models are being introduced to better understand the pathogenesis and new drug effects on diabetes. Diabetes mellitus is growing at an alarming rate. The main cause of this problem is aging, urbanization and increasing privilege leading to obesity and physical inactivity (Patil *et al.*, 2011) [18]. Diabetes mellitus when not appropriately managed precipitates a number of debilitating complications that affects the heart, kidney, eyes, and nervous system, resulting to other secondary effects like hyperlipidemia, oxidative stress, stroke, keto-acidosis and non-ketotic hyperosmolar coma. Despite the present management procedures the prevalence has been projected to increase to about 64% by 2025, that is affecting 53.1 million people; while others argue that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 370 million (Linda, *et al*., 2002; Shaw, *et al.*, 2010; Prabhakar and Doble, 2011; Rowley and Bezold 2012) [15, 25, 20, 21].
Currently available therapy (hypoglycemic agents) which focuses on α-glucosidase regulation for diabetes includes insulin and various oral hypoglycemic agents such as sulfonylureas,

metformin, glucosidase inhibitors, troglitazone, etc. Despite the use of these drugs, the management of diabetes without any side effects is still a challenge for medical system. This has leads researchers to search for safer, cost effective and low side effect antidiabetic drugs from plants used in traditional system (Patil *et al.*,2011; Maiti *et al.*, 2004) [16, 18]. Moreover plants extracts containing bioactive components have been shown to provide unlimited opportunities for new drug discoveries due to their multivariate chemical diversity. Studies have shown that over 80% of the world's population relies on traditional medicine for their primary healthcare needs including various ailments like diabetes mellitus (Cosa *et al.*, 2006; Duraipandiyan, *et al.*, 2006; Shaw, *et al.*, 2010) [3, 4, 25]. Much of the current research work on medicinal plants for the treatment of diabetes however involves phytochemical and pharmacological analyses to identify and isolate bioactive principles.

Considering the fact that plants continue to be used in their native form for medicines, there is an inevitable necessity to evaluate antidiabetic plants not only in terms of their pharmacological parameters, but vis-à-vis the demand for quality control and standardization .This development has also created a pressing need for appropriate methodologies for comprehensive characterization of plants beyond analytical studies of bioactive components.

Spectroscopy plays a major role in phytochemical characterization of medicinal plants. Techniques such as UV-Visible spectroscopy, Infrared spectroscopy (IR) and Scanning Electron Microscopy (SEM) are used widely in phytochemistry. Each technique uses different frequencies of the Electromagnetic spectrum to excite the samples and provide information on different aspects of their chemical nature (Pavi et al., 2009) [19]

Ultraviolet and visible (UV-Vis) absorption spectroscopy for instance, is a the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range (Kemp, 1975; Sharma, 2007) [13, 24]. Infrared (IR) spectroscopy is used to determine functional groups in molecules. It measures the vibrations of atoms,and based on this it is possible to determine the functional groups of compounds (Yadav, 2007) [29]. Although these spectroscopic methods are traditionally used for studying single molecules, is ideally suited for metabolomics and has been applied in various studies of plants and whole extracts (Kim et al., 2010; Ghatak et al,2014 and Jayasundar et al., 2014) [6, 12, 14] Scanning electron microscopy (SEM) uses a focused beam of high energy electrons to generate a variety of signals at the surface of solid specimen. The electrons interact with atoms in the sample, producing various signals that contains information about the samples surface topography and composition (Hollas, 2003) [8].

Our earlier works on the assessment of the anti-diabetes potentials of *Murraya koenigii, Hibiscus cannabinus, Vernonia amygdalina and Telfairia occidentalis* leave extract yielded captivating results which indicated a competing hypoglycaemic activity with a standard drug (James *et al*; 2016a,b) [10, 11]. Although Trojan *et al.*, (2011) [27] noted that research on plant finding should not only be tailored toward the mechanism of action of these plants extract but serious consideration be given in establishing their bioactive constituents.

This study is therefore an exploratory effort at extending our previous finding to characterizing the partially purified plants (*Murraya koenigii, Hibiscus cannabinus, Vernonia amygdalina and Telfairia occidentalis*) extract using the spectroscopic techniques of UV-Visible spectroscopy, Infrared spectroscopy (IR) and Scanning Electron Microscopy (SEM). Apart from fuelling the literature in this line of research, findings from present study may tend to elucidate further the spectral pattern of these plants extract and their inherent properties in the quest for the management of diabetes mellitus.

Methodology

Experimental Design

The plant samples were sourced from various gardens around Kaduna metropolis. There authentication and identification was conducted at the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The sample was extracted using ethanol as the solvent. The extract of each of the plant was fractionated by passing the extract through a column pack with silica gel as stationary phase. The fraction of the plants extract was subjected to characterization.

Chemicals and Reagents

Disodium phosphate (2331-BDH); Monosodium phosphate (1470-BDH); Silica Gel (60-200 mesh, Qualikens); Absolute ethanol (JHD); Sodium hydroxide (G3209 2006- Sigma Aldrich); Hydrochloric acid (B777- Sigma Aldrich). All other chemicals and reagents used were of analytical grade.

Plant Preparation and extraction

Plant preparation and extraction was carryout using standard method. Fresh leaves of *Murraya koenigii, Hibiscus cannabinus, Vernonia amygdalina and Telfairia occidentalis* were dried at room temperature and grounded into powder with pestle and mortal; after which each powder was transferred into an air tight plastic contain and then stored at 8^{o}C for further analysis.

For each leaf extract, 200g of the previously prepared powdered plant was separately transferred into a beaker and soaked in 800 ml of 70 % ethanol, sealed with an aluminum foil and was allowed to stand for 24hrs at room temperature as described by Gohil *et al.*, (2010) [31]. After 24hrs the mixture was filtered using muslin cloth and re-filtered with the aid of a 2-folded Whatman No. 1 filter paper to obtain the ethanolic extract. The ethanolic extract was subjected to rotatory evaporator (R-205D-NYC) and the solid crude extract obtained was weighed and then transferred into a sample vial and stored at 8^{o}C in the refrigerator.

Preparation of chromatography column (silica gel)

The Fractionation or Cromatography column (500mm-Pyrex) was washed and rinsed with deionized water, a plastic funnel was placed on top of the column to serves as the packing and buffer reservoir. The chromatography column was filled to about one third with 0.2M phosphate buffer (pH 6.3). A slurry of 103g (160 ml) of silica gel was made by adding 350ml of phosphate. The slurry mixture was introduce into the column and allowed to equilibrate. After which the excess buffer in the column was removed.

Fractionation of plants crude extract

Each ethanolic extracts of *Murraya koenigii, Hibiscus cannabinus, Vernonia amygdalina and Telfairia occidentalis* were dissolved separately in 25 % dimethyl sulphoxide (DMSO) and further dissolved with 30ml of 0.2M phosphate buffer (pH 6.3). The reconstituted solution was loaded into

the prepared column above and allowed to move through the inlet and the outlet of the column and was collected as eluent, the time taken for 30ml to pass through the column and collected as the eluent was noted as the flow rate/30ml. Three fractions was require, therefore if 100ml is loaded the flow rate/100ml was divided by three to get the exact time for the collection of each of the fraction base on the number of layers formed in the column, the retention time, volume and the color was noted.

Characterization

Characterization was carried out after fractionation with column chromatography. The fraction so obtained were subjected to the following techniques which involves UV-Visible spectroscopy, Infrared spectroscopy (IR) and Scanning electron microscopy (SEM).

1. Spectroscopy studies of the fraction of the plant extract

The various fractions obtained were subjected to spectroscopic studies at different wavelength. Exactly 5.5ml each of the plant extracts fractions were placed in a test tube and 4.5ml of phosphate buffer was added to dilute the solutions. The absorbance of the extracts was then measured at the wavelength of 200-700nm at the interval of 5nm using a spectrophotometer (UV-752, Pec medical, USA). The absorbance versus the wavelength was plotted to yield each spectrum for the fractions, and the maximum wavelength determined from the spectra.

2. Infrared spectroscopy

Aliquot of 5ml sample was measured according to standard procedure. Each KBr containing fractions were scanned at 4mm/s at a resolution of 2cm over a wave number region of 400-4500cm^{-1}. The characteristic peaks were recorded using Perkin Elmer Spectrophotometer (model 180).

3. Scanning electron microscopy

Scanning electron microscopy (SEM) were performed on the various plant fractions, the fraction was mounted on aluminum stubs with double sticky tape and coated with 20 nm of gold. The samples were viewed and photographed on a Phenom world (Eioid Honven-Netherland) at accelerating voltage of 10 kV using back scattered electron detectors.

Results

Partial purification and characterization of anti-diabetic properties of fractions of ethanolic leave extracts of *Murraya koenigii, Habiscus cannabinus, Vernonia amydalina and Telfairia occidentalis* was conducted and the ethanolic leaves extract of the 200g powdered plants yielded 8.68g, 9.65g, 10.11g and 13.07g for *M. koenigii*, *T. occidentalis*, *V. amygdalina* and *H. cannabinus* respectively.

The plant extracts were fractionated in a column chromatography and various fractions were collected at different retention time as presented in table1.

Ultra-violent spectroscopy studies, shows fractions of *H. cannabinus* HC1 to absorbs maximally (λ_{max}) at wavelength of 400nm, while HC2 absorbs maximally at of 390nm and HC3 fraction at 340nm (see figure1). The fractions of *V. amygdalina* VA1 absorbed maximally at 340nm with a minor peak at 260nm, VA2 λ_{max} is 340nm with a minor peak at 240nm and VA3 absorb maximally at 360nm with a minor peak of 240nm while VA4 λ_{max} is 340nm with a minor peak of 250nm (see figure 2). Also, the fractions of *M. koenigii* absorbed maximally at 235nm and 340nm for MK1, 320nm for MK2 and 335nmm for MK3 (see figure 3). Then fractions of *T. occidentalis* absorbed maximally at 385nm for TO1, 365nm for TO2 and 355nm forTO3 (see figure 4).

Further characterization by IR spectroscopy showed that MK1(figure 5) fraction of *Murraya koenigii* consists of a C-I stretch of Alkyl halides (418.14cm^{-1}), a strong C=C stretch of Aromatic compounds (1489.39cm^{-1}), a strong N-H bend of Amides (1521.53cm^{-1}), a C=O stretch (1635.82cm^{-1}),strong C=C stretch of Alkanes (2342.57 cm^{-1}), and O-H stretch of Alcohol (3853.37cm^{-1}). Fraction of MK2 (figure 6) consists of a C-Br stretch of Alkyl halides (544.74cm^{-1}), a =C-H bend of Alkenes (951.00cm^{-1}), A H-C-H bend of Alkanes (1418.53cm^{-1}), C=C stretch of Aromatic compound (1456.72cm^{--1}), N-O stretch of Nitro compounds (1507.02cm^{-1}), weak N-H stretch of Amides (3421.83cm^{-1}) and MK3 (figure 7) consists of a C-H bend of Alkenes(950.71cm^{-1}), a ring C-C stretch of Aromatic compound (1506.25cm^{-1}),

Table 1: Various Retention time, volume and colour of fractions obtained from column chromatography.

Fractions	Retention Time (min)	Volume (ml)	Colour
MK1	17	61	Dark brown
MK2	19	55	Reddish brown
MK3	22	62	Light Yellow
TO1	109	104	Dark brown
TO2	134	48	Orange
TO3	140	66	Light brown
HC1	12	48	Dark brown
HC2	14	54	Brown
HC3	17	73	Yellow
VA1	33	36	Yellow
VA2	34	53	Dark brown
VA3	36	48	Brown
VA4	41	65	Orange

The MK1, MK2, MK3 represent 1st, 2nd and 3rd fractions of *Murraya koenigii* respectively; TO1, TO2 and TO3 represent 1st, 2nd and 3rd fraction of *Telfairia occidentalis* respectively; VA1, VA2, VA3 and VA4 represent 1st, 2nd, 3rd and 4th fraction of *Vernonia amygdalina* respectively; while HC1, HC2 and HC3 represent 1st, 2nd and 3rd fraction of *Hibiscus cannabinus* respectively. Fractions were obtained from column chromatography. And the retention time is the time taken for the plants extract solution to move from the inlet of the column, separate and come out of the column as eluent.

C=O stretch and N-H bend of Amides (1652.03cm^{-1} and 1539.40cm^{-1}), a C=O stretch of Aldehyde (1732.73cm^{-1}). MK3 also contains benzene ring with several bands of overtone at peak 2088.81cm^{-1}and an O-H stretching of Alcohols and phenols (3899.06cm^{-1}).

Fraction of *Telfairia occidentalis* TO1 (figure 8) consists of a C-Br stretch of Alkyl halides (668.05cm^{-1}), a C-O stretch of Alcohol (1077.75cm^{-1}), C=O stretch of Amides (1634.63cm^{-1}), =C-H stretch of Alkenes (2089.93cm^{-1}), a C-H stretch of Alkane (2921.93cm^{-1}), and an N-H stretch of Amides (3445.08cm^{-1}). TO2 (figure 9) consists of a C-I of Alkyl halides (418.73cm^{-1}), a =C-H bend of Alkynes (668.84cm^{-1}), C=O stretch of carboxylic acid (1684.19cm^{-1}), N-H stretch of Amines (3447.41cm^{-1}) and O-H stretch of Alcohol and phenols (3711.40cm^{-1}). TO3 (figure 10) consists of, C=O stretch of Amides (1078.71cm^{-1}), C-H bend of Alkanes

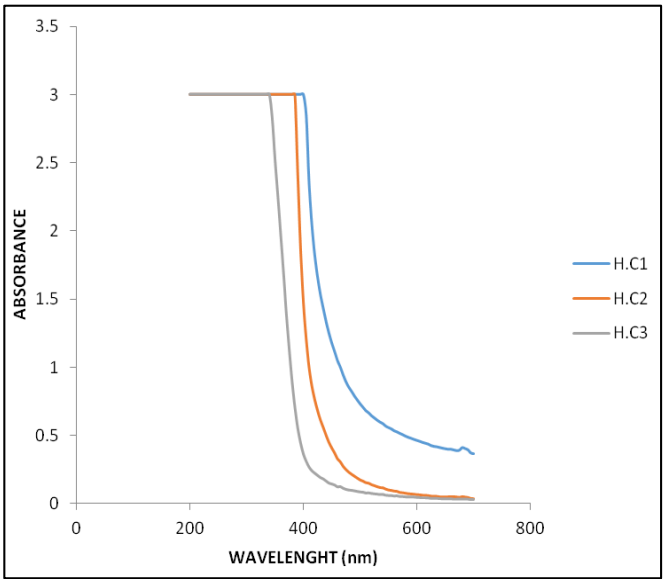

Fig 1: The spectrum of three fraction of ethanolic leaf extract of *Hibiscus cannabinus*.

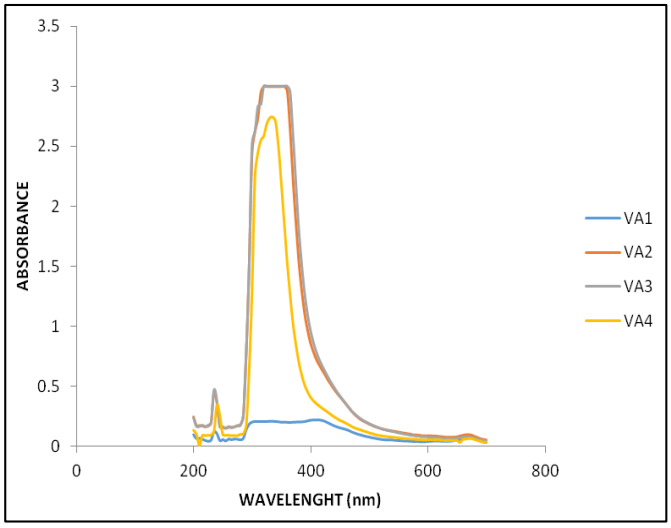

Fig 2: The spectrum of three fraction of ethanolic leaf extract of *Vernonia amygdalina*

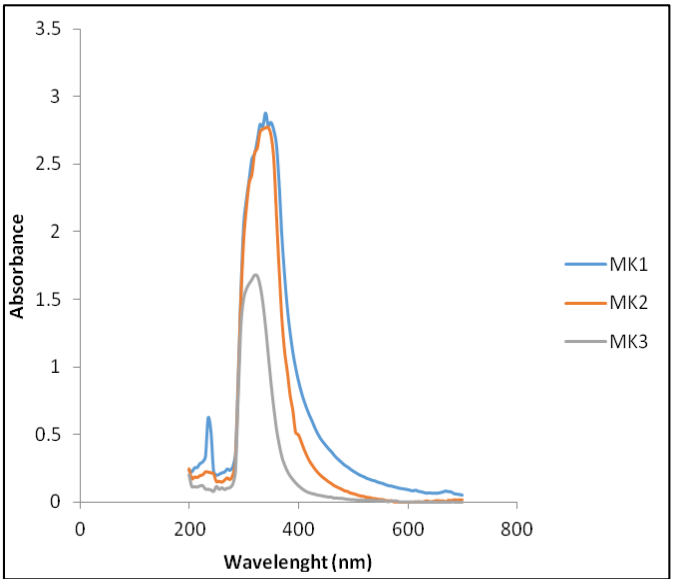

Fig 3: The spectrum of fraction of ethanolic leaf extract of *Murraya. koenigii*.

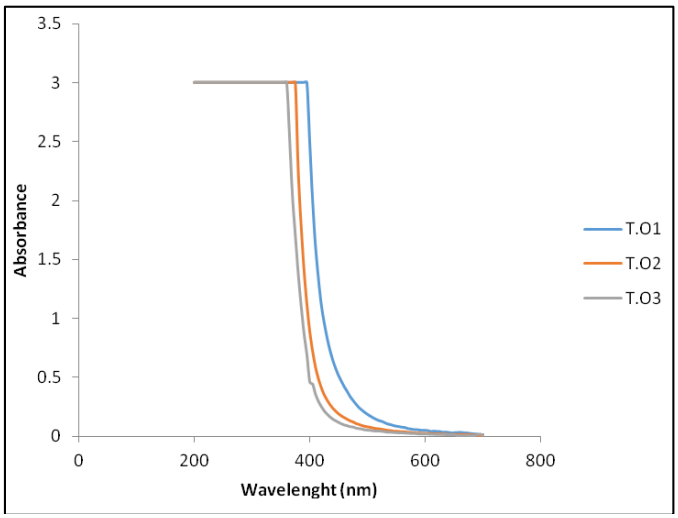

Fig 4: The spectrum of fraction of ethanolic leaf extract of *Telfairia occidentalis*

(1456.19cm^{-1}), a ring C=C stretch of Aromatic compound (1506.37cm^{-1}), and an N-H stretch of Amines (3432.49cm^{-1}).

The Infrared spectrum of *H. cannabinus* fraction HC1 (figure 11) indicates the presence of functional group such as O-H stretch of phenol and alcohol (3445.21cm^{-1}), C-H stretch of Alkanes (2923.88cm^{-1}), C≡C stretch of Alkynes (2359.13cm^{-1}) C=O stretch of Ketones (1738.76cm^{-1}) C=O stretch of aldehyde (1682.68cm^{-1}) ring C=C stretch of aromatic compound (1557.73cm^{-1}) and C=C stretch of Aromatic compound (1455.79cm^{-1}). Also, additional transmittance was observed at the finger print region (1500-450cm^{-1}) which indicate the presence of alkyl halide (668.34cm^{-1}). However, fraction HC2 (figure 12) indicate the presence of functional group such as O-H stretch of Alcohol (3304.32cm^{-1}), N-H stretch of 1^{o} amine (1635.55cm^{-1}), N-H stretch of Amine (1436.41cm^{-1}), C-N stretch of Aromatic amine (1319.10cm^{-1}) and C-F stretch of alkyl halide (1012.22cm^{-1}). While fraction HC3 (figure 13) indicate the presence of functional group such as O-H stretch of Alcohol (3435.75cm^{-1}), C-H stretch of Alkanes (2927.79cm^{-1}), N-H stretch of 1^{o} Amines (1635.73cm^{-1}); with finger print region which may indicate the presence of alkyl halides.

Vernonia amygdalina VA1 fraction IR indicate the presence of functional group such as O-H stretch of Alcohol and phenols (3439.81cm^{-1}), C-H stretch of Alkanes (2923.71cm^{-1}), N-H stretch of 1^{o} Amines (1634.65cm^{-1}) and C=C stretch of Aromatic compound (1455.89cm^{-1}); with finger print region which may also indicate the presence of alkyl halides (see figure 14). The Infrared spectrum of the VA2 (figure 15) indicate the presence functional group such as O-H stretch of phenol and alcohol (3646.20cm^{-1}),O-H stretch of Alcohols (3445.23cm^{-1}), C=C stretch of Aromatic compound (2341.38cm^{-1}), N-H stretch of 2^{o} amines (1557.75cm^{-1}) and N-H stretch of 1^{o} amines (1456.09cm^{-1}). Also, VA3 fraction indicate functional group such as O-H stretch of phenol and alcohol (3427.84cm^{-1}), C-H stretch of Alkanes (2924.16cm^{-1}), N=O stretch of Nitro compound (1635.02cm^{-1}) and C-H stretch of Alkanes (1456.66cm^{-1}) (see figure 16). However, fraction VA4 (see figure 17)suggest the presence of functional group such as O-H stretch of phenol and alcohol (3444.84cm^{-1}),C-H stretch of Alkanes (2923.66cm^{-1}), C=C stretch of Alkenes (2359.28cm^{-1}), ring C=C stretch of Aromatic compound (1558.70cm^{-1}) and N-H bend of primary amine (1489.15cm^{-1}).

Finally, characterization using scanning electron microscopy (SEM) to study the morphology of the compounds present in the fractions shows structural morphology of the micrograph. The SEM of MK1 is a mixture of cubic and spherical shape and MK2 is circular and irregular in shape, while the SEM of TO1 is a mixture of spherical and oval shape and that of TO3 is oval in shape. While the SEM of VA1 and VA3 are Oval in shape, and that of HC3 is a mixture of cylindrical and oval shape; but that of HC2 is a mixture of cylindrical and cubic in shape (See fig 18-21).

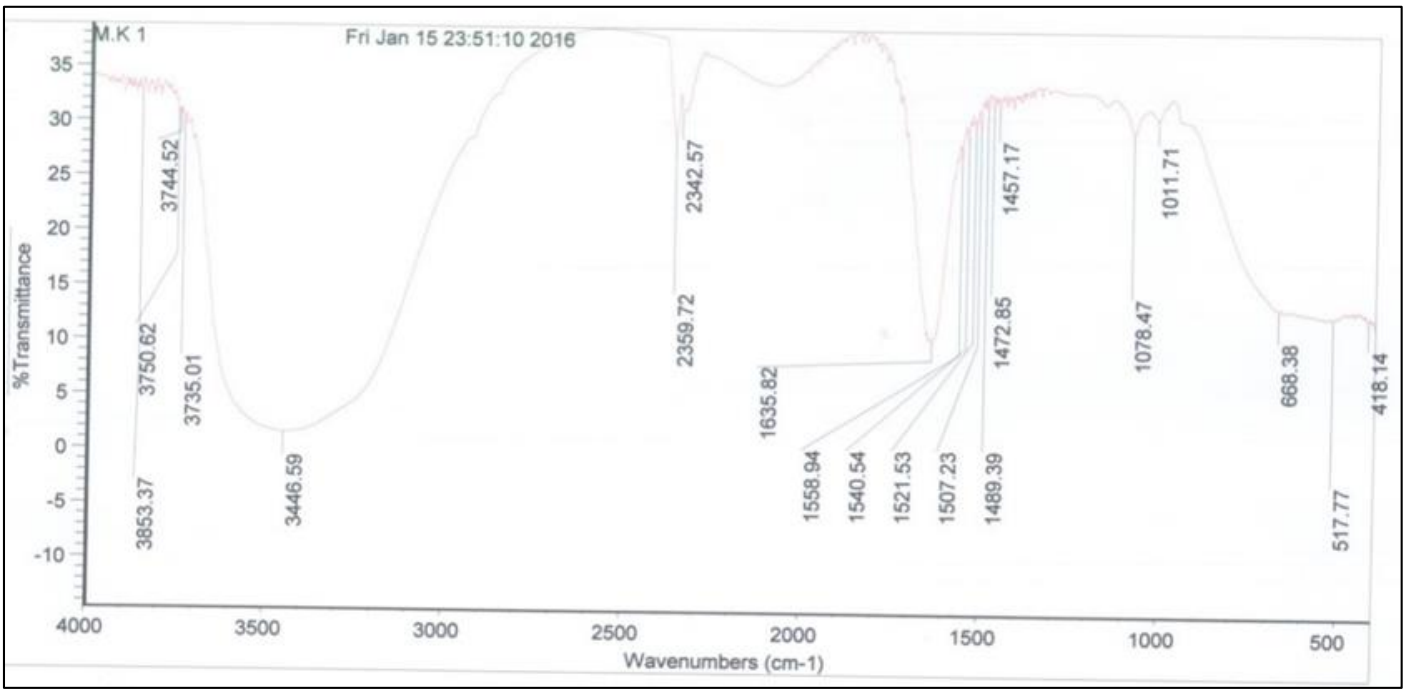

Fig 5: IR Spectrum of *Murraya koenigii* Fraction 1 (MK1)

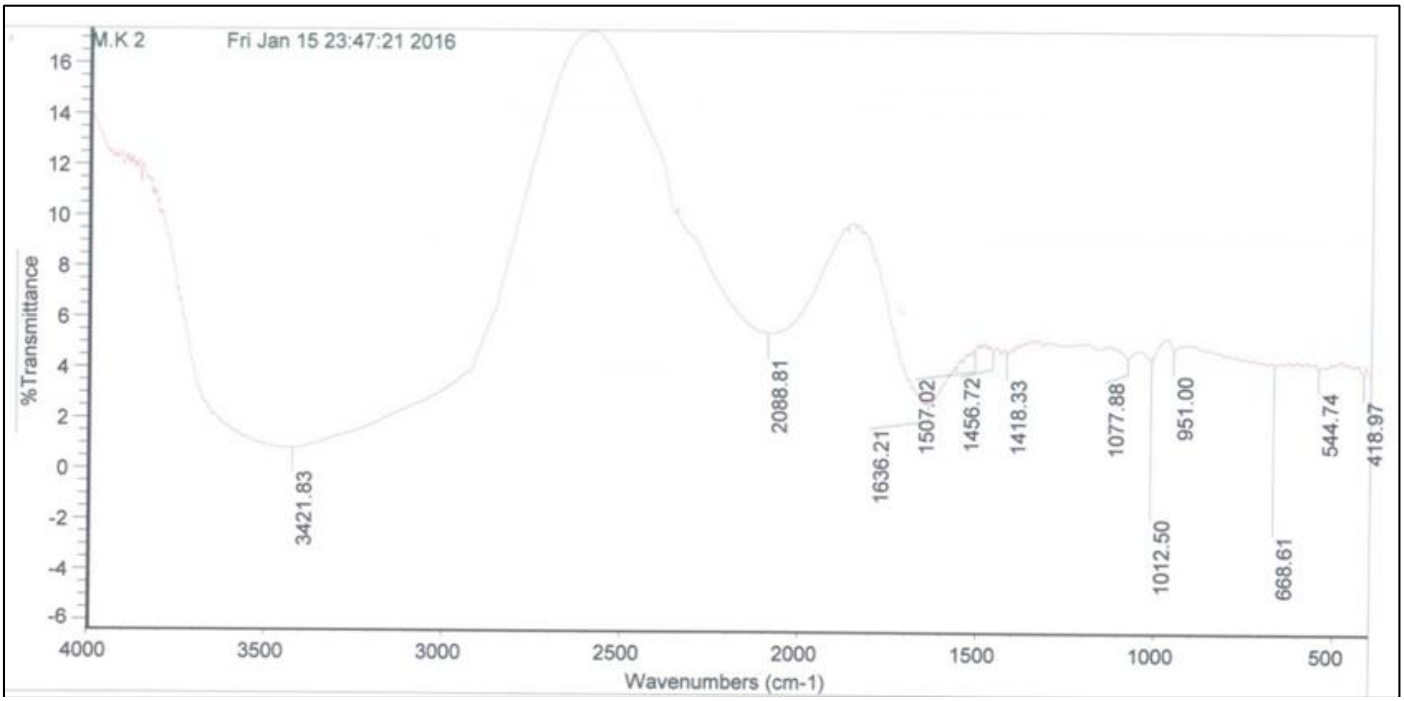

Fig 6: IR Spectrum of *Murraya koenigii* Fraction 2 (MK2)

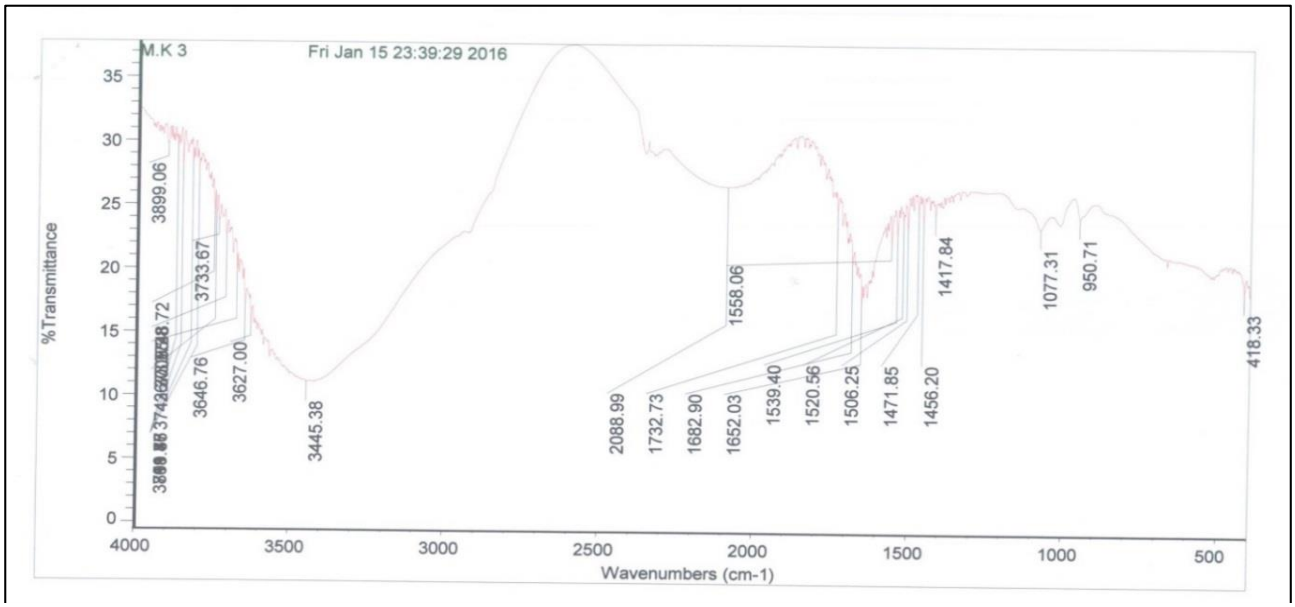

Fig 7: IR Spectrum of *Murraya koenigii* Fraction 3 (MK3)

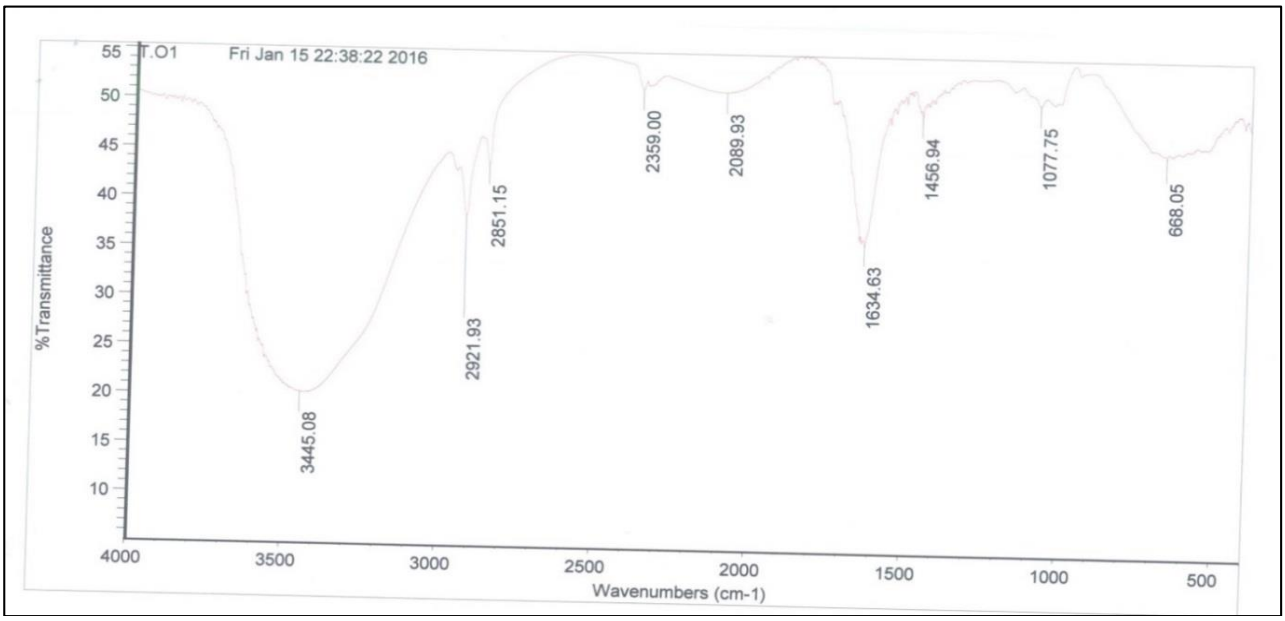

Fig 8: IR Spectrum of *Telfairia occidentalis* Fraction 1 (TO1)

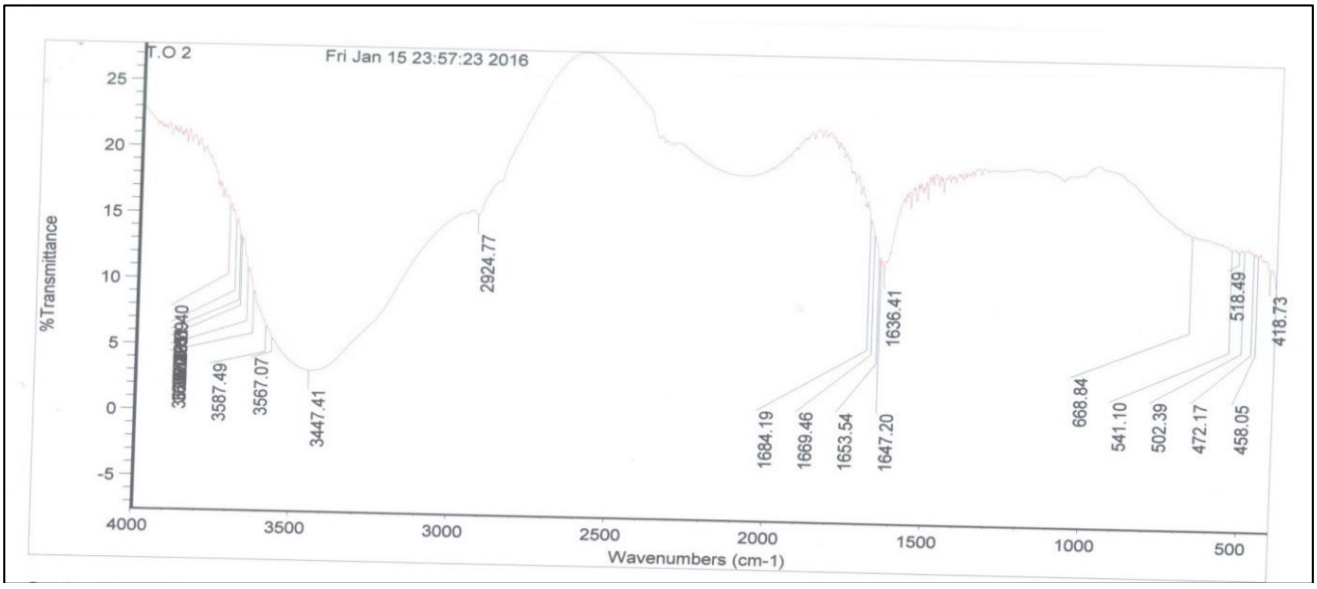

Fig 9: IR Spectrum of *Telfairia occidentalis* Fraction 2 (TO2)

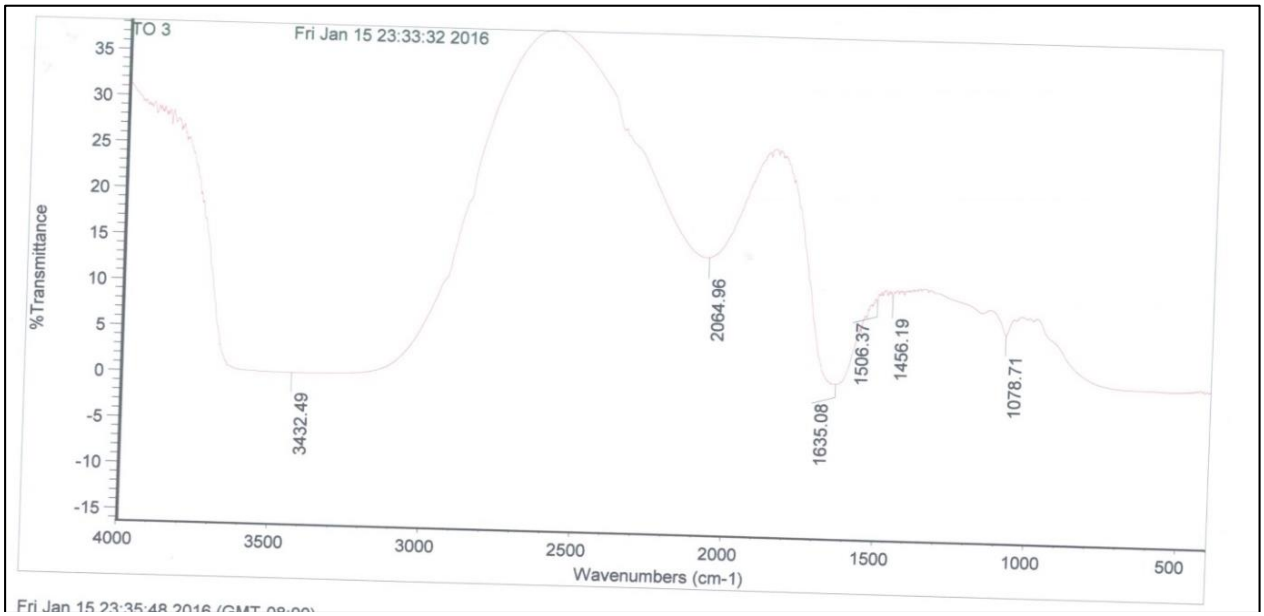

Fig 10: IR Spectrum of *Telfairia occidentalis* Fraction 3 (TO3)

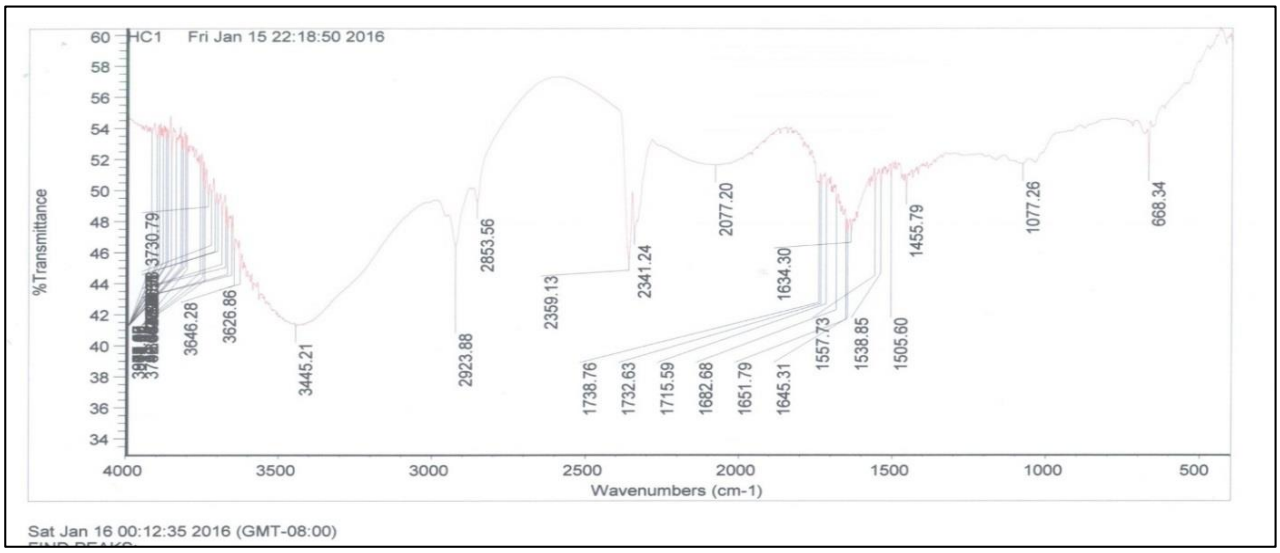

Fig 11: IR Spectrum of *Hibiscus cannabinus* Fraction 1 (HC1)

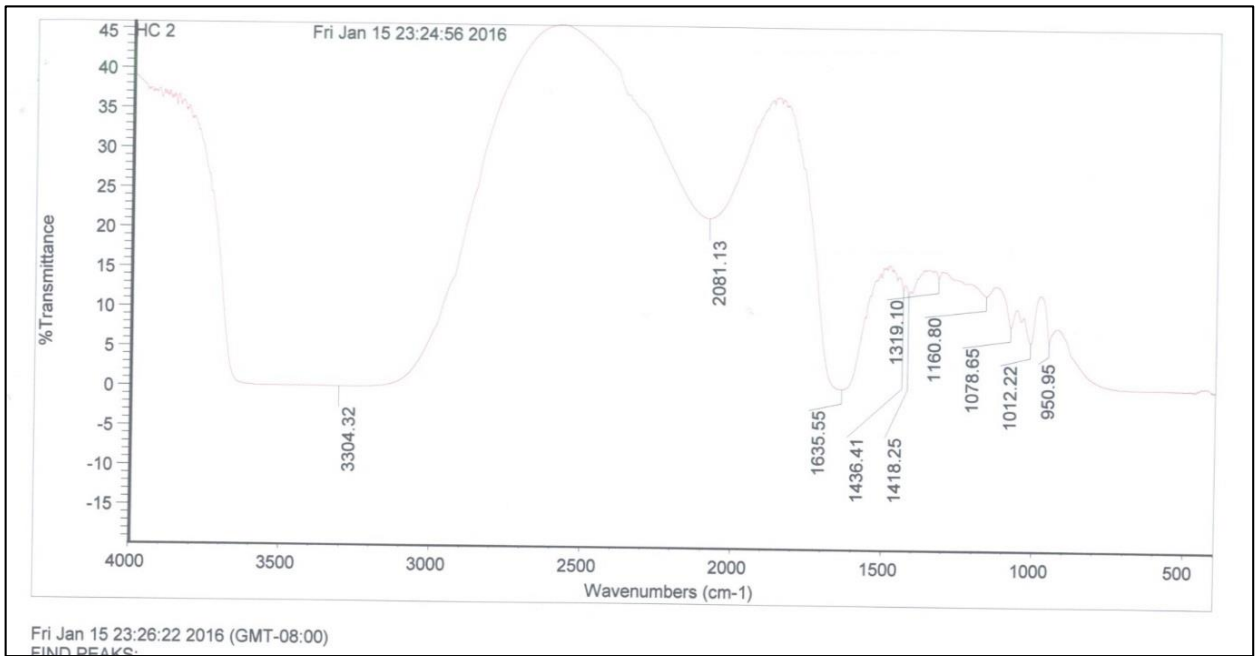

Fig 12: IR Spectrum of *Hibiscus cannabinus* Fraction 2 (HC2)

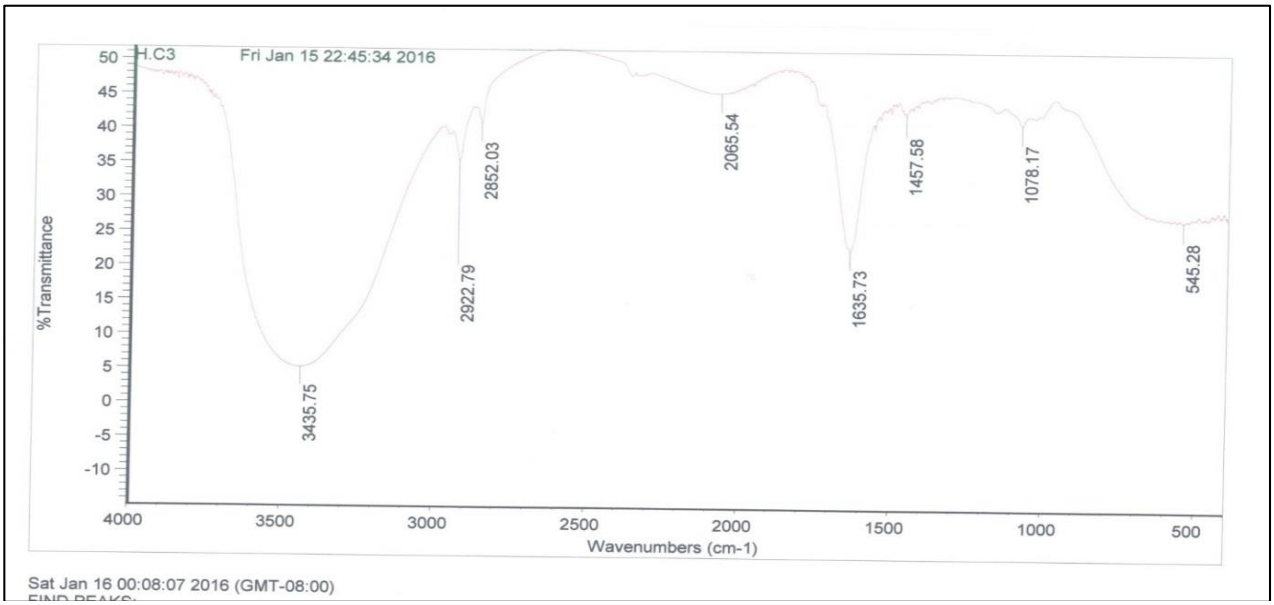

Fig 13: IR Spectrum of *Hibiscus cannabinus* Fraction 3 (HC3)

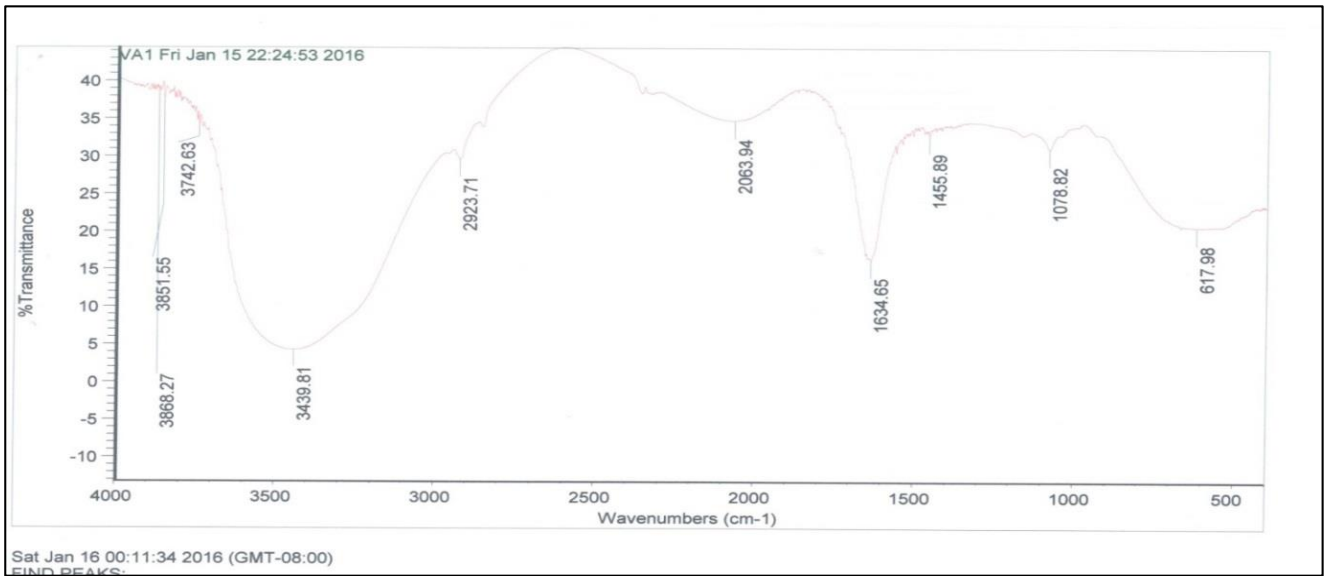

Fig 14: IR Spectrum of *Vernonia amygdalina* Fraction 1 (VA1)

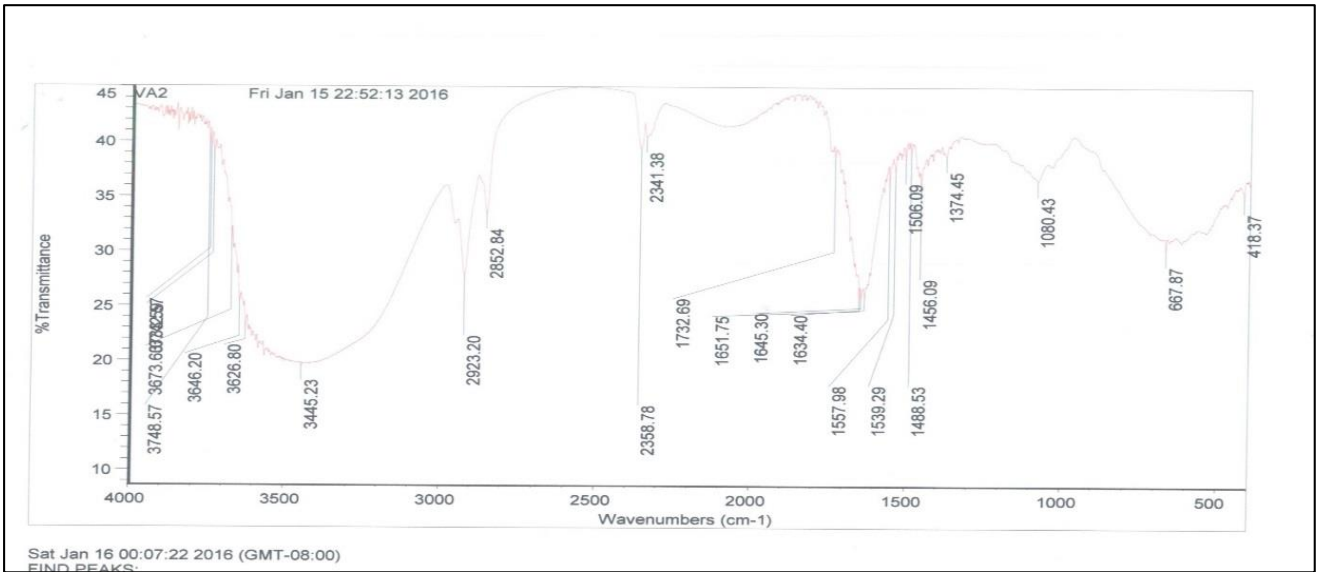

Fig 15: IR Spectrum of *Vernonia amygdalina* Fraction 2 (VA2)

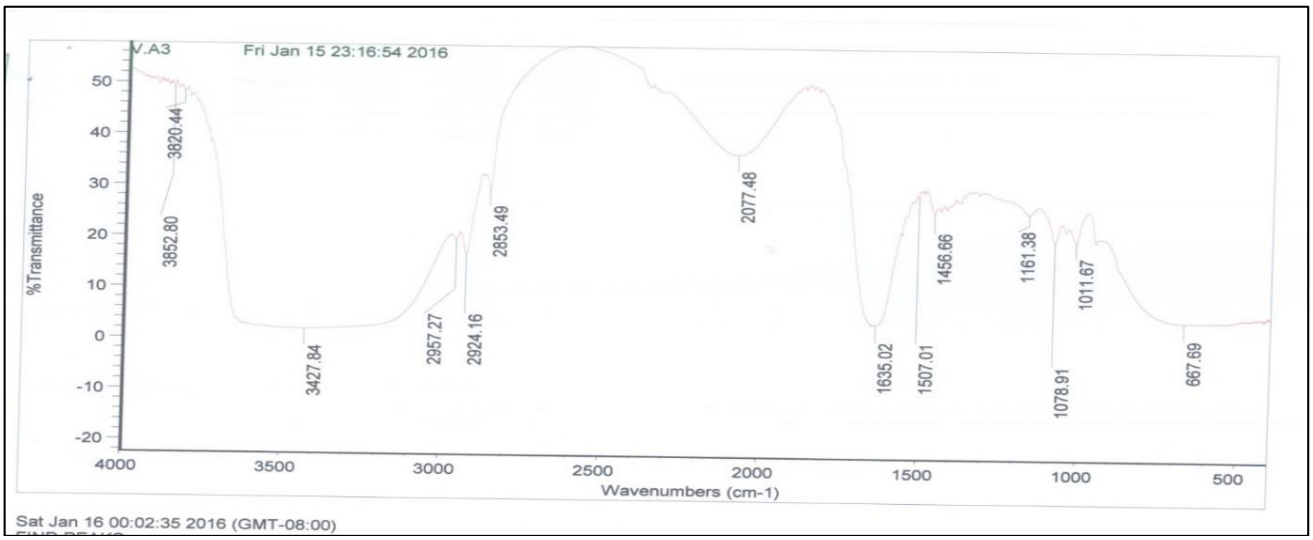

Fig 16: IR Spectrum of *Vernonia amygdalina* Fraction 3 (VA3)

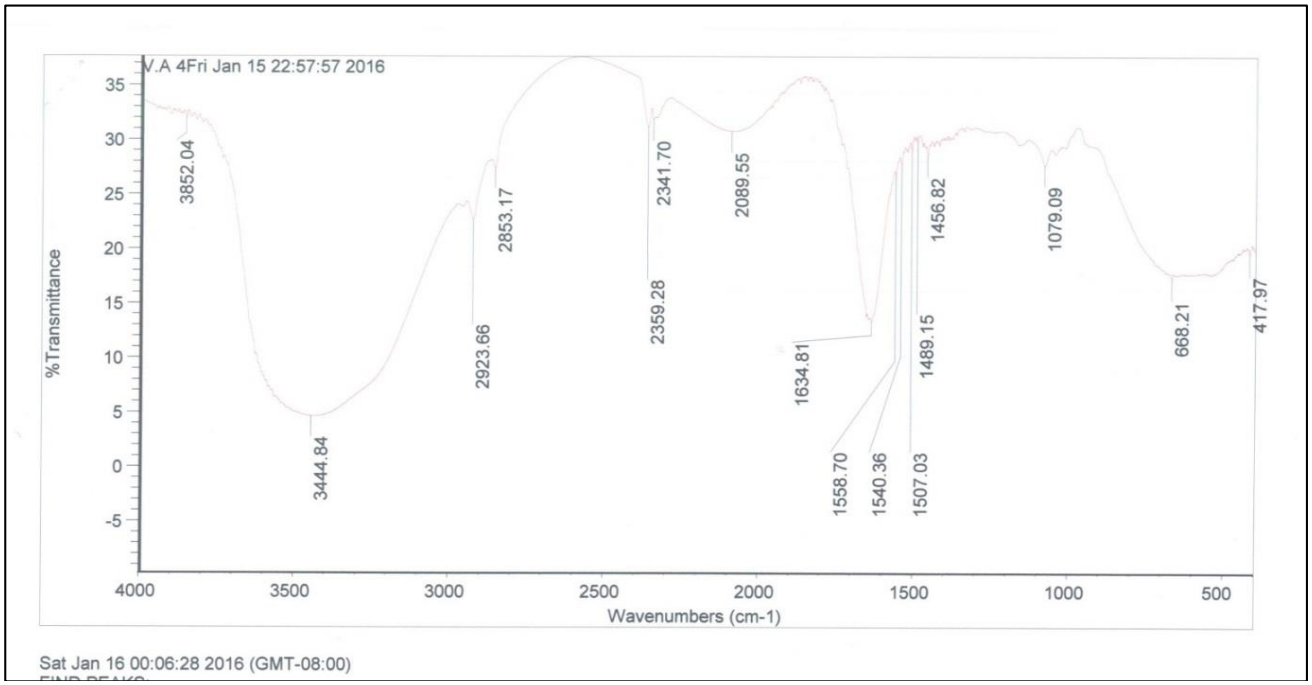

Fig 17: IR Spectrum of *Vernonia amygdalina* Fraction 4 (VA4)

Also, the fractions HC1, HC3, VA1 and VA3 SEM results (see figure 22 – 26) shows structural morphology of the micrograph. The SEM of VA1 and VA3 are Oval in shape, while the SEM of HC3 is a mixture of cylindrical and oval shape. The SEM of HC2 is a mixture of cylindrical and cubic in shape.

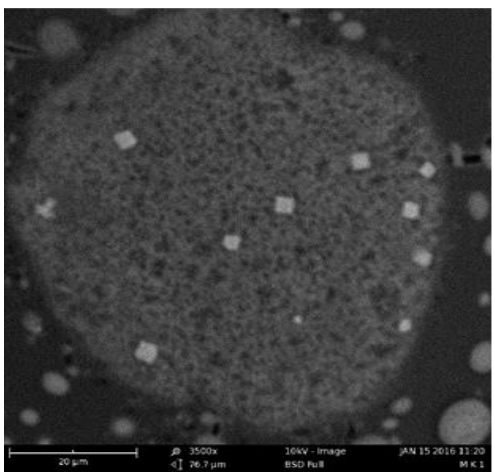

Fig 18: Scanned image of MK1

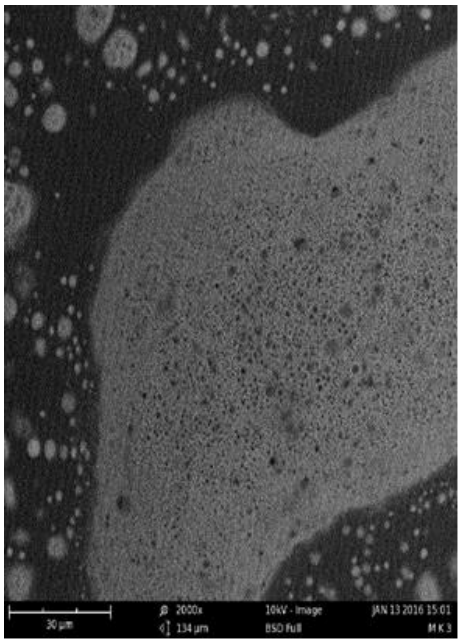

Fig 19: Scanned image of MK3

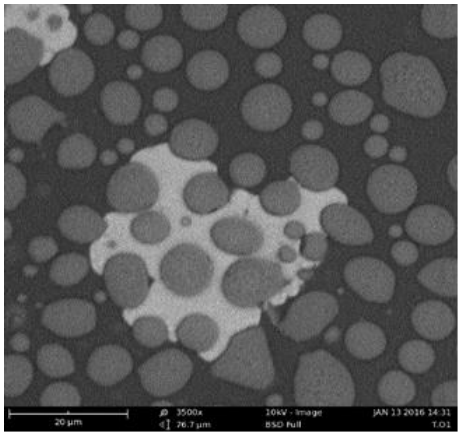

Fig 20: Scanned image of TO1

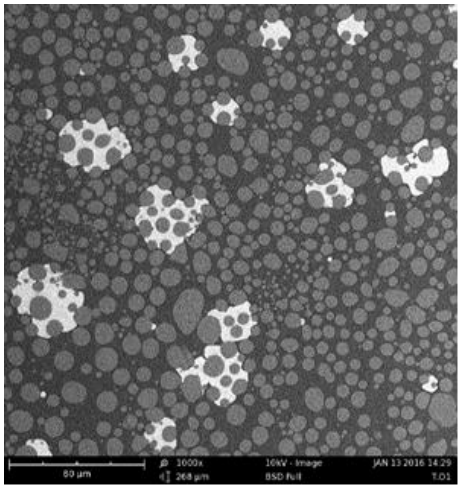

Fig 21: Scanned image of TO3

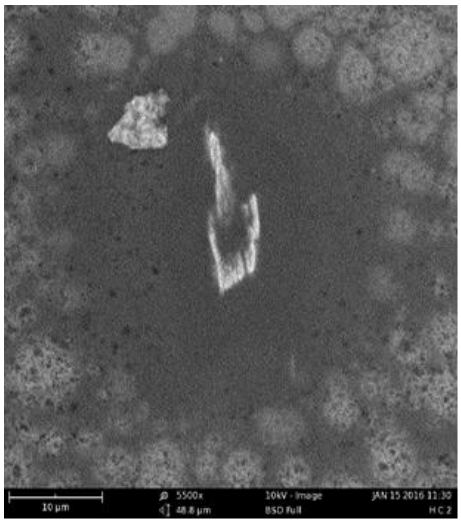

Fig 22: Scanning electron microscope image of H.C2

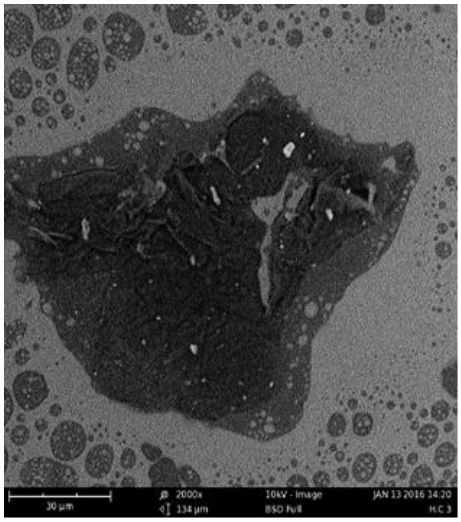

Fig 23: Scanning electron microscope of HC3

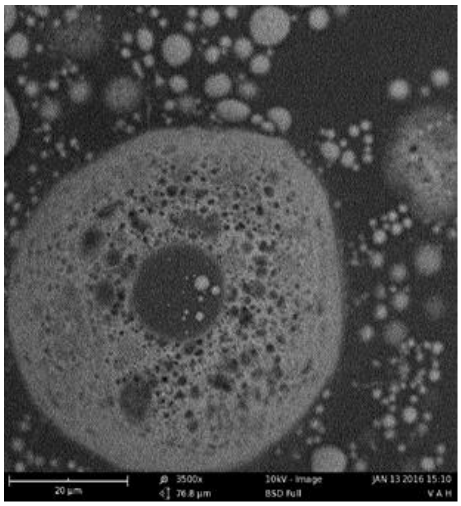

Fig 24: Scanning electron microscope image of V.A1

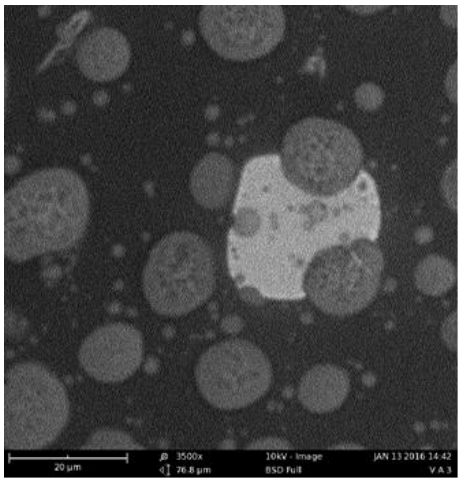

Fig 26: Scanning electron microscope image of V.A3

Discussion

There is a revival of interest in traditional medical systems universally. This has prompted a growing interest in understanding in the use of plants for antidiabetic therapy. This study is therefore an attempt to explore the use of structuro-functional information deduced from spectroscopic techniques (UV-VIS, IR, SEM) to further elucidate the bioactive constituents previously isolated from these plants (James et al,2016a,b) [10].

The spectroscopic studies of the fraction showed that the fractions obtained from column chromatography absorbed UV light at different wavelength. This absorption of UV light by the fraction indicates that the component present in each of the fractions contains conjugated double bonds in their structures. Conjugation of double bond with another double bond causes absorption band to shift to longer wavelength with greater density (Bailey and Day, 1989) [2]. The body of academic literatures have shown that substances like Murrayanol, Hispidulin flavonoids, Mahanimbine alkaloid, flavonoids, Mahanine alkaloids, oxalic acid, phenolic compounds are present in the fractions of *Murraya koenigii* and *Telfairia occidentalis* (Anil *et al*, 2013; Harish *et al*, 2012 and Yun-lian *et al*,2000) [1, 7, 30]. Hence the fractions obtained from *Murraya koenigii* may contain the presence of a phenol ring with an OH stretch, alkane group, alkyl halides, N-H bend of amides, a double bond stretch of aldehyde and a benzene ring which suggest the presence of the possible compounds Murrayanol, Hispidulin, flavonoids, Saponins, Phenolic compound, chibulagic acid, Altagic acid and also Mahanimbine alkaloid.

Considering the extract from *Vernonia amygdalina* studies suggest the present of phenolic and saponin compounds or with benzene related compound that has functional group(s) of OH, alkanes side chain, alcohol, carbonyl and aldehyde (Robert and Boyd, 2008). These groups and their parent compounds are found to be present in phytochemicals of *Vernonia amygdalina;* such phytochemicals that were reported to be in the plant by James (2012) [9]. In addition, the study also shows that *H.cannabinus* contain the presence of compound such as flavonoids and conjugated aromatic compound which have been implicated in medicinal chemistry (Bailey and Day, 1989; Harish, *et al*., 2012) [2, 7].

From this study the extract from *Murraya koenigii* suggested the presence of the possible compounds like alkaloid, Murrayanol and flavonoids. These compounds are found to be present in phytochemicals of *Murraya koenigii* (James, 2012) [9]. Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity. However, when ingested by animals, they affect glucagon, thyroid stimulating hormone and inhibit certain enzymatic activities (Eyong, *et al.,* 2011) [5]. This may also affect α- amylase that hydrolyses glycosidic bonds in carbohydrate digestion in the gastrointestinal tract. The present study shows the presence of glycoside which has been suggested to reduce the plasma glucose of normal and streptozotocin induced diabetic rats (Taiwo, *et al.*, 2009) [26].

Results also suggested the presence Saponins and phenolic compounds; which Eyong, *et al.,* (2011) [5] also reported that the bioactive compound (saponin) reduces the uptake of cholesterol and glucose at the gut through intra-lumenal physicochemical interaction. The structure of saponin has a sugar attached to its carbon 3 with a hydroxyl group (OH), it also contains phenols which may be in agreement with the infrared spectrum of the fraction (James, 2012) [9]. The most important role of phenolic compound may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections (James, 2012) [9].

Also, results from the fractions obtained from *Telfairia occidentalis* shows that there could be the presence of the compounds Flavonoids, Mahanine alkaloids, oxalic acid, phenolic compounds and saponin. Osinubi and Sanusi (2007) [17] suggested that flavonoids stimulate the release and production of insulin from β-cell of islet of langerhan in the pancreas. Flavonoids apart from their ability to scavenge free radicals, they also possess antioxidant properties (Harish, *et al*., 2012) [7], and have the ability to inhibit α-amylase as studies by Bailey and Day (1989) [2].

Conclusion

Plant chemistry has seen tremendous progress in recent years owing to the advancement in the field of natural product technologies and analytical techniques. Although whole extracts and complex mixtures do not fit the conventional model of single active molecule, the growing interest in multitargeting and synergism in phytomedicines have opened new vistas in phytochemistry research (Wagner, 2011; Saw et al.,2012) [28, 23]. In this our exploratory report, spectroscopic characterization of fractions of partially purified ethanolic extracts of *Murraya koenigii, Telfairia occidentalis Habiscus cannabinus* and *Vernonia amygdalina* is a further confirmation of our previous studies concerning physiochemical properties of the antidiabetic substances of these plants (James et al., 2017a,b) [10, 11]. Findings from the identified spectral of specific functional groups that absorbs and transmit light at different wavelength is indicative that the plant extracts possess the characteristics so discussed. In addition, the ability of the plant extract to initiate the stimulation and production of insulin and the subsequent inhibition of α-amylase by the glycoside may further suggest that the active compound could be of therapeutic importance to abate diabetes mellitus or hyperglycemia that arise from compounds that are phenolic in nature or may have flavonoids and glycoside as their basic compound. On our part, effort is being geared toward characterizing the extract further by using NMR and GC/MS so as to determined molecular mass of the fractions and also the mechanism of action of the components in the extracts.

To continue to elucidate these properties in the extracts under investigation, further purification of the fractions using other chromatographic techniques like thin layer chromatography

(TLC), gas chromatography (GC) and High performance liquid chromatography (HPLC) should be employed to have a more pure isolate. These parameters with previous studies put together will go a long way in identifying a lead compound for the development and design of affordable and efficacious drug in the quest for the management of diabetes mellitus.

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