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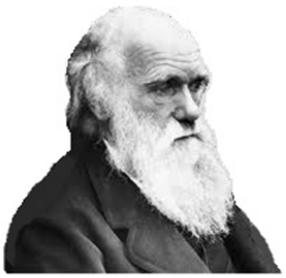

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Trace metal, FTIR and phytochemical analysis of *Viscum album* leaves harvested from *Pentaclethra macrophylla*

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ABSTRACT

The leaves of mistletoe (*Viscum album*) harvested from oil bean tree (*Pentaclethra macrophylla*) in Imo State South Eastern Nigeria was studied. The study was aimed at carrying out trace metal, Fourier Transform Infra-red (FTIR) and phytochemical analysis of ethanol leave extracts of *Viscum album* harvested from *Pentaclethra macrophylla*. The sample for trace metal analysis was digested with a mixture of concentrated HNO_3 and $HClO_4$ at a ratio of 1:1 for 24 hours. Ethanol was then used to extract the sample meant for FTIR and phytochemical analysis. Herein, trace metal determination indicates the presence of Mg, Zn, Fe, Cu and Cr, and the concentration of Ca and Mn is at < 0.00 mg/kg. The result of the phytochemical screening demonstrates the presence of flavonoids, alkaloids, phenols, saponins and tannins. FTIR analysis confirmed the presence of alcohols, amides, aromatics and carbonyl compounds in ethanol extracts of *Viscum album* harvested from *Pentaclethra macrophylla.* The presence of some trace metals and phytochemicals, as well as some important functional groups in the plant sample, as indicated by the result of the study, authenticates its use in traditional medicine.

***Keywords*:** *Viscum album*, *Pentaclethra macrophylla*, trace metal, FTIR, phytochemicals

1. INTRODUCTION

African mistletoes (*Viscum album*) are considered as parasitic flowering plants that attaches to the stem of another plant (host). The plant gets its water, minerals, nitrogen, carbon

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and other dissolved compounds from the host. They may adversely affect the growth and fruiting of their hosts (Solar *et al*, 1998). The areas close to point of attachment to the host plant could be killed especially when there is heavy infestation on the host plant. *Viscum album* growth rate is slow, their first leaves are produced only after two years and flowering from 5 to 7 years of maturity (Calder, 1983). There is variation in the length of the shoots from some inches to several feet

In the South-Eastern part of Nigeria mistletoes is called 'apari' (Tizhe *et al*, 2016). It is mainly found growing on tree crops like cocoa (*Theobroma cacao* L), kola (*Cola nitida* or *Cola acuminata*), coffee (*Coffea arabica* L.), oil bean tree (*Pentaclethra macrophylla)* and bush mango (*Irvingia gabonensis*). The plant can also be found growing on citrus plants like orange (*Citrus* sp.) and guava (*Psidium guajava* L.) (Ogunmefun *et. al*., 2013; Ishiwu *et al*., 2013).

The plant is used for different purposes by different cultures; the Greek and Romans use it as medicinal plant, while North Americans and Germans use it ceremonially (Bowman 1992). It is seen as medicinal plant in Nigeria, hence its use as herbal remedy for curing ailments such as diabetes, dysentery, wounds, diarrhoea, cancer, inflammations, hypertension in human and animal (Akinmoladum *et al*., 2007).

The chemical composition of mistletoe varies and depends on the host plant (Luczkiewics *et al*, 2001). Studies of mistletoe from different host plants and region in Nigeria is well documented (Abubakar *et al*., 2007; Ogunmefun *et al*., 2013; Ishiwu *et al*., 2013). Ogunmefun et al., (2013), extracted the milled mistletoe leaves with methanol using cold extraction method and found out that it contain major cations like potassium, magnesium, calcium and sodium in varying quantities, which were relative to the individual host plants. Ishiwu *et al*. (2013) showed that there was significant differences in the mineral content of the samples ($P < 0.05$) except in sodium content which was very low in all the samples. Taiga (2013) reported the presence of flavonoids, tannins, phenolic compounds, alkaloids and saponins in *Viscum album* collected from guava (*Psidium guajava*), kolanut (*Cola nitida*), orange (*Citrus*), pear (*Persea americana*) and cocoa (*Theobroma cacao*)

However, information regarding the functional groups presence in different extracts of mistletoe leaves is lacking as far as it could be established. These functional groups could be responsible for the many usefulness of this plant. This study therefore contributes to information regarding to mistletoe leaves. This achieved by performing trace metals, Fourier Transform Infra-Red analysis as well as phytochemical analysis of mistletoes leaves harvested from oil bean tree in Imo state, South Eastern Nigeria. The need for this study owns to the observed difference in the chemical content of *Viscum album* due to biochemical variations within species, geographical locations, methods of extraction and solvent used.

2. MATERIALS AND METHOD

2. 1. Sample collection preparation

Fresh mistletoe (*Viscum album*) leaves were harvested from oil bean tree (*Pentaclethra macrophylla)* in a forest located at Ngali Logara in Ngor Okpala Local Government Area of Imo State, South-Eastern part of Nigeria. The oil bean tree (Figure 1) is very popular in Nigeria, known locally as Ugba in the south eastern part of Nigeria. Oil bean is a tropical tree which belongs to the Leguminosae family (Mimosoideae). The plant is native to tropical Africa, though specie of the plant exists in tropical South and Central America. The oil bean tree is a

wild plant that grows naturally in the southern rain forest zone of West Africa, up to a height of 21 m.

Viscum album leaves were harvested from *Pentaclethra macrophylla* within the month of October, 2016. The harvested *Viscum album* leaves were identified at the Department of Plant Science and Biotechnology, Imo State University, Owerri. The dust particles on the samples were cleaned by washing it with deionized water. Samples were air dried for two weeks, pulverized into fine powder using pestle and mortar and stored in a previously cleaned plastic bottle in a refrigerator.

Figure 1. Mistletoe on oil bean tree (*Pentaclethra macrophylla)*

2. 2. Sample extraction

50 g of the fine powder was accurately weighed using an electronic weighing balance (Nanbei NBT-A200) into a properly washed 250 ml flask. 100 mL of ethanol was introduced into the flask containing the fine powdered sample. The flask was covered and allowed to stand for 24 hours under ambient temperature (Ikpa *et al*., 2014). This followed filtration and the filtrate was used for phytochemical and FTIR analysis.

2. 3. Trace metal determination

2g of the powdered sample was weighed and transferred into a 100 ml standard flask and 20 ml of concentrated nitric acid and perchloric acid were added at 1:1 ratio. The mixture was allowed to stand for 24 hours before filtration. The flask was washed with 15 ml deionized

water and made up to the mark with deionized. This was used for the trace metal determination using Hanna multi-parameter bench photometer HI 83200 (Duru *et al*., 2017).

2. 4. Phytochemical analysis

Phytochemical screening of leave extracts of *Viscum album* from *Pentaclethra macrophylla* was carried out according to standard methods as described by Harborne (1973), Nwankwo and Egbuonu (2011), Tiwari et al. (2011), Ikpa *et al*.(2014). The ethanol extracts of the *Viscum album* leaves were screened for saponins, alkaloids, flavonoids, tannins and phenols.

2. 5. Functional group determination

The FTIR analysis was done using Shimadzu FTIR- 8400S Fourier Transform Infrared Spectrophotometer at the National Research Institute for Chemical Technology (NARICT) Zaria Nigeria.

2. 6. Quality control

All the chemicals, reagents and solvents used in the determinations were of analytical grade. They were purchased from Finlab, Owerri, hence no further purification was required in the course of the analysis. The laboratory glass wares as well as the plastic wares used in the study were properly washed with soap. Soaked with 10% nitric acid overnight before rinsing again with deionized water

3. RESULTS AND DISCUSSION

3. 1. Phytochemical analysis

Table 1. Results of qualitative phytochemical screening of ethanol extract of *Viscum album*

Parameter	*Viscum album*
Flavonoids	++
Alkaloids	+++
Phenols	+
Saponins	++
Tannins	++

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

The qualitative phytochemical content of the leaves of *Viscum album* are shown in Table 1. Table 1 indicates that flavonoids, alkaloids, phenol, saponins and tannins were present in the ethanol extract of *Viscum album* at varying intensities. Alkaloids were very deeply present;

phenols were slightly present while flavonoids, saponins and tannins were also deeply present in the ethanol extract of *Viscum album.* Ogunmefun *et al*. (2013) reported the presence of alkaloids and saponins in mistletoe leaves harvested from *Irvingia gabonensis*, *Theobroma cacao* and *Kola nitida* respectively using the Dragendoff's and ferric chloride methods. They also reported the absence of alkaloids using Meyer's method. The present result is comparable to Yusuf *et al*. (2013) who reported that *Viscum album* extracts from cola nut tree contain phenols, alkaloids, saponins, flavonoids, terpenoid and phytate. These phytochemicals have been found to confer antimicrobial properties on the plant, help defend against environmental challenges and also provide humans with protection against various diseases as well (Yusuf et al., 2013; Ogunmefun *et al*., 2013).

3. 2. Trace metal analysis

The result of trace metal analysis of ethanol extract of *Viscum album* from oil bean is presented in Table 2. The concentrations of Ca and Mn observed in the sample were very low (< 0.001 mg/kg). The current results of Ca and Mn are comparable to reported concentrations; 0.00214 ±0.004 mg/kg, 0.00226 ±0.001 mg/kg and 0.00214 ±0.003 mg/kg for samples of mistletoe leaves growing on oil bean, avocado pear and kola nut tree respectively (Ishiwu et. al., 2013). Calcium enhances tissues resistance which makes it possible for stem of plants to stand upright. Manganese plays the role of a cofactor for enzymes. Though, high concentration of Mn above certain thresholds could result to neurological disorders.

Table 2. Trace metal analysis of *Viscum album* from *Pentaclethra macrophylla*

Parameter	Concentration (mg/kg)
Calcium (Ca)	<0.001
Magnesium (Mg)	2.40
Zinc (Zn)	0.30
Manganese (Mn)	<0.001
Iron (Fe)	36.00
Copper (Cu)	5.10
Chromium (Cr)	0.35

The level of zinc in the mistletoe leaf from oil bean (0.30 mg/kg) is below the WHO standard for medicinal leaf (60 mg/kg). Zinc is an essential component of many proteins in plants, even though it could be toxic when the concentration is in excess. The current study recorded low Zn concentration when compared to report of leaves from *B. monnieri* (4.80 ±0.0907 mg/kg) and *H. indicus* (4.18 ± 0.0212 mg/kg) (Kulhari *et al*., 2013).

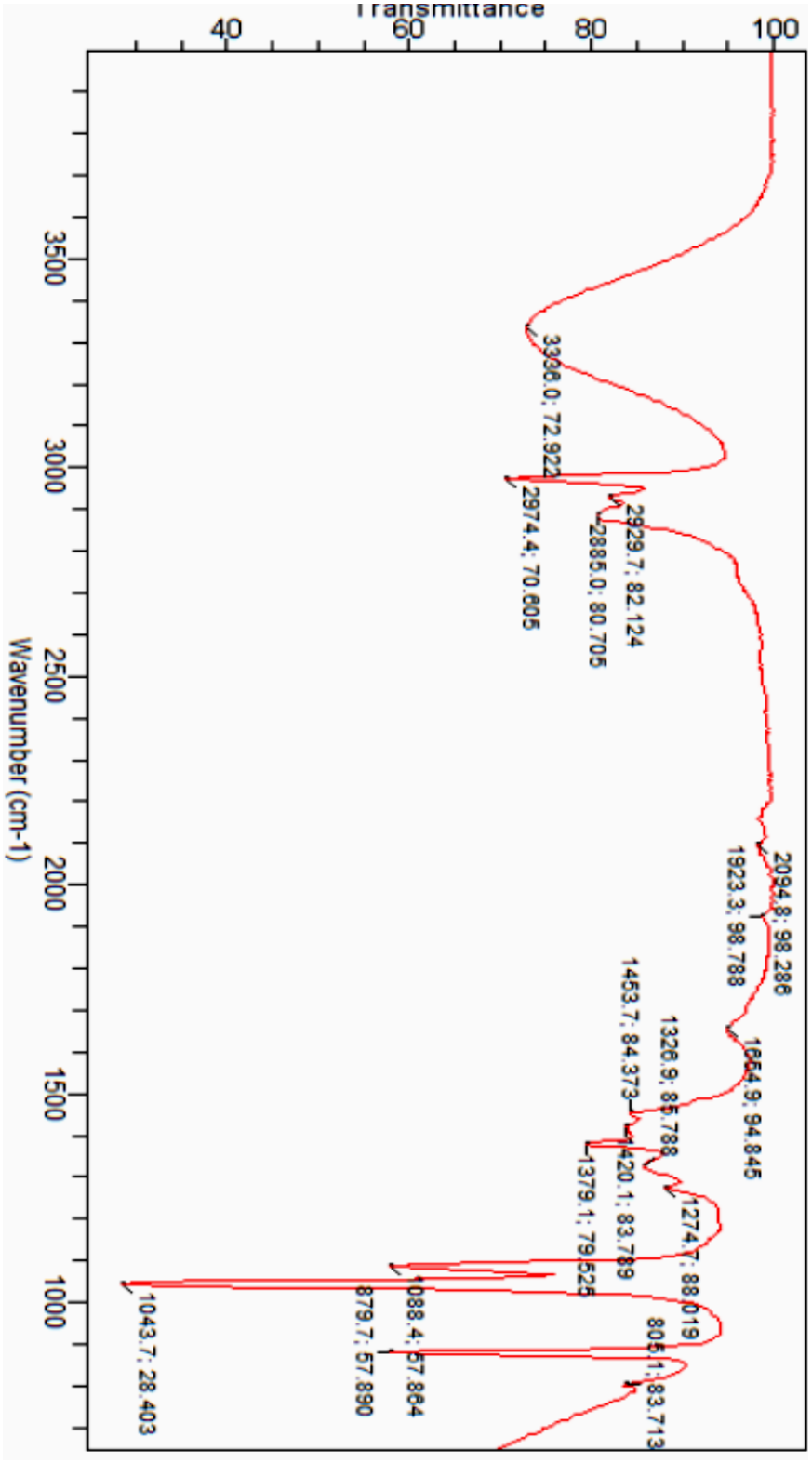

Figure 2. FTIR peaks of ethanol extracts of *Viscum album*

The present study recorded magnesium concentration of 2.40 mg/kg. The concentration is high when compared to magnesium concentrations of 0.00081±0.003 mg/kg, 0.00092 ±0.003 mg/kg and 0.00021 ±0.002 mg/kg respectively for samples of mistletoe growing on oil bean, avocado pear and kola nut tree respectively as earlier reported (Ishiwu *et. al*., 2013). Mg is important in the heart as it helps to fight heart diseases. It is vital for strengthening of bones and needed to promote enzymatic activities in human bodies (McLean, 1994).

Fe concentration observed in the present study is 36.00 mg/kg. Elevated concentration of Fe was recorded in the present study when compared to concentration found in mistletoe leaves from oil bean tree (0.00127 ± 0.003 mg/kg), avocado pear tree (0.00124 ± 0.005) and kola tree (0.00142 ± 0.006 mg/kg) grown in Enugu, Nigeria (Ishiwu et al., 2013). High concentration of Fe has been reported in *A. nilotica* stem samples (25.30 ± 0.1464 ppm) and *W. somnifera* leaf samples (17.44 ± 0.0202 ppm) collected respectively from Jhunjhunu and Bahadurgarh, India (Kulhari et al., 2013).

The observed elevated concentration of Fe recorded in this study could be attributed to the fact that Fe is one the most abundant element in the earth crust (Ibe et al., 2016). Fe is one of the important trace metals. Mixture of iron and protein plays essential role in the metabolism of living things. Iron is also important in many enzymatic systems like chlorophyll synthesis in plant. However, in human overdose of more than 40 mg/kg can cause moderate gastro-intestinal intoxication and may also be lethal (Spanierman, 2011). However, the observed concentration in this study is within the WHO permissible limits (48 mg/kg) for herbal materials (WHO, 2007).

Table 3. The functional groups in ethanol extract of *Viscum album* leaves from *Pentaclethra macrophylla*

S/N	Wave number cm^{-1}	Functional group
1	3324.8	Alcohol (O-H, broad)
2	2974.4	Alcohol (O-H, stretch)
3	2929.7-2885.0	Alkyl (CH_3-)
4	2154.4	Alkynyl (C=C)
5	1654.9	Amide (C=O)
6	1453.7-1420.1	Aromatics (C=C)
8	1274.7	Carbonyl group (C-O)
9	1088.4	Amine (C-N)
10	1043.7	Ether (C-O)
11	879.7-805.1	Aromatic (C-H)

Chromium (Cr) becomes critical for plant at concentrations between 5-30 mg/kg which adversely affect plant growth and yield. The permissible limit for Cr in raw herbal materials is 2.0 mg/kg (WHO, 2007). However, the Cr concentrations obtained in this study (0.35 mg/kg) is below the permissible limits. Variable concentration of Cr has been reported in medicinal plants harvested from different areas in Ogbomoso, South-western Nigeria (Ajasa *et al*., 2004). Princewill-Ogbonna and Ogbonna (2011) noted that fast urbanization and industrialization as well as vehicular emissions are factors that contribute to the high level of metallic elements in the soil and plants grown on it.

Copper content found in the mistletoe leaf was 5.10 mg/kg which is below the limit (36 mg/kg), the WHO limit for herbal material (WHO, 2007). Copper occur naturally in most vegetables and plays a definitive role in the intrinsic mechanisms, regulating vital biological processes. Copper is involved in hemopoiesis and in maintenance of vascular and skeletal integrity in addition to the structure and function of central nervous system. The presence of these metals in the leave extract could be from the soil on which the oil bean is grown. Since plants have the tendency to absorb and accumulate metals from the soil (Ibe *et. al*., 2017).

3. 3. FTIR analysis

The FT-IR spectrum was used to identify the functional groups of the active components present in the extract based on the peaks values in the region of IR radiation. When the extract was passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio as presented in Figure 2 and interpreted in Table 3. Eleven major characteristics band and their functional groups were detected including 3224.8 cm^{-1} (-OH broad), 2974.4 cm^{-1} (-OH stretching vibrations), 2929.7-2885.0 cm^{-1} (-CH_3 stretching vibrations). Ring and side group vibrations of C=C at characteristics band of 2154.4 and range of 1453.7-1420.1 cm^{-1}, stretching vibrations of C-O at characteristics band of 1274.7 and 1043.7, at band 1088.4 (-CN stretching vibration), 1654.9 cm^{-1} (-C=O broad vibration) and 879.7-805.1 cm^{-1} (-CH ring and side group vibrations). The results of FT-IR analysis confirmed the presence of alcohol, aromatics, alkynyl, aromatic carboxylic acid, carbonyl group, alkyl halide, amide, amine, and ether. Natural products are known to contain compounds possessing alkyl, aromatic and hydroxyl groups (Duru et al, 2015). The alcohol present could be benzyl alcohol which occur naturally in plant and used as a general solvent for inks, waxes, shellacs, paints, lacquers, and epoxy resin coatings. There are limited studies regarding FTIR analysis of mistletoe plant. However, similar result was reported in a study on Mulberry mistletoe leaf by Thoa and Cuong (2017), who confirmed that the leaf contains phenol, alcohol, alkane, alkyne, aromatics, hydrocarbons and amines functional groups. The groups suggest the mistletoe plant possess antioxidant (Sangeetha *et al*., 2014; Subashini *et al.* 2015) and also antimicrobial properties (Jabamalairaj et al. 2015), which is common with green plants.

4. CONCLUSIONS

Medicinal plants are sources of a large number of active components of herbal and modern medicine. The present investigation clearly demonstrated the variation in trace metal concentration in ethanol extract of *Viscum album* from *Pentaclethra macrophylla*. However, the permissible limits have not been prescribed for many metals which are considered micronutrients in herbal materials. Furthermore, the FTIR analysis conducted showed that the

mistletoe extract from the oil bean tree could be utilized in medicinal and industrial uses. FTIR analysis confirmed the presence of alcohols, amides, aromatics and carbonyl compounds in ethanol extract of *Viscum album* harvested from *Pentaclethra macrophylla.* The presence of these trace metals and phytochemicals as well as the functional groups may have been responsible for the medicinal uses of *Viscum album*.

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