Phytochemical Screening and Fourier Transform Infrared Spectroscopy (FT-IR) Analysis of *Vernonia amygdalina* Del. (Bitter leaf) Methanol Leaf Extract

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Abstract
Vernonia amygdalina is a tropical African plant of the *Asteraceae* family and is occasionally cultivated for its medicinal uses, which include treatment of diarrhea, skin wounds, fever mastitis and worms infection. The aim of this study is to test for the phytochemical constituents and FT-IR analysis of the leaf extract of *V. amygdalina.* The result of qualitative phytochemical analysis revealed the presence of important metabolites such as saponin, alkaloid, tannin, phenol, flavonoid, glycoside and terpenoids. The present FT-IR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols on the basis of identification of functional groups of OH, C-H and C=O which signifies the presence of these compounds in the methanol extracts of *V. amygdalina*.

Keywords: *Vernonia amygdalina,* Phytochemicals, FT-IR Analysis, Methanol Leaf Extract

INTRODUCTION

The search for natural products to cure diseases has received considerable attentions in which medicinal plants have been the most important source (Okwu, 2001). These plants are believed to be an essential source of new chemical substances with potential therapeutic effects (Kuhn and Winston, 2000) and due to the crucial role that plant-derived compounds have played in drug discovery and treatment of several diseases, the isolation of new bioactive compounds from medicinal plants based on ethnomedicinal data appears to be a very promising approach (Newman, 2008). Traditional medicine products have played and are still playing greater roles in the lives of the people across the world in the face of the global upsurge of drug resistance, toxicity, adverse effects and increasing costs of synthetic products (Mbi and Bilikha, 1998). Poly-herbal therapies have the synergistic and antagonistic pharmacological agents within themselves that work together in a dynamic way to produce therapeutic efficacy with minimum side effects (Tiwari and Rao, 2002; Alexandros, 2007; Ebong, 2008).

The *V. amygdalina* Del, commonly known as bitter leaf is a shrub of 2-5 m tall with petiolate green leaves of about 6mm diameter. It belongs to the family *Astaraceae* and it is the most widely cultivated species of the genus *Vernonia* which has about 1,000 species of shrubs (Muanya, 2013). It is vegetatively cultivated by stem cutting at an angle of 450^0 and popular in most of West Africa countries including Nigeria, Cameroon, Gabon and Congo Democratic Republic. The plant is frequently found in gardens (Schiffers, 2000).

True to its name, bitter leaf is bitter to taste but surprisingly delicious in meals (Abosi and Raseroka, 2003). Bitter leaf is called Omjunso in East Africa especially Tanzania, Onugbo in Igbo-Eastern Nigeria, Ewuro (Yoruba), and Shuwaka (Hausa). The branches are brittle and break off easily. It has grey or brown coloured bark; the bark has a rough texture and is flaked. The leaves are elliptical and up to 20cm long. The leaves are oblongate, lance-like in shape, veined are bear, soft hairs on the underside. The bark is green or brownish dark with rough surface. The leaves are green with characteristic odour and bitter taste. The flowers are white, small, tiny and clustered. The small fruits have slightly hairy small nuts inside. It can reach 23ft in height when fully grown (Mbinglo, 1998; Remison, 2005; Muanya, 2013). It is a multipurpose plant that has a lot of potential valuable uses yet to be harnessed by the rural population of Nigeria. The leaves are used for popular bitter leaf soup and all parts of the plant are pharmacologically useful (Ojiako and Nwanjo 2006). The roots and the leaves are used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort (Igwe, 2015).

Many authors describe the medicinal and biological activities of *V. amygdalina* (Ijeh and Ejike, 2011; Atangwho, 2010). The leaves of *V. amygdalina* are crushed to make a paste to be rubbed on the body of livestock for destroying ectoparasites (Asawalam, 2008; Alawa, 2006). It is one of the traditionally used antifertility plants in Ethiopia. Preliminary study also confirmed that the plant has antifertility effect (Asawalam, 2008). The plant is well known for its antidiabetic and antihypertensive properties. The common and documented medicinal uses include the treatment of schistomiasis, amoebic dysentery, and gastrointestinal problems. It is also used in the treatment of headache, fever, malaria, venereal diseases, wounds, hepatitis, and diabetes (Oyugi, 2011; Ayoola, 2008). Plants are composed of secondary metabolites which are believed to be responsible for their various therapeutics and toxicological properties and can be detected through phytochemical screening. These active constituents are organic compounds which are not involved in the process of growth, development, reproduction of an organism, or other primary functions. Therefore in the absence of these compounds, a plant will not die immediately, but will result in long term impairment in other functions, such as defensive mechanisms or aesthetic appearance, or in some cases of impact. Where a primary metabolites are necessary for functioning of the plant. This study is aimed at screening the phytochemical constituents of this plant material and conduct FT-IR analysis in order to have better understanding of its chemical profile which is very crucial in drug development.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh sample of the leaves of *V. amygdalina* were collected from the field in July, 2019 from Adamawa state, Nigeria. The identity of the plant was confirmed by a botanist at the plant science Department of Modibbo Adama University of Technology, Yola.

Preparation of Plant Material

The samples of the plant parts were thoroughly washed, chopped into pieces and air dried at room temperature of about 33^{o}C for a period of two weeks before powdering. The dried leaves were coarsely pounded using pestle and mortar and stored in a tightly covered glass jars for further studies.

Extraction of Plant Material

Exactly 50g of the powdered material of the leaves was soaked in 500ml of methanol for 72 hours at room temperature. Extracts were first filtered through Whatman No. 1 filter paper. After filtration, the extract was concentrated to dryness and kept in a refrigerator.

Phytochemical Screening

Qualitative preliminary phytochemical analysis of all the solvent extracts was conducted in accordance with the standard procedure (Harborne, 1992) to detect the nature of phytoconstituents and their presence in plant parts. The tests for saponins, tannins, flavanoids, glycoside, alkaloids and phenols were carried out by standard qualitative methods (Trease, 2002; Sood and Kaur, 2012).

Fourier Transform Infrared Spectrophotometer (FT-IR)

Dried powder of the leaves extract of *V. amygdalina* was used for FT-IR analysis. 10 mg of the powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of the extracts was loaded in FT-IR spectroscope, with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} (Kalsi, 2007).

RESULTS AND DISCUSSION

Result of phytochemical screening of methanol leaf extract of *V. amygdalina* shows the presence of the following constituents: Saponin, Alkaloid, Tannin, Phenol, Flavonoid, Glycoside and Terpenoid. The FT-IR analysis data interpretation are shown in table 1.

Table 1: FT-IR analysis data interpretation of the methanolic leaf extract of *V. amygdalina.*

Type of Chemical bond	Absorption Frequency (cm^{-1})	Intensity	Remark an assignment
OH group (alcohol)	3436.69	S, B	OH stretching, H-bonded
CH Alkanes	2928.20	M	C-H stretching alkanes
CH Alkanes	2861.80	M	C-H stretching alkanes
Ester group	1743.75	S	C=O ester stretching
Aromatic C=C group	1644.48	S	C=C stretching
Methylene group	1462.26	M	C-H bending
OH group (alcohol)	1376.92	M	OH stretching
C-O Carboxylic Acid	1212.97	S	C-O ester stretching

Key**:** S=Strong, B=Broad, M=Medium.

FT-IR Spectral Analysis

The bitter leaf spectra show the identity of O-H stretching of intermolecular bonded alcohol at 3436 cm^{-1}. The double peak at 2928 cm-1 and 2861 cm^{-1} indicate the presence of C=O stretching of ester takes place at 1743cm^{-1}. The peak at 1644 cm^{-1} elucidate the occurrence of C=C stretching. The fingerprint region of the spectra accounts for the presence of methylene C-H bending, O-H bending of alcohol, and C-O stretching at 1462 cm^{-1}, 1376 cm^{-1} and 1212 cm^{-1} respectively.

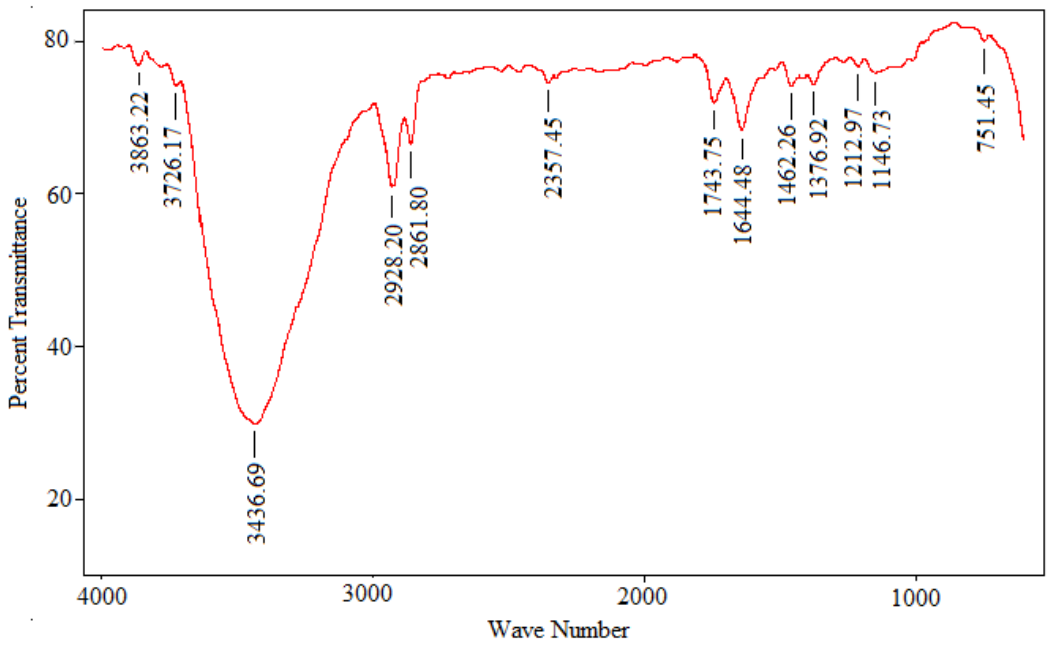

Figure 1: FT-IR spectrum of methanolic leaf extract.

The therapeutic effect of medicinal plants is attributed to the possession of important chemical constituents known as phytochemical (Nyamai and Arika, 2016). The outcome of this study revealed the presence of saponin, alkaloid, tannin, phenol, flavonoid, glycoside and terpenoids which is in agreements with the work of Aguoru (2017), Maku (2007) and Wakirwa (2013). These observations therefore support the use of *V. amygdalina* in herbal care remedies. A wide array of phytochemical, including anti-nutritional factors, has been shown to be present in *V. amygdalina* (Awe, 1999; Oboh, 2009; Yeap, 2010).

Alkaloids are synthesized form of amino acids. They have multiplicity of host-mediated biological activities, including antimicrobial, cytotoxic, analgesic and antipyretic activities (Tylor, 2011; Semwal *et al*., 2011; Hanita, *et al*., 2013). Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine), antibacterial (e.g., chelerythrine) and antihyperglycemic activities (e.g., piperine) (Namadina, *et al.*, 2019). Flavonoids are phenolic compounds and possessed the properties of antibacterial, antioxidant, anticancer, anti-inflammatory, antipyretic and analgesic activities (Ali *et al*., 2013; Magdelena *et al*., 2013; Emad, 2013; Elzbieta *et al*., 2013). The biological function of flavonoid includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxins and tumors (Okwu, 2004). Saponins are glycosidic triterpenoids and they are widely found in plants. They have the pharmacological properties like anti-inflammatory and antipyretic activities (Emmanuel *et al*., 2012; Adiukwu *et al*., 2013; Atangwho, *et al.*, 2010). The tannin-containing plants extracts can be used as astringents, anti-diarrhea, diuretics, treatment of stomach and duodenal tumours and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Mamta, 2013). Tannin is one of the major active ingredients found in plant based medicines (Haslam, 1996). They possess antiviral, antibacterial, and antitumor activity (Haslam, 1996; Khanbabaee and Van Ree, 2001). Tannin has been reported to selectively inhibit Human Immune Virus (HIV) replication (Kashiwada *et al*., 1992). Phenols exhibit antimicrobial,

anthelmintic, antiapoptotic and antidiarrhoeal activities (Cowan, 1999). Terpenes have been reported to have antimicrobial, antioxidant, anticancer and Antiparasitic activities (Sankhadip *et al*., 2010; Chonthicha *et al*., 2013; Oboh and Enobhayisobo, 2009; Ifedaya *et al*., 2013; Ayoola *et al.,* 2008).

Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. The wavelength of light absorbed is salient feature of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined (Geethu, *et al.,* 2014). The FT-IR spectrum showed in figure-1 of the methanolic leaf extract of *V. amygdalina* has bands and wave numbers of 3436.69 cm^{-1} to 2861.80 cm^{-1} as the prominent peaks. The peak at a frequency of 3436.69 cm^{-1} to 1146.73 cm^{-1} were strong, broad and medium. The present FT-IR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the methanol extracts of *V. amygdalina* and is also in agreement with previous studies conducted on other plants (Muruganantham, 2009; Meenambal, 2012; Vijayalakshmi, 2012; Janakiraman, 2011). Many works conducted revealed that the FT-IR spectrum is an effective tool for differentiating, classifying and discriminating closely related plants and other organisms.

CONCLUSION

The leaves of *Vernonia amygdalina* is well known for its ethnomedicinal properties. The common and documented medicinal uses include the treatment of schistomiasis, amoebic dysentery, and gastrointestinal problems. From the present study, the leaf extracts showed an abundant production of phytochemicals as secondary metabolite. Through FT-IR analysis, it was found out that the methanol extracts of the plant material contained 8 peaks based on the differences of polarity. The identification of functional groups of OH, C-H and C=O confirmed the presence of alcohol, alkanes and ester compounds in this plant specimen. Further extensive study of chemical composition of this herbal species may pave way for identification and isolation of significant bioactive compounds with pronounced antioxidant and other therapeutic activities for drug development.

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