GC-MS CHARACTERIZED BIOACTIVE CONSTITUENTS AND ANTIOXIDANT CAPACITIES OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *Rauvolfia vomitoria*: A COMPARATIVE STUDY

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ABSTRACT

The comparative analysis of the bioactive constituents and antioxidant capacities of the aqueous and ethanolic extracts of *Rauvolfia vomitoria* leaves was carried out in this study. Bioactive compounds in the leaf extracts were characterized using Gas Chromatography-Mass Spectrometry (GC-MS), while the antioxidant strengths were determined following standard biochemical procedures. The results showed percentage yields of 18.8%, and 6% respectively. Proteins, phenol and terpenes were present in both extracts; saponin, phlobatanin and alkaloids were present only in the aqueous extract; while flavonoids and steroids were present only in the ethanolic extract, with variations only in the alkaloids and reducing sugar amounts. The GC-MS revealed the presence of different compounds (with varying relative area percent) belonging to the groups of alkanols, esters, fatty acids, alkanes etc in the extracts. Also, the plant's extracts showed moderate free radical mopping and antioxidant capacities relative to the standard compound (ascorbic acid) in a pattern suggesting distilled water as solvent of choice for the extraction of this plant's leaves as evident in their IC_{50} and EC_{50} values. The results of this study provide scientific insights into the ethnotherapeutic properties of this plant's leaves as well as useful information for drug development.

Key words: *Rauvolfia vomitoria*; extraction solvents; phytochemical constituents; antioxidant capacity; ethnomedicine.

INTRODUCTION

The high use of medicinal herbs as alternative therapy in most societies is due to the manifestation of little or no side effects arising from their use, high efficacy and the rising cost of orthodox drugs that are needed for the maintenance of health (Hoareau and DaSilva, 1999; Nwangwu *et al.*, 2009; Anigboro *et al.*, 2019; Anigboro *et al.*, 2021; Anigboro *et al.*, 2022). They also serve protective functions in plants as regards diseases and also contribute to the coloration, flavor and scent of plants. Generally, phytochemicals are chemicals that protect plants not only from pathogenic attack but also environmental hazards such as drought, pollution, stress and UV exposure (Gibson *et al*., 1998; Koche *et al.*, 2016). Noteworthy, dietary consumption has vividly shown that phytochemicals play a crucial role in the protection of human health (Mecha *et al*., 1980; Tonukari *et al.*, 2013; Cavalli and Santos, 2015; Koche *et al.*, 2016).

Rauvolfia vomitoria (Figure 1) is a small tree plant belonging to the family Apocynaceae and is found in most tropical forests of South America, Asia and Africa (Mecha *et al*., 1980; Ehiagbonare, 2004). In the traditional healthcare system, *R. vomitoria* is used in the management of ailments such as; mental disorder, hypertension, dysentery, jaundice, cerebral cramps and gastrointestinal disorders (Kutalek and Prince, 2007). Several studies have reported antioxidant, antipyretic, antiglycemic, anticonvulsant, analgesic, antipsychotic, and sedative properties in *R. vomitoria* (Amole *et al.*, 2006; Akpanabiatu *et al*., 2009; Amole *et al.*, 2009; Eluwa *et al*, 2009; Bisong *et al*, 2010; Bisong *et al*., 2012). Further reports of this plant include antitumor (Jun *et al*., 2013), antimalarial (Omoya and Falusi, 2019), anthelmintic (Tekwu *et al.*, 2017) and other medicinal properties (Fapojuwomi and Asinwa, 2013; Chinonye *et al.*, 2021).

With this plant's high pharmacological relevance over the years, there is need to carry out the comparative evaluation of the phytochemical constituents and antioxidant activities of the aqueous and ethanolic leaf extracts. This study will provide knowledge of the most suitable solvent(s) for the therapeutic use of this plant leaves together with valuable information on some inherent prevalent bioactive compounds that could be harnessed for possible development of drugs for further clinical evaluations.

Figure 1: Picture of *Rauvolfia vomitoria* Leaves.

MATERIALS AND METHODS

Reagents and Authentication of Plant Material

All chemicals and reagents used for the experimental analyses were of analytical quality. Leaves of *Rauvolfia vomitoria* were collected from a bush in Benin City, Edo State, and authenticated at the Plant Biology and Biotechnology Department, University of Benin, Nigeria, with voucher specimen (UBH-R421) deposited in the University Herbarium.

Extracts Preparation

The fresh leaves of *Rauvolfia vomitoria* were rinsed to remove debris, air-dried and ground to coarse powder (using an electric blender) from which 100 g was extracted with 800 ml of distilled water and ethanol separately using cold maceration for 48 hours (with stirring after 24 hours). The extracts were sequentially filtered with double-layered cheese cloth and filter paper, after which the resulting filtrates were concentrated at 50 ^{o}C in a rotary evaporator for 2 hours and was evaporated to dryness in a water-bath maintained at the same temperature, to obtain the aqueous and ethanolic extracts that were used for biochemical analysis. Percentage yields of the extraction processes were calculated using the equation 1.0:

$$\text{Yeild (\%)} = \frac{\text{Weight of extract obtained}}{\text{Weight of ground leaves used}} \text{ x } 100$$

…… Equation 1.0

Qualitative Screening for Bioactive Compounds

Preliminary screening of the various leaf extracts was carried out using the standard methods described by Borokini and Omotayo (2012); and Njoku and Obi (2009) to check for the presence of saponins, phenol, tannins, flavonoids, phlobatannin, terpenes, steroids, proteins, reducing sugars and alkaloids.

Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

The GC-MS determination of the specific bioactive compounds that are present in the aqueous and ethanolic leaf extracts of *Rauvolfia vomitoria* was carried out followed by identification of the bioactive constituents of the extracts using the spectra of characterised reference chemical compounds in the National Institute of Standards and Technology (NIST) library as described by Olasehinde *et al*. (2019).

Quantitative Analysis of Bioactive Constituents

The determination of total flavonoids (Jia *et al*., 1999), reducing sugar (Miller, 1959), proteins (Gornall *et al*., 1949), alkaloids (Shamsa *et al*., 2008), phenols and tannins (Singleton and Rossi, 1965) were done using the respective standard methods, which were also reported by Anigboro *et al.* (2022). A calibration curve was constructed (with varying concentrations of respective

standard compounds) from which the results were expressed as milligram equivalents/g of the extracts.

In-vitro Determination of Free Radical Scavenging and Antioxidants Activities

1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Ability

Using the method of Manzocco *et al.* (1998), the DPPH radical inhibitory activity of the aqueous and ethanolic leaf extracts of *Rauvolfia vomitoria* was carried out by reacting the leaf extracts as well as ascorbic acid samples (0.2 ml, 0.25 – 4.0 mg/ml) with DPPH solution (2.0 ml; 0.3 mM). The optical densities of the mixtures were measured at 517 nm (after incubation in the dark for 30 minutes) and the percentage inhibition of DPPH radical were calculated using the equation 2.0:

$$\text{Radical inhibition } (\%) = \frac{C-S}{C}$$

…… Equation 2.0

Where C and S are the optical densities of the control and that in the presence of the extract or the standard compound.

Nitric Oxide Radical ($NO^{\bullet}$) Scavenging Activity

Employing the procedure reported by of Marcocci *et al.* (1994), the mopping activity of the leaf extracts as well as ascorbic acid samples was evaluated by reacting sodium nitroprusside (2.0 ml; 10 mM) dissolved in phosphate buffer saline (0.5 ml, 10 mM, pH 7.4) with the various samples (0.5 ml, 0.25 – 4.0 mg/ml) followed by incubation 150 minutes at 25 ^{0}C. Thereafter, 1.0 ml of the incubated solution was mixed with sulfanilic acid solution (2.0 ml, 0.33% in 20% glacial acetic acid), incubated for 5 minutes before adding naphthylethylenediamine dichloride (2.0 ml, 0.1% w/v) and allowed to stand again at the same temperature for 30 minutes. The optical densities were measured at 546 nm against blank and the percentage inhibition of $NO^{\bullet}$ was calculated using the equation 2.0.

Reducing Power (RP) Determination

Following the standard method reported by Oyaizu (1986), the RP of the various leaf extracts as well as ascorbic acid samples was evaluated by mixing phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and $K_3Fe(CN)_6$ (2.5 ml, 1% w/v) with the various samples (1.0 ml, 0.25 – 4.00 mg/ml) followed by incubation at 50 ^{0}C for 20 minutes and addition of Trichloroacetic acid (2.5 ml, 10% w/v). The resulting mixture was centrifuged at 3000 rpm for 10 minutes, the supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 mL, 0.1% w/v) thereafter the absorbance was measured at 700 nm against blank (that contained sodium phosphate buffer and distilled water).

Total Antioxidant Capacity

The standard method of Prieto *et al*. (1999) was used for the determination of total antioxidant capacity of the samples. The working reagent solution (2.0 mL) was mixed with the samples (0.2 mL) and incubated at 95 °C for 90 minutes. After cooling to room temperature, the absorbance of the reaction mixture in each tube was measured spectrophotometrically at 695 nm against a blank.

Statistical Analysis

All data were subjected to statistical analysis using SPSS-PC Programme package while Microsoft Excel was used for graphical presentation. The result values were expressed as Means ± Standard deviation (n = 3) while Student T-Test and One-way Analysis of Variance (ANOVA) was used to test for differences between groups at 0.05 level of significance ($p<0.05$).

RESULTS AND DISCUSSION

The utilization of herbs in the treatment of disease is sought-after in developing countries for numerous reasons (Nwangwu *et al.*, 2009; Anigboro *et al.*, 2019). The high use of medicinal herbs as alternative therapy in most African societies is due to the fewer side effects, high efficacy and the rise in the cost of orthodox drugs required for the control of diseases (Hoareau and DaSilva, 1999; Anigboro *et al.*, 2021). Due to this high use of herbal medication, so much research is currently ongoing into the pharmacological activities, together with their active constituents that control biological processes and reverse disease state (Ugochukwu *et al.*, 2003). The solvent of choice is a major determinant of the type of bioactive constituents obtained in the extraction of plant material which in turn will determine the pharmacological effect.

The analysis of the *Rauvolfia vomitoria*'s aqueous and ethanolic leaf extracts showed that the percentage yields were 18.8 %, and 6.0 % respectively. Proteins, phenol and terpenes were present in both extracts; saponin, phlobatanin and alkaloids were present only in the aqueous extract; while flavonoids and steroids were present only in the ethanolic extract (Tables 1) with variations only in the alkaloids and reducing sugar amounts (Table 2).

Table 1: Percentage Yield and Qualitative Bioactive Constituents of *Rauvolfia vomitoria* Leaf Extracts

	***Rauvolfia vomitoria* Leaf Extracts**	
	Aqueous	**Ethanolic**
Yield (%)	18.8	6.0
Bioactive Compounds		
Saponin	+	—
Phlobatanin	+	—
Flavonoids	—	+
Tannins	—	—
Alkaloids	+	—
Terpenes	+	+
Phenols	+	+
Steroids	—	+
Reducing sugars	—	—
Proteins	+	+

KEY: (+) = Present; (–) = Not present.

Table 2: Quantitative Bioactive Compounds of *Rauvolfia vomitoria* Leaf Extracts

Bioactive Compounds	***Rauvolfia vomitoria* Leaf Extracts**	
(mg/g DW)	**Aqueous**	**Ethanolic**
Phenol (GAE)	7.99 ± 1.10^{a}	9.43 ± 0.88^{a}
Tannins (TAE)	5.37 ± 0.82^{a}	6.45 ± 0.66^{a}
Flavoniods (CE)	7.98 ± 0.11^{a}	10.08 ± 1.70^{a}
Reducing Sugars (GE)	127.75 ± 1.34^{a}	77.33 ± 4.66^{b}
Proteins (BSAE)	0.41 ± 0.05^{a}	0.38 ± 0.13^{a}
Alkaloids (AE)	18.07 ± 5.79^{a}	33.91 ± 1.20^{b}

Values are represented as Means ± Standard Deviation (n = 3). Values with different superscript alphabets on the same row under the same plant, differ significantly ($p<0.05$).
KEY: GAE, TAE, CE, GE, BSAE and AE represent: Gallic acid, Tannic acid, Catechin, Glucose, Bovine Serum Albumin and Atropine Equivalents respectively.

Determination of specific compounds in the extracts using GC-MS revealed the presence of compounds (with varying relative area percent) belonging to the groups of alkanol, esters, fatty acids, alkanes etc. These variations are a

reflection of the solvent type used in the extraction process (Tables 3a and 3b). Several of these compounds including acetic acid, oxirane, cyclopropane and oxalic acid have been reported as having pharmacological relevance in disease prevention and treatment (Robertson, 2011; Tonukari *et al*., 2013, Anigboro *et al*., 2014, Tonukari *et al*., 2015; Yamashita *et al*., 2016; Cahyono *et al*., 2020; Anigboro *et al*., 2022). Alkaloids, terpenoids and phenolics have been numerously reported as possessing anti-inflammatory and antioxidant properties (Ekanayake *et al*., 2000; Tawaha *et al*., 2007; Joan *et al*., 2012; Aganbi *et al*., 2017). Toluene, silane and other volatile organic compounds (VOCs) which were present in the extracts may be due to the plant ability to absorb these compounds from the environment (Sriprapat *et al*., 2014).

Table 3a: GC-MS Identified Compounds in Aqueous Leaf Extract of *Rauvolfia vomitoria.*

Peak	RT (min)	Area (%)	Name of Compound	Mol. Wt.	CAS Number
1	3.4188	4.4679	Toluene	92.063	000108-88-3
2	4.5461	1.6065	Acetic acid, butyl ester	116.084	000123-86-4
3	5.1583	0.489	Silane,(3-chloropropyl) methoxydimethyl-	166.058	018171-14-7
4	5.2499	2.8431	2-Hexanol, 2-methyl-	116.12	000625-23-0
5	5.8221	0.0371	1-(2-Adamantylidene)semicarbazide	207.137	065814-27-9
6	6.0395	0.0939	Benzenemethanol, 3-methoxy-4-nitro-	183.053	080866-88-2
7	6.1082	4.896	Cyclohexanone	98.073	000108-94-1
8	6.4229	8.1714	Ethanol, 2-butoxy-	118.099	000111-76-2
9	6.5259	0.2136	Propanoic acid, 2-methylpropyl ester	130.099	000540-42-1
10	6.6804	0.2634	2,3-Heptadien-5-yne, 2,4-dimethyl-	120.094	041898-89-9
11	7.2526	0.46	Benzene, propyl-	120.094	000103-65-1
12	7.4185	1.8771	Benzene, 1,2,4-trimethyl-	120.094	000095-63-6
13	7.5616	0.7145	Benzene, 1,2,3-trimethyl-	120.094	000526-73-8
14	7.7504	0.5538	Benzene, 1-ethyl-3-methyl-	120.094	000620-14-4
15	8.746	0.2852	5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione	207.072	086071-95-6
16	9.0321	0.2815	3,4-Furandicarboxylic acid	156.006	003387-26-6
17	9.1637	0.0828	Benzaldehyde,2-nitro-, diaminomethylidenhydrazone	207.076	102632-31-5

18	9.4155	0.0812	5,6,7-Trinitro-1,4-benzodioxane	271.008	135399-56-3
19	9.7874	0.4373	Oxalic acid, allyl hexadecyl ester	354.277	1000309-24-4
20	9.9648	0.0429	5-Cyclopropyl-2H-pyrazole-3-carbaldehyde	136.064	1000409-95-9
21	10.4455	0.067	Cyclopropane, 1,1'-ethenylidenebis-	108.094	000822-93-5
22	10.9776	0.1992	1-(6-Purinyl)-2-pyrrolidinecarboxylic acid	233.091	091129-94-1
23	12.3051	0.1482	[a]1-Hexen-3-yne, 2-methyl-	94.078	023056-94-2
24	12.4768	0.092	5-Hexen-3-yn-2-ol, 2-methyl-	110.073	000690-94-8
25	13.1978	0.2148	2-Phenethyl-.beta.-phenylpropionate	254.131	028049-10-7
26	13.7242	0.2011	[a]1,3,4-Hexatriene, 3-methoxy-	110.073	053783-88-3
27	13.8673	0.0792	Oxirane, 2-(chloromethyl)-2-(1-methylethenyl)-	132.034	121505-32-6
28	15.0517	0.1136	1-Nonen-4-yne	122.11	031508-12-0
29	15.5381	0.227	2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a-methyl-, (S)-	164.12	004087-39-2
30	15.7327	0.2728	3-Nonynoic acid	154.099	056630-33-2
31	18.6738	0.0705	11-(2-Cyclopenten-1-yl)undecanoic acid, (+)-	252.209	000459-67-6
32	19.4062	0.0777	Histamine, 5-nitro-N-trifluoroacetyl-	252.047	1000129-60-1
33	19.9326	0.1336	Oleic Acid	282.256	000112-80-1
34	20.1386	1.2507	Pentadecanoic acid, 14-methyl-, methyl ester	270.256	005129-60-2
35	21.5005	0.2194	Dodecahydropyrido [1,2-b]isoquinolin-6-one	207.162	108873-36-5
36	21.8266	3.2405	9-Octadecenal, (Z)-	266.261	002423-10-1
37	22.0555	0.3442	Dodecanoic acid, 10-methyl-, methyl ester	228.209	005129-65-7
38	22.2787	0.4333	Pyrido [2,3-d]pyrimidine, 4-phenyl-	207.08	028732-75-4
39	22.6678	0.4379	[a]1,1,1,3,5,5,5-Heptamethyltrisiloxane	222.093	001873-88-7
40	23.2743	1.1577	Ethane, 1-(4,4,4-trifluoro-1,3-dithiobutyl)-2-(3,3,3-trifluoro-1,2-dithiopropyl)-	307.926	1000226-87-3
41	23.692	0.1927	2-Myristynoyl-glycinamide	280.215	1000111-57-7

42	23.9209	0.7219	3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone	207.083	1000195-15-1
43	24.207	0.4456	[a]Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	207.09	1000129-52-1
44	24.3844	0.228	[a][1,2,4]Triazolo[1,5-a]pyrimidine-6-carbox ylic acid, 4,7-dihydro-7-imino-, ethyl ester	207.076	1000351-62-2
45	25.1397	9.2017	3-Quinolinecarboxylic acid, 6,8-difluoro -4-hydroxy-, ethyl ester	253.055	1000362-34-6
46	25.5002	2.8205	[a]Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11, 13,13-tetradecamethyl-	504.152	019095-23-9
47	25.626	1.673	Methyl 2-[1-(4-methylphenyl)hydrazino]propanoate	208.121	1000305-34-3
48	26.1983	7.6218	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	250.121	1000161-21-8
49	26.6446	4.8131	Acetamide, N-(4-fluorophenyl)-2,2,2-trifluoro-	207.031	1000307-30-8
50	26.8964	2.9812	Arsenous acid, tris(trimethylsilyl) ester	342.048	055429-29-3
51	27.0795	0.9196	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	282.089	093103-70-9
52	27.2797	1.372	N,N-Dimethyl-4-nitroso-3-(trimethylsilyl)aniline	222.119	017993-84-9
53	27.4285	0.8495	1H-Indole, 5-methyl-2-phenyl-	207.105	013228-36-9
54	27.5715	1.0244	2-Ethylacridine	207.105	055751-83-2
55	27.7318	1.1777	[a]1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	222.162	001020-31-1
56	27.9721	1.395	Methyltris(trimethylsiloxy)silane	310.127	017928-28-8
57	28.2639	0.516	[a]Silicic acid, diethyl bis(trimethylsilyl) ester	296.13	003555-45-1
58	28.5157	0.581	[a]1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	207.047	1000404-25-4

59	28.8533	2.8914	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	222.141	014923-29-6
60	29.0536	1.0833	[a]Cyclotrisiloxane, hexamethyl-	222.056	000541-05-9
61	29.1737	0.8913	Benzo[h]quinoline, 2,4-dimethyl-	207.105	000605-67-4
62	29.3912	0.2985	Benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl-	207.072	1000258-63-4

KEY: RT = Retention time; Mol. Wt. = molecular weight; CAS = Chemical Abstracts Service. Compounds preceded with superscript alphabet were present in both extracts.

Table 3b: GC-MS Identified Compounds in Ethanolic Extract of *Rauvolfia vomitoria*.

Peak	RT (min)	Area (%)	Name of Compound	Mol. Wt.	CAS Number
1	3.3505	2.2107	2,2-Dimethoxybutane	118.099	003453-99-4
2	3.6538	0.0981	9,12-Octadecadienoyl chloride, (Z,Z)-	298.206	007459-33-8
3	3.7682	0.7237	Pyridine, 2,5-dimethyl-	107.073	000589-93-5
4	4.0658	0.2216	Tricyclo[3.3.1.1(3,7)]decanone, 4-(acetyloxy)-, (1.alpha.,3.beta.,4.beta.,5.alpha.,7.beta.)-	208.11	055821-13-1
5	4.2546	0.0451	Cyclopentanepropanol, 2-methylene-	140.12	053544-48-2
6	4.4663	0.3907	2,5-Cyclooctadien-1-one	122.073	010061-05-9
7	4.9527	0.2506	2,6,7-Trimethyl-(1,2,4)-triazolo(2,3-b)(1,2,4)-triazine	163.086	061139-77-3
8	5.1873	0.5149	3,6,9,12,15-Pentaoxanonadecan-1-ol	294.204	001786-94-3
9	5.3017	0.4115	Methyl allyl diglycolcarbonate	248.09	087292-24-8
10	5.9884	0.05	Phenol, 4-(2-amino-5-nitrophenyliminomethyl)-2-methoxy-	287.091	306744-56-9
11	6.429	0.2773	Benzene, [(methylenecyclopropyl)sulfonyl]-	194.04	078656-83-4
12	6.7437	0.1228	3,8-Dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl-	138.068	053966-43-1
13	7.3788	0.0555	Bicyclo[5.2.0]non-1-ene	122.11	065811-17-8
14	7.9625	0.1124	2-Amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid	193.085	1000287-12-4
15	8.3973	0.4222	3-Octen-1-yne, (Z)-	108.094	042091-89-4

16	8.9467	0.153	6H-1,2,5-Oxadiazolo[3,4-E]indole-6,8a-diol, 4,5,5a,7,8,8a-hexahydro-, 3-oxide	213.075	331853-25-9
17	9.2671	0.0652	Hydrazinecarboxamide, 2-(2,6-cyclooctadien-1-ylidene)-	179.106	068344-44-5
18	9.7878	0.5915	Decane	142.172	000124-18-5
19	10.0682	0.0985	8-Nonynoic acid	154.099	030964-01-3
20	10.1712	0.0844	Cyclohexylacetylene	108.094	000931-48-6
21	10.6003	0.2161	Spiro[2.5]octane-1,1-dicarbonitrile	160.1	068352-24-9
22	11.3385	0.1157	2-Pentyn-1-ol	84.058	006261-22-9
23	12.0423	0.1322	1-{[4-(Pentyloxy)benzoyl]oxy}pyridin-2(1H)-one	301.131	1000404-27-2
24	12.111	0.2149	[a]1,3,4-Hexatriene, 3-methoxy-	110.073	053783-88-3
25	12.5573	0.2658	2-Decanynoic acid	168.115	001851-90-7
26	12.8548	0.0783	1,1-Dichloro-2-methyl-3-(4,4-diformyl-1,3-butadien-1-yl)cyclopropane	232.006	132607-16-0
27	13.1753	0.8119	Butanedioic acid, 2,3-dihydroxy-, diethyl ester, [S-(R*,R*)]-	206.079	013811-71-7
28	14.9491	0.058	[a]1-Hexen-3-yne, 2-methyl-	94.078	023056-94-2
29	15.1551	0.2289	1,6-Cyclodecanediol	172.146	091108-69-9
30	15.6987	0.1058	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano-	313.204	1000294-64-4
31	16.288	0.0926	7-Norcarancarboxylic acid,methyl ester	154.099	1000222-16-2
32	18.0905	0.0828	1-Cyclohexene-1-methanol	112.089	004845-04-9
33	19.0461	0.5217	Oxirane, tetradecyl-	240.245	007320-37-8
34	19.5038	0.1016	2-Methyl-Z,Z-3,13-octadecadienol	280.277	1000130-90-5
35	19.7041	0.2047	1,14-Tetradecanediol	230.225	019812-64-7
36	19.8929	0.0641	3-Tridecen-1-yne, (E)-	178.172	074744-41-5
37	20.1447	0.7681	Hexadecanoic acid, methyl ester	270.256	000112-39-0
38	21.661	0.205	1,3-Butadiene-1-carboxylic acid	98.037	000626-99-3
39	21.8327	1.2268	Z,Z-4,16-Octadecadien-1-ol acetate	308.272	1000130-95-7
40	21.9586	1.9109	Neophytadiene	278.297	000504-96-1
41	22.0616	0.2018	cis-10-Heptadecenoic acid, methyl ester	282.256	1000333-62-1

42	22.9199	0.2383	[a]1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one	207.047	1000404-25-4
43	23.3033	0.4836	[a]Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	207.09	1000129-52-1
44	24.4191	2.7056	1,2-Benzisothiazol-3-amine, TMS derivative	222.065	1000332-66-2
45	24.5793	0.3813	[a][1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	207.076	1000351-62-2
46	24.8311	1.2327	7-Methyl -2-phenyl-1H-indole	207.105	059541-82-1
47	25.2431	3.2347	[a]1,1,1,3,5,5,5-Heptamethyltrisiloxane	222.093	001873-88-7
48	25.6436	1.7984	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	578.171	019095-24-0
49	26.2158	5.9573	Methyl 3-bromo-1-adamantaneacetate	286.057	014575-01-0
50	26.4904	0.9084	2-Ethylacridine	207.105	055751-83-2
51	26.8338	1.5074	[a]Silicic acid, diethyl bis(trimethylsilyl) ester	296.13	003555-45-1
52	26.9539	0.5197	3H-indole, 2-methyl-3-phenyl-	207.105	1000400-55-3
53	27.3659	0.4198	[a]Cyclotrisiloxane, hexamethyl-	222.056	000541-05-9
54	27.8637	6.2377	[a]1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	222.162	001020-31-1
55	28.064	0.6567	[a]Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	504.152	019095-23-9
56	28.2757	8.7863	Olean-12-en-3-ol, acetate, (3.beta.)-	468.397	001616-93-9
57	29.1283	38.0942	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-	468.397	001259-10-5

KEY: RT = Retention time; Mol. Wt. = molecular weight; CAS = Chemical Abstracts Service. Compounds preceded with superscript alphabet were present in both extracts.

Also, the spectrophotometric methods used in the assessment of the free radical quenching reducing power and assessment of the total antioxidant potentials of the extracts' samples in comparison with a standard antioxidant compound (Ascorbic

acid) revealed (Figures 2, 3, 4 and 5) that the leaf extracts possessed moderate antioxidant properties though lower than that of the standard compound, where the aqueous extract showed an appreciable and higher capacity than the ethanolic extract as evident in their IC_{50} and EC_{50} values.

Free radical scavenging and antioxidant capacity are of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases together with the potential for substantial savings in the cost of health care delivery (Alam *et al*., 2013; Anigboro *et al.*, 2019). Similar antioxidative ability was observed in several leaf extracts by Anigboro and his research teams (2018, 2019 and 2020), who suggested thereafter their possible pharmacological relevance against diseases implicated by oxidative stress. According to Cavalli and Santos (2015), oxidative stress in living systems can be combated by both endogenous antioxidant defense systems and ingested or administered bioactive compounds. The observed antioxidant property of the extract samples in this study may be attributed to the bioactive constituents of the plant's leaves. However, variation of the antioxidative capacities of the samples in the different *in vitro* antioxidant techniques employed in this study, may be as a result of the dependency of the sensitivity of the method on different components of the samples under investigation.

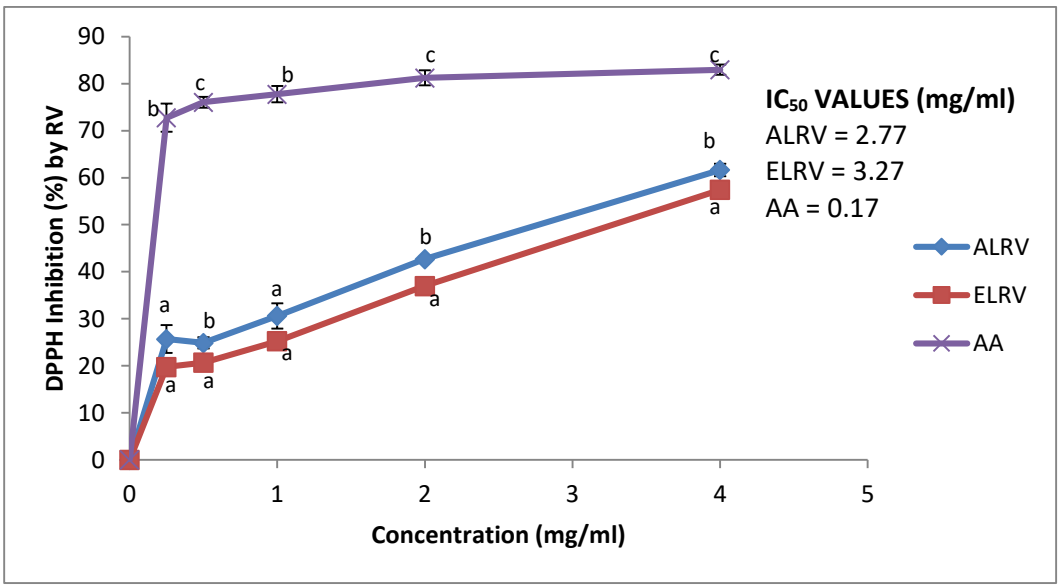

Figure 2: Inhibition of DPPH Radical by Leaf Extracts of *Rauvolfia vomitoria.*
Values are means ± standard deviations (n = 3). Data points (along a common concentration value) containing a similar attached letter are statistically the same ($p>0.05$).
KEY: AA = Ascorbic acid (standard compound), ALRV and ELRV represent aqueous and ethanolic leaf extracts of *Rauvolfia vomitoria* respectively.

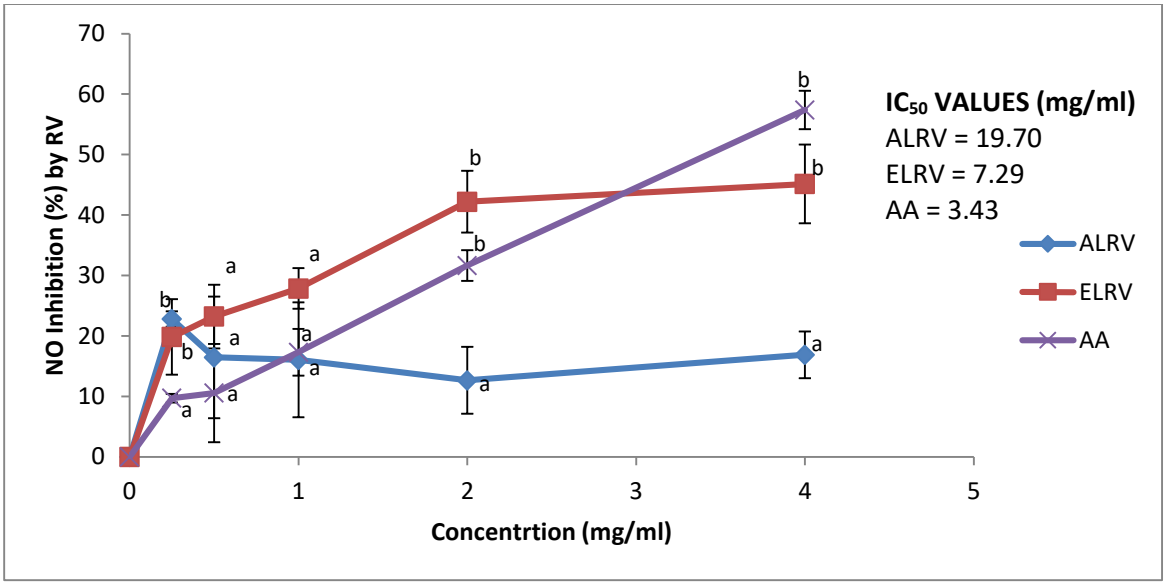

Figure 3: Inhibition of NO˙ Radical by Leaf Extracts of *Rauvolfia vomitoria.*
Values are means ± standard deviations (n = 3). Data points (along a common concentration value) containing a similar attached letter are statistically the same (p>0.05).
KEY: AA = Ascorbic acid (standard compound), ALRV and ELRV represent aqueous and ethanolic leaf extracts of *Rauvolfia vomitoria* respectively.

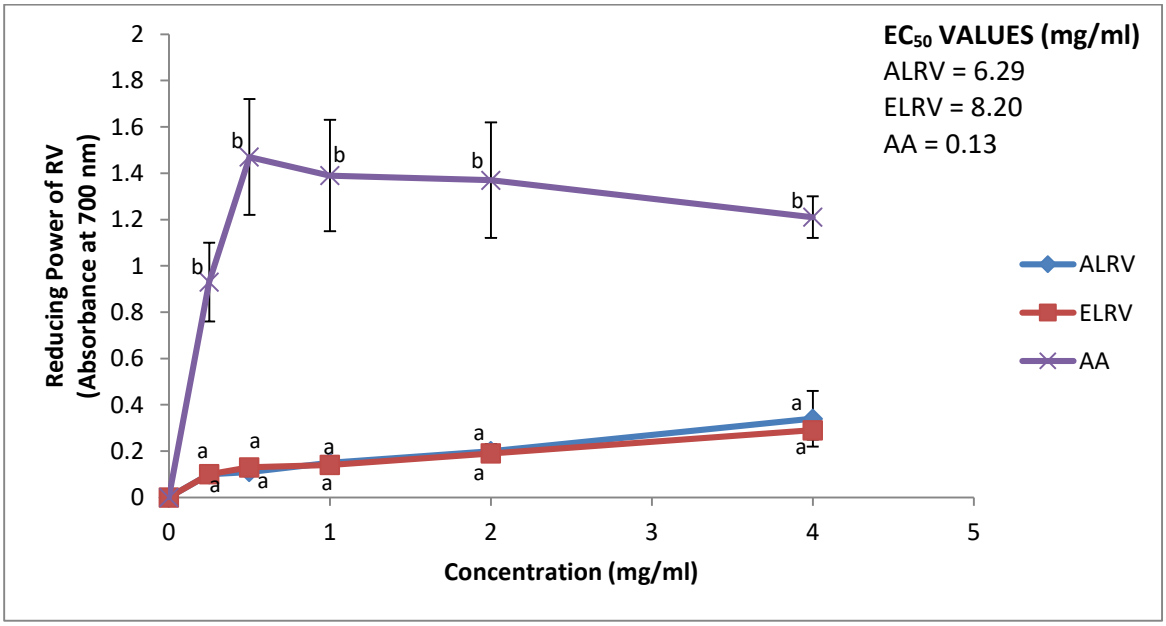

Figure 4: Reducing Power of *Rauvolfia vomitoria* Leaf Extracts.
Values are means ± standard deviations (n = 3). Data points (along a common concentration value) containing a similar attached letter are statistically the same (p>0.05).
KEY: AA = Ascorbic acid (standard compound), ALRV and ELRV represent aqueous and ethanolic leaf extracts of *Rauvolfia vomitoria* respectively.

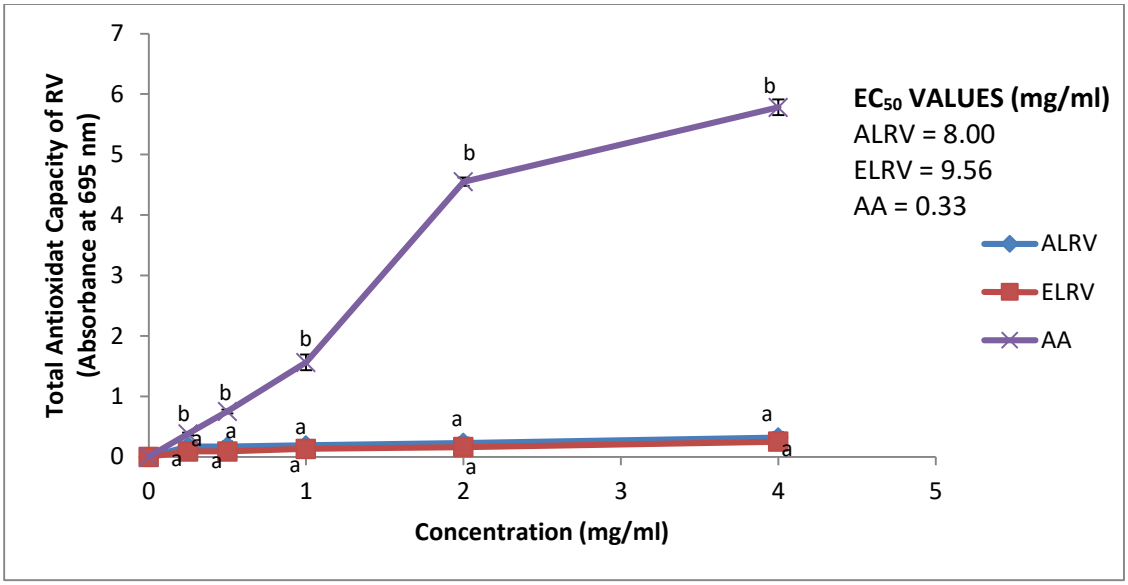

Figure 5: Total Antioxidant Capacity of *Rauvolfia vomitoria* Leaf Extracts.
Values are means ± standard deviations (n = 3). Data points (along a common concentration value) containing a similar attached letter are statistically the same ($p>0.05$).
KEY: AA = Ascorbic acid (standard compound), ALRV and ELRV represent aqueous and ethanolic leaf extracts of *Rauvolfia vomitoria* respectively.

CONCLUSION

The different solvents' extracts possessed different bioactive constituents as well as varying levels of moderate antioxidant capacity in comparison with the standard antioxidant compound (Ascorbic acid) used in this study. These variations are a reflection of the solvent type used in the extraction process, suggesting distilled water as solvent of choice for the extraction of this plant's leaves. The result from this study provide scientific insights into the ethnotherapeutic use of these plants leaves as well as useful information for possible development of novel drugs for management of oxidative stress-associated health conditions.

Conflict of Interests

The authors declare that they have no known competing interests that could appear to influence the work reported in this paper.

Authors' Contribution to the Manuscript

The experiment was conceived and designed by A.A. Anigboro (AAA), O.J. Avwioroko (OJA) and N.J. Tonukari (NJT); O. Akeghware (OA), O. Oborirhovo (OO), OJA, B.J. Okafor (BJO) and F.O. Ovowa (FOO) carried out the experimental work; OA, OO, BJO and OJA analyzed the data; OO, AAA, OA and NJT contributed reagents, materials and softwares for analysis; OJA, OA, AAA and BJO undertook the manuscript writing and

proofreading; while AAA, OJA and NJT supervised the experiments and revised the manuscript.

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