Nutritional, Phytochemical and Fourier Transform Infra-Red Spectroscopy Profiling of *Vernonia amygdalina* Seeds

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Abstract: Nutritional composition, phytochemicals and Fourier transform infra-red spectroscopy profiling of *Vernonia amygdalina* seeds were investigated using standard procedures. The proximate composition (%) of the *Vernonia amygdalina* seeds was found to be; ash (9.828 ± 0.195), moisture (5.447 ± 0.070), crude fat (9.480 ± 0.480), crude fibre (23.124 ± 0.780), protein (25.235 ± 0.000) and carbohydrate contents (26.886 ± 0.000). The seeds also contains appreciable amount of vitamins (mg/g); Vitamin C (50.807 ± 0.832), vitamin B₁ (0.096 ± 0.001) and vitamin B₂ (0.055 ± 0.005). The mineral content of the sample (mg/kg) were sodium (30.400), potassium (85.100), calcium (61.200), magnesium (6.185), iron (1.072), zinc (0.311) and manganese (0.273). The phytochemical analyses showed that total phenol, saponins, alkaloid, flavonoids, glycosides and tannins were present while terpenoids and anthraquinones were absent. The results from the infrared spectrum characterize the bioactive compounds present in the *Vernonia amygdalina* seeds sample. It is advantageous to incorporate *Vernonia amygdalina* seeds as food supplement for human and livestock benefits.

Keywords: Infra-red Spectroscopy, minerals, phytochemicals, seeds, *Vernonia amygdalina*, vitamins

INTRODUCTION

Vernonia amygdalina commonly known as bitter leaf is a shrub in the family of Asteraceae (Ijeh and Ejike, 2011). It is a vegetable used for preparing the popular bitter leaf soup. Although this plant has been around for hundreds of years, only a few people know of its medicinal and healing properties great for diabetic patients (Kokwaro, 2009). Bitter leaf as the name implies, is a plant that is often known for its bitter taste, but underneath that bitterness, are useful and numerous health benefits, that is highly needed by our body. The plant precisely in Nigeria is very a common vegetable that is abundantly grown almost everywhere in the country. It is popularly known as Ewuro amongst the Yorubas, Onugbu by the Igbos and called Shiwaka by the Hausas.

This shrub has been used by traditional locals to treat different ailments and diseases due to the medicinal value and healing potencies present in this vegetable (Huffman and Seifu, 1989). However the vegetable can be prepared by juicing it with blender, juicer or it can be done in the local way by adding little water to the leaves rubbing it on palm and squeezing the juice out of it. Another way of preparing it is by cooking it as a soup (Bitter leaf soup is a popular delicacy amongst the Southern people of Nigeria). But it works

effectively when it's juiced. *Vernonia amygdalina* has also been fed to broilers, where it was able to replace 300g/kg of maize-based diet without affecting feed intake, body weight gain and feed efficiency (Teguia *et al.*, 1993; Bonsi *et al.*, 1995)

The aim of this study is to investigate the chemical composition, phytochemicals, minerals element and vitamins, and bioactive compounds by means of infra red spectroscopy embedded in *Vernonia amygdalina* seeds.

MATERIALS AND METHODS

Sample collection and preparation.

The *Vernonia amygdalina* seeds used for this study were procured during the dry season from a garden in Egbedore Local Government Area, Osun State, Nigeria. The seeds sample was air dried at room- temperature for about three weeks, after which it was grinded with mortar and pestle into a fine powdered and sieved with a 1.5mm plastic sieve. It was kept in an airtight container for subsequent uses.

Methodology

Aqueous extraction

The aqueous extract of the sample was prepared by soaking 100g of dried powdered samples in 200ml of distilled water for 12- hour. The extracts were filtered using whatman filter paper no 42 (125mm).

Phytochemical analysis

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures as to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Determination of Vitamin C (Ascorbic acid)

About 0.5g of the sample was weighed, macerated with 10mls of 0.4% oxalic acid in a test tube for 10 minutes, centrifuged for 5 minutes and the solution filtered. 1ml of the filtrate was transferred into a dry test tube in duplicates, 9mls of 2.6 dichlorophenol indophenols was added and absorbance was taken at 15 seconds and 30 seconds interval at 520nm (AOAC, 1990).

Determination of Vitamin B₁ and B₂

These vitamins B₁ and B₂, were determined by spectrophotometric method as described by Khalil and Mann, (1990).

Fourier Transform Infra-Red Spectroscopy Analysis (FTIR)

About 2mg of the sample and 200mg KBr was dried and ground. The mixture was squeezed to form transparent pellets which was measured directly (Perkins,1986).

Proximate and Mineral Analyses

These were carried out according to the method described by the Association of Official Analytical Chemist (1990).

RESULTS AND DISCUSSION

Table 1: Proximate Composition of *Vernonia amygdalina* Seeds

Parameters	Composition (%)
Ash content	9.828±0.195
Moisture content	5.447 ± 0.070
Crude fat content	9.480 ± 0.480
Crude fibre content	23.124 ± 0.780
Protein. Content	25.235 ± 0.000
Carbohydrate content	26.886 ± 0.000

Table 2: Minerals Composition of *Vernonia amygdalina* Seeds

Parameters	Compositions (mg/kg)
Sodium	30.400±0.02
Potassium	85.100±0.001
Calcium	61.200±0.004
Magnesium	6.185±0.001
Iron	1.072±0.001
Zinc	0.311±0.003
Lead	NA
Manganese	0.273±0.002

Key: NA-not detected

Table 3: Qualitative Phytochemical Composition of *Vernonia amygdalina* Seeds

Parameters	Constituents
Tannins	+ve
Total Phenols	+ve
Alkaloids	+ve
Flavonoids	+ve
Terpenoids	-ve
Saponins	+ve
Glycosides	+ve

Key: +ve - presence of constituent
-ve – absence of constituent

Table 4: Quantitative Phytochemical Composition of *Vernonia amygdalina* Seeds

Parameters	Compositions
Total phenols	12.799 $\pm$ 0.013
Saponins	7.037 $\pm$ 0.020
Alkaloids	3.756 $\pm$ 0.000
Flavonoids	5.996 $\pm$ 0.000
Glycosides (mg/kg)	5.060 $\pm$ 0.000
Tannins (mg/g)	1.209 $\pm$ 0.000

Tables 5: Vitamin Composition of *Vernonia amygdalina* Seeds

Parameters	Compositions (mg/g)
Vitamin C	50.807 $\pm$ 0.832
Vitamin B ₁	0.096 $\pm$ 0.001
Vitamin B ₂	0.055 $\pm$ 0.005

Table 6: Infrared Vibration Bands and Functional Compounds of *Vernonia amygdalina* Seeds

Wave number cm^{-1}	Functional compounds
3395.0	OH group
2925.37	Alkanes with strong intensity nitriles, thiols, methylene group.
	C = O Strong intensity saturated aldehyde
1727.67	C = C alkene
1649.00	C – OH Bending vibration; COOH carboxylic group
1429.00	Aromatics, phenyl group OH -
1377.00	Phosphate (str).
1257.25	Amines, esters, P – OR esters.
1038.36

Fig 1: Infrared spectrum of *Vernonia amygdalina* seeds extracts using Fourier Transform Infra-red spectroscopy

The proximate compositions (%) of *Vernonia amygdalina* seeds were shown in table 1. The nutrient in food is of great importance, has it accounts for the establishment of nutritional composition. The *Vernonia amygdalina* seeds showed a higher value of ash content ($9.828 \pm 0.195\%$) when compared with water melon seeds (6.000 ± 0.10). This is an indication of higher mineral contents (Anwar *et al.*, 2008). The crude fat content of the *Vernonia amygdalina* seeds is ($9.480 \pm 0.480\%$) while the value is lower when compared with that of bitter lemon seeds which is (34.00 ± 0.00) as reported by Matthew *et al.*, (2014). Healthwise, fat plays a vital role in hair and skin, which insulate the body organs against shock, maintaining body temperature and promoting healthy cell function. It is also essential in enhancing the diets as they increase the pleasant taste of food (Omotoso, 2006). But *Vernonia amygdalina* seeds cannot be regarded as oil seeds. The result shows that *Vernonia amygdalina* seeds have a higher value of crude fibre ($23.124 \pm 0.780\%$) than that of *Citrus maxima* peels (2.58 ± 0.41) as reported by Peace and Happiness, (2018). The physiological role of crude fibre in the body is to maintain an internal distention for poor peristaltic movement of the intestinal tract (Oduor *et al.*, 2008). The protein content in the *Vernonia amygdalina* seeds is ($25.235 \pm 0.000\%$) when compared with watermelon seeds content (21.500 ± 0.13) as reported by Matthew *et al.*, (2014). The high protein content in the sample showed that it could be regarded as good source of protein; hence it can be modified into protein concentrate feeds for livestock. The higher level of protein in the samples indicates that it contributes significantly to the daily protein requirements of 23 - 56g for humans as stipulated by the NRC (1980). The carbohydrate value of the sample is $26.886 \pm 0.000\%$ thus it is rich in energy that is also good for the body.

The mineral element of the sample is shown in table 2. The level of sodium in the sample is 30.400 ± 0.02 . This value is compared with the value obtained for *Citrus maxima* peels (274.77 ± 5.07) as reported by Peace and Happiness (2018). Sodium helps in maintaining body fluid. The value obtained for potassium in the sample is 85.100 ± 0.001 and high when compared with that reported by Chinazum *et al.*, (2017) for *Vigna aconitifolia* (51.57 ± 0.02). Potassium is important in the repair of cellular water balance, body pH regulation and also associated with carbohydrate and protein metabolism (Shils, 1973). According to Shils (1973), more intake of potassium can reduce blood pressure up to 3.2 mmHg value, which implies that it can reduce death rate to about 8%. The calcium content of the sample is (61.200 ± 0.004), which is higher than the value obtained from pawpaw seeds (5.46 ± 0.03) as reported by Matthew *et al.*, (2014). Calcium is a structural component of bones and teeth. It also plays a vital role in enzyme activation, blood clotting, muscle contraction and cellular processes (Gropper *et al.*, 2005). Magnesium content in the sample is 6.185 ± 0.001 which is higher than 3.21 ± 0.12 for cherry seeds as reported by Matthew *et al.*, (2014). Magnesium is an enzyme activator in biochemical and physiological processes (Schrauzer, 2000). Iron in seeds is necessary in formation of haemoglobin and normal functioning of the central nervous system. There is no much difference in iron of *Vernonia amygdalina* seeds (1.072 ± 0.001) and that of *Vigna aconitifolia* seeds (1.490 ± 0.00). Iron increases the amount of blood in human especially in anaemic conditions and also it is necessary for cognitive growth, proper functioning of the immune system, energy metabolism and temperature regulation (Baynes, 2000). The

zinc content of the *Vernonia amygdalina* seeds is lower (0.311 ± 0.003) to that of the zinc content in water melon seeds (5.52 ± 0.20) as reported by Matthew *et al.*, (2014). Zinc is important for the regulation of many biochemical processes and physiological functions of living tissues (Berry, 1998). The manganese content of *Vernonia amygdalina* seeds is 0.273 ± 0.002 . Manganese helps in metabolism of carbohydrates and proteins. It is also essential for proper brain function as well as strengthening of weak bones and painful joints (Franklin, 2000).

The results reveal the presence and absence of some certain phytochemicals in tables 3 and 4. Phenol is important in reducing risk of heart disease by controlling cholesterol accumulation (Meyer *et al.*, 1997). Phenolics in functional food have antioxidant effect and its mechanism involves free radical scavenging and metal chelating activities (Wang *et al.*, 2014). Saponins helps to reduce cancer risk as anti – oxidant, reduce bone loss and immunity booster (Sodipo *et al.*, 2000). Saponins has relationship with sex hormones like oxytocin. Oxytocin is a sex hormone involved in controlling the onset of labour in women and the subsequent release of milk (Sodipo *et al.*, 2000). Alkaloids are secondary plant metabolite with great pharmacological significance (Adeolu and Enesi, 2013). Flavonoids in the plant is rich and it has the potency to minimize inflammation in the arteries. It also guides the body's cell from dangerous free radicals that comes from smoke and other environmental contaminant (Maria *et al.*, 2009). Tannins are involved in healing wounds and inflamed mucus membrane. They also exhibit astringent and antimicrobial potentials (Braids *et al.*, 2012).

The vitamins compositions of *Vernonia amygdalina* seeds were shown in table 5. Vitamin C also known as ascorbic acid is an enzyme co-factor required for synthesis of important biochemicals (Hunt *et al.*, 1980). Vitamin C is also a potent antioxidant which reduces oxidative stress. It is required for maintenance of normal connective tissue and involved in wound healing (Chaney, 2006). Its deficiency results to capillary fragility, scurvy and anemia (Chaney, 2006). Among the investigated vitamins, vitamins C had the highest concentration. The content of vitamin C in *Vernonia amygdalina* seeds is 50.807 ± 0.832 and higher than the content in Moth bean seeds (42.250 ± 0.010). The availability of vitamin C in *Vernonia amygdalina* seeds suggest that its of high capacity and plays a mediated roles in fighting against germs in the body. Vitamins B complexes are classified as energy releasing water soluble vitamins, they are very essential for regulation of normal human metabolism especially in minutes measure. Vitamin B₁ is also known as thiamine and the value of Vitamin B₁ content in *Vernonia amygdalina* seeds is (0.096 ± 0.001) lower than the content in Moth bean (0.239 ± 0.000). The deficiency of vitamin B₁ causes the disease known as beriberi (Hunt *et al.*, 1980). Vitamin B₂ also known as riboflavin. The content of B₂ in *Vernonia amygdalina* seeds (0.055 ± 0.005) is lower than Moth beans (0.450 ± 0.000). Deficiency of riboflavin does not result to a specific disease; however there is the possibility of underestimating its importance. The symptoms of riboflavin deficiency include inflammation of the tongue, and lesions in the eyes and lips (Hunts *et al.*, 1980; Taylor, 1972). Vitamin

B complexes help the body produce the energy it needs to function better. It also assists the body in the formation of red blood cells which supply oxygen to the body tissues (Hunts *et al.*, 1980) and Taylor, 1972).

Table 6 depicts the infrared spectrum of *Vernonia amygdalina* seeds. The result of the infrared spectrum showed that the functional group region which ranges from 4000 – 1450cm⁻¹ which usually contained relatively few peaks associated with stretching vibrations. Absorptions peak 3395 cm⁻¹ is characterized with OH (hydroxyl) functional group, 2925.37cm⁻¹ showed an alkanes having strong intensity with CH₃, CH₂ and CH, nitriles, thiols and methylene group. However, absorption at 1727.67cm⁻¹ showed a saturated carbonyl compounds having strong intensity. Also the absorption at 1649.00cm⁻¹ indicated the presence of a carbon – carbon double bond (C=C); an alkene group. The peak at 3395cm⁻¹ has already been attributed to hydroxyl (OH⁻) group. This confirms the presence of large amount of saponin compound in *Vernonia amygdalina* seeds. Compounds such as alkaloids, saponins, tannins and flavonoids have been reported to have inhibitory properties (Uyoh *et al.*, 2013). Thiols functional group at the peak 2925.37cm⁻¹ are group of thiophenes which are organo – sulphur, heterocyclic compound also known as thio furan having potential therapeutic in the treatment of ailments. However, at the finger print region, ranging from 1450 – 400cm⁻¹ characterized with bending vibration within the molecule. This region is important because each different compound produces its own unique pattern of peaks as shown in the infrared spectrum in figure 1. At this finger print region, peak at 1429cm⁻¹ showed carbonyl bonded for hydroxyl group (C – OH) ; carboxylic group absorption at 1377cm⁻¹ depicts the presence of aromatics phenyl group. Phosphate group at 1251.25cm⁻¹ having strong intensity. In addition, the absorption at 1038.36cm⁻¹ showed the presence of amines and esters. The carboxylic group of acetohydroxamic acid is one of the inhibitors studied as the potential therapeutic drug in the treatment of ulcer caused by *Helicobacter pylori* and has been suggested as a means of preventing the various related pathological conditions (NBMC, 2017). Also the presence of hydrogen atom attached to the carbon (C – H) in the ring by a substituent group (OH, NH₂, NH, COOH) improves inhibition (Debi *et al.*, 2013). The natural phenolic compound is important due to the health benefits it confers to the body (Gil *et al.*, 2002). The presence of phenols; an aromatic compound in the *Vernonia amygdalina* seeds conferred medicinal property on the plant's seeds. Phenols and flavonoids are primary antioxidants due to their hydroxyl group (OH⁻) directly bonded to the benzene ring. Thus allowing them to easily donate electron to electron deficient free radicals in order to reduce their menace in biological system (Uyoh *et al.*, 2013). Moreover, esters are traditionally formed from the esterification of alcohol with fatty acids (Vans Der Slijs *et al.*, 2002). The significance of ester contribution to development of aroma in well-known short chain esters are highly volatile precursors. Amines have also been found to be flavor precursors and hence the associated flavor with *Vernonia amygdalina* seeds (Smith, 1982). However, the presence of phosphorus in the form of phosphonate and P-OR esters in the extract of *Vernonia* seeds depict the medicinal importance of the seeds under investigation. Phosphorus is commonly found in the body as phosphate and it is primary essential minerals for growth and repair of worn – out cells and tissue. According to the University of Maryland Medical Centre, all

body cell contained about 85% phosphorus occurring in bones and teeth. The ratio of calcium – phosphorus enhances bone formation and is required for variety of biochemical processes including energy production and pH regulation for a balanced body metabolism (Gropper *et al.*, 2005).

CONCLUSION

Vernonia amygdalina possesses various bioactivities with low or absence of side effects. With its great health promotion effect, it may be more advantageous to incorporate *Vernonia amygdalina* seeds into health supplement for human benefits rather than using it solely as feed for the poultry industry. *Vernonia amygdalina* seeds have been promising boosting immunity and preventing infections. In general, the regular consumption of prebiotics enhanced immune functioning; it improved colonic integrity, decreased incidence and duration of intestinal infections, and improved digestion.

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