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Proximate and Fourier transform infrared (FTIR) spectroscopic analysis of phytochemicals from hexane extracts of root and leaf of *Rauvolfia vomitoria* (Afzel)

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Abstract

This research, aimed to determine phytochemical constituents and identify functional groups of the secondary metabolites of *Rauvolfia vomitoria* (Afzel*)* root and leaf. Soxhlet extraction was carried out using n-hexane. Proximate analyses using standard procedures revealed that 100 g of root contained 64.5 g of carbohydrates, 14.48 g of fibre, some crude fat and proteins. Phytochemical screening confirmed the presence of various phytochemicals such as alkaloids, saponins, cardiac glycosides, terpenoids, steroids, phenolics, tannins and flavonoids. Quantitatively analyses revealed that 100 g of dry root/leaf contained 161.3 mg/147.65 mg alkaloids, 10.70 mg/17.68 mg saponins, 30.01 mg/44.16 mg reducing sugar and 17.92 mg/10.79 mg cardiac glycosides. Others detected are 9.45 mg/13.92 mg steroids, 18.63 mg/14.10 mg tannins and 77.01 mg/107.16 mg phenolics, respectively. FTIR spectroscopy showed peaks with diverse functional groups, including N-H (alkaloids), C-O (triterpenoid), O-H (flavonoids), C=C (phenolics) and C=O (steroids). The present study demonstrated the plant parts could serve as good sources of macronutrients.

Key words: *Rauvolfia vomitoria*, FTIR, Proximate analysis, Functional groups, Phytochemicals

Introduction

Rauvolfia vomitoria, the tropical plant species known as the "poison devil's pepper", belongs to the genus *Rauvolfia* and the family, Apocynaceae. It is a widely used medicinal plant that is found all over the world, particularly in West African and Asian countries (Huang *et al.*, 2022). The plant is native to China, Bangladesh, the Himalayas and Puerto Rico. It is also found in Eastern Senegal, Sudan, Tanzania and the southern region of Angola (Balogun and Ashafa 2019).

In its natural habitat, *R. vomitoria* grows to 8 meters (26 feet) in height. Except for the mature wood, all parts of the plant contains latex (Najjiaa *et al.,* 2017). Most parts of the plants are frequently used in traditional therapies and possess various chemicals useful to the pharmaceutical industries (Balogun and Ashafa 2019). A study in 2013 reported on the anti – ovarian cancer activity of *R. vomitoria* root extract and concluded that the plant had the ability to decrease cell growth in the tested ovarian cancer cell lines (Yu *et al.*, 2013). Similarly, extract from the plant reduced the growth of human pancreatic cancer cell at an inhibitory concentration (IC_{50}) of 125–325 g/ml (Dong *et al.*, 2018). In another article, the plant extract has been used to repress colorectal cancer cells and promote apoptosis (Wang *et al.*, 2021).

In African traditional folk medicine, *R. vomitoria* has been used to treat a variety of diseases including diarrhoea, malaria, convulsion, diabetes, cancer, rheumatism, jaundice, venereal diseases, male infertility, snakebites (Balogun and Ashafa 2019) and sickle cell (Gbolade, 2012; Abere *et al.*, 2014. The ethnomedicine use of the leaves, root and stem bark of *R. vomitoria* has been documented in Côte d'Ivoire (N'doua *et al.*, 2016), Democratic Republic of Congo (Mushagalusa *et al.*, 2021; Kasali *et al.*, 2021), Gabon (Ngoua-Meye-Misso *et al* 2019) and South Africa (Mudau *et al.*, 2020). In Nigeria, several authors have attempted to demonstrate the efficacy of the root components of the plants for the management of certain conditions including mental disorders (Eluwa *et al.*, 2009; Ekong *et al.,* 2016) and hypertension (Owoade *et al.*, 2021; Omoya *et al.,* 2019).

The extracts from various parts of the plant have been extensively studied and findings have revealed that *R. vomitoria* is rich in reducing sugars, alkaloids, flavonoids, glycosides, saponins, and phenols. Apart from these phytochemicals, the alkaloids and polyphenolic chemical constituents such as ajmaline, alstonine, reserpine, rescinnamine, serpentine, serpentinine, and yohimbine, have been credited with its efficacy (Ekong *et al.,* 2020). Alkaloids are assumed to be responsible for sedative and anti-hypertensive properties of the plant (Yu *et al.,* 2013; Wang *et al.*, 2021; Eluwa *et al.*, 2009).

Taken together, these studies suggest that *R. vomitoria* extract may be promising alternative for the treatment of some diseases. Nonetheless, most studies in Nigeria are centered on the medicinal effects without recourse to analysis of the bioactive molecules or nutritive values. We hypothesise that the plant ecology may change under various environmental conditions and could conversely affect the phytoprofiles. Hence, the present study was carried out to investigate the proximate and phytocomponents of the leaf and root extracts of the plant in order to ascertain the functional groups that may be responsible for the plant use in the management of the ailments in Nigeria. Previous work had been carried out on some plants linking the functional groups with bioactivity like the phenolics and flavonoids which have high antioxidant properties as reported by Osibote *et al.*, 2020.

Materials and Methods

Collection of *Rauvolfia vomitoria*

R. vomitoria leaves and roots were obtained from the premises of a public secondary school surrounded by two communities in the Alimosho local government area of Lagos State, Nigeria (Obiefuna *et al.,* 2021). The plant identification and authentication were carried out at herbarium of the Botany Department. The assigned voucher number was LUH 8624.

Preparation of the leaf and root extracts

In brief, the leaf and the root were air dried at room temperature (25-27 ^{o}C) for two weeks and pulverised by milling. The masses of the pulverized leaves and roots were 100 g and 1000 g respectively. The plant extracts were prepared with Soxhlet method, 1 kg of the root and 100 g of the leaf was extracted in 1 l of n-hexane in the flask and later evaporated to concentrate the extract. n-hexane is used though non polar, it is best in soxhlet extraction because all oils are soluble in n-hexane. To obtain a crude extract, the liquid extract of each was evaporated at 55 ^{o}C with reduced pressure using rotary evaporator (Buch Rotavapour, R200). The extracts were separated with chromatography.

Proximate analysis

Using methods recommended by the Association of Official Analytical Chemists (AOAC, 2006), the moisture content, total ash, crude fat, crude protein, carbohydrates and fibre were measured. All analyses were done in duplicates.

Phytochemical screening

Using the methods of (Ejikeme *et al.*, 2014), the crude extracts of the plants were qualitatively and quantitatively estimated for the presence of phytochemicals including alkaloids, saponins, reducing sugars, anthraquinones, cardiac glycosides, terpenoids, triterpenoids, steroids, phenolic compounds, flavanoids and tannins.

Thin Layer Chromatography

Each n-hexane extract from the root and the leaf was subjected to analytical thin-layer

chromatography using a variety of solvent systems until the extract-separating solvent system was identified (Cannell,1998). The components were separated with chloroform:methanol (95:5, v/v) as the resolving solvent system giving two components each. The highest peak heights of retention factor (Rf) were: for the root 0.52, 0.85 and leaf Rf 0.68, 0.88. The columns were used to separate the components and thin layer chromatography (tlc) was used for identification using an ultraviolet light with a wavelength of 254 nm.

Fourier transform infrared (FTIR) spectroscopic analysis

The infrared spectra of the plant extracts was evaluated with FTIR-8400S (Shimadzu Deutchland GmbH) spectrophotometer. Leaf and root extracts (0.01 g each) were measured and homogenized into pellets using 0.01 g anhydrous Potassium bromide (KBr), and thin films were formed by applying pressure (Okereke, 2017). The data on infrared transmittance was gathered over a wavelength of 500 - 4000 cm^{-1}. KBr pellets was used as the control and analysed in duplicate. The functional groups present in the sample were identified by comparing the wave numbers obtained with data of standard reference

Results

Table 1: Proximate composition of *Rauvolfia vomitoria* root

S/N	Macronutrients	% composition
1	Carbohydrates	64.50
2	Crude Fibre1	4.48
3	Moisture Content	9.23
4	Ash Value	5.37
5	Lipid/Fat	4.20
6	Protein	2.20

Qualitative phytochemical analysis of *R. vomitoria*

The qualitative phytochemical assessment of the *R. vomitoria* leaves and roots revealed the presence of secondary metabolites including alkaloids, saponins, reducing sugars, cardiac glycosides, terpenoids, steroids, phenolics, flavonoids, and tannins (Table 2). The metabolites seemed to be present in sufficient quantities. However, neither extract contained any anthraquinone.

Table 2: Preliminary qualitative phytochemical analysis of the extracts of leaf and root parts of *R. vomitoria*

		Inference	
Constituents	**Methods**	**Leaf**	**Root**
	Mayer's Test	+	+
Alkaloids	Dragendorff's Test	+	+
Saponins	Frothing Test	+	+
Reducing Sugar	Fehling's Test	+	+
Anthraquinone	Borntrager's Test	-	-
Cardiac glycosides	Keller Killani's Test	+	+
Terpenoid	Liebermann Burchards	+	+
Triterpenoid	Liebermann Burchards	+	+
Steroids	Salkowski's Test	+	+
Phenolic compounds	Lead acetate Test	+	+
Flavonoids	Shinoda's Test	+	+
Tannins	Ferric Chloride Test	+	+

Key: + Detected, - Not detected

Quantitative detection of the phytochemical components

Table 3 displays the outcome of the quantitative phytochemical analysis of *R. vomitoria* extracts. The total alkaloid content per 100 g of sample in root part varied widely and higher than the leaf. Conversely, the leaf showed the presence of substantial levels of phenolics (107.16 ± 0.40), reducing sugar (44.16 ± 0.29), steroids (13.92 ± 0.04), and flavonoids (124.47 ± 1.17). The level of phytochemicals may vary at the leaf and root because they are at different positions on the plant, one exposed to various temperature and the other under the soil.

Table 3: Quantitative phytochemical composition of root and leaf of *R. vomitoria*

	Plant Extracts (mg/100g ± SD, of dry weight)	
Identified Compounds	Leaf	Root
Alkaloid	147.65 ± 1.89	161.31 ± 1.03
Saponins	17.68 ± 0.055	10.70 ± 0.34
Reducing Sugar	44.16 ± 0.29	30.01 ± 0.13
Cardiac Glycoside	10.79 ± 0.02	17.92 ± 0.00
Steroids	13.92 ± 0.04	9.45 ± 0.03
Tannins	14.10 ± 0.01	18.63 ± 0.05
Phenolic	107.16 ± 0.40	77.01 ± 0.08
Flavonoids	124.47 ± 1.17	55.49 ± 0.29

Tests were performed in duplicate and represented as mean ±standard error (SD)

Identification of functional group

The FTIR wave numbers of the interpreted peak values and probable functional groups present in the leaf and root extracts are shown in the Table 3. The infrared spectra are in the wave numbers, ranging from 3381.20 cm^{-1} to 353.45 cm^{-1} for the n-hexane extract of root and 3789.90 cm^{-1} to 360.07 cm^{-1} for the n-hexane leaf extract. The absorption spectra of the root extract exhibited a peak 3790 cm^{-1} representing the presence of alkaloids (N-H stretch). Likewise, leaf extract of *R. vomitoria* exhibited a peak at 3381.20 cm^{-1} presenting the presence of flavonoids (OH stretching).

Table 4: Functional groups in the leaf and root extracts of *R. vomitoria* FTIR technique

Fraction	**Bands present**	**Functional groups assigned**	**Likely Class of Compound**
n-Hexane leaf extract of *R. vomitoria*	3790cm^{-1}	N-H Stretching	Alkaloids
	3436cm^{-1}	OH stretching	
	2918cm^{-1}	SP_3 C-H stretching	
	2850cm^{-1}	SP_3 C-H stretching	-OH present in Flavonoids
	2728cm^{-1}	=C-H Stretching	
	1731 cm^{-1}	C=O stretching	Steroids
	1712cm^{-1}	C=O Stretching.	
	1643cm^{-1}	C=C Stretching	Phenolics
	1555cm^{-1}	N-H bending	
	1463cm^{-1}	C-H bending	
	1377cm^{-1}	C-H bending	
	1241cm^{-1}	C-O stretching	
	1174cm^{-1}	C-O stretching	

	1038cm^{-1}	C-O stretching	
	979cm^{-1}	=C-H bending	
	883 cm^{-1}	=C-H bending	
	839 cm^{-1}	=C-H bending	
	720 cm^{-1}	=C-H bending	
	3381cm^{-1}	OH Stretching	
	3005cm^{-1}	SP_2 C-H Stretching	
	2925cm^{-1}	SP_3 C-H Stretching	Flavonoids
	2862cm^{-1}	SP_3 C-H Stretching	
	2728cm^{-1}	=C–H Stretching	
	1744cm^{-1}	C=O Stretching	
	1631cm^{-1}	Aromatic C=C stretching	
	1590cm^{-1}	Aromatic C=C stretching	
	1564cm^{-1}	Aromatic C=C stretching	Phenolics
	1465cm^{-1}	Aromatic C=C stretching	
n-Hexane Root extract of *R. vomitoria*	1417cm^{-1}	Aromatic C=C stretching	
	1378cm^{-1}	SP_3 C-H bending	
	1334cm^{-1}	SP_3 C-H bending	
	1227cm^{-1}	C- O Stretching	Triterpenoids-oxygenated terpenes
	1174cm^{-1}	C- O Stretching	
	1131cm^{-1}	C- O Stretching	
	1034cm^{-1}	SP_3 C-H bending	
	864 cm^{-1}	=C-H bending	
	824 cm^{-1}	=C-H bending	
	723 cm^{-1}	Aromatic out of plane wagging	

Discussion

The proximate analysis in the current study revealed that *R. vomitoria* has nutritional properties and may be a viable source of macronutrients for the human diet. Our findings were in agreement with the range of carbohydrate contents of the seeds (Ajayi and Ojelere, 2013) and root (Alagbe, 2021). In many diets, carbohydrates play a pivotal role and the digestible forms serve as primary source of energy. *R. vomitoria* might thus be a great source of carbohydrates supply to both humans and animals. Alagbe, 2021 suggested that root extract of the plant could be useful as a nutritional supplement and phytogenic feed additive in young rabbit diets. Moreover, the plant might be regarded as a valuable source of dietary fibre in human nutrition considering its crude fibre content (14.48 %). Our findings significantly concur with the 7.40 % estimate provided for the seeds of the same plant species (Ajayi and Ojelere, 2013) but lower than the values reported by Alagbe, (2021) and (Chukwu *et al.,* 2015). Similarly, when compared with the finding of the authors, the protein, lipid and moisture contents appeared to be low for the roots and leaves samples examined in this study. These variances might be due to fact that the plants were collected from different geographical locations.

It has been assumed that environmental factors that affect growth conditions can interfere with the metabolic pathways in plants and consequently on the total concentration of bioactive compounds. The overall secondary metabolites extracted from the leaves and roots of *R. vomitoria* in our study are consistent with those of other authors (Akanji *et al.,* 2013; Balogun and Akintunde, 2022), though with slightly higher concentrations in some cases. The preliminary phytochemical screening of the leaf and root of *R. vomitoria* revealed the presence of

secondary metabolites, including alkaloids, phenols, flavonoids, reducing sugars, cardiac glycosides, terpenoids, steroids and saponins which are consistent with those described in the literature (Adebayo *et al.,* 2014).

There are several biological and therapeutic features associated with these secondary metabolites. The high alkaloid content in the leaf and root conforms to the findings of other authors (Dewick, 2002). The fact that the plant uses the root and leaf as a storage area may be the reason for the high alkaloid content in those sections (Benbolt *et al*., 2012). The high levels of phenolic and flavonoid compounds found in the leaves and roots the plant may be responsible for its antioxidant qualities, suggesting the relevance of the plants in the management of ailments that have been associated with the Reactive Oxygen Species such as cancer and inflammation (Yu *et al*., 2013; Ezeani, 2018; Fang *et al*., 2021).

The therapeutic effects of bioactive chemicals found in medicinal plants are mediated by functional groups and molecular shapes. The FTIR analysis of the n-hexane extracts of root and leaf of *R. vomitoria* showed bands corresponding to N-H, which can represent the functional groups present in alkaloids, C=O and C=C functional groups present in triterpenoids, C-O, O-H in steroids,-O-H in flavonoids and phenolics. These functional groups confirm the presence of the secondary metabolites described here that underlie the bioactivities of the plants. It is also not impossible that these compounds could be working synergistically which may have contributed to its therapeutic outcomes as observed in other studies (Huang *et al*., 2022; Dong *et al.,* 2018). These biomolecules could therefore be identified, developed, and optimised through extensive scientific research in order to provide useful innovative medications for the treatment of diseases.

Conclusion

The results of the FTIR analysis correspond with the phytochemicals identified in the qualitative and quantitative study of the plant extracts. These phytochemicals include phenolics, reducing sugar, flavonoids, tannins, and terpenoids. They are known for their bioactivities and medicinal values in the management of malaria, inflammation and reactive oxygen species-related diseases. This provides scientific support for the therapeutic use of *R. vomitoria* in folklore medicine.

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