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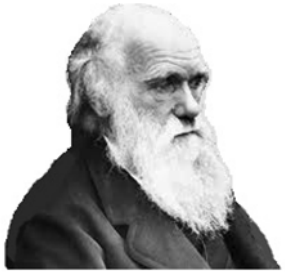

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Infrared Studies and Mineral Element Analysis of the leaf of *Vernonia amygdalina*

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ABSTRACT

Used in traditional medicine, infrared characterization of column eluates and elemental analysis of *V. amygdalina* was carried out to establish preliminary views of the chemical and mineral element composition of the leaf of this plant. In the five pure eluates obtained, we found that they contained saturated aliphatic esters, nitro-groups conjugated with benzene ring, secondary amines/nitrogen heterocycles, 1,3-disubstituted benzene and oxazine/oxazoline fragments, respectively. Moreover, we established that potassium, magnesium and sodium were the major minerals in the leaf, while copper, zinc, iron, nickel, manganese and chromium occurred in trace amounts. Research attention should therefore be focused on this plant for the full exploration and exploitation of its obvious antibiotic and mineral values.

Keywords**:** *V. amygdalina*, Infrared, Elemental analysis, Aliphatic esters, Secondary amines, Oxazine

1. INTRODUCTION

Vernonia amygdalina belongs to the Kingdom Plantae. It is an angiosperm, of the order *Asterales*, of the family *Asteraceae*, genus *Vernonia*, and *s*pecies *V. amygdalina.* Its binomial name is *Vernonia amygdalina* (Akah and Ekekwe 1995). It is called bitter leaf in English, "oriwo" in Edo, "ewuro" in Yoruba, "shikawa" in Hausa and "olugbu" in Igbo (Oboh and Masodje 2009). It occurs as a small shrub with height ranging from 2-5 m. It has a rough stem with young branches and petiolate green leaves of about 6 mm diameter. The leaves are

elliptic in shape, short, acuminate at the apex and slightly chordate at the base. It has a characteristic odor and bitter taste (Ijeh and Ejike, 2011; Nwobegu and Egbuna 2002).

Pharmacological studies have shown that the leaf extract has both hypoglycemic and hypolipidaemic properties in experimental animals and so could be potentially useful in the management of diabetes mellitus (Akah and Okafor 1992; Ebong et al., 2008). Aqueous extracts from the leaf is used as tonics for the treatment of various illnesses (Igile, et al., 1995). It is effective against amoebic dysentery (Moundipa, 2000) and has antimicrobial activities (Akinpelu, 1999).

The wide medicinal applications of *V. amygdalina* threaten the availability of this plant in the near future. The synthetic production of related medicinal chemical compounds from *V. amygdalina* would serve to reduce the pressure on this all important plant. Reports in literature have focused on the phytochemical properties (Oshim et al., 2016; Offor, 2014; Eyong et al., 2011) and medicinal applications (Eyong et al., 2011; Oguwike et al., 2013) of the parts of *V. amygdalina*. Information on the specific chemical compounds or molecular fragments in the leaves and other parts of this plant is scarce.

This study would therefore isolate and characterize some molecular fragments in the crude ethanolic extract of *V. amygdalina* leaf. Various Mineral elements in the leaves of the plant would also be identified and quantified.

2. MATERIALS AND METHODS

2. 1. Reagents and Preparation

Absolute ethanol, chloroform, Petroleum ether, HNO_3 and $HClO_4$ were of analytical grades and purchased from Finlab Owerri, Nigeria. Reagents used in the mineral element analysis were prepared with deionized water.

2. 2. Preparation of Plant Material

Fresh leaves of *V. amygdalina* were collected from a garden located at Umunweke Amaulu Ihitte Mbieri Mbaitoli Local Government Area of Imo State Nigeria. They were identified at the Department of Plant Science and Biotechnology, Imo State University, Owerri. The fresh leaf samples were placed on newsprint paper and air-dried at room temperature. Thereafter, the dried leaves were blended using a manual blender and the powdered sample was stored in a polythene bag prior to use.

2. 3. Extraction of Crude Phytochemicals from Leaves

Hundred grams (100 g) of powdered leaf sample were weighed and transferred into a 500 mL beaker. 300 mL of absolute ethanol (98 %) was added to the beaker containing the powdered sample and left to stand for 72 h at room temperature. Thereafter, the mixture was filtered with a white cotton cloth, and then refiltered using a Whatman filter paper (No. 1). The filtrate was concentrated to a gel form at 35 °C on a water bath. The concentrated extract now dark green in colour, was partitioned in 1:1 chloroform-water mixture in a separatory funnel. The lower chloroform layer was collected and allowed to dry at room temperature. It was stored in a refrigerator until required.

2. 4. Column Chromatographic Separation of Crude Extract Components

The column was packed with silica gel 60-200 mesh using the slurry method. 1 g of the chloroform extract was redissolved in 20 mL of chloroform and 10 g of silica gel was added and mixed thoroughly with the extract. It was allowed to dry to a free flowing powder. It was then introduced into the loaded column and eluted with 100 mL each of the following solvent mixtures: Petroleum ether-Chloroform (1:0, 9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, 1:4, 1:9 0:1 v/v). The eluting solvent was collected at 100 mL volumes and each allowed to evaporated to about 5 mL volume at room temperature to concentrate any eluted compound. Each concentrate was then spotted on TLC plates. After developing the plates, eluates that gave a single spot were characterized.

2. 5. Analysis of Mineral Elements in the Leaves

Dried leaves were prepared for elemental analysis by following the procedure according to Karla (1998). Powdered leaf sample of weight 0.5 g was transferred into a 50 mL digestion tube. 6.0 mL of HNO_3 was pipetted into the tube and the mixture was allowed to predigest at room temperature overnight. It was then placed in a digestion block port for 60 min at 150 °C. After cooling the tube to room temperature, 2.0 mL of $HC1O_4$ was added and the tube was returned into the digestion block port at a block temperature of 215 °C for 2 h. It was then removed and allowed to cool in a fume hood. The solution was mixed with 10 mL of deionized water using a vortex stirrer, and diluted to 100 mL in a volumetric flask. The final solution was filtered to remove all particulate matter in the digest prior to analysis. Elemental analysis of plant digest was done using Atomic Adsorption Spectrophotometer (AAS).

2. 6. Instrumentation

Eluates were characterized using FTIR- 8400S Fourier Transform Infrared Spectrophotometer by SHIMADZU, JAPAN. Mineral elements in the leaves were determined using GBC Scientific SensAA Dual Atomic Absorption Spectrophotometer.

3. RESULTS AND DISCUSSION

The order of elution of phytocompounds that gave single spots on TLC plates is shown in Figure 1.

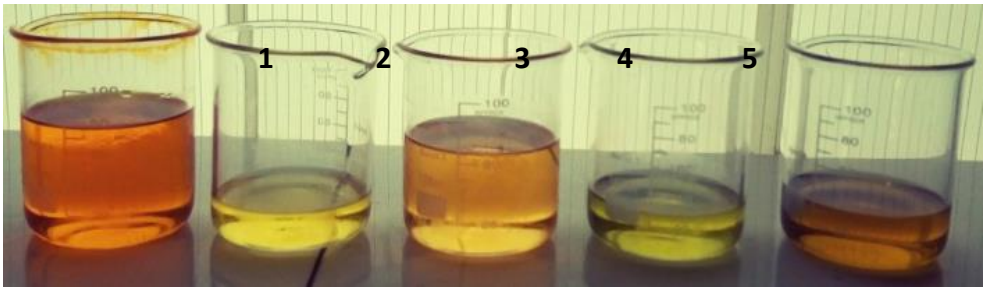
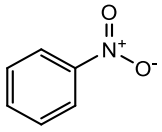
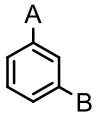


Figure 1. Pure eluates from the leaves of *V. amygdalina*

The solubilities of the five pure eluates were between hexane and hexane-chloroform mixtures in the ratio 4:1, 3:2, 2:3 and 1:9 respectively. Under white light, eluates showed distinct colours which appeared as amber, pale yellow, orange, lemon green and deep yellow respectively. These colours were stable for more than four weeks under room conditions. Infrared absorption bands of the eluates were determined and interpreted following Mistry (2009). Molecular fragments observed in FTIR scans of the eluates are shown in Table 1.

Table 1. FTIR characterization of eluates from the leaves of *V. amygdalina*

Elution Order	Elution Mixture	Colour	Absorption Band	Proposed Molecular Fragments
1	H_{100}	Amber	1750-1735 s.	Saturated aliphatic esters RCOOR
2	H_{80} C_{20} (4:1)	Pale yellow	Multiple bands at 1500 and 1377 s.	Nitro-group conjugated with benzene ring 
3	H_{60} C_{40} (3:2)	Orange	3300-3500 w. 1580-1550 w.	Secondary amine stretch Nitrogen heterocyles, C=C + C=N Str.
4	H_{40} C_{60} (2:3)	Lemon green	680-725 m.	1,3-disubstituted benzene 
5	H_{10} C_{90} (1:9)	Deep yellow	1690-1670 v. 1275-1200 v.	Oxazine  Oxazoline  Conjugated ether ROR Str.

H = Hexane, C = Chloroform, s = strong, w = weak, m = medium, v = variable

The oily and volatile amber coloured pure hexane eluate gave a strong absorption band at 1750-1735 cm^{-1}, indicating the presence of saturated aliphatic esters. The oily nature of this product indicates that they are likely to be fatty acid methyl esters (FAME). FAME has been extracted from a variety of plants and has shown antimicrobial activities (Chandrasekaran et al., 2008). Fatty acid methyl esters from ten species of marine macroalgae belonging to the classes Chlorophyceae, Phaeophyceae and Rhodophyceae have been reported to possess antibacterial activity (Anantharaj et al., 2004). Kabara (1978) reported that fatty acids such as oleic, palmitic, stearic, myristic, linoleic and linolenic acids show activity against *Clostridium perfringens* and *Staphylococcus pyogens*.

Hexane-chloroform 4:1 mixture gave a pale yellow eluate with a strong multiple absorption bands at 1500 and 1377 cm^{-1} indicating the presence of a nitro group. Since these two peaks are at lower wavenumbers than usual, the nitro group is possibly conjugated with a benzene ring. Few aromatic nitro-compounds occur in plants. A typical example is chloramphenicol, an antibiotics used in the treatment of a number of bacterial infections.

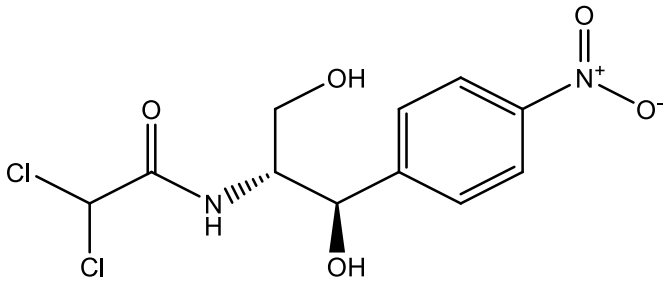


Figure 2. Structure of chloramphenicol

An orange coloured eluate was obtained in hexane-chloroform 3:2 mixture and gave weak adsorption peak at 3300-3500 cm^{-1} and 1580-1550 cm^{-1}. These peaks were assigned to secondary amines and nitrogen heterocycles respectively. Many nitrogen heterocyles are secondary amines. These compounds in plants are mostly alkaloids. True alkaloids contain nitrogen in the heterocycle and originate from amino acids (Plemenkov, 2001). Alkaloids play an important role in plant defense systems against pathogens and animals. Medicinally active compounds in plants used in traditional medicine are mostly alkaloids and scientific studies have verified that many of them have anti-inflammatory, anti-microbial, anti-parasitic and anti-cancer effects (Reza and Abbas, 2007; Patel et al., 2012).

A lemon-green eluate was obtained in hexane-chloroform 2:3 mixture. This eluate gave a weak absorption band at 680-725 cm^{-1} which was assigned to 1,3-disubstituted benzene. Benzene ring is a basic skeletal component in natural products and its disubstituted forms are ubiquitous in phytochemicals. The good antimicrobial and antiviral activities of compounds carrying the 1,3-disubstituted benzene moiety have been reported (Choudhury et al., 2009; Maruyama et al., 2003).

The deep yellow coloured eluate from a 1:9 hexane-chloroform mixture gave a variable absorption band at 1275-1200 cm^{-1} and was assigned to a conjugated ether (ROR stretch). Another variable absorption band at 1690-1670 cm^{-1} was assigned to oxazine and oxazoline

based derivatives. The oxazoline and oxazine rings are important constituents of numerous bioactive natural products and pharmaceuticals. Oxazine containing compounds have been shown to have anticoagulant properties (Kumar et al., 1985). Oxazoline derivatives have shown antimicrobial activity (Waschinski and Tiller, 2005), acaricidal/insecticidal activity (Yu et al., 2015), anti-tuberculosis activity (Moraski et al., 2010) and anti-malaria activity (Gordey et al., 2011).

Mineral elements and their concentrations in the leaf of *V. amygdalina* are shown in Table 2.

Table 2. Mineral Elemental Analysis of the Leaf of *V. amygdalina*

Element	Concentration (g/kg)
Sodium (Na)	0.11
Magnesium (Mg)	0.66
Potassium (K)	1.84
Calcium (Ca)	ND
Copper (Cu)	0.03
Zinc (Zn)	0.01
Iron (Fe)	0.02
Nickel (Ni)	0.01
Manganese (Mn)	0.02
Cobalt (Co)	ND
Chromium (Cr)	0.01

ND = Not Detected

The major minerals detected were in the order K > Mg > Na. Potassium is the third most abundant mineral in the body and is an electrolyte that helps with proper nerve and muscle functions including the beating of the heart. Magnesium is the sixth most abundant mineral in the body and it works with calcium to assist in muscle contraction, blood clotting, and the regulation of blood pressure and lung function. Sodium is the fifth most abundant minerals in the body, and is an electrolyte that helps to maintain fluid and electrolyte balance (Soetan et al., 2010).

Cu, Zn, Fe, Ni, Mn and Cr occurred in trace amounts. Copper colors hair and skin and helps form the protective shield around nerve fibers. Zinc is the second most abundant trace mineral. It assists in enzyme reactions necessary for blood clotting, and is essential to taste, vision, and wound healing. Iron is the most abundant trace mineral. It is found in protein-rich

foods, and helps proteins take in, carry and release oxygen throughout the body. Nickel is a cofactor for enzyme functioning in nitrogen metabolism. Manganese assists with bone formation and metabolic functions. Chromium enhances the activity of insulin and helps to maintain insulin levels (Soetan et al., 2010).

4. CONCLUSIONS

Column chromatographic eluates from the crude extract of *V. amygdalina* leaf contain molecular fragments which are the core moieties found in many drug compounds. This leaf can therefore serve as a natural source of antibiotics for man. It is also rich in major and trace minerals elements and as such a multi-mineral element source for the humans system.

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