

Original Research Article
Comparative analysis of the Phytochemicals, Minerals, Antioxidant and Antimicrobial activities of Aqueous and Ethanolic extracts of *Rauvolfia vomtoria* leafs

ABSTRACT

In this study, the phytochemicals, B vitamins, minerals, antioxidants and antimicrobial activities of aqueous and ethanolic extracts of *Rauvolfia vomtoria* leaves were determined and compared. Qualitative and quantitative phytochemical analysis, mineral and B vitamin composition, *in vitro* antioxidant capacity and antimicrobial properties of both extracts were determined using standard protocols. Precise analysis of *R. vomtoria* revealed that the moisture content of the leaves was 8.90%, ash content was 7.70% and crude fiber content was 20.00%. The carbohydrate content of the leaves was 41%, crude protein content was 17.00% and total fat content was 5.28%. Cardiac glycosides, tannins, phenols, saponins, phytates, steroids, terpenoids and alkaloids were detected in both aqueous and ethanolic extracts. However, significantly higher concentrations of saponins, tannins, flavonoids, steroids, alkaloids, cardiac glycosides and phytates were detected in the ethanolic extract compared to the aqueous extract. There was no significant difference in the amount of phenols and terpenoids in the two extracts ($p > 0.05$). The thiamine content in the aqueous extract was 0.20 whereas that in the ethanolic extract was 0.27. The riboflavin content was higher in the ethanolic extract (1.80) than in the aqueous extract (1.40). Similarly, the niacin content of the ethanolic extract was 0.81 whereas that of the aqueous extract was 0.81. There was no significant difference in the calcium, magnesium and phosphorus values recorded for the two extracts ($p > 0.05$). In contrast, there was a significant difference in the sodium, potassium, zinc, iron and copper contents of the two extracts with the aqueous extract containing higher amounts of potassium and zinc. The aqueous and ethanolic extracts had zones of inhibition against *Enterobacter faecalis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Cetrobacter freundii* and *Shigella flexnerii*. However, the zone of inhibition of the ethanolic extract against the tested organisms was higher than that of the aqueous extract. At a concentration of 10%, the aqueous and ethanolic extracts showed 87.29% and 92.03% DPPH scavenging activity, respectively. The percentage of inhibitory activity of the extracts against DPPH radical increased in a dose-dependent manner, with the ethanolic extract showing the highest DPPH radical inhibitory activity at a concentration of 50%. The results confirm that ethanol is a suitable solvent for the extraction of biologically active molecules from *R. vomtoria* leaves.

Keywords: Phytochemicals, Minerals, Vitamin B, Antioxidant, *Rauvolfia vomtoria* leafs

1. INTRODUCTION

The study of medicinal plants and traditional medicine has long fascinated herbalists around the world. Traditional medicine focuses on improving overall health, preventing diseases, regulating the immune system, and providing individualized care through a holistic approach [1-2]. It is noteworthy that more than 60% of medicines are of plant origin. The reason is that they act gently on the human body, have minimal or negligible side effects, are cheap to produce, and are easily available. As a result, medicinal plants have great benefits for human well-being [3-4]. However, only 5–10% of the 250,000 species of higher plants have been phytochemically studied, and only a fraction of them have been exposed to biological or pharmacological screening. The plant kingdom still serves as a vast reservoir for new molecules to be discovered [5].

Medicinal plants are known as important natural reservoirs of secondary metabolites, and therefore, great efforts have been made in the research and development of phytomedicines, consisting of flavonoids, vitamins, alkaloids, tannins, and terpenoids, which have biological activities such as detoxifying toxic enzymes, inhibiting cell damage, regulating gene expression, and antibacterial and anti-inflammatory effects [5-8]. Several lines of research suggest that ingesting natural antioxidants can reduce the risk of various health complications such as cancer, neurodegenerative diseases, and diabetes [9-13]. The positive role of natural antioxidants in human health is mainly due to their reducing effect, which quenches free radicals such as reactive oxygen and nitrogen species (ROS/RNS) and prevents oxidative damage to cells caused by their action, due to their tendency to release hydrogen [14].

Free radicals are highly reactive chemical species, especially hydroxyl radicals (-OH) and superoxide ions (O_2^-), which react with important biological compounds such as phospholipids, proteins, and nucleic acids, causing oxidative damage to healthy body cells [15]. Various studies have confirmed the inhibitory effect of natural antioxidants in plant extracts under *in vitro* conditions against the harmful effects of free radicals [16-19]. Recently, there has also been an increased interest in natural therapeutic agents, as they are less toxic than chemicals widely used in the medical, food, and cosmetic industries [20-21].

One of the medicinal plants found in the humid tropics is *Rauwolfia*, a tropical shrub with white or greenish flowers. The plant *Rauwolfia vomitoria* belongs to the family *Ethnocarpaceae*. *Rauwolfia vomitoria* is also known as snake wood, snake root, stirstick, and colloquial names "Asofeyeje" in Yoruba, "Ira" in Igbo, "Wadda" in Hausa, "Akata" in Bini, and "Utwenyin" in Efik.

It is found mainly in the forests of southern Nigeria [22]. Research has shown that the herbal preparation made from the alkaloid extract of *R. vomitoria* has been used in traditional African folk medicine as an antihypertensive agent [23-24], to treat neurological disorders [25], as an antioxidant, anti-inflammatory and anti-cancer properties [26-28].

Different plant extracts have different properties: methanol extracts can be used as antimalarial drugs [29]; aqueous extracts of *Rauwolfia vomitoria* can be used to treat typhoid and jaundice [30]; and aqueous methanol extracts of *R. vomitoria* leaves are also used as a treatment for sickle cell anemia [31]. Choosing the best solvent for phytochemical extraction is important because phytochemicals exist with a variety of chemical structures and polarities that can affect their solubility in the selected solvent. Plants contain a variety of phytochemicals with different solubilities depending on the charge and polarity of the molecule [32]. Optimal solution

extraction optimizes the yield of phytochemicals and antioxidants [33-34]. Water is commonly used for phytochemical digestion with methanol, ethanol, acetone, and combinations of these organic solvents with water [35]. Sultana *et al.* [36] reported that higher phenolic and antioxidant activity contents of plant materials were obtained from aqueous organic solvents than from anhydrous solvents, therefore, it is important to investigate suitable extraction solvents to optimize antioxidant activity of plants [37].

Considering the rapid emergence of drug-resistant bacterial strains, the need for new and effective treatments is crucial. Given the widespread use of *R. vomitoria* in herbal medicine, scientific research into its phytochemicals and their effects in biological systems cannot be overemphasized. Therefore, in this study, we compared the phytochemical, antioxidant, and antibacterial activities of aqueous and ethanolic extracts of *R. vomitoria* leaves.

2. MATERIAL AND METHODS

2.1 Chemical and reagents

All reagents and chemicals including rutin, gallic acid, potassium ferricyanide ($K_3Fe(CN)_6$), Folin-Ciocalteu, quercetin, aluminum chloride ($AlCl_3$), sulphuric acid (H_2SO_4), oxalic acid, ferric chloride ($FeCl_3 \cdot 7H_2O$), trichloroacetic acid (TCA), hydrochloric acid (HCl), Na_2CO_3 , catechin, ammonium molybdate, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid diammonium salt) (ABTS) were manufactured by Sigma-Aldrich, England. All the reagents used were of analytical grade. The bacterial strains including; *Staphylococcus aureus*, ATCC 29213, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* 25922, *Bacillus pumilus* ATCC 14884, *Pseudomonas aeruginosa* ATCC

19582, *Staphylococcus epidermis* ATCC 12228 were gotten from the Department of Biochemistry and Microbiology, University of Fort Hare in South Africa.

2.2 Collection and Identification of Plant Materials and Preparation of Extract

Fresh plant leaves of *Rauwolfia vomitoria* were obtained from Ngor Okpalain Imo State, Nigeria. These plant samples were identified and authenticated at the Department of Crop Science & Biotechnology, Faculty of Agriculture and Veterinary Medicine, Imo State University where the TSN: 565450 was issued. *Rauwolfia vomitoria* leaves were washed using a running tap water to remove accumulated dirt on the surface of the leaves. The leaves were air dried at room temperature, ground into fine powder and weighed. 124g and 81g of the resulted sample were extracted with distilled water and 100% ethanol respectively [26]. The percentage yield of the aqueous extraction was 8.17%, and ethanol extraction was 24.9%.

2.3 Phytochemical Analysis

2.3.1 Qualitative tests:

The qualitative analysis of the following phyto molecules; saponin, tannin, reducing sugars, flavonoids, alkaloids, phenols, terpenoids and Cardiac glycosides were investigated in the crude aqueous and ethanol extract according to documented standard methods [38-40].

2.3.2 Quantitative tests:

The quantitative determination of saponin, phenols, flavonoids, tannins, alkaloids and terpenoids were carried out according to standard method as stated by Harborne [41] (1973), phytates and

steroid contents were determined according to the method described Okeke and Elekwa [42], while glycosides were determined according to the method described by Ezeonu and Ejikeme [43].

2.4 Antioxidant Assays

DPPH radical scavenging activity was assayed according to the method described by Koksal *et al* [44]. 1 mL each of 0.33 M DPPH in methanol, methanol and extraction fraction (10 – 50 mg/mL) were dispensed into a 10 mL test tube, mixed and incubated for 10 mins in a dark cabinet. A positive control (ascorbic acid solution) and a negative control (DPPH solution) were also assayed alongside. The scavenging activity was measured at an absorbance of 517 nm and percentage of scavenging inhibition calculated according to the equation as below:

$$\text{Scavenging inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

A_{control}

Where A_{control} = Absorbance of negative control; A_{sample} = Absorbance of extract fraction or ascorbic acid

2.5 Determination of proximate

The crude protein, fat, fibre, ash, moisture and carbohydrate content of *Rauvolfia vomitoria* leaves were assayed according to the procedures of AOAC [45].

2.5 Investigation on Antimicrobial Activities

The antimicrobial activity was determined by agar diffusion method.

Preliminary dilutions of the ethanol and aqueous extracts were prepared by making serial two fold dilutions. One in ten dilution of the extract and molten Mueller–Hinton agar were prepared, allowed to equilibrate at 50 °C in a water bath, mixed thoroughly and poured aseptically into Petri dishes to a depth of 3 to 4 mm. Mueller–Hinton agar plates without the plant extracts was used as controls. The bacteria isolates were standardized using the 0.5 McFarland turbidity standards. Standardized (0.5 McFarland turbidity standards) bacteria strains were inoculated in sterile plates of Mueller-Hinton agar. With the aid of Cork borer, 6mm wells were bored in the Mueller-hinton agar. Using standardized pipettes, 107 CFU/mL of the extracts were placed in each respective wells and then incubated at 37⁰C for 24h. A plate inoculated with distilled water to ensure there was no contamination and Mueller–Hinton agar plates without the plant extracts were used as controls. The width zone diameter of inhibition were measured and recorded in millimeters [46].

2.6 FTIR Analysis

This analysis was conducted using fourier transform infrared (FTIR) spectroscopic technique to determine the different functional groups present in the aqueous extract *Rauvolfia vomitoria* leaf.

2.7 Statistical Analysis

Differences between the means were calculated by using student t-test, and the significant levels of the data were calculated by analysis of variance according to Steels and Torrie [47].

3. RESULTS

3.1 Proximate Analysis of *Rauvolfia vomitoria* leafs

The Proximate Analysis of *Rauvolfia vomitoria* leafs is presented in Table 1. The leaf has moisture content of 8.90%, Ash content of 7.70% and Crude fibre of 20.00%. Carbohydrate

content of the leaf stood as 41% while the crude protein and total fat contents were 17.00% and 5.28% respectively.

Table 1: Proximate Analysis of *Rauvolfia vomtoria* leaves

Parameters	Value
Moisture Content (%)	8.90±0.23
Ash Content (%)	7.70±0.13
Crude Fibre (%)	20.00±0.10
Total Fat (%)	5.28±0.46
Crude Protein (%)	17.00±0.69
Carbohydrate (%)	41.00±0.25
Metabolisable Energy (Kcal/Dm ³)	1218.±9.70

Values are presented as Mean ± SD of three determinations.

3.2 Qualitative Phytochemical screening of Aqueous and Ethanolic Extracts of *Rauvolfia vomtoria* leaves

The result of the qualitative phytochemical screening of aqueous and ethanolic Extracts of *Rauvolfia vomtoria* leaves is shown in Table 2. Cardiac glycoside, tannins, phenols, saponins, and phytates were detected as well as steroids, terpenoids and alkaloids.

Table 2: Qualitative Phytochemical Analysis of Aqueous and Ethanolic Extracts of *Rauvolfia vomtoria* leaves

Phytochemical Parameter	Aqueous Extract	Ethanol Extract
Saponins	+	+
Tannins	++	+++
Phenols	+	+
Flavonoids	+	++
Steroids	+	++
Terpenoids	+	+
Cardiac glycosides	+	+
Alkaloids	+	+
Phytate	+	++

Key: Present = +; Absent = -

3.3 Quantitative Phytochemical screening of Aqueous and Ethanolic Extracts of *Rauvolfia vomtoria* leafs

The result of the quantitative phytochemical screening of aqueous and ethanolic Extracts of *Rauvolfia vomtoria* leafs is shown in Table 3. Significantly higher levels of saponins, tannins, flavonoids, steroids, alkaloids, cardiac glycosides and phytate were found in the ethanol extract compared to the aqueous extract. There was no significant difference ($p > 0.05$) in the quantity of phenols and terpenoids in the two extracts.

Table 3: Quantitative Phytochemical Analysis of Aqueous and Ethanolic Extracts of *Rauvolfia vomtoria* leafs

Phytochemical Parameter (mg/g)	Aqueous Extract	Ethanol Extract
Saponins	8.50±0.32 ^a	9.40±0.10 ^b
Tannins	12.00±0.23 ^a	18.00±0.09 ^b
Phenols	4.20±0.09 ^a	4.70±0.02 ^a
Flavonoids	3.10±0.12 ^a	12.00±0.16 ^b
Steroids	3.40±0.19 ^a	12.00±0.21 ^b
Terpenoids	2.80±0.071 ^a	3.00±0.032 ^a
Cardiac glycosides	3.60±0.11 ^a	4.60±0.12 ^b
Alkaloids	3.40±0.078 ^a	4.70±0.022 ^b
Phytate	8.70±0.03 ^a	14.00±0.24 ^b

Values are presented as Mean ± SD of three determinations. Values with different superscripts in the same row differ significantly ($p < 0.05$)

3.4 Vitamin B Composition of Aqueous and Ethanol Extracts of *Rauvolfia vomtoria* leafs

The Vitamin B composition of the aqueous and ethanolic extracts of *Rauvolfia vomtoria* leafs is shown in Table 4. Thiamine content in the aqueous extract was 0.20 while that in the ethanol extract was 0.27. Riboflavin was higher in the ethanol extract (1.80) than the content in the aqueous extract (1.40). Similarly, Niacin in the ethanol extract was 0.81 while that in the aqueous extract was 0.81. No significant difference ($p > 0.05$) was found in the vitamin B composition of the two extracts.

Table 4: Vitamin B Composition Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* leaves

Vitamin	Aqueous Extract	Ethanol Extract
Thiamine B1 (mg/100 g)	0.20±0.015 ^a	0.27±0.015 ^a
Riboflavin B2 (mg/100 g)	1.40±0.049 ^a	1.80±0.13 ^a
Niacin B3 (mg/100 g)	0.81±0.031 ^a	0.84±0.046 ^a

Values are presented as Mean ± SD of three determinations. Values with different superscripts in the same row differ significantly (p<0.05).

3.5 Mineral Contents of Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* leaves

Table 5 shows the mineral contents of aqueous and ethanolic extracts of *Rauvolfia vomitoria* leaves. There was no significant difference (p>0.05) in the values of calcium, magnesium and phosphorus recorded for the two extracts. Whereas there was significant difference in the sodium, potassium, zinc, iron and copper contents of the two extracts with the aqueous extract having higher quantity of potassium and zinc. Sodium, iron and copper were higher in the ethanol extract.

Table 5: Mineral Contents of Aqueous and Ethanol Extracts of *Rauvolfia vomitoria* leaves

Mineral (mg/100 g)	Aqueous Extract	Ethanol Extract
Calcium	18.00±0.19 ^a	19.00±0.38 ^a
Magnesium	130.00±0.77 ^a	129.00±2.00 ^a
Phosphorus	72.00±0.37 ^a	72.00±0.99 ^a
Sodium	14.00±0.44 ^a	16.00±0.17 ^b
Potassium	295.00±1.30 ^a	283.00±3.40 ^b
Zinc	0.93±0.044 ^a	0.79±0.036 ^b
Iron	53.00±2.40 ^a	57.00±0.50 ^b
Copper	0.50±0.010 ^a	0.58±0.032 ^b

Values are presented as Mean ± SD of three determinations. Values with different superscripts in the same row differ significantly (p<0.05).

3.6 Anti-microbial Effects of Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* Leaves

Table 6 shows the anti-microbial Effects of Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* Leafs. The aqueous and ethanolic extracts had zones of inhibition suggesting susceptibilities of the organisms ranging from 10.20 to 19.50 at the various concentrations tested. In all the tests, the ethanolic extract had higher inhibition against test organisms compared to the aqueous extract.

Table 6: Anti-microbial Effects of Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* Leafs (Zone of inhibition in mm).

Test Organisms	Ethanolic Extract					Aqueous Extract				
	12.5	25	50	75	100	12.5	25	50	75	100
<i>Enterobacter faecalis</i>	14.30	13.90	16.20	11.70	16.40	11.80	11.10	12.70	12.50	15.40
<i>Klebsiella pneumoniae</i>	11.40	12.10	13.80	16.10	19.50	10.00	12.00	15.20	15.00	18.10
<i>Salmonella typhii</i>	10.20	13.60	15.20	13.70	16.60	12.30	15.90	17.10	18.50	19.30
<i>Cetrobacterfreundi</i>	11.50	12.10	15.80	15.30	15.50	9.80	11.40	12.40	13.10	14.00
<i>Shigella flexneri</i>	13.40	16.10	11.90	15.50	17.20	9.80	11.90	12.30	11.70	13.10

3.7 *In vitro* Antioxidant Activities of Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* Leafs

The *in vitro* antioxidant activities of aqueous and ethanolic extracts of *Rauvolfia vomitoria* leaves is presented in Table 7. At 10% concentration, the aqueous and ethanolic extracts had DPPH scavenging activities of 87.29% and 92.03%. There was a dose-dependent increase in percentage

inhibitory activity of the extracts against DPPH radical. The ethanolic extract showed the highest DPPH radical inhibitory activity at concentration of 50%.

With regards to FRAP and nitric oxide (NO) inhibition, inhibitory activity of the extracts reduced their concentrations increased.

Table 7: *In vitro* Antioxidant Activities of Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* Leafs

Antioxidant Assays	Ethanolic Extract					Aqueous Extract				
	10%	25%	50%	75%	100%	10%	25%	50%	75%	100%
DPPH	87.29	97.13	98.84	80.94	77.06	92.03	97.28	98.04	80.94	76.95
FRAP	17.71	1.49	0.04	0.00	0.00	13.60	41.56	0.04	0.00	0.00
NOSA	119.93	41.61	22.36	0.34	28.00	19.91	41.56	22.21	0.96	27.87

3.8 FTIR Analysis of Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* Leafs

The qualitative analysis of phytochemicals revealed that both aqueous and ethanolic extracts of *Rauvolfia vomitoria* leaf contained diverse phytochemical compounds. Therefore, to further investigate these extracts, FTIR analysis was conducted to identify the functional groups present in the natural compounds present in the extracts. Fourier transform infrared (FTIR) spectroscopic technique was used to identify the presence of functional groups in aqueous (Fig 1 and Table 8) and ethanolic (Fig 2 and Table 9) extracts of *Rauvolfia vomitoria* leaf

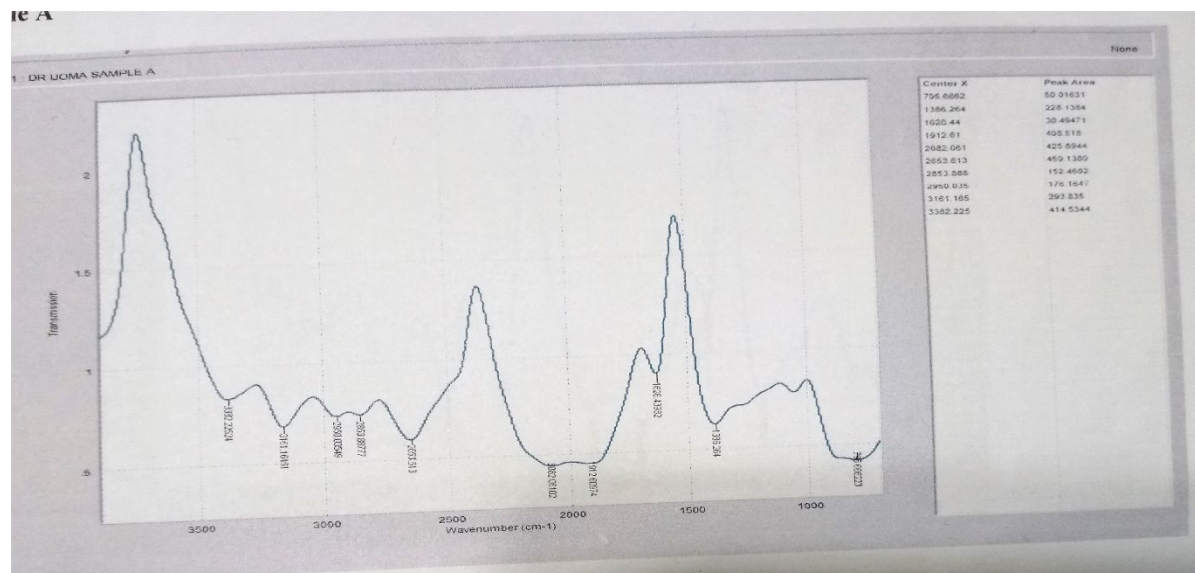


Figure 1: FTIR spectra of Aqueous Extract of *Rauvolfia vomitoria* Leaves

Table 8: FTIR spectra of Aqueous Extract of *Rauvolfia vomitoria* Leaves

S/N	Wavelength (cm ⁻¹)	Functional group	Compounds
1	795.6662	C-Cl	Chloro Cl symmetric stretch
2	1385.264	H ₂ C=CH	Ethene CH anti-symmetric stretch
3	1628.440	RNH ₃	1 ⁰ amine NH stretch
4	1912.610	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
5	2082.061	RCOOH	Carboxylic acid C=O stretch
6	2653.613	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
7	2853.888	CH ₂	Methylene CH stretch
8	2950.035	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
9	3161.165	RCHOH	1 ⁰ alcohol OH stretch
10	3382.255	R ₂ CHOH	2 ⁰ alcohol OH stretch

From the table of results above, the peak values around 795.6662 cm⁻¹ was due to C-Cl stretching vibration of halogenous compounds. The height around 1385.264 cm⁻¹ was assigned to C=C stretching vibration of vinylidene compound. The medium band around 1628.440 cm⁻¹ was due to N-H stretching vibration of 1⁰ amine compound. The absorbance around 1912.610 cm⁻¹ and 2950.035 cm⁻¹ were due to SCN stretching vibration of thiocyanate compound. The band around 2082.061 cm⁻¹ was due to C=O anti-symmetric stretching vibration of carboxylic acid. The weak

band around 2653.613 cm^{-1} and 2853.888 cm^{-1} were assigned to C-H stretching vibration of methylene compounds. The strong band around 3161.165 cm^{-1} was and 3382.225 cm^{-1} were due to OH stretching vibration of 1° and 2° alcoholic compounds respectively.

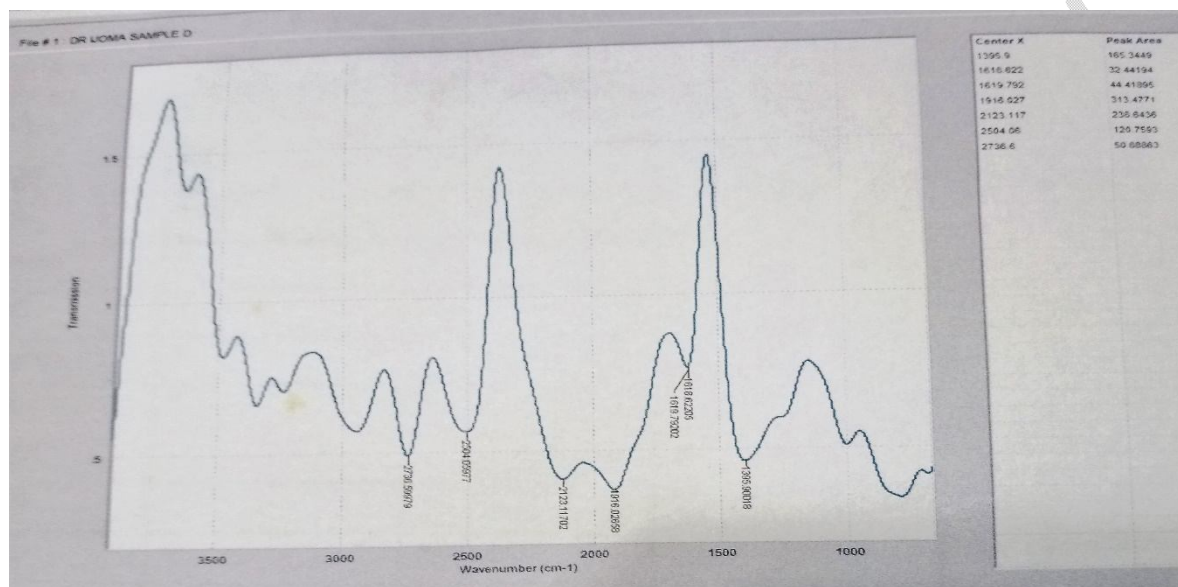


Figure 2: FTIR spectra of Ethanolic Extract of *Rauwolfia vomitoria* Leaves

Table 9: FTIR spectra of Ethanolic Extract of *Rauwolfia vomitoria* Leaves

S/N	Wavelength (cm^{-1})	Functional group	Compounds
1	1395.900	$\text{H}_2\text{C}-\text{CH}$	Ethene CH anti-symmetric stretch
2	1618.622	RNH_3	1° amine NH stretch
3	1619.792	RNH_3	1° amine NH stretch
4	1916.027	$\text{R}-\text{S}-\text{C}\equiv\text{N}$	Thiocyanate SCN antisymmetric stretch
5	2123.117	RCOOH	Carboxylic acid C=O stretch
6	2504.060	RCOOH	Carboxylic acid C=O stretch
7	2736.600	CH_2	Methylene CH stretch

From the table of results above, the absorbance around 1395.900 cm^{-1} was due to C=C stretching vibration of ethene compounds. The medium band around 1618.622 cm^{-1} and 1619.792 cm^{-1} corresponds to N-H stretching vibration of 1° amine compounds. The absorbance around

1916.027 cm^{-1} was due to SCN stretching vibration of thiocyanate compound. The band around 2123.117 cm^{-1} was assigned to C=O anti-symmetric stretching vibration of carboxylic acid, whereas the wavelength around 2504.060 cm^{-1} was assigned to CN anti-symmetric stretching vibration of nitrile compound. The weak band around 2736.600 cm^{-1} was assigned to CH stretching vibration of methylene compounds.

4. DISCUSSION

Since biologically active compounds occur naturally in very small concentrations, the choice of an extraction method and the corresponding suitable solvent is an important step in the drug discovery process [46]. This work compared the phytochemicals, minerals, antioxidant and antimicrobial activities of aqueous and ethanolic extracts of *Rauwolfia vomitoria* leaves.

The result of proximate content of *Rauwolfia vomitoria* leaf (Table 1) shows that the plant is rich in carbohydrates, moisture, protein, ash, fats and fiber and it is in consonance with the report of [48]. This indicates that *Rauwolfia vomitoria* leaf is a good source of carbohydrate with high energy values for human and livestock [48].

The availability of phytochemicals depends on the solubility of the compounds in the solvent [33]. The results of the qualitative phytochemical screening of *Rauwolfia vomitoria* are shown in Table 2. Cardiac glycosides, tannins, phenols, saponins, and phytates, as well as steroids, terpenoids, and alkaloids were detected in the aqueous and ethanolic extracts of *Rauwolfia vomitoria* leaves (Table 2). This is consistent with the results of [5, 24, 33, 49]. Secondary metabolites are mainly produced by plants as products of primary metabolism and as part of plant defense mechanisms. Phytochemicals such as alkaloids, tannins, and flavonoids are examples of plant-produced phytochemicals that are believed to be responsible for the healing properties

of plants [50]. Phenolic compounds are associated with antioxidant activity due to their ability to scavenge free radicals [51]. Compounds such as flavonoids, resins, saponins, and tannins have been shown to have healing properties against most pathogenic bacteria [52]. Moreover, these phytochemicals act as the best antioxidants and protect cells from free radical damage, such as carotenoids, polyphenols, etc., or minimize the risk of cancer by inhibiting tumor development, hormonal stimulation, and antibacterial activity [53]. Flavonoids are a group of hydroxylated phenols, whose beneficial effects are associated with antioxidant, antibacterial, anticancer, and anti-inflammatory properties [54]. Saponins have medicinal properties such as anti-inflammatory, cytotoxic anticancer, antibacterial, and activities [55]. Alkaloids have also been shown to exhibit antioxidant properties by reducing oxidative damage caused by hydrogen peroxide [56].

In this study, significantly higher concentrations of saponins, tannins, flavonoids, steroids, alkaloids, cardiac glycosides, and phytates were found in the ethanol extracts compared to the aqueous extracts (Table 5). Similar results were also reported [37, 57]. The solubility of glycosides and phenols in the extraction solvent depends on the functional groups attached to the main structure of these phytochemicals, as well as the molecular size and length of the hydrocarbons [58]. Furthermore, the solvation potential determines the solubility of phytochemicals. The variation in phytochemical content may be due to the difference in the extractability of different solvents. It has been said that ethanol is suitable for the extraction of compounds with a wide range of polarities, whereas water is suitable for the extraction of very polar compounds [59]. As a result, higher amounts of flavonoids and phenolic compounds were found in the ethanol samples than in the water extracts; hence, better activity. This is consistent with previous reports that ethanol is more suitable for the extraction of phenolic compounds in plants [60]. Phytochemicals in plants have been reported to have strong biological activities,

including antibacterial activity [61]. Previous qualitative and quantitative studies have similarly reported the presence of tannins, saponins, and flavonoids in *R. Thevomitus* remains on the leaves, although in different concentrations [62-63].

Plant extracts are believed to have various protective functions due to their content of phytochemicals that contribute significantly to antioxidant and antibacterial activity [64-65]. Oxidative stress leads to the production of free radicals in the body and plays a key role in the development of chronic degenerative diseases such as cancer, arthritis, aging, autoimmune diseases, cardiovascular diseases, and neurodegenerative diseases. Antioxidants play a key role in inactivating and scavenging free radicals, thus reducing the risk of these chronic diseases. The aqueous and ethanolic extracts in this study had zones of inhibition indicating microbial susceptibility, with the ethanolic extract showing higher antibacterial activity (Table 6). Ethanol solvent can extract a large amount of polar metabolites, which, in turn, contain important antibacterial agents [66]. Furthermore, the percentage of inhibitory activity of the extracts against DPPH radical increased in a dose-dependent manner, which is consistent with the previous report by Helan and Vignesh [2]. The ethanol extract showed the highest DPPH radical inhibitory activity at a concentration of 50% (Table 7). The presence of glycosides, flavonoids, and phenolic compounds may be responsible for the high antioxidant properties of the ethanol extract. This may be due to the presence of polar antioxidant compounds.

The significant association between phenolics and bioactivity was consistent with previous evidence suggesting the important contribution of phenolics and flavonoids [67]. Higher levels of flavonoids, essential biocompounds required for radical scavenging activity, are also associated with improved DPPH radical scavenging activity [32, 37]. The DPPH test is a common spectrophotometric technique for measuring antioxidant activity. The advantage of this

method is that antioxidant activity is usually measured at ambient temperature, thus eliminating the risk of thermal degradation of the tested molecules. Radical scavenging is a known mechanism by which lipid oxidation is inhibited by antioxidants [68]. Differences in the polarity of antioxidants may be the reason for the variation in activity observed. Moreover, solvent polarity is known to play an important role in enhancing the solubility of phenolic biocompounds [69].

In this study, the antioxidant activity of *Rauwolfia vomitoria* leaf extracts was also evaluated using the FRAP method, which is based on the ability to reduce ferric ions to their ferrous form. The results showed that the ethanolic extract had significantly higher FRAP values than the aqueous extract. Similar results were also reported by Tourabiet *et al.* [70]. Flavonoids and phenolic compounds are the two most important and widespread polyphenols found in medicinal plants [71]. Both phenols and flavonoids are known for their free radical scavenging activity. This is due to the predominance of hydroxyl groups that have the inherent ability to donate hydrogen atoms to free radicals, thus stopping the accumulation of free radicals in the body [49]. The study also found that the extract caused NO inhibition, suggesting its potential utility as a treatment for inflammatory diseases and vascular dysfunction caused by NO dysregulation, such as cancer. The relationship between NO inhibition and the antiproliferative activity of medicinal plants was reported by Carabajal *et al.* [72].

The results of mineral composition of *Rauwolfia vomitoria* shown in Table 4 are consistent with the report by Ugwu *et al.* [48], where the three highest minerals are K > Mg > P > Na. These results are in line with a previously published study [73], which showed the presence of significant amounts of minerals in medicinal plants. Minerals are necessary for normal growth, muscle activity and skeletal development, cellular activity and oxygen transport (copper and iron), chemical reactions in the body and intestinal absorption (magnesium), fluid balance and

neurotransmission (sodium and potassium) [73]. Their presence is necessary for the maintenance of certain vital physicochemical processes. Although they do not provide energy, they play a key role in many body activities. Minerals are involved in maintaining acid-base balance and regulating body fluids. Some are cofactors in enzyme reactions. Minerals are used in the formation of hemoglobin and thyroxine. Some minerals play a role in antioxidant functions. They also transport gases and aid in muscle contraction [74].

The vitamin B composition of aqueous and ethanolic extracts of *Rauvolfia vomitoria* leaves (Table 3) is in agreement with the papers of Ugwu *et al.* [24] and Alagbe [75] who demonstrated the presence of vitamin B in the leaf and root extracts of *Rauvolfia vomitoria*, respectively. This is also in agreement with the study of Traber [76]. Vitamins are essential for human health, growth, development, reproduction and maintenance, and vitamin deficiency poses serious threats to health [77]. They are diverse in nature compared to fats, carbohydrates and proteins, and are distinguished from other groups by their organic nature, and their classification depends on their chemical properties and functions [78]. Vitamin B1 is essential for the normal functioning of the nervous system, digestive system and brain [79]. Vitamin B2 provides antioxidant protection and promotes iron metabolism in the body [80]. Vitamin B12 also plays an important role in energy metabolism and other biological processes, while Vitamin B3 is responsible for antioxidant defense. Vitamins found in food have been shown to have a positive effect on health. Epidemiological studies have shown that high vitamin intakes are associated with a lower incidence of some cancers and cardiovascular diseases.

The results of this study showed that both the aqueous and ethanolic leaf extracts contained significant amounts of vitamins that are part of the nutritional requirements of humans and

livestock, suggesting that these leaves may be useful as feed supplements to improve the health and growth of humans and livestock [48; 81] (Ugwuet *al.*, 2022; Zorodduet *al.*, 2019).

5. CONCLUSION

In this work the phytochemicals, minerals, vitamins, antioxidant and antimicrobial activities of aqueous and ethanolic extracts of *Rauvolfia vomtoria* leaves were compared using standard methods. In many of the parameters examined, the ethanolic extract had better performance than the aqueous extract. The results confirm that ethanol is a better solvent than water for extraction of biological active molecules from *R. vomtoria* leaf.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

REFERENCES

1. Efekemo O, Orororo OC. Effect of mixture of leaf extract of *Ocimum gratissimum* and *Vernonia amygdalina* on cadmium induced hepatotoxicity in wistar rats. *J Biochemistry Intl.* 2022; 9(4):73-81
2. Helan CJ, Vignesh T. In vitro and In silico Study of Ethanolic Leaf Extracts of *Rauvolfia Canescens*. *Research Journal of Chemistry and Environ.* 2016; 20 (8):31-37.
3. Ashvini YP, Gunjan PM, Aaysha RS, Wrushali AP, Jagdish VM, Ravindra LB. Ethnopharmacological review of traditional medicinal plants as immunomodulator. *World J Bio Pharm Health Sci.* 2021; 6 (2): 043-055.
4. Tin MH, Kyi KS, Hazwan H. Comparative Study on Phytochemical Screening and Antioxidant Activity of Aqueous Extract from Various Parts of *Bauhinia purpurea*. *Bioactivities*, 2023; 1 (1):24-31.

5. Ajayi, OA. Phytochemical and GC-MS analysis of bioactive components in ethanolic extract of *Rauvolfia vomitoria* Leaves. J Chem Soc Nig.2021; 46 (4): 0656 – 0660
6. Gengatharan, A.; Dykes, G.A.; Choo, W.S. Betalains: Natural plant pigments with potential application in functional foods. LWT Food Sci. Technol. 2015, 64, 645–649.
7. Gandía-Herrero, F.; Escribano, J.; García-Carmona, F. Biological activities of plant pigments betalains. Cri. Rev. Food Sci. Nutr. 2016, 56, 937–945.
8. Onobrudu, D.A., Ikewuchi, J.C. and Onyeike, E.N. (2016). Moderation of ocular markers of oxidative stress of selenite induced cataractous pups by *Boswellia dalzielii* Hutch extract. Global Advanced Research Journal of Medicinal Plant 4(2): 007 – 011
9. Orororo, O. C, Asagba, S. O., Egbune, E. O. and Efejene, O. I. (2022). Sperm parameters and histological changes in testes of cadmium-exposed rats treated with *Hibiscus sabdarrifa*L. anthocyanins. *Sokoto Journal of Medical Laboratory Science*; 7(3):114-122.
10. Pohl, F.; Thoo Lin, P.K. The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases: In vitro, in vivo and clinical trials. *Molecules* 2018, 23, 3283.
11. Hrelia, S.; Angeloni, C. New Mechanisms of Action of Natural Antioxidants in Health and Disease. *Antioxidants* 2020, 9, 344.
12. Onobrudu, D.A. Onyeike, E.N. and Ikewuchi, J.C. (2017). Effect of methanolic leaf extract of *Boswellia dalzielii* hutch on ocular ATPase profiles of selenite-induced cataract in wister pup. *Journal of Complementary and Alternative Medical Research* 2(2): 1-7
13. Orororo, O. C. and Asagba, S. O. (2022). Treatment with *Hibiscus sabdarrifa* L Anthocyanins Improve Hematological Parameters in Rats Exposed to Cadmium. *Journal of Exploratory Research in Pharmacology* 2022 vol. 000(000)| 000-000 Epub DOI: 10.14218/JERP.2022.00003
14. Ekakitie, L. I., Orororo O. C. and Okpoghono, J., (2021). Changes in haematological parameters of aluminium-exposed rats treated with natural bee honey. *IOSR Journal of Environmental Science, Toxicology and Food Technology*; 15(5):22-25
15. Kumar, S. Free Radicals and Antioxidants: Human and Food System. *Adv. Appl. Sci. Res.* 2011, 2, 129–135.
16. Izzo, S.; Naponelli, V.; Bettuzzi, S. Flavonoids as Epigenetic Modulators for Prostate Cancer Prevention. *Nutrients* 2020, 12, 1010.
17. Carrera, I.; Martínez, O.; Cacabelos, R. Neuroprotection with Natural Antioxidants and Nutraceuticals in the Context of Brain Cell Degeneration: The Epigenetic Connection. *Curr. Top. Med. Chem.* 2019, 19, 2999–3011.
18. Orororo, O. C., Asagba, S. O, Tonukari, N. J., Okandeji, O. J., and Mbanugo J. J. (2018). Comparative Assessment of the Antioxidant Properties of *Hibiscus sabdarrifa*L Anthocyanins and its Aqueous Extract in Cadmium-exposed Rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*; 9(3): 836-843
19. Onobrudu, D.A. (2017). Saponins and polyphenolics of methanol leaf extract of *Boswellia dalzielii* Hutch. *Archives of current Research International* 8(3): 1- 6
20. Majeed, M.; Pirzadah, T.B.; Mir, M.A.; Hakeem, K.R.; Alharby, H.F.; Alsamadany, H.; Bamagoos, A.A.; Rehman, R.U. Comparative Study on Phytochemical Profile and Antioxidant Activity of an Epiphyte, *Viscum album* L. (White Berry Mistletoe), Derived from Different Host Trees. *Plants* 2021, 10, 1191.

21. Naz, R.; Roberts, T.H.; Bano, A.; Nosheen, A.; Yasmin, H.; Hassan, M.N.; Anwar, Z. GC-MS analysis, antimicrobial, antioxidant, antilipoxygenase and cytotoxic activities of *Jacaranda mimosifolia* methanol leaf extracts and fractions. *PLoS ONE* 2020, 15, e0236319.
22. Asoro^{1*}, I. I. Ebuehi¹ O. A. T. and Igwo-Ezikpe¹ M. N. GC-MS Analysis of the *Rauwolfia vomitoria* Ethanol Extracts. *European Journal of Medicinal Plants* 32(6): 34-45, 2021; Article no.EJMP.68096
23. Lobay D. *Rauwolfia* in the Treatment of Hypertension. *Integrative medicine (Encinitas, Calif.)*. 2015;14(3):40–46.
24. Ugwu Okechukwu P. C., 1Okon M. B., 1Nweze T. K., 2Mba A. N., 3Ozioko Paul C. and 1Nweke O. L (2019). Phytochemical Analysis of Ethanol Leaf Extract of *Rauwolfia vomitoria*.. *INOSR APPLIED SCIENCES* 5(1): 35-40, 2019. 35
25. Ezekwesili-Ofili JO, Okaka ANC. *Herbal Medicines in African Traditional Medicine*; 2019. (4): 649–652.
26. Youmbie DDB, Dzeufiet DPD, Nkwengoua ZE, Zingue S, Mezui C, Bibi FAO, Tankeu NF, Pieme CA, Dimo T. Anti-Inflammatory and Antioxidant Effects of the Stem Bark Aqueous Extract of *Rauwolfia Vomitoria* (Apocynaceae) In Female Wistar Rats. *European Journal of Pharmaceutical and Medical Research*. 2015;2(7):64-73.
27. Oboh G, Adebayo AA, Ademosun AO (2019). HPLC phenolic fingerprinting, antioxidant and anti-phosphodiesterase-5 properties of *Rauwolfia vomitoria* extract. *J Basic Clin PhysiolPharmacol*. 30:/jbcpp.2019.30.issue-5/jbcpp-2019-0059/jbcpp-2019-0059.xml
28. Dong R, Chen P, Chen Q (2018). Inhibition of pancreatic cancer stem cells by *Rauwolfia vomitoria* extract. *Oncol Rep*. 40: 3144 - 3154.
29. Tlhapi DB, Ramaite IDI, Van Ree T, Anokwuru CP, Orazio T-S, Hoppe HC. Isolation, chemical profile and antimalarial activities of bioactive compounds from *Rauwolfiacaffra*Sond. *Molecules*. 2019; 24:39.
30. Aquaisua A, Mbadugha C, Bassey E, Ekong M, Ekanem T, Akpanabiatu M. Effects of *Rauwolfia vomitoria* on the cerebellar histology, body and brain weights of albino wistar rats. *Journal of Experimental and Clinical Anatomy, Rauwolfia vomitoria*. 2017;16(1):41.
31. Tavs A Abere, Ogechi KO, Freddy OA, Gerald IE. Antisickling and Toxicological Evaluation of the Leaves of *Rauwolfia vomitoria* Afzel (Apocynaceae). *Journal of Science and Practice of Pharmacy*. 2014; 1(1):11-15.
32. Okoro IO. Effects of Extraction Solvents on the Antioxidant and Phytochemical Activities of *Manihot Esculenta* Leaves. *Iranian Journal of Toxicology*. 2020; 14(1):51-58.
33. Uba, Garba Hambali Dauda¹, Kabiru Musa Aujara¹, and Umar Ali² Solvent Extraction and its Effects on the Phytochemical Yield and Antioxidant Capacity of *Commiphora africana*(Bursaceae). *Bioremediation Science and Technology Research*, 2020, Vol 8, No 2, 8-11
34. Pham H, Nguyen V, Vuong Q, Bowyer M, Scarlett C. Effect of Extraction Solvents and Drying Methods on the Physicochemical and Antioxidant Properties of *Helictereshirsuta*Lour. Leaves. *Technologies*. 2015;3(4):285–301.
35. Sallau M, Tajuddeen N, Ndukwe G, Musa A, Dambatta B, Sani Y. Phytochemical and Antimicrobial Properties of *Commiphora Pedunculata* (ENGL) Stem Extracts. *Bayero J Pure Appl Sci*. 2014;7(1):101.
36. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* 2009; 14: 2167-80.

37. Akinmoladun, Afolabi C. Olubusola Elizabeth Falaiye, Olubukola Benedicta Ojo* , Abimbola Adeoti, Zainab Abiola Amoo and Mary Tolulope Olaleye. Effect of extraction technique, solvent polarity, and plant matrix on the antioxidant properties of *Chrysophyllum albidum* G. Don (African Star Apple). *Bulletin of the National Research Centre* (2022) 46:40:1-9
38. Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. *LWT-Food Sci Technol.* 46:548 –555.
39. Trease, G.E. and Evans, W.C. (1989). *Pharmacognosy*. 11th Edition, Bailliere Tindall, London. 45 - 50
40. Sofowora E.A. (1993). *Medicinal plants and traditional medicines in Africa*. 2nd Edition, spectrum Books Ltd, Ibandan, Nigeria. 10 -19
41. Harborne JD (1973) *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall London pp: 279.
42. Okeke, C.U. and Elekwa, I. (2003). Phytochemical study of the extract of *Gongronemalatifolium* Benth. *Journal of Health and Visual Sciences* 5(1): 47 – 55
43. Ezeonu, C.S. and Ejikeme, C.M. (2016). Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwood. *New Journal of Science* 1: 1- 9
44. Koksai E, Bursal E, Dikici E, Tozoglu F, Gulcin I (2011). Antioxidant activity of *Melissa officinalis* leaves. *J Med Plants Res.* 5: 217–222.
45. AOAC (2000) Association of Official Analytical Chemists. *Official Methods of Analysis* 19th Edition Washington DC, USA pp: 69-77.
46. Chigayo K, Mojapelo PE, Bessong P, Gumbo JR. The preliminary assessment of antimicrobial activity of HPLC separated extracts of *Kirkiawilmsii*. *Afr J Tradit Complement AlternMed* 2014; 11: 275-81.
47. Steel RG, Torrie JH (1981). *Principles and Procedures of Statistics: A Biometrical Approach*. 2nd Edn., McGraw Hill Book Co., New York.
48. Ugwu Okechukwu Paul Chima, 2Nweke O.L., 1Alia Lilian Chinazaekperem, 1Okon Michael Ben, 1Onyeke Stanley C. 3,4Eze Chukwuebuka G. and 5Eze Ifeoma Martha. The Mineral Composition of Methanol Leaf Extract of *Rauwolfia vomitoria* *INOSR APPLIED SCIENCES* 8(1):71-75,2022
49. Samuel T.A.1, James A.B.1, Adebisin O.2, Okafor I.1 and Iwalokun, B.A3 Phytochemical Investigation and Anti-Proliferative Effects of *Rauwolfia vomitoria*, *Calliandra portoricensis* and *Anthocleista djalonensis* on Human Breast Cancer (MCF-7) Cell Line *Afr. J. Biomed. Res. Vol. 24 (September, 2021); 403- 411*
50. Bhandary SK, Kumari N, Bhat VS, Sharmila K, Bekal MP. Preliminary phytochemical screening of various extracts of *Punica granatum* peel, whole fruit and seeds. *Nitte Univ J Health Sci* 2012; 2: 34-8.
51. Maria John KM, Ayyanar M, Arumugam T, Enkhtaivan G, Jin K, Kim DH. Phytochemical screening and antioxidant activity of different solvent extracts from *Strychnos minor* Dennst leaves. *Asian Pac J Trop Dis* 2015; 5: 204-9.
52. Khanam Z, Wen CS, Bhat UIH. Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). *J King Saud Uni Sci* 2015; 27: 23-
53. Mezzomo N, Ferreira SRS. Carotenoids Functionality, Sources, and Processing by Supercritical Technology: A Review. *J Chem* .2016;2016:1–16.

54. Khanam UKS, Oba S, Yanase E, *et al.*: Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables. *J Funct Foods*. 2012; 4(4): 979–987.
55. Ashour AS, El Aziz MMA, Melad ASG, *et al.*: A review on saponins from medicinal plants: chemistry, isolation, and determination. *J Nanomed Res*. 2019; 8(1): 6–12.
56. Kibiti CM, Afolayan AJ: Preliminary phytochemical screening and biological activities of *Bulbine abyssinica* caused in the folk medicine in the Eastern Cape Province, South Africa. *Evid Based Complement Alternat Med*. 2015; 2015: 617607.
57. Salami, T. A*, Aderinboye, R. Y*, Akinbode, R. M*, Adebayo, K. O* and Fasae, O. A+. Potentials of *Rauwolfia vomitoria* leaves as an antimethanogenic feed additive for ruminants. Proc. 49th Conf., Nig. Soc. for Anim. Prod. 24 – 27 March, 2024, Univ. of Ibadan, Nigeria, 1905
58. Thavamoney N, Sivanadian L, Tee LH, Khoo HE, Prasad KN, Kong KW. Extraction and recovery of phytochemical components and antioxidative properties in fruit parts of *Dacryodes rostrata* influenced by different solvents. *J Food Sci Technol*. 2018;55(7):2523–32.
59. Sun C, Wu ZS, Wang Z, *et al.*: Effect of ethanol/water solvents on phenolic profiles and antioxidant properties of Beijing Propolis extracts. *Evid Based Complement Alternat Med*. 2015; 2015: 595393.
60. Jimoh MO, Afolayan AJ, Lewu FB: Antioxidant and phytochemical activities of *Amaranthus caudatus* L. harvested from different soils at various growth stages. *Sci Rep*. 2019; 9(1): 12965.
61. Reddy, B. S., Vijayakumar, G., Balasubramaniam, G. A., Sivaramam, S. and Kathirel, S. (2020). Efficacy of neostigmine and azithromycin in buffaloes with functional ileus. *Buffalo Bulletin*, 38
62. Ojo, O. O., Ajayi S. S. and Owolabi, L. O. (2012). Phytochemical screening, anti-nutrient composition, proximate analyses and the antimicrobial activities of the aqueous and organic extracts of bark of *Rauwolfia vomitoria* and leaves of *Peperomia pellucida*. *International Research Journal of Biochemistry and Bioinformatics* 2(6): 127–134.
63. Chinonye, I., Chijioko, C. Iwuji, S., Obiagwu, I., Lynda, U. O., Ukaoma, A., Chijioko-Okere, M. and Ezekoye, M. (2022). Chemical and medicinal properties of *Rauwolfia vomitoria* (AFZEL) harvested from the South Eastern Nigeria. *Asian Journal of Chemical Sciences*, 10: 56–71.
64. Abifarin TO, Otunola GA, Afolayan AJ: Cytotoxicity evaluation and anti-inflammatory potentials of *Cucumis africanus* L.f. leaves. *Med Plants-Inter J PhytomedRelat Indus*. 2020; 12(1): 48–52.
65. Kuspradini H, Wulandari I, Putri AS, *et al.*: Phytochemical, antioxidant and antimicrobial properties of *Litsea angulata* extracts. *F1000 Res*. 2018; 7: 1839.
66. Paiva P.M.G., Gomes F.S., Napoleão T.H., Sá R.A., Correia M.T.S. and Coelho L.C.B.B., Antimicrobial activity of secondary metabolites and lectins from plants. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, Méndez A. (Ed.), 396-406 (2010)
67. Compaoré M, Meda RN-T, Bakasso S, Vlase L, Kiendrebeogo M. Antioxidative, anti-inflammatory potentials and phytochemical profile of *Commiphora africana* (A. Rich.) Engl. (Burseraceae) and *Loeseneriella africana* (Willd.) (Celastraceae) stem leaves extracts. *Asian Pac J Trop Biomed*. 2016;6(8):665–70.

68. Dvorakova M, Moreira MM, Dostalek P, Skulilova Z, Guido LF, Barros AA. Characterization of monomeric and oligomeric flavan-3-ols from barley and malt by liquid chromatography-ultraviolet detection-electrospray ionization mass spectrometry. *J Chromatogr A*. 2008; 1189(1-2):398-405.
69. Naczki M, Shahidi F. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J Pharm Biomed Anal*. 2006;41(5):1523-42.
70. Tourabi Meryem 1*, Amira Metouekel 2, Asmae E. L. ghouizi 1,3, Mohamed Jeddi 4, Ghizlane Nouiouara 1, Hassan Laaroussi 1, Md. Eram Hosen 5, Kawtar Fikri Benbrahim 4, Mohammed Bourhia 6, Ahmad Mohammad Salamatullah 7, Hiba Allah Nafidi 8, Gezahign Fentahun Wondmie 9*, Badiaa Lyoussi 1 & Elhoussine Derwich 1,1 Efficacy of various extracting solvents on phytochemical composition, and biological properties of *Mentha longifolia* L. leaf extracts. *Scientific Reports* | (2023) 13:18028 |
71. Neveu V, Perez-Jiménez J, Vos F, Crespy V, du Chaffaut L, Mennen L, Knox C, Eisner R, Cruz J, Wishart D, Scalbert A. (2010): Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxford). bap024. doi: 10.1093/database/bap024.
72. Carabajal MPA, Piloto-Ferrer J, Nicollela HD, Squarisi IS, Prado Guissone AP, Esperandim TR, Tavares DC, Isla MI, Zampini IC (2020). Antigenotoxic, antiproliferative and antimetastatic properties of a combination of native medicinal plants from Argentina. *J Ethnopharmacol*. 20: 113479.
73. Moneim, A. and Sulieman, E. (2019). *Rauwolfia vomitoria*: Chemical Composition. In: Mariod A. (eds) Wild Fruits: Composition, Nutritional Value and Products. *Springer*, pp 285-299
74. Murray, R. K., Gramer D.K., Mayes P.A. and Rodwell V.W. (2000). Harper's Biochemistry, 25th edition, McGraw Hill, health Profession Division, USA. 25.
75. Alagbe JO. Dietary Supplementation of Rauwolfia Vomitoria Root Extract as A Phyto-genic Feed Additive in Growing Rabbit Diets: Growth Performance and Caecal Microbial Population. *Con Dai & Vet Sci* 4(2)- 2021.
76. Traber, M. G. (2014). Vitamin E inadequacy in humans: causes and consequences. *Advances in Nutrition*, 5(5):503-14.
77. Muhammad Amir Maqbool, Muhammad Aslam, Waseem Akbar, Zubair Iqbal (2017) Biological importance of vitamins for human health: A review. *J Agric Basic Sci* 2(3): 50-58.
78. Asensi Fabado MA, Munne-Bosch S (2010) Vitamins in plants: occurrence, biosynthesis and antioxidant function. *Trends Plant Sci* 15(10): 582-592.
79. Keogh JB, Cleanthous X, Wycherley TP, (2012) Increased vitamin B1e intake may be required to maintain vitamin B1e status during weight loss in patients with type 2 diabetes. *Diabetes Res Clin Pract* 98: 40-42.
80. Lanska DJ (2010) Chapter 30: historical aspects of the major neurological vitamin deficiency disorders: the water-soluble B vitamins. *Handbook Clin Neurol* 95: 445-476.
81. Zoroddu M, Aashet J, Crisponi G, Medici S, Peana M, Nurchi V. The essential metals for humans: a brief overview. *J Inorganic Biochem*. 2019; 195: 120-129.