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Phytochemical, FTIR and Elemental Studies of African Mistlotoe (*Viscun album*) Leaves on *Cola nitida* from South-Eastern Nigeria

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ABSTRACT

In the recent time there is an increase in the use of medicinal plants for treatment of ailments. This is owning to availability and low cost of herbal materials, which has increased the use of plants such as *Viscum album* for medicinal purposes. These plants may contain mineral elements, important phytochemicals and functional groups for healthy leaving, but increased use of this plant could lead to magnification of the mineral elements. Therefore, leaves of mistletoe (*Viscum album*) harvested from kola nut tree (*Cola nitida*) in Imo state, South Eastern Nigeria was studied. The study was aimed at determining the elemental content, phytochemicals as well as Fourier Transform Infra-red (FTIR) analysis of petroleum ether and chloroform extract of *Viscum album* harvested from *Cola nitida*. The sample for elemental analysis was digested with mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$). Petroleum ether and chloroform were used as solvents to extract the sample used for phytochemical and FTIR analysis. Elemental content analysis indicates that Ca, Cr, Cu, Fe, Mg, Mn and Zn were present at varying concentrations. The elemental content is in the order Fe > Mn > Cu > Mg >Zn > Cr > Ca. The presence of flavonoids, alkaloids, phenols, saponins and tannins were confirmed by the phytochemical screening. FTIR analysis indicates that alcohols, amides, aromatics and carbonyl compounds are present in the chloroform and petroleum ether extract of *Viscum album*

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harvested from *cola nitida*, though at different intensities. The presence of some essential minerals as well as some phytochemicals and some important functional groups must have necessitated the use of this plant for medicinal purposes.

Keywords**:** *Viscum album, Cola nitida,* Phytochemicals, Functional groups, elemental content

1. INTRODUCTION

Nigeria and Africa as a whole are blessed with several accessible plants that possess medicinal qualities [1]. African mistletoe (*Viscum album*) is one of such plants with medicinal properties [2]. It is known as 'awuruse' in Igbo language of the South Eastern part of Nigeria. *Viscum album* (1b) is a member of Loranthaceae family, commonly called mistletoe [3, 4]. *Viscum album* is usually a parasitic plant that can grow on different types of host trees; the once that grow on plants that are fit for human consumption are used for their medicinal qualities [5]. Mistletoe being parasitic plant attaches its root on the branches and trunks of other trees other than the soil from where it gets nutrient for survival [6], and the evergreen leaves are photosynthetic [7]. Mistletoe can grow on different types of host plants such as cocoa (*Theobroma cacao*), cola nut (*Cola nitida*) and cush mango (*Irvingia gabonensis*), orange (*Citrus* sp.) and guava (*Psidium guajava* L.) and coffee (*Coffea arabica* L.) [8]. The use of *Viscum album* in curing different diseases such as cancer, hypertension, nervousness, insomnia and diabetes owns to the fact that it grows on different plant with several nutritional values [9].

Phytochemicals refer to chemical compounds with some biological importance, even though there nutritive values are not established [10] (Brown and Arthur, 2001), though they possess disease protective properties [11]. Phytochemicals occur naturally in plants, and are responsible for colour and some other properties that affect plant organs [12, 13]. There is variation in the chemical composition of *Viscum album* which is a function of the type of host tree [14] (Luczkiewics *et al*, 2001). Studies of mistletoe on different host trees have been reported in South-Western Nigeria [8], Northern Nigeria [15], and in Enugu, South-Eastern Nigeria [16]. The presence of some important terpenoids, fatty acids and natural antioxidants such as vitamin E has been reported in *Viscum album* [17]. The chemical properties of *Viscum album* including phytochemicals as well as the mineral content has been reported [16]. The presence of Ca, Cu, Fe and Zn as mineral nutrient contained in *Viscum album* is well documented [18]. Analysis of heavy metal levels in medicinal plants used frequently in North Western India revealed the presence of Mn, Cr, Pb, Fe, Cd, Co, Zn and Ni [19]. Phytochemical study of mistletoe plant from guava (*Psidium guajava*), kolanut (*Cola nitida*), orange (*Citrus*), pear (*Persea americana*) and cocoa (*Theobroma cacao*) showed the presence of alkaloid, phenolic compounds, tanins, saponins, phytate, oxalate and trypsin inhibitors [6].

So many different studies have established the microbial and medicinal importance of *Viscum album*. Only few studies have reported the phytochemical and elemental content of *Viscum album*. Hence there is paucity of FTIR report of mistletoe in as much as is yet to be ascertained. The functional groups in different extracts of the plant and mineral contents are possibly the basis for its medicinal properties. There is an increase in the use of herbal materials for treatment of illness due to its cheap cost. The constant use of this plant could lead to the accumulation of mineral elements above recommended level. This is in addition to the fact that chemical properties of *Viscum album* could be influenced by factors like type of host,

geographical location and solvent used for extraction and digestion. Hence the need for this work which is aimed at conducting phytochemical, FTIR and elemental (calcium (Ca) chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn) and zinc (Zn)) studies of mistletoe (*Viscun album*) leaves from Cola nut (*Cola nitida)* tree in Imo State, South-Eastern Nigeria.

2. MATERIALS AND METHOD

2. 1. Sample collection and preparation

Fresh mistletoe leaves were collected from *Cola nitida* in a wild forest located in Aboh Mbaise Local Government Area of Imo state South-Eastern Nigeria. The plant leaves were collected in the month of October, 2016. Cola nut plant belongs to the family Sterculiaceae. It has three species which is native to the tropical rainforest zone of Africa. Only two out of the three species; namely *Cola nitida* (Figure 1b) and *Cola acuminate* are common among the Yorubas of South Western Nigeria and Igbos of South Eastern [20]

The *Vascum album* leaves were taken to the Department of Plant Science and Biotechnology, Imo State University, Owerri, where it was identified. The mistletoe leaves were first wash with deionized water to remove dust particles. Samples were then allowed to air dry for a period of two weeks before being grinded into fine powder. The powdered sample was then stored in air tight plastic container.

(a)


(b)

Figure 1. (a) Mistletoe (*Viscum album*), (b) cola nut tree (*Cola nitida*)

2. 2. Sample extraction

About 70 g of the pulverized sample was accurately weighed using Nanbei NBT-A200 and soaked separately with petroleum ether and chloroform in 250 ml flask. The soaked samples were allowed to stand for 48 hours before filtration with filter paper. The filtrate was concentrated by allowing the solvents used in the extraction to evaporate. The extract was covered properly and stored in a refrigerator from where the sample was used for phytochemical and FTIR analysis.

2. 3. Elemental content determination

A mixture of concentrated nitric acid and perchloric acid in the ratio of 1:1(20 ml) in a 100 ml flask was used to digest 2g of the sample meant for elemental content determination. After 48 hours, the mixture was filtered with whatmann filter paper (0.45 μ pore size) into 100 ml flask and made up to the mark with deionized water. The elemental contents was then determined with Hanna multiparameter bench photometer HI 83200 [21].

2. 4. Phytochemical analysis

The *Viscum album* leaves extract from *Cola nitida* were qualitatively screened for phytochemicals using standard methods as described by [13, 22-25].

Alkaloids: Iodine in potassium iodide (Wagner's reagent) was used to treat 0.5 g of the *Viscum album leaves extract.* On formation of brownish red precipitate, presence of alkaloids was confirmed.

Saponins: 2 ml of water was used to shake 0.5g of the sample extract and persistence of the foam for ten minutes proved the presence of saponins.

Tanins: 2 ml of the leaves extract was mixed with water and heated on a water bath. The mixture was filtered after 5mins and 2 drops of 15% ferric chloride was added. The presence of tannins was indicated by the formation of dark green solution.

Flavonoids: 5 ml of dilute ammonia was added to 1ml of the leaves extract followed by addition of 1ml of concentrated sulphuric acid. Formation of yellow colour shows the presence of flavonoids.

Phenols: 2g of the leaves extract were treated with 4 drops of ferric chloride solution. The presence of phenols was confirmed by formation of blue – black coloured solution.

2. 5. FTIR Analysis

The functional groups present in the *Viscum album* leaves extract were determined with Shimadzu FTIR- 8400S Fourier Transform Infrared Spectrophotometer at the National Research Institute for Chemical Technology (NARICT) Zaria, Nigeria.

2. 6. Quality control

Analytical grade reagents, chemicals and solvents used in the study were purchased from Finlab, Owerri, and were not further purified for the analysis. The glass wares and plastic containers were washed with detergent and rinsed with deionized water. They were further soaked overnight with 10% nitric acid and rinse again with deionized water.

3. RESULTS AND DISCUSSION

3. 1. Phytochemical analysis

Table 1 shows the qualitative phytochemical content of *Viscum album* leaves extract. The result in Table 1 indicates that alkaloids, flavonoids, phenol, saponins and tannins were present in both the petroleum ether and chloroform extracts of *Viscum album* at different intensities.

Alkaloids were very deeply present in both petroleum ether and chloroform extracts. Flavonoids were deeply present in both petroleum ether and chloroform extracts. Saponins and tanins were deeply present in petroleum ether extract but slightly present in the chloroform extract. The result indicates that phenols were slightly present in both petroleum ether and chloroform extracts. The presence of alkaloids and saponins has been reported in mistletoe leaves harvested from *Irvingia gabonensis, Theobroma cacao* and *cola nitida* respectively (Ogunmefun *et. al.*, 2013). Yusuf and coworkers reported the presence of phenol, alkaloid, saponins, flavonoids, terpenoid and phytate in *Viscum album* extracts from *Cola nitida*, which is comparable with the result of the present study[2]. Reported of high content of tanins in

mistletoe leaves harvested from *Theobroma cacao, Pentaclethra macrophylla* and *cola nitida* is documented [16]. The use of this plant for its medicinal properties could be due to the assumed role of these phytochemicals as they are believed to protect humans against some diseases [8].

Table 1. Results of qualitative phytochemical screening of of *Viscun album*

Parameter	Petroleum ether	Chloroform
Flavonoids	++	++
Alkaloids	+++	+++
Phenols	+	+
Saponins	++	+
Tannins	++	+

*'+' slightly present, '++' deeply present, '+++' very deeply present

3. 2. Elemental content analysis

Table 2 is the result of elemental content analysis of *Viscum album* harvested from *Cola nitida*. The result indicates that all the metals (Ca, Cu, Cr, Fe, Mg, Mn and Zn) analyzed were detected. Concentration of the metals are in the order Fe > Mn > Cu > Mg >Zn > Cr > Ca. Elevated concentration of Fe (28 mg/kg) was recorded in the study. The high concentration of Fe could be associated with its abundance in the soil where the mistletoe host is grown since plants have the capacity to accumulate and absorb metallic elements from the soil [26]. The occurrence of elevated concentration of Fe in plant materials and soil is well documented [27, 28]. High concentration of Fe has been reported in mistletoe host plants [18]. Fe is considered as essential mineral element as its presence is crucial to enable living things to metabolize. It is imperative to state that its overdose could be lethal and may result to death in infants. Its toxicity in the gastro-intestine sets in at concentration above 20 mg/kg. Concentration of Fe in human body above 40 mg/kg could be moderately toxic, while Fe absorption exceeding 60 mg/kg may be poisonous as it could lead to death [29]. Calcium concentration recorded in the study is 0.004 mg/kg. Low concentration of calcium was recorded in the study, though it is comparable with the observed concentration of mistletoe harvested from *Theobroma cacao, Pentaclethra macrophylla* and *cola nitida* [16]. It has been noted that the required daily intake for adult is about 800 mg/day [30]. Calcium is vital for proper teeth and bone development as well as in muscle metabolism [31]. Calcium and iron are important in energy metabolism central nervous system and formation of hemoglobin [32]. It has been reported that 1 mg/g Ca should be contained in calcium rich foods and 0.5 mg/g in foods with low calcium content [33]. Calcium also improves resistance of plant tissues and makes the stems to be well erected [34].

Table 2 indicates that magnesium (Mg) concentration recorded in the study is 0.30 mg/kg. Magnesium concentration recorded in this study is not elevated when compared with magnesium level reported in samples of mistletoe leaves obtained from oil bean, avocado pear

and kola nut [16]. Magnesium is one of the essential mineral elements considered important in the prevention of heart diseases. It is required in the body for proper functioning of the enzymes as well as strengthening of the bones in human body [35].

Table 2. Result of elemental content of *Viscum album* from *Pentaclethra macrophylla*

Parameters	Concentration (mg/kg)
Calcium (Ca)	0.004
Magnesium (Mg)	1.400
Zinc (Zn)	1.260
Manganese (Mn)	5.000
Iron (Fe)	27.10
Copper (Cu)	1.500
Chromium (Cr)	0.170

The result shown in Table 2 suggests that the concentration of Manganese (Mn) recorded in the study is 1.40 mg/kg. A related study on the heavy metal level of regularly used medicinal plants revealed higher concentration of manganese [19]. Manganese is a vital mineral element which is less toxic, but could lead to neurological problem when the level is above 5 mg/m^3 [19]. Zinc concentration recorded in the study is 1.26 mg/kg. The concentration of zinc obtained in the present study is low compared to values reported in leaves of *B. monnieri* and *H. indicus* [19]. Elevated concentration (29.30 mg/kg and 4.20 mg/kg) of Zn has been reported in *Ficus capensis* leaves and bark respectively [13]. However, Zn concentration recorded in this study is higher than the value reported for *Viscum album* leaves harvested from *Pentaclethra macrophylla* [34], but lower than the recommended 60 mg/kg for medicinal plants [36]. Zinc is a crucial trace mineral element which is considered important in many plant proteins, but could be toxic when the concentration exceeds certain threshold (Kulhari *et al*., 2013; Ibe *et al*., 2019). Zn is known for its antioxidant activities and protection against quick muscle and skin aging in humans. Zn is vital in most biological and metabolic activities of living things and its toxicity occurs only when recommended dosage is exceeded [37, 38]. Chromium (Cr) concentration recorded this study is 0.170 mg/kg. The observed concentration of Cr in the present study is below the permissible Cr limit (2.0 mg/kg) for herbal materials (WHO, 2007). Cr concentrations at 5–30 mg/kg could adversely affect the growth and yield of plants. Long term exposure to Cr can lead to kidney and liver damage as well as circulatory and nervous tissue problem [39]. Copper (Cu) concentration of 1.57 mg/kg was recorded in the study. Copper is a trace mineral element that exists in plants naturally and takes part in some definitive intrinsic mechanisms. It is also considered important in vital biological process regulation. Also copper is believed to be involved in the formation of red blood cells, vascular and skeletal integrity and the central nervous system [34]

3. 3. FTIR analysis

The FTIR results are presented in Table 3, Figures 2 and 3. The results indicate seven and eight characteristic functional groups and peaks respectively for chloroform and petroleum ether extracts as shown in Table 3. In the case of petroleum ether extract (Figure 2), peaks were recorded at 3389.2 cm^{-1}, 2922.4 cm^{-1}, 2855.1 cm^{-1}, 2202.9 cm^{-1}, 2154.4 cm^{-1}, 1733.2 cm^{-1}, 1610.2 cm^{-1}, 1461.1 $cm^{-1}$and 1043.7. The peaks at 3389.2 cm^{-1}, 2922.4 cm^{-1} and 2855.1 cm^{-1} are peculiar to OH group and CH_2 stretching vibration. The peaks at 2202.9 cm^{-1}, 2154.4 cm^{-1} and 1733.2 cm^{-1} represents C=C stretching and C=O stretching, while 1610.2 cm^{-1}, 1461.1 cm^{-1} and 1043 represents the C≡C group, C=C aromatic group and C=O group for ketones. The chloroform extract (Figure 3) showed peaks at 3406.8 cm^{-1}, 2922.2 cm^{-1}, 2855.1 cm^{-1}, 1613.9 cm^{-1}, 1733.2 cm^{-1}, 1461.1 cm^{-1} and 723.1 cm^{-1}. The peaks are characteristic of OH stretching vibration in alcohol (3406.8 cm^{-1}), C-H stretching vibration in alkane (2922.2 cm^{-1}), C=O stretching vibration in carbonyl compounds (1733.2 cm^{-1}), C=C in aromatics (1461.1 cm^{-1}) and the peak at 723.1 cm^{-1} depicts substituted aromatic compound.

Table 3. The functional groups in the *Viscum album* leaf extracts *from Cola nitida*

	Petroleum ether extract		Chloroform extract	
S/N	Wave number cm^{-1}	Functional group	Wave number cm^{-1}	Functional group
1	3389.2	Alcohol (O-H, broad)	3406.8	Alcohol (O-H, broad)
2	2922.4	Alkane (C-H Stretch)	2922.2	Alkane (C-H) Stretch
3	2855.1	Alkyl (C-H Stretch)	2855.1	Alkyl (C-H Stretch)
4	2202.9	Alkene (C=C)	1733.2	Aldehyde (C=O)
5	2154.4	Alkene (C=C)	1613.9	α,β-unsaturated ketone
6	1733.2	Carbonyl (C=O, stretch)		
7	1610.2	Alkyne (C≡C, stretch)		
8	1461.1	Aromatic (C=C)	1461.1	Aromatic (C=C)
9	1043.7	Carbonyl (C=O, stretch	723.1	Sub. aromatic comp.

The FT-IR results proved the presence of aromatic compounds, alcohols, phenols alkyl groups, alkanes, aromatics, carboxylic acids, amides, amine, carbonyl compounds and alkynes and ethers in the leaf extracts. Natural products are known to contain organic compounds such as alkanes, alkyl groups, aromatics and hydroxyl groups which exist in products of natural origin [1, 34]. The presence of some of these functional groups possibly could be responsible for the medicinal properties of *Viscum album*.

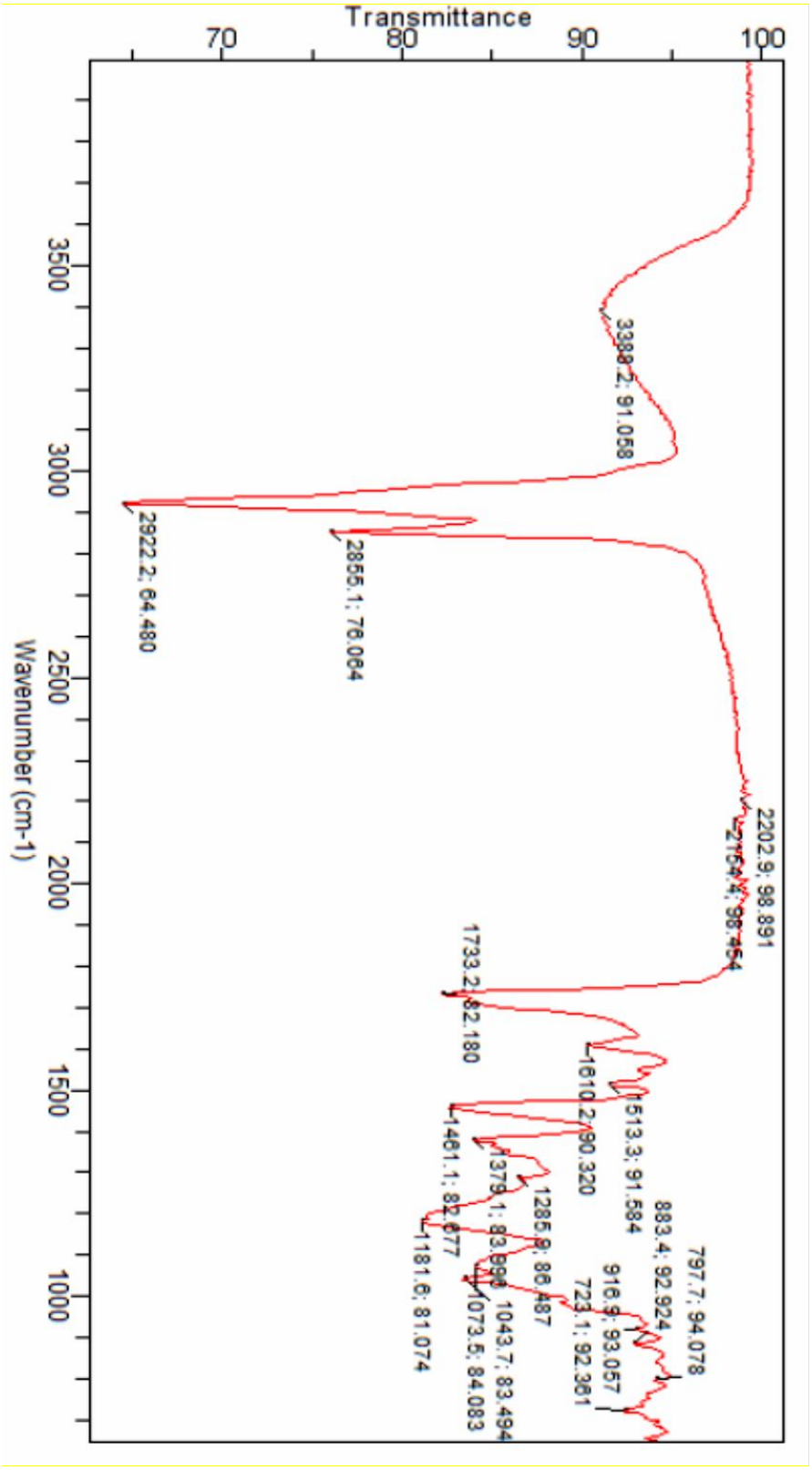

Figure 2. FTIR spectrum of Petroleum ether extracts of *Viscum album*

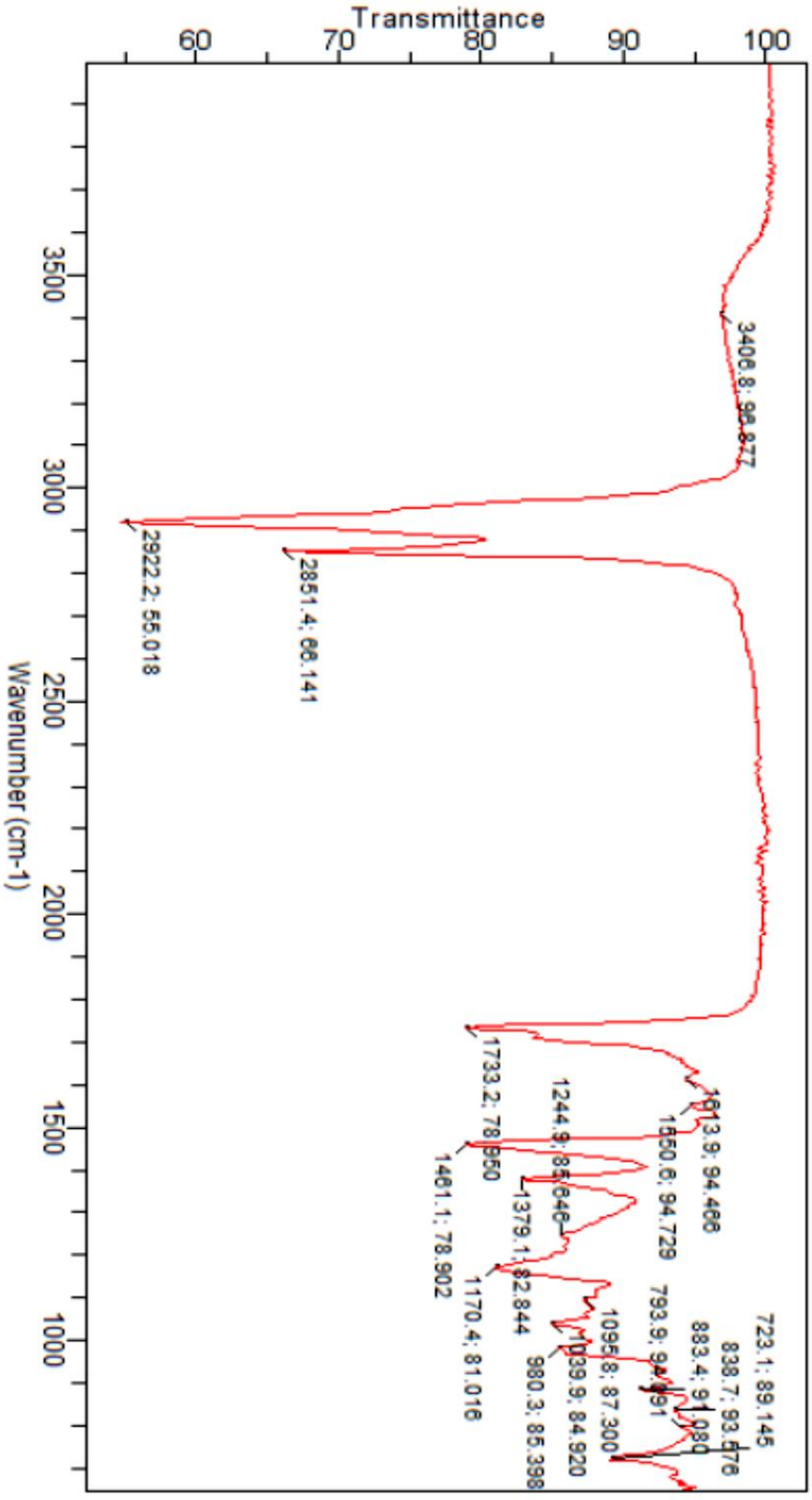

Figure 3. FTIR spectrum of chloroform extracts of *Viscum album*

4. CONCLUSIONS

Materials of plant origin such as *Viscum album* have proved to be major source of active components in most orthodox and traditional medicine. The study established the presence of vital mineral elements (Ca, Cr, Cu, Fe, Mg, Mn and Zn) at varying concentrations in *Viscum album* collected from *Cola nitida,* which are within the permissible limit for herbal materials, though limits are yet to be established for most metallic elements in herbal materials. Some of these minerals like calcium and magnesium are major constituent of cell membranes, adenosine triphosphate and deoxyribonucleic acid, and important in bone formation. Mineral elements such as copper iron, manganese and zinc play specific role in human body such as hemoglobin formation, sexual development, and as enzyme cofactor. The petroleum ether and chloroform extracts of *Viscum album* harvested from *Cola nitida* indicates the occurrence of alkaloids, flavonoids, phenol, saponins and tannins in the extracts. FTIR spectrum revealed several characteristic peaks which are specific for alkane, alkyne, alkene, alcohols aromatic compounds and carbonyl compounds. The presence of these phytochemicals, essential mineral elements and some important functional groups must have necessitated the used of this plant for medicinal purposes.

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