



Phytochemical Screening and Gcms Analyses of Leaf Extracts of *Artemisia Annua* Var. *Chiknensis*. (Cbge/Chna/09/Ltns/G) and *Vernonia Amygdalina* Del.

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ABSTRACT

The research was aimed at identifying phytochemicals in the aqueous and methanolic leaf extracts of *Artemisia annua* (*A.annua*) and *Vernonia amygdalina*. Preliminary phytochemical studies and GCMS analysis were carried out on the leaf extracts of *A.annua* and *V.amygdalina*. The preliminary screening showed the presence of alkaloids, flavonoids, tannins, saponins, steroids, phenols, glycosides and terpenes. Phenols and glycosides were present in large quantities compared to others while alkaloids had the least in terms of occurrence in both leaf extracts of *A.annua* and *V.amygdalina*. The GCMS analysis of the methanolic leaf extract of *A.annua* showed that it contained 33 chemicals with their chemical formula, chemical structure, molecular weight and percentage abundance only 23 showed various % abundance. Of all these chemicals, Deoxyqinghaosu (Deoxyartemisinin) had (7.6%), Oxireno[4,5]cyclopenta[1,2-c]pyran-2(1aH)-one, hexahydro-5a,6-dihydroxy-1a-methyl-, (1a α ,1b β ,5a β ,6a β ,6a α)- (3%), 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-(2.6%), 2,6-Adamantanedione, 4-iodo-, (1R)-(2.2%). The remaining others had a range of % abundance of between 0.2 -1.8%. The GCMS analysis on aq. A.A showed 37 peaks, with a total of 4 compounds with their names, formula and molecular weights. Only two compounds showed % abundance and include 1,3,5-Trioxane (8.1%) and Propanoic acid, 2-methoxy-, methyl ester (0.4%). 41 compounds were revealed by analysis of the methanolic leaf extract of *Vernonia amygdalina*. Compounds with relatively high % abundance are ethyl oleate (6.7%), 3-O-Methyl-D-glucose (4%) and 9,12,15-Octadecatrienoic acid, methyl ester, (3.2%). On the other hand, others had various relatively lower % abundance. Total of 36 compounds were revealed by the analysis on the aqueous leaf extract of *Vernonia amygdalina*. However, only 24 showed various % abundance and include 3-O-Methyl-D-glucose (4.9%), hexadecenoic acid (3%) and -Amino-5-mercapto-4-methyl-1,2,4-triazole has relatively (2.8%) higher % abundance. All others were much lower in % abundance. They are (SR)- or (RS)-4-methyl-2,3-pentanediol (0.06%), 2-Methyl-6-methylene-octa-1,7-dien-3-ol (0.07%), 7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin (0.07%), 1-Naphthalenepropanol, α -ethenyldecahydro-2,4-dihydroxy- α ,2,5,5,8a-pentamethyl-, [1R-(1 α (R*),2 β ,4 α ,4a β ,8a α)]-(0.07%), and 1-Cyclohexylnonene (0.08%) had relatively low % abundance. These compounds have been reported to possess biological activities such as antioxidant property, anti-obesity effects, antidiabetic effects, anti-inflammatory property, antimicrobial activity and many other effects. They are therefore of enormous application in pharmaceutical and other related industries to meet diverse health challenges.

Key words: Phytochemical Screening, GCMS Analysis, *Artemisia annua*

1.0 Introduction

World Health Organization (WHO, 2019) confirmed that 80% of the worldwide populace depend on herbal remedy for their primary healthcare.

Medicinal plants contain phytochemicals which display a wide range of activity including treatment of numerous diseases with minimal side effects relative to synthetic drugs. This has facilitated their use in primary health care delivery system in some countries including Nigeria (Awuchi, 2019). These plants do not have any activity without the presence of certain bioactive chemicals as shown by several researchers (Mercy *et al.*, 2017; Oladeji *et al.*, 2019). Hence plants are reservoirs of these important phytochemicals and must be studied or evaluated to determine their presence, quantity, chemical formula, molecular weights, and other parameters to unravel their identity and authenticate their potency.

Their identification is a key to understanding the various activity they have in the treatment of diseases (Mercy *et al.*, 2017; Oladeji *et al.*, 2019).

Although Ogbonna *et al.* (2017) and Wang *et al.* (2020) have reported on *Artemisia annua* and *Vernonia amygdalina*, the need for more research on these plants cannot be over emphasized due to their usefulness in tackling health and other challenges around the world

This work aims at identifying the bioactive constituents in and *Vernonia amygdalina* and *Artemisia annua* (CBGE/CHNA/09/LTNGS/G) a genetically improved variety of *A.annua* using qualitative and GCMS analyses.

2.0 Materials and Methods

2.1 Preparation of Plant Material and Extraction

Vernonia amygdalina were purchased from a farm behind Modern Market in Makurdi. It was afterward identified and authenticated in the Taxonomy Unit of the Department of Botany of Joseph Sarwuan Tarka University, Makurdi,

Artemisia annua (CBGE/CHNA/09/LTNGS/G) was obtained from the Biotechnology Farm of the Centre for Biotechnology and Genetic engineering, Department of Plant Science and Biotechnology, University of Jos, Jos Nigeria.

Both plant samples were shade-dried at room temperature of between 25°C to 28°C while occasionally being stirred to avoid rot and to facilitate the drying process which occurred within a week.

2.2 Preparation of Extracts.

Four hundred grams (400g) of powdered (dried) leaves of each plant was put in a conical flask containing 2000ml of sterile distilled water. The flask was heated with Bunsen flame for few minutes and was allowed to cool to room temperature, It was aseptically filtered using Whatman filter paper (No1) to separate the residue from the filtrate.

2.3 Qualitative Phytochemical Screening.

The presence of the following bioactive components was evaluated according to the protocol described by Sofowora (1993)

Test for Flavonoids: Sodium hydroxide test was used. Here, 5 ml of the extracts was poured into a test tube and 3 drops of 10% NaOH was added into the test tube. A yellow color showed presence of flavonoid.

Test for Alkaloids: Meyers test was employed. Five ml of the extracts of *Artemisia annua* was poured into test tube after which three drops of Meyers reagent was added. Appearance of creamy color indicated a positive test.

Test for Glycosides: 5ml of the extract was dispensed inside a test tube. Then, one ml of glacial acetic acid containing traces of Ferric Chloride solution was dissolved and moved into a dry clean test tube. One ml of C.H₂SO₄ was added along the side of the tube to form a lower film at the lowermost of the test tube. A thin Purple brown ring indicated dextrose sugar while a pale -green color in the upper acetic acid coat specified presence of cardiac glycoside.

Test for Tannins: Ferric Chloride test by Sofowora (1993) was employed. Five ml of the extract was discharged into a test tube. Then 3-5 drops of Ferric Chloride solution were added to the extract. A green- brown color is the occurrence of tannin while a blue or brownish -blue color is the presence of hydrolysable tannin.

Test for Saponins: Frothing Test by Sofowora (1993) was used. Ten ml of the extract of *A.annua* was prepared with ten ml of distilled water and vigorously shaken for about 30 seconds. Emergence of froth which persist for few minutes showed the presence of saponin.

Test for Steroid: Lieberman Burchardt test was employed (Sofowora, 1993). One ml of the extract of *A.annua* was poured into a test tube after which one ml of chloroform and 2-3 ml of Acetic Anhydride were added. Also added was 1-2 drops of C.H₂SO₄. A dark green color showed steroid is present

Test for Phenol: Five ml of Ferric Chloride solution was added to five ml of the extract of *A.annua* inside a test tube.. Emergence of a blue green color showed the presence of phenol.

Test for Terpenes: The method according to Alamzed, *et al.* (2013) was used to evaluate for presence of Terpenes. Here, a mixture of 2 mL chloroform and 3 mL conc. H₂ SO₄ were poured into a test tube containing about 0.2g of the extracts of *A.annua*. The emergence of a red colored upper layer indicated the presence of terpenes.

The entire procedure was repeated for qualitative test of *V.amygdalina*.

2.4 GC-MS analyses of the aqueous and methanolic extracts of *A.annua* and *V.amygdalina*

Gas Chromatography- Mass Spectrometry (GC-MS) analysis of the various extracts of *A.annua* and *Vernonia amygdalina* were performed with GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemical Components in the plant extracts was done with a QP2010 gas chromatography by means of Thermal Desorption System, TD 20 plus a Mass Spectroscopy (Shimadzu). An ionization voltage of 70eV was employed. Gas Chromatography was performed in the temperature programming mode through a Restek column (0.25 mm, 60 m, XTI-5). A starting column temperature was 80oC for 1min, and then raised linearly at 70oC 60 seconds to 220°C, held for 3 min followed by another linear raise in temperature from 10oC

min-1 to 290oC for 10 min. The temperature of the injection port was 290°C and the GC-MS interface was stayed at 290°C. Each of the plant extracts were separately injected through an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min-1.

2.5 Identification of compounds:

The identification of bioactive mixtures was done through comparative assessment of retention time and fragmentation pattern, with the data deposited in the GC-MS processor Reference library in National Research Institute for Chemical Technology (NARICT) in addition to those published in research works. Information from such other sources were correlated with the data obtained from the bioactive components during the GCMS analysis. Hence, the nomenclature, molecular formula, molecular weight, molecular structure(s) and percentage abundance of the phytochemicals of *A. annua* and *V. amygdalina* were established.

3.0 Results and Discussions

3.1 Results

Table 1: Qualitative Test of Aqueous and Methanolic leaf extracts of *A.annua* and *V. amygdalina*

S/N	Phytochemical Components	<i>A.annua</i>		<i>V.amygdalina</i>	
		Aqueous	Meth.	Aqueous	Meth.
1.	Alkaloids	++	+	+++	-
2.	Flavonoids	+++	+++	-	+
3.	Tannins	+++	+++	+++	++
4.	Saponin	++	++	+++	+++
5.	Steroids	++	+++	-	+++
6.	Phenols	+++	+++	+++	+++
7.	Glycosides	+++	+++	+++	+++
8.	Terpenes	++	+	+++	+

KEY:

+ = Trace amount

++ = Moderate amount +++ = Large amount - = Absent

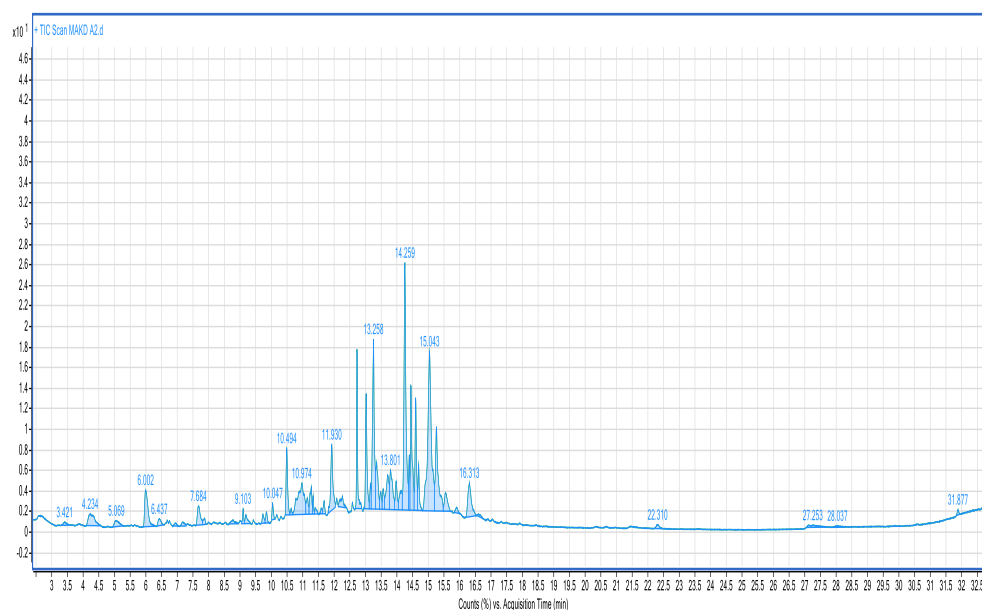


Figure 1 :Chromatogram of methanolic leaf extracts of *Artemisia annua*

33 compound revealed by the analysis of the methanolic leaf extracts of *Artemisia annua*

Table 2a: GCMS of methanolic leaf extract of *A. annua* showing compounds with and without % abundance

PEAK	COMPOUND IDENTIFIED	FORMULAR	MW	ABUNDANCE (%)
1	I. Artemisyl propionate	C13H22O2	210	0.2
	II. Azetidine, 1,2-dimethyl-	C5H11N	85	
2	I. 4,5-Dihydro-2(1H)-pentalenone	C8H8O	120	1.1
	II. 1,2-Benzenedimethanol	C8H10O2	138	
	III. Benzaldehyde	C11H14O2	178	
3	I. Pyrazine	C5H6N2O	110	0.4
	II. 4,5-Dihydro-2(1H)-pentalenone	C8H8O	120	
	III. Pyridine, 2-(1H-tetrazol-5-yl)-	C6H5N5	147	
4	I. Silane, trifluoro(2-methyl-2-butenyl)-	C5H9F3Si	154	1.8
	II. Tutin	C15H18O6	294	
	III. 2-Cyclohexene-1-thione, 3,5,5-trimethyl-	C9H14S	154	
5	I. 2(3H)-Benzofuranone, 3-methyl-	C9H8O2	148	0.3
7	I. Terbulatine	C12H19NO3	225	1.8

KEY: MW= Molecular Weight

Table 2b : GCMS of methanolic leaf extract of *A. annua* showing compounds with and without % abundance

PEAK	COMPOUND IDENTIFIED	FORMULAR	MW	ABUNDANCE (%)
9	I. Coumarin	C9H6O2	146	0.8
	<u>I.</u> 1,2-Naphthalenedione, hydroxy-	6- C10H6O3	174	0.2
	<u>II.</u> 1,5-Naphthyridin-4-ol	C8H6N2O	146	
12	i. Phenethylamine, p, α -dimethyl-	C10H15N	149	0.3
13	I. 2,3-Dimethylamphetamine	C11H17N	163	0.2
15	I. Camphor	C10H16O	152	0.3
18	I. Astypyrone	C9H12O5	200	3
20	I. 6-(3-Methyl-3-cyclohexenyl)-2-methyl-2,6-heptadienol	C15H24O	220	0.8
25	I. 2,6-Adamantanedione, 4-iodo-, (1R)-	C10H11IO2	289	2.2
26	I. α -Methoxy- β , β -dimethylstyrene	C11H14O	162	0.5
28	I. caryophyllene	C15H24	204	2.6

33	I.	photocitral B	C10H16O	152	0.4
34	<u>I.</u>	2,3-Dehydro-4-oxo- β -ionone	C13H16O2	204	1.5
35	I.	Retinal	C20H28O	284	1.7
36	I.	2,4-Dimethyl-7-oxo-4,7-dihydro-triazolo(3,2-c)triazine	C6H7N5O	165	0.8
38	I.	Deoxyqinghaosu	C15H22O4	266	7.6
42	I.	Tetradecanoic acid	C16H32O2	256	0.9
47	i.	Avocadynone	C17H30O3	282	1.8

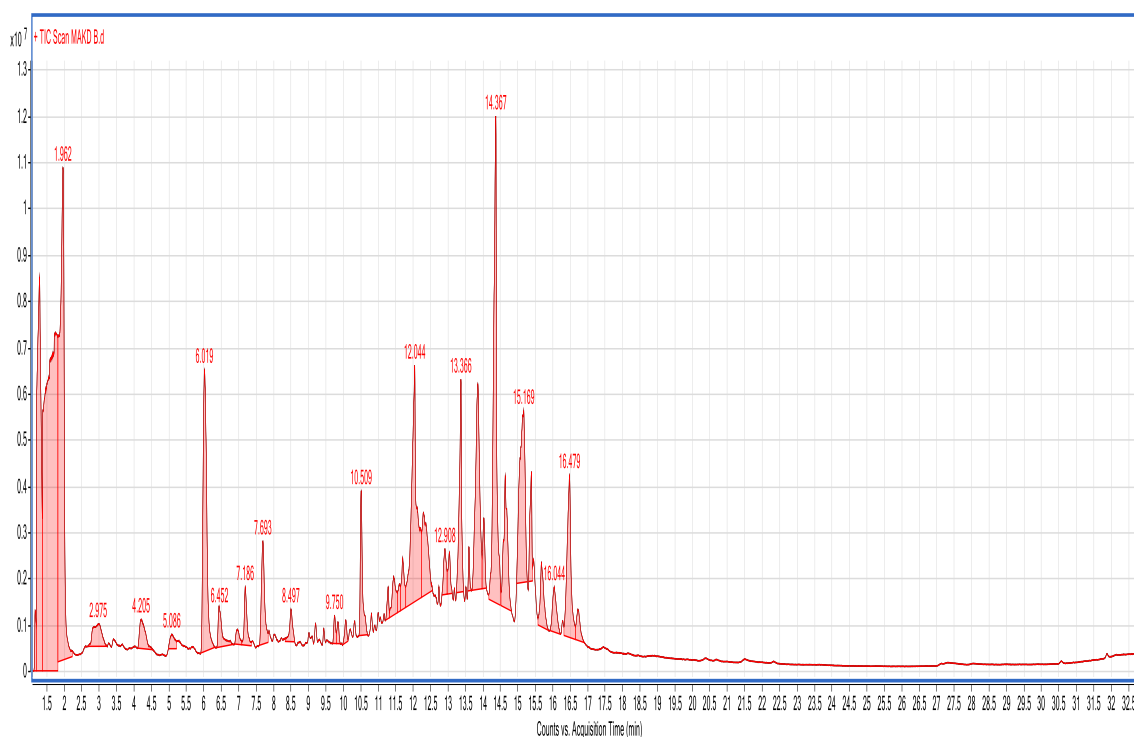


Figure2 : Chromatogram of the aqueous leaf extract of *A.annua*

Table : GCMS of aqueous leaf extract of *A.annua*

PEAK		COMPOUND IDENTIFIED	FORMULAR	MW	ABUNDANCE (%)
1	I.	Erythrolinic acid	C5H10O3	118	0.4
	II.	Propanoic acid, 2-methoxy-	C4H8O3	104	
	III.	Ethanol, 1-methoxy-, acetate	C5H10O3	118	
2	I.	1,3,5-Trioxane	C3H6O3	90	8.1

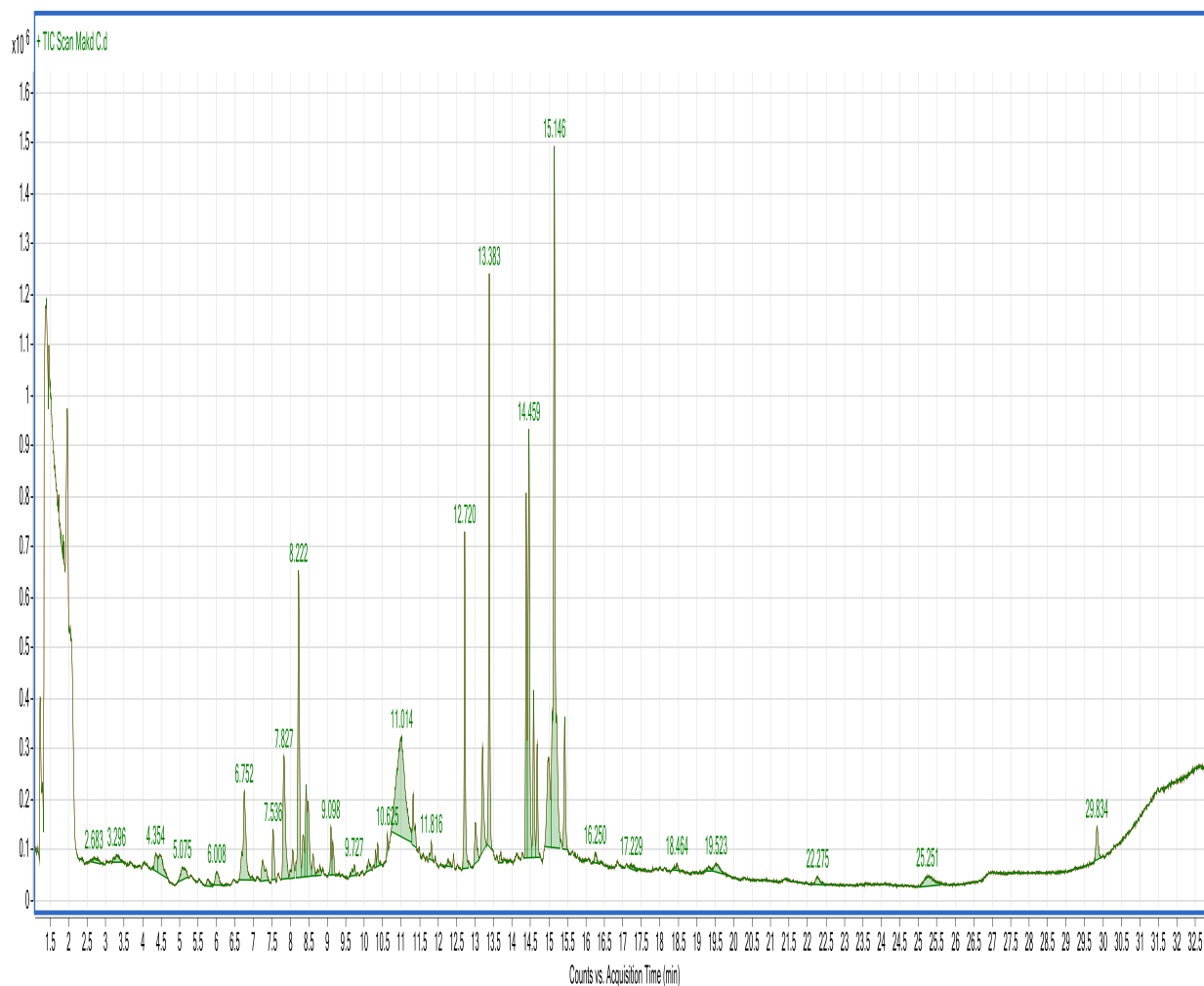


Figure 3 : Chromatogram of the methanolic leaf extract of *V.amygdalina*

59 compound revealed by analysis of sample C

The compound with the highest percentage abundance is

Table 4a: GCMS of methanolic extract of *V. amygdalina*

PEAK	COMPOUND IDENTIFIED	FORMULAR	MW	ABUNDANCE (%)
1	I. 3-Heptadecenal	C17H32O	252	0.1
	II. Chloroacetic acid, 2-tetradecyl ester	C16H31ClO2	290	
2	I. 1,3,8-p-Menthatriene	C10H14	134	0.1
	II. Benzene, 2-ethyl-1,4-dimethyl-	C10H14	134	
3	i. Octanoic acid, ethyl ester	C10H20O2	172	0.2
4	I. 1-methyl-1-indanol	C10H12O	148	0.4
	Acetoxyacetic acid, nonyl ester	C13H24O4	244	
5	I. Benzeneethanamine, $\alpha,3,4$ -trimethyl-	C11H17N	163	0.2
7	I. 3-Selenetanol, 3-(4-methoxyphenyl)-	C10H12O2Se	244	0.2
	II. 4-Acetoxy-3-methoxystyrene	C11H12O3	192	
	iii. Benzeneacetaldehyde, 2-methoxy-	C9H10O2	150	

8	i.	Ethyl 9-decenoate	C ₁₂ H ₂₂ O ₂	198	1.3
	ii.	9-Decenoic acid	C ₁₀ H ₁₈ O ₂	170	
9	I.	Cyclomenol	C ₁₄ H ₂₀ O	204	0.3
	II.	(Z,Z)- α -Farnesene	C ₁₅ H ₂₄	204	
11	i.	α -Guaiene	C ₁₅ H ₂₄	204	1.3
13	I.	β -Bisabolene	C ₁₅ H ₂₄	204	2.3

Table 4b:GCMS of methanolic leaf extract of *V. amygdalina*

PEAK		COMPOUND IDENTIFIED	FORMULAR	MW	ABUNDANCE (%)
14	I.	Deconexent	C ₂₂ H ₃₂ O ₂	328	0.3
	II.	Naprosyn	C ₁₄ H ₁₄ O ₃	230	
	III.	Methyl Montanate	C ₂₉ H ₅₈ O ₂	438	
15	I.	Carophylliene	C ₁₅ H ₂₄	204	0.6
16	I.	Carpacin	C ₁₁ H ₁₂ O ₃	192	0.6
17	I.	Jasmolin	C ₂₁ H ₃₀ O ₃ S	362	0.1
18	i.	Aleve	C ₁₄ H ₁₄ O ₃	230	0.1
21	I.	Diosphenol	C ₁₀ H ₁₆ O ₂	168	0.1
25	I.	Octadecanoic acid, 11-methyl-, methyl ester	C ₂₀ H ₄₀ O ₂	312	0.07
26	I.	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	194	4
30	I.	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	1.8
31	I.	Cyclohexanemethyl propanoate	C ₁₀ H ₁₈ O ₂	170	0.4
32	I.	Undecanoic acid	C ₁₁ H ₂₂ O ₂	186	0.9
33	I.	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	2.8

Table 4c: GCMS of methanolic leaf extract of *V.amgdalina*

PEAK		COMPOUND IDENTIFIED	FORMULAR	MW	ABUNDANCE (%)
35	I.	methyl linoleate	C ₁₉ H ₃₄ O ₂	294	1.9
36		methyl linolenate	C ₁₉ H ₃₂ O ₂	292	3.2
37	I.	Phytol	C ₂₀ H ₄₀ O	296	1
38	I.	Palmitic Acid	C ₁₆ H ₃₂ O ₂	256	0.6
40	I.	Ethyl Oleate	C ₂₀ H ₃₈ O ₂	310	6.7
41	I.	Arachic	C ₂₀ H ₄₀ O ₂	312	0.9
45	I.	Dodecanal	C ₁₂ H ₂₄ O	184	0.07
48	I.	1-n-butyladamantane	C ₁₄ H ₂₄	192	0.4

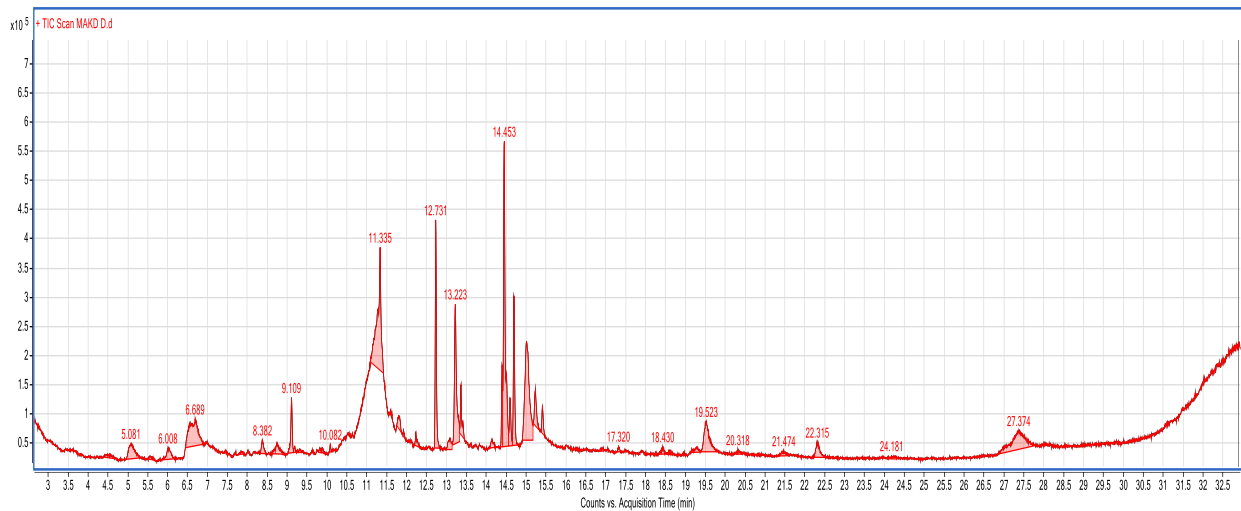


Figure 4: Chromatogram of the aqueous leaf extract of *V.amygdalina*

Total of 36 compounds revealed by the analysis on sample D. However, only 22 showed various % abundance

The compound with the highest percentage abundance is 3-O-Methyl-d-glucose with 4.9%

Table 5a : GCMS of aqueous leaf extract of *A.amygdalina*

PEAK	COMPOUND IDENTIFIED	FORMULAR	MW	ABUNDANCE (%)
2	I. Chloromethyl 2-chloropropanoate	C4H6Cl2O2	156	1
	II. 4-isopropylphenylacetic acid	C11H14O2	178	
	III. Benzaldehyde, 3-methyl-	C8H8O	120	
4	I. 4-Acetoxy-3-methoxystyrene	C11H12O3	192	0.5
	II. 3-Selenetanol, 3-(4-methoxyphenyl)-	C10H12O2Se	244	
	III. Benzeneacetaldehyde, 2-methoxy-	C9H10O2	150	
6	I. 2-Methyl-6-methylene-octa-1,7-dien-3-ol	C10H16O	152	0.07
	II. Dimethoxyphenol	C8H10O3	154	
7	I. Acetamide, N,N'-2,6-pyrazinediylbis-	C8H10N4O2	194	0.3
	II. 7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin	C14H14O3	230	
8	I. 1-Cyclohexyl-1-(4-ethylcyclohexyl)ethane	C16H30	222	0.5
	II. 1,3-Dimethyl-5-n-decylcyclohexane	C18H36	252	
	III. 9-Oxononanoic acid	C9H16O3	172	
9	I. 1-Nonadecene	C19H38	266	0.9
14	I. Naprosyn	C14H14O3	230	0.07
15	I. 3-O-Methyl-d-glucose	C7H14O6	194	4.9
	II. 3-Methylmannoside	C7H14O6	194	
	III. D-Fructose, 3-O-methyl-	C7H14O6	194	

Table 5b: GCMS of aqueous leaf extract of *A.amygdalina*

COMPOUND IDENTIFIED	FORMULA	MW	%ABUNDANCE
16 i. Hexanidiol	C6H14O2	118	0.06
ii. 2,2,4-Trimethyl-3-pentanol	C8H18O	130	
18 i. Auricolic acid	C20H36O3	324	0.07
20 i. Hexadecanoic acid, methyl ester	C17H34O2	270	3
21 i. Ambroxan	C16H28O	263	0.4
27 i. Phytol	C20H40O	296	0.8
35 i. 1-Cyclohexylnonene	C15H28	208	0.08
ii. 6-Nonenal, (Z)-	C9H16O	140	
36 i. 1-Cyclohexylnonene	C15H28	208	0.2
ii. Tetradecanal	C14H28O	212	
38 i. 1,15-Pentadecanediol	C15H32O2	244	0.1
42 i. 2-thioethyl-1h-tetrazole	C3H6N4S	130	2.8
ii. 1α, 4$\alpha$$\beta$, 8$\alpha\alpha$-Decahydro-1-naphthalenol	C10H18O	154	

3.2 Discussion

Phytochemical components are naturally occurring in plant. They are responsible for health, colour, flavour, aroma and other important features.

From Table 1, the qualitative analysis of the leaf extracts of *A.annua* and *V.amygdalina* revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, phenols, glycosides and terpenes.

Phenols and glycosides were present in large quantities compared to others while alkaloids had the least in terms of occurrence in both leaf extracts of *A.annua* and *V.amygdalina*. In any case, few phytochemicals showed either trace as with Alkaloids in *A. annua* methanolic, flavonoids in *V.amygdalina* methanolic, terpenes in *A.annua* methanolic and in *V.amygdalina* methanolic or even absent as in the case of alkaloids in *V.amygdalina* methanolic, and flavonoids and steroids in *V.amygdalina* aqueous.

Similar result was reported by Ogbonna *et al.* (2010) and Okete *et al.*(2015) after evaluation of *A.annua* and *V.amygdalina*.

A. annua and *V.amygdalina* have a wide range of phytochemicals (Hwang *et al.*,2016; Ekier *et al.*2021). The nature of these phytochemicals vary according to different factors such as the environment where it was cultivated (Nageeb *et al.*, 2014).

Other supporting report such as the one from Luo *et al* (2017) also revealed the presence most of these phytochemicals unveiled in this work.

Approximately six hundred phytochemicals have been identified in *A.annua*. Some notable ones include

numerous sesquiterpenoids, triterpenoids, monoterpenoids, steroids, flavonoids, coumarins, alkaloids and benzenoids. Although there hasn't been notable morphological variation in *A.annua* from different regions of the world, variations on the basis of chemical components and possible health related uses have been reported(Qui *et al.*, 2018).

The GCMS analysis of the various leaf extracts showed the various phytochemicals present with their retention time, molecular formula, molecular weight and peak area % as presented on the Chromatograms and Tables.

The GCMS analysis of the methanolic leaf extract of *A.annua* showed that it contained 33 chemicals with their chemical formula, chemical structure, molecular weight and percentage abundance **only 23 showed various % abundance**. Of all these chemicals, Deoxyqinghaosu(DeoxyArtemisinin) had (7.6%), Oxireno[4,5]cyclopenta[1,2-c]pyran-2(1aH)-one, hexahydro-5a,6-dihydroxy-1a-methyl-, (1 $\alpha\alpha$,1b β ,5a β ,6 α ,6 $\alpha\alpha$)- (3%), I 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-(2.6%), 2,6-Adamantanedione, 4-iodo-, (1R)-(2.2%).

The remaining others had a range of % abundance of between 0.2 -1.8%.

The phytochemicals have been described to have different effects such as antioxidant property, anti-obesity effects, antidiabetic effects, anti-inflammatory property, antimicrobial activity and many other effects (Gbinidu and Nimenibo, 2019).

On the other hand, the GCMS analysis on aq. *A. annua* showed 37 peaks, with a total of 4 compounds with their names, formula and molecular weights. Only two compounds showed % abundance and include 1,3,5-Trioxane (8.1%) and Propanoic acid, 2-methoxy-, methyl ester (0.4)

A large variation in the percentage abundance of artemisinin and other phytochemicals has been detected in the leaves of diverse samples of *Artemisia annua*. The variation may occur due to factors such as extract method and equipment used in the evaluation, stage of growth of plant samples, the time of collection and preparation of the samples. Furthermore, an environmental factor such as temperature and availability of nutrient may account for the variations.

Although of interest among the phytochemical components of *A. annua* is artemisinin, and even though artemisinin was not found among the phytochemicals revealed in this research, its analogues such as deoxy artemisinin, Oxireno, and cyclopenta among others were found in varying yields.

Past work reported 0.01 -1.4% artemisinin from wild variety (Jansen, 2006), 1.2% **Ferreira, 2010**), 4.6% (Ogbonna *et al.*, 2017). However, at different growth stages of *A. annua* the concentration of AA and DHAA may surpass those of artemisinin but at maturity, artemisinin overtakes in concentration relative to those other phytochemicals.

In a study by Nagy *et al.* (2021) on *A. annua* extract, they detected the presence of artemisinin and its analogues such as ascaridole, artemisia ketone, casticin, deoxyartemisinin, arteannuic acid, artemetin, dihydroartemisinic acid

A total of 41 compounds were revealed by GCMS analysis of the methanolic leaf extract of *Vernonia amygdalina*. Compounds with relatively high % abundance are ethyl oleate (6.7%), 3-O-Methyl -d-glucose (4%) and 9,12,15-Octadecatrienoic acid, methyl ester, (3.2%). Meanwhile, other components had various relatively lower % abundance.

This result is a contrast of the one obtained by Adeoye *et al.* (2018) and Igbiduru and Nimenibo (2019) who reported lesser number of phytochemicals in *Vernonia amygdalina*

This variance may be due to the equipment used, method of extraction and geographical location among other factors.

These phytochemicals have various activities such as antioxidant, antidiabetic and other effects (Igwe *et al.*, 2015; Igbiduru and Nimenibo 2019).

Total of 36 compounds revealed by the analysis on the aqueous leaf extract of *Vernonia amygdalina*. However, only 24 had various % abundance as presented on Table 5a and 5b

3-O-Methyl-d-glucose (4.9%), hexadecenoic acid (3%) and -Amino-5-mercapto-4-methyl-1,2,4-triazole has relatively (2.8%) higher % abundance. All others were much lower in % abundance.

Igwe *et al.* (2015) on the other hand identified the presence of eleven phytochemicals in GCMS analysis of *Vernonia amygdalina* ethanolic leaf extract obtained from Umudike, Nigeria. They consist of, 3, 5-bis (1, 1 dimethylethyl (Phenol) ; Tetradecanoic , (Eicosanoic acid); 9, 12-octadecadienoic acid (Linoleic acid); 3, 7- dimethyldodecan-1-ol (Phytol); 6-octadecenoic acid (Oleic acid); octadecanoic acid (Stearic acid); Cholest-5, 3- ol, 5-acetate (Cholestane) and 1,2-Benzenedicarboxylic acid (Di-n-octyl phthalate).

[Olusola-Makinde et al.](#) (2021) reported that GC-MS on the aqueous extract of *V. amygdalina* yielded higher percentage abundance of 11.89 when compared to ethanol extract (5.37%). It revealed the presence of butanoic acid, squalene, palmitaldehyde, octadecanoic acid, Z-hexadecanoic acid ethyl ester, oxirane, tetradecyl, 3- methyl-2-phenylindole, n-heneicosane, phytol, methyl-2-O-benzyl-d-arabinofuranoside, cholest-5-en-3-ol acetate; with hexadecanoic acid ethyl ester and 1,1-diethoxy-3-methylbutane having the highest percentage composition of 24.37% and 13.42% in aqueous and ethanol extract respectively

Conclusion

The research has shown that *A. annua* and *V. amygdalina* possess several phytochemicals whose formula structure and activity have been brought to lime light may facilitate their incorporation in drug formulation. The methanolic extract produced high abundance of the deoxyartemisinin and few other phytochemicals than the aqueous extract. *V. amygdalina* from Makurdi appears to generally produce very high yield of the phytochemicals. Although all solvents are useful in extracting these components, an understanding of the use of appropriate solvent may enhance obtaining relatively higher percentage abundance.

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References

Adeoye, A.T., Akinrinde, A.S., Oyagbemi, A.A., Omobowale, T.O., Adedapo, A.D.A., Ayodele, E.A., Yakubu, M.A., Adedapo, A.A. (2018). Phytochemical Analgesic, in-Vitro Antioxidant and GCMS Analysis of *Vernonia amygdalina* leaves. *African Journal of Biomedical Resources* Vol.21:303-312

Alamzeb, M., Khan, M.R., Ali, S., Shah, S.Q., & Mamoon, U.R. (2013). Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*, *Bangladesh Journal of Pharmacology*, 8(2): 107-109. <https://doi.org/10.3329/bjp.v8i2.13551>, Accessed: 19.01.2018.

Awuchi, C.G. Medicinal plants: The medicinal, food and Nutritional Biochemistry and Uses. *International Journal Advance Academy Research*, 5(11) (2019), pp.220- 241.

Ekiert, H., Świątkowska, J., Klin, P., Rzeplia, A and Szopa, A (2021) *Artemisia annua* – Importance in Traditional Medicine and Current State of Knowledge on the Chemistry, Biological Activity and Possible Applications. *Planta Med* 2021; 87: 584–599 |

Ferreira, J. F., and Luthria, D. L. (2010). Drying affects artemisinin, dihydroartemisinic acid, artemisinic acid, and the antioxidant capacity of *Artemisia annua* L. leaves. *Journal of Agriculture and Food Chemistry*. 58, 1691–1698. doi: 10.1021/jf903222j

Hwang DI, Won KJ, Kim DY, Yoon SW, Park JH, Kim B, Lee HM(2016). Anti-adipocyte differentiation activity and chemical composition of essential oil from *Artemisia annua*. *Natural Products Communication* 11: 539–542

Igbinidu GO and Nimenibo Uadia R (2019) GC-MS analysis, phytochemical screening and In vitro alpha amylase and alpha glucosidase inhibitory activities of *Vernonia amygdalina* root extract and fractions. *Journal of Pharmacognosy and Phytochemistry* 2019; 8(4): 2125-2131

Igwe, K.K.I., Okafor, P.N and Ijeh and I.I (2015) GC-MS analysis of phytochemicals in *Vernonia amygdalina* Del leaves and its contractile potential in mammary tissue in female albino Wistar rats. *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)* e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 11 Ver. I PP 25-30 www.iosrjournals.org DOI:

Jansen, F.H., 2006. The herbal tea approach for artemisinin as a therapy for malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 100, 285–286.

Luo, X., Jiang, Y., Fronczek, F. R., Lin, C., Izevbogie, E. B., Lee, S. and Lee, K. S. (2017). Isolation and Structure Determination of a Sesquiterpene Lactone (Vernodalinalin) from *Vernonia amygdalina* Extracts. *Pharmaceutical Biology*. 49(5): 464–470.

Mercy, A.G., Light, W.F., & Gospel, S.A. (2017). Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta region of Nigeria. *Journal of food and Nutrition Sciences*, 5(5): 198-205 <https://doi.org/10.11648/j.jfns.20170505.16>, Accessed: 08.09.2018.

Nageeb A, Al-Tawashi A, Mohammad Emwas AH, Abdel-Halim Al-Talla Z, Al-Rifai N. Comparison of *Artemisia annua* bioactivities between traditional medicine and chemical extracts. *Curr Bioact Compd* 2014; 9: 324–332

Nagy, C., Pesti, A., András, M., Vasas, G and Gáspár, A (2021)

Determination of artemisinin and its analogs in *Artemisia annua* extracts by capillary electrophoresis – Mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* Volume 202, 5 August 2021, 114131

Okeke CU, Ezeabara CA, Okoronkwo OF, Udechukwu CD, Uka CJ (2015). Determination of Nutritional and phytochemical compositions of two variants of bitter leaf (*Vernonia amygdalina* Del). *Journal of Human Nutrition and Food Science* 3(3):1065.

Ogbonna, C.I.C., Ajayi, J.A., Nwufe, B.T., Ajala, B.A., Ogbonna, A.I., Agbo, E.B., Oyawoye, O.M., Ameh, J.B and Akpojita, F. (2010) Insecticidal activity of *Artemisia annua* L. (CBGE/CHNA/09/LTNGS/G) ethanolic leaf and seed extracts on *Anopheles gambiae*

Nigerian Journal of Biotechnology Vol. 21 :18 – 24 ISSN: 0189 17131 Available online at <http://www.ajol.info/index.php/njb/index> and www.biotechsocietynigeria.org

C. I. C. Ogbonna, A. I. Ogbonna*, I. A. Onyimba2, J. U. Itelima1, A. F. Umar3, N. Onyezili4 and M. F. Istifanus (2017) Combined Anti-diabetic Effects of Extracts of *Artemisia annua* Combined Anti-diabetic Effects of Extracts of *Artemisia annua* var. *chiknensis* (CBGE/CHNA/09/LTNGS/G) and Each of Three Other Plants (*Momordica charantia* Linn. *Vernonia amygdalina* Del. and *Aegle marmelos* Correa) Traditionally Used in Nigeria for the Treatment of Diabetes (CBGE/CHNA/09/LTNGS/G) and Each of Three Other Plants (*Momordica charantia* Linn. *Vernonia amygdalina* Del. and *Aegle marmelos* Correa) Traditionally Used in Nigeria for the Treatment of Diabetes

Journal of Scientific Research and Reports 16(2): 1-12, 2017; Article no.JSRR.36375 ISSN: 2320-0227

Oladeji, O.S., Odelade K.A., & Oloke, K. (2019). Phytochemical screening and anti-microbial investigation of *Moringa oleifera* leaf extract. *African Journal of Science and Technology, Innovation, and Development*, 12(1): 79-84. <https://doi.org/10.1080/20421338.2019.1589082>, Accessed: 04.11.2020.

Olusola-Makinde, O., Olabanji, O.B and , T.A (2021) Evaluation of the bioactive compounds of *Vernonia amygdalina* Delile extracts and their antibacterial potentials on water- related bacteria *Bulletin of the National Research Centre* volume 45, Article number: 191

Qiu, F.; Wu, S.; Lu, X.; Zhang, C.; Li, J.; Gong, M.; Wang, M (2018). Quality Evaluation of the Artemisinin-Producing Plant *Artemisia Annua* L. Based on Simultaneous Quantification of Artemisinin and Six Synergistic Components and Hierarchical Cluster Analysis. *Ind. Crop. Prod.* 2018, 118, 131–141. [Google Scholar] [CrossRef]

Soforowa,A(1993). Screening Plants for Bioactive Agents in Medicinal Plants and Traditional Medicine in Africa, 2nd Edition, Spectrum Books Ltd. Sunshine House ,Ibadan, Nigeria. Pp.42-44, 221-229, 246-249, 304-306, 331-332, 391-327.

Wang, D., Cui, L., Chang, X., Guan, D.(2020).Biosynthesis and characterization of Zinc Oxide Nanoparticles from *Artemisia annua* and Investigate Their Effect on Proliferation, Osteogenic Differentiation and Mineralization in Human Osteoblast-like MG-63 Cells. *Journal Photochemistry Photobiology. B*, 141, 854-859.

WHO, (2019). Artemisia pollen is the main vector for airborne endotoxin. *Journal of Allergy and Clinical Immunology*, 143(1), 369-377.