Original Research Article

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

ABSTRACT

Inthisstudy, the phytochemicals, B vitamins, minerals, antioxidants and antimicrobial activities of aqueous and ethanolic extracts of *Rauvolfia vomtoria*leaveswere determined andcompared.Qualitative and quantitative phytochemical analysis,mineraland 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 theleaveswas 8.90%, ash content was 7.70% and crudefibercontentwas 20.00%.Thecarbohydrate content of the leaveswas41%, crude protein contentwas17.00% and total fat contentwas5.28%.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($p>0.05$).Thethiamine content in the aqueous extract was 0.20 whereas that in the ethanolic extract was 0.27.Theriboflavincontent was higher in the ethanolic extract (1.80) than in the aqueous extract (1.40).Similarly, the niacincontentoftheethanolic extract was 0.81 whereas that of the aqueous extract was 0.81.There was no significant difference in the calcium, magnesium and phosphorus valuesrecorded for the two extracts($p>0.05$).Incontrast, 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 freundiiand Shigella flexlerii*. However, thezoneofinhibitionof the ethanolic extract againstthetestedorganismswas higher thanthatof the aqueous extract.At aconcentrationof10%, the aqueous and ethanolic extracts showed87.29%and92.03% DPPH scavenging activity,respectively.Thepercentage of inhibitory activity of the extracts against DPPH radical increasedinadose-dependentmanner, with the ethanolic extract showing the highest DPPH radical inhibitory activity at a concentration of 50%.Theresultsconfirm that ethanol is a suitable solvent for the extraction of biologically active molecules from *R.vomtoria*leaves.

Keywords: Phytochemicals, Minerals, Vitamin B, Antioxidant, *Rauvolfiavomtoria*leafs

1. INTRODUCTION

Thestudyof medicinal plants and traditional medicinehas long fascinated herbalists aroundtheworld.Traditional medicinefocuses on improving overall health,

preventingdiseases,regulatingthe immune system, and providingindividualized care throughaholisticapproach[1-2].It is noteworthy that morethan 60% of medicinesareofplantorigin.Thereasonisthattheyactgently on the human body, have minimal or negligible side effects, arecheaptoproduce, and areeasilyavailable.Asaresult, medicinal plants havegreatbenefits for human well-being [3-4].However, only5–10%ofthe250,000 species of higher plants have been phytochemically studied, and only a fraction of themhave 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 havebeenmadein the research and development of phytomedicines,consistingof flavonoids, vitamins, alkaloids, tannins, and terpenoids,which have biological activities such as detoxifying toxic enzymes, inhibitingcell 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 suchascancer, neurodegenerative diseases, and diabetes [9-13].Thepositiverole of natural antioxidants in human health ismainlydueto their reducing effect,whichquenches free radicals such as reactive oxygen and nitrogen species (ROS/RNS) andpreventsoxidativedamagetocellscausedbytheiraction, due to their tendency toreleasehydrogen [14].

Free radicals are highly reactive chemical species, especially hydroxyl radicals ($^{-}$OH) and superoxide ions($O2^{-}$),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 beenanincreased interest in natural therapeuticagents,astheyarelesstoxic than chemicalswidelyused in themedical, food, and cosmetic industries [20-21].

One of the medicinal plants foundin 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 alsoknownassnakewood,snakeroot,stirstick, and colloquialnames"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 hasshown that the herbal preparationismadefromthe alkaloid extract of*R.vomitoria*has been used in traditional African folk medicine as an antihypertensive agent[23-24], totreatneurologicaldisorders[25], as anantioxidant, anti-inflammatory and anti-cancer properties [26-28.

Different plant extracts have differentproperties:methanolextracts can be used as antimalarial drugs[29];aqueousextracts of *Rauwolfia vomitoria* can be used to treat typhoid and jaundice [30];andaqueousmethanolextracts of *R.vomitoria* leaves are also used as atreatmentforsicklecellanemia[31].Choosing the best solvent for phytochemical extraction is important becausephytochemicalsexistwithavariety of chemical structures and polarities that can affect their solubility in the selected solvent.Plants contain avarietyof phytochemicals with different solubilities depending on thecharge and polarity ofthemolecule[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 materialswere obtained from aqueous organic solvents than

fromanhydroussolvents,therefore, it is important to investigate suitable extraction solventstooptimize antioxidant activity ofplants[37].

Considering the rapid emergence of drug-resistantbacterialstrains,theneed for new and effective treatmentsiscrucial.Given the widespread use of *R. vomitoria* in herbal medicine, scientific researchinto 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. vomtoria*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 ($FeCl_3.7H_20$), trichloroacetic acid (TCA), hydrochloric acid (HCl), Na_2CO_3, catechin, ammonium molybdate,2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylobenzothiazoline-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 plantleavesof*Rauwolfia vomitoria were*obtainedfrom Ngor Okpalain Imo State, Nigeria.Theseplantsamples 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.*Rauvolfia vomitoria* leaves were washed using a running tap water to remove accumulated dirton 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 wereextracted 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 analysisof 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 ofsaponin, phenols, flavonoids, tannins, alkaloids and terpenoids were carried out according to standard method as stated by Harborne [41] (1973), phytates and steroid contentswere determined according to the method described Okeke and Elekwa[42], whileglycosides was 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] 1mL each of 0.33M DPPH in methanol, methanol and extraction fraction (10 – 50 mg/mL) were dispensed into a 10ml test tube, mixed and incubated for 10 mins in a dark carbinet. A positive control (ascorbic acid solution) and a negative control (DPPH solution) was 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:

Scavenging inhibition (%) = Acontrol – Asample x 100

Acontrol

Where Acontrol = Absorbance of negative control; Asample = 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 wereplaced in eachrespectivewellsand then incubated at 37^0C 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 vomtoria* leafs

The Proximate Analysis of *Rauvolfia vomtoria* 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* leafs

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* leafs

The result of the qualitative phytochemical screening of aqueous and ethanolic Extracts of *Rauvolfia vomtoria* leafsis 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* leafs

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* leafsis 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 Compositionof 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 vomtoria* leafs

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 vomtoria* leafs

Table 5 shows the mineral contents of aqueous and ethanolic extracts of *Rauvolfia vomtoria* leafs. 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 vomtoria* leafs

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*Leafs

Table 6 shows the anti-microbial Effects of Aqueous and Ethanolic Extracts of *Rauvolfia vomitoria* Leafs. The aqueous andethanol extracts had zones of inhibition suggestingsusceptibilities 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**
Enterobacter faecalis	14.30	13.90	16.20	11.70	16.40	11.80	11.10	12.70	12.50	15.40
Klebsiella pneumoniae	11.40	12.10	13.80	16.10	19.50	10.00	12.00	15.20	15.00	18.10
Salmonella typhii	10.20	13.60	15.20	13.70	16.60	12.30	15.90	17.10	18.50	19.30
Cetrobacterfreundi	11.50	12.10	15.80	15.30	15.50	9.80	11.40	12.40	13.10	14.00
Shigella flexreri	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 ofAqueous and Ethanolic Extracts of *Rauvolfia vomitoria*Leafs

The *in vitro* antioxidant activities of aqueous and ethanolic extracts of *Rauvolfia vomitoria* leafs 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 increasein 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 extractsreduced their concentrations increased.

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

Antioxidant		Ethanolic Extract				Aqueous Extract				
Assays	**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* leafscontained 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* leafs

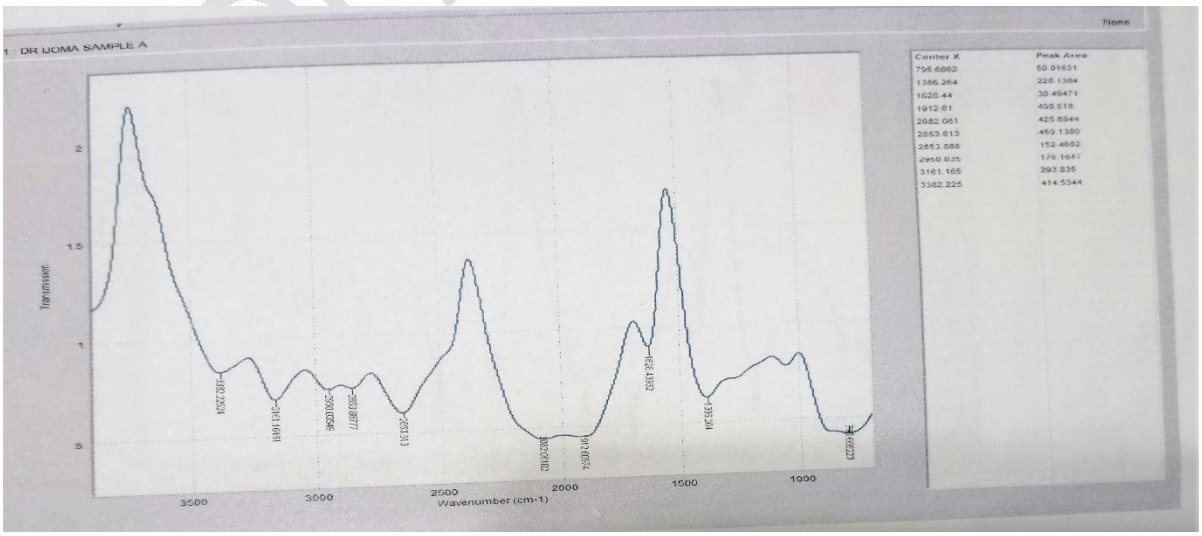

Figure 1: FTIR spectra of Aqueous Extract of*Rauvolfia vomitoria*Leafs

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

S/N	Wavelength (cm^{-1})	Functional group	Compounds
1	795.6662	C-Cl	Chloro Cl symmetric stretch
2	1385.264	$H_2C=CH$	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	RC00H	Carboxylic acid C0 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	RCH0H	1^0 alcohol 0H stretch
10	3382.255	R2CH0H	2^0 alcohol 0H 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 1^0 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 C00 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 0H stretching vibration of 1^0 and 2^0 alcoholic compounds respectively.

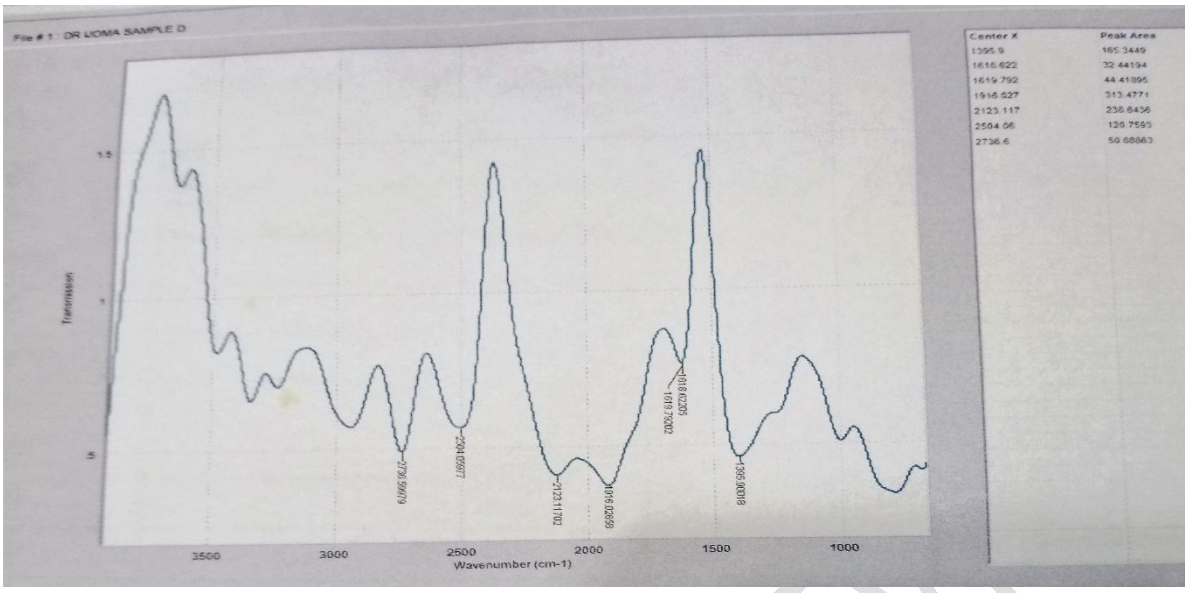

Figure 2: FTIR spectra of Ethanolic Extract of*Rauvolfia vomitoria*Leafs

Table 9: FTIR spectra of Ethanolic Extract of*Rauvolfia vomitoria*Leafs

S/N	Wavelength (cm^{-1})	Functional group	Compounds
1	1395.900	H_2C-CH	Ethene CH anti-symmetric stretch
2	1618.622	RNH_3	1^0 amine NH stretch
3	1619.792	RNH_3	1^0 amine NH stretch
4	1916.027	R-S-C≡N	Thiocyanate SCN antisymmetric stretch
5	2123.117	RC00H	Carboxylic acid C0 stretch
6	2504.060	RC00H	Carboxylic acid C0 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 1^0 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 C00 anti-symmetric stretching vibration of carboxylic acid, whereas the wavelength around 2504.060cm^{-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 verysmall concentrations, the choice of an extraction method and thecorresponding suitable solvent is an important step in the drugdiscovery process[46].This work compared the phytochemicals, minerals, antioxidant and antimicrobial activities of aqueous and ethanolic extracts of *Rauvolfia vomtoria* leafs.

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* leafis 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 Rauvolfiavomtoriaare shown in Table2.Cardiac glycosides, tannins, phenols, saponins, and phytates, as well as steroids, terpenoids, and alkaloids were detected in the aqueous and ethanolic extracts of Rauvolfiavomtorialeaves (Table 2).This isconsistent with the results of [5, 24, 33, 49].Secondary metabolites are mainly produced by plants as products of primary metabolism and as part of plantdefensemechanisms.Phytochemicals such as alkaloids, tannins, and flavonoids are examples of plant-producedphytochemicalsthatarebelievedtoberesponsiblefor the healing properties ofplants[50].Phenolic compounds are associated with antioxidant activity due to their abilitytoscavenge free radicals[51].Compounds such as flavonoids, resins, saponins, and tannins have been shown to have healing properties against most pathogenicbacteria[52].Moreover, these

phytochemicals act as the best antioxidants and protect cells from free radical damage, suchas 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 associatedwith 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 resultswere also reported [37, 57]. The solubility of glycosides and phenols in theextractionsolvent depends on the functional groups attached to the main structure of these phytochemicals, aswellas the molecular size and length of the hydrocarbons [58].Furthermore, the solvation potential determines the solubility of phytochemicals.The variation in phytochemicalcontentmaybedueto the difference in the extractability of different solvents.It has been saidthatethanolis suitable for the extraction of compounds with a wide range of polarities,whereas water is suitable for theextractionof very polar compounds [59].As a result, higher amounts of flavonoids and phenolic compounds were found in the ethanol samples thanin 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, includingantibacterialactivity[61].Previous qualitative and quantitative studies have similarly reported the presence of tannins,saponins, and flavonoids in R.Thevomitusremainsontheleaves, although indifferent concentrations [62-63].

Plant extracts arebelieved to have various protective functions due to their contentofphytochemicalsthatcontributesignificantly to antioxidant and antibacterialactivity[64-65].Oxidative stress leads to the production of free radicals in the bodyand 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,thusreducing the risk of these chronic diseases. The aqueous and ethanolic extracts in this study had zones of inhibition indicatingmicrobialsusceptibility, with the ethanolic extract showing higher antibacterial activity (Table 6).Ethanol solvent can extract alargeamount of polar metabolites,which, in turn,containimportant antibacterial agents [66].Furthermore,the percentage of inhibitory activity of the extracts against DPPH radicalincreasedinadose-dependentmanner, which is consistent with theprevious 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 oftheethanol 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 measuringantioxidantactivity.The advantage of this method is that antioxidant activity is usually measured at ambient temperature, thuseliminating the risk of thermaldegradation of the tested molecules.Radical scavenging is a known mechanismby which lipid oxidation is inhibited by antioxidants [68].Differences in the polarity

of antioxidants may be the reason for the variation in activityobserved.Moreover, solvent polarity is known to play animportant role in enhancingthe solubility of phenolic biocompounds[69].

In this study, the antioxidant activity of Rauvolfiavomtorialeaf extracts was also evaluatedusing the FRAP method, which is based on the ability to reduce ferric ions to their ferrous form.The results showed that theethanolicextract had significantly higher FRAP values than the aqueous extract.Similar resultswere also reported by Tourabi*et al*. [70].Flavonoids and phenolic compounds are the two mostimportant and widespread polyphenols found in medicinal plants [71].Both phenols and flavonoids are known for their free radical scavenging activity.Thisis due to thepredominance of hydroxyl groups thathavethe inherent ability to donate hydrogen atoms to free radicals,thusstoppingtheaccumulationoffree radicals in thebody[49].Thestudy also found thattheextractcaused NO inhibition, suggestingits potential utility as a treatmentfor 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 areconsistent with the report by Ugwu *et al*.[48], where the three highest minerals are K>Mg>P>Na.These results areinline with apreviouslypublishedstudy[73], which showed the presence of significantamountsof minerals in medicinal plants.Minerals are necessary for normal growth, muscleactivity 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 donotprovide energy, they play akeyrole in many body activities.Minerals are involved in maintaining acid-base balance and

regulating body fluids.Some are cofactors in enzymereactions.Minerals are used in theformationof hemoglobin and thyroxine.Some minerals play arole in antioxidant functions.They also transport gases and aid in muscle contraction[74].

The vitamin B composition of aqueous and ethanolic extracts of Rauvolfiavomtorialeaves (Table 3) isinagreement with thepapersof Ugwu *et al*.[24] and Alagbe[75] who demonstrated the presence of vitaminBin the leaf and root extractsofRauvolfiavomitoria, respectively.Thisis also inagreement with the study of Traber[76].Vitamins are essential for human health, growth, development, reproduction and maintenance, and vitamindeficiencyposes serious threatsto health [77].They are diverse in nature compared to fats, carbohydrates and proteins, and aredistinguished 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.Vitaminsfoundinfoodhavebeenshown to have a positive effect on health.Epidemiological studies have shown that high vitamin intakes are associated with alower 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 partofthe 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] (Ugwu*et al*., 2022; Zoroddu*et al*., 2019).

5. CONCLUSION

In this work the phytochemicals, minerals, vitamins, antioxidant and antimicrobial activities of aqueous and ethanolic extracts of *Rauvolfia vomtoria* leafs 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.The extracts are thus excellent sources of antioxidants that can decrease disease risks occasioned by free radicals. These properties should be explored by pharmaceutical companies in making products. However, more *in vivo* studies should be conducted to determine the antioxidant prowess of the extracts. In addition, the effect of environment and plant source on the phytochemicals should be investigated.

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 *Ocimumgratissimum*and *Vernonia amygdalina* on cadmium induced hepatotoxicity in wistar rats. JBiochemIntl. 2022; 9(4):73-81
2. Helan CJ, Vignesh T. In vitro and In silico Study of Ethanolic Leaf Extracts of RauvolfiaCanescens.Research Journal of Chemistry and Environ. 2016; 20 (8):31-37.
3. Ashvini YP, Gunjan PM, Aayesha RS, Wrushali AP, Jagdish VM, RavindraLB. 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 GA, Choo WS.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 DA, Ikewuchi JC, Onyeike EN. Moderation of ocular markers of oxidative stress of selenite induced cataractous pups by Boswellia dalzielii Hutch extract. Global AdvRes J MedPlant. 2016; 4(2): 007 – 011
9. Orororo OC, Asagba SO, Egbune EO,Efejene OI. Sperm parameters and histological changes in testes of cadmium-exposed rats treated with *Hibiscus sabdarrifa*L. anthocyanins. Sokoto J Med Lab Sci. 2022; 7(3):114-122.
10. Pohl F, Thoo, Lin, PK. 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, 23:32-43.
11. Hrelia S, Angeloni C. New Mechanisms of Action of Natural Antioxidants in Health and Disease. Antioxidants. 2020, 9:34-44.
12. Onobrudu DA,Onyeke EN,Ikewuchi JC. Effect of methanolic leaf extract of Boswellia dalzielii hutch on ocular ATPase profiles of selenite-induced cataract in wister pup. J Complemen Alt Med Res. 2017; 2(2): 1-7
13. Orororo OC,Asagba SO. Treatment with *Hibiscus sabdarrifa* L Anthocyanins Improve Hematological Parameters in Rats Exposed to Cadmium. J Expl Res Pharmaco. 2022; 3:1-10
14. Ekakitie LI, Orororo OC, Okpoghono J. Changes in haematological parameters of aluminium-exposed rats treated with natural bee honey. IOSR J Environ SciToxico Food Techno; 2021; 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, 12:10-20.
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 OC, Asagba SO, Tonukari NJ, Okandeji OJ, Mbanugo JJ. Comparative Assessment of the Antioxidant Properties of *Hibiscus sabdarrifa*L Anthocyanins and its Aqueous Extract in Cadmium-exposed Rats. Res J Pharma Bio Chem Sci.2018; 9(3): 836-843
19. Onobrudu DA. Saponins and polyphenolics of methanol leaf extract of Boswellia dalzielii Hutch. Arch Current Res Intl.2017; 8(3): 1- 6
20. Majeed M, Pirzadah TB, Mir MA, Hakeem KR, Alharby HF, Alsamadany H, Bamagoos AA, Rehman RU. 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 TH, Bano A, Nosheen A, Yasmin H, Hassan MN, 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 II, Ebuehi OAT,Igwo-Ezikpe MN. GC-MS Analysis of the *Rauwolfia vomitoria* Ethanol Extracts. Eur J MedPlants.2021; 32(6): 34-45.
23. Lobay D. Rauwolfia in the Treatment of Hypertension. Integrative Med (Encinitas, Calif.). 2015;14(3):40–46.
24. Ugwu OPC, Okon MB, Nweze TK, Mba AN, Ozioko PC,Nweke OL. Phytochemical Analysis of Ethanol Leaf Extract of *Rauwolfia vomitoria*.. INOSR Appl Sci. 2019;5(1): 35-40.
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. Eur J PharmaMed Res. 2015;2(7):64-73.
27. Oboh G, Adebayo AA, Ademosun AO. HPLC phenolic fingerprinting, antioxidant and anti-phosphodiesterase-5 properties of *Rauwolfia vomitoria* extract. J Basic Clin PhysiolPharmacol. 2019; 30:/j/jbcpp.2019.30.issue-5/jbcpp-2019-0059/jbcpp-2019-0059.xml.
28. Dong R, Chen P, Chen Q. Inhibition of pancreatic cancer stem cells by *Rauwolfia vomitoria* extract. Oncol Rep. 2018; 40: 3144-3154.
29. Tlhapi DB, Ramaite IDI, Van Ree T, Anokwuru CP, Orazio TS, Hoppe HC. Isolation, chemical profile and antimalarial activities of bioactive compounds from *Rauvolfiacaffra*Sond. Molecules. 2019; 24:39.
30. Aquaisua A, Mbadugha C, Bassey E, Ekong M, Ekanem T, Akpanabiatu M. Effects of *Rauvolfia vomitoria* on the cerebellar histology, body and brain weights of albino wistar rats. J ExpClin Ana. 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). J Sci Prac Pharm. 2014; 1(1):11-15.
32. Okoro IO. Effects of Extraction Solvents on the Antioxidant and Phytochemical Activities of *Manihot Esculenta* Leaves. Iranian J Toxico. 2020; 14(1):51-58.
33. Uba GH, Dauda KM, Aujara N, Umar A. Solvent Extraction and its Effects on the Phytochemical Yield and Antioxidant Capacity of *Commiphoraafricana*(Burseraceae). Bioremed Sci Tech Res. 2020; 8(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 AC, Olubusola EF, Olubukola BO, Abimbola A, Zainab AA, Olaleye MT. Effect of extraction technique, solvent polarity, and plant matrix on the antioxidant properties

of *Chrysophyllum albidum* G. Don (African Star Apple). Bull National Res Centre. 2022; 46:40:1-9
38. Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT-Food Sci Technol. 2012; 46:548 –555.
39. Trease GE, Evans WC Pharmacognosy. 11th Edition, Bailliere Tindall, London,1989; 45 - 50
40. Sofowora EA. Medicinal plants and traditional mdicines in Africa. 2nd Edition, spectrum Books Ltd, Ibadan, Nigeria. 1993; 10 -19
41. Harborne JD. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall London, 1973; 279.
42. Okeke CU, Elekwa I. Phytochemical study of the extract of *Gongronemalatifolium* Benth. J Health Vis Sci. 2003; 5(1): 47 – 55
43. Ezeonu CS, Ejikeme CM. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwood. New J Sci. 2016;1: 1- 9
44. Koksal E, Bursal E, Dikici E, Tozoglu F, Gulcin I.Antioxidant activity of Melissa officinalis leaves. J Med Plants Res. 2011; 5: 217–222.
45. AOAC (2000) Association of Official Analytical Chemists. Official Methods of Analysis 19th Edition Washington DC, USA; 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. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York, 1981.
48. Ugwu OP, Chima NO, Alia LC, Okon MB, Onyeke SC, Eze CG, Eze IM. The Mineral Composition of Methanol Leaf Extract of *Rauwolfia vomitoria* INOSR Appl Sci. 8(1):71-75,2022
49. Samuel TA, James AB, Adebesin O, Okafor I, Iwalokun BA. Phytochemical Investigation and Anti-Proliferative Effects of Rauvolfia vomitoria, Calliandra portoriscensis and Anthocleistadjalonensis on Human Breast Cancer (MCF-7) Cell Line.Afr J Biomed Res. 2021; 24: 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 JKM, 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: 204-209.
52. Khanam Z, Wen CS, Bhat IUIH. 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. 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. 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 *Bulbineabyssinica*used in the folk medicine in the Eastern Cape Province, South Africa. Evid Based Complement Alternat Med. 2015; 2015: 617-607.
57. Salami TA, Aderinboye RY, Akinbode RM, Adebayo KO,Fasae OA. Potentials of *Rauvolfia 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. 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 BS, Vijayakumar G, Balasubramaniam GA, Sivaramam S, Kathirel S. Efficacy of neostigmine and azithromycin in buffaloes with functional ileus. Buffalo Bulletin, 2020; 38
62. Ojo OO, Ajayi SS, Owolabi LO. Phytochemical screening, anti-nutrient composition, proximate analyses and the antimicrobial activities of the aqueous and organic extracts of bark of Rauvolfia vomitoria and leaves of Peperomia pellucida. Inte Res JBiochemBioinfor. 2012; 2(6): 127–134.
63. Chinonye I, Chijioke C, Iwuji S, Obiagwu I, Lynda UO, Ukaoma A, Chijioke-Okere M,Ezekoye M. Chemical and medicinal properties of Rauwolfia vomitoria (AFZEL) harvested from the South Eastern Nigeria. Asian J Chem Sci.2022;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. Phytochemical, antioxidant and antimicrobial properties of *Litseaangulata*extracts. F1000 Res.2018; 7: 18-39.
66. Paiva PMG, Gomes FS, Napoleão TH, Sá RA, Correia MTS, Coelho LCBB. Antimicrobial activity of secondary metabolites and lectins from plants. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Méndez A. (Ed.). 2010; 396-406
67. Compaoré M, Meda RN-T, Bakasso S, Vlase L, Kiendrebeogo M. Antioxidative, anti-inflammatory potentials and phytochemical profile of Commiphoraafricana (A. Rich.) Engl. (Burseraceae) and Loeseneriellaafricana (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. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: ccurrence, extraction and analysis. J Pharm Biomed Anal. 2006;41(5):1523-42.
70. Tourabi M, Amira M, Asmae EL,ghouizi H, Mohamed J, Ghizlane N, Hassan L, Eram H, Kawtar FB, Mohammed B, Ahmad MS, Hiba ·Allah N, Gezahign FW, Badiaa L,Elhoussine

D. Efficacy of various extracting solvents on phytochemical composition, and biological properties of *Mentha longifolia* L. leaf extracts. Sci 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. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. Database (Oxford). 2010: 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). *Rauwolfiavomitoria*: 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 Rauvolfia Vomitoria Root Extract as A Phytogenic Feed Additive in Growing Rabbit Diets: Growth Performance and CaecalMicrobial Population. Con Dai Vet Sci 4(2)- 2021.
76. Traber MG. Vitamin E inadequacy in humans: causes and consequences. Adv Nutri. 2014; 5(5):503-14.
77. Muhammad AM, Muhammad A, Waseem A, Zubair I. Biological importance of vitamins for human health: A review. J Agric Basic Sci. 2017; 2(3): 50-58.
78. AsensiFabado MA, Munne´-Bosch S. Vitamins in plants: occurrence, biosynthesis and antioxidant function. Trends Plant Sci. 2010; 15(10): 582-592.
79. Keogh JB, Cleanthous X, Wycherley TP. Increased vitamin B1e intake may berequired to maintain vitamin B1e status during weight loss in patients with type 2diabetes. Diabetes Res Clin Pract. 2012; 98: 40-42.
80. Lanska DJ. Chapter 30: historical aspects of the major neurological vitamin deficiency disorders: the water-soluble B vitamins. Handbook Clin Neurol. 2010; 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.