BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 13, Number 1, April 2024 | Pages: 297-303 | DOI: 10.14421/biomedich.2024.131.297-303 ISSN 2540-9328 (online)

Exploring Allelochemical Compositions in The Roots of Two Varieties of *Anacardium occidentale* (Cashew)

Nnenna Ejije Okoronkwo*, Jude Chibuzor Igwe, Nkemakolam Bright Nwosu, Precious Onyinyechi Chukwu, Udoka Ifunnaya Chukwukere, Perculiar C. Ihedigbo
Pure and Industrial Chemistry Department, Faculty of Physical Science, Abia State University Uturu, Abia State, 08035626322, Nigeria.

Corresponding author*
ne.okoronkwo@abiastateuniversity.edu.ng, nwosunkemakolambright@gmail.com

Manuscript received: 27 May, 2024. Revision accepted: 14 July, 2024. Published: 26 August, 2024.

Abstract

Allelochemicals are chemicals released by plants that affect other plants and pests. The chemicals are provided by different parts of the plant or released through the natural decomposition of the associated plants. Anacardium occidentale has been reported to exhibit allelopathy. The bark and inner parts of the roots of the two varieties (red and yellow) of Anacardium occidental (Linn) were qualitatively and quantitatively analysed for the presence of allelochemicals. The samples were also subjected to Gas Chromatography-Mass Spectrometry (GC/MS) analysis. The qualitative results showed that flavonoids, tannins, and phenols were present in both the bark and inner parts of the roots, whereas alkaloids, saponins, and terpenoids were present only in the bark of the roots. The quantitative results showed that while the root bark of the red variety had the highest saponin content, that of the yellow variety had the highest phenol content. The GC/MS results for both samples showed the presence of different compounds, and one of the compound peaks observed in the inner root was not identified; 9,12– octadecadienoic acid (fatty acid) was the compound with the highest composition identified in the yellow variety. Similar compounds were identified in the root bark of the red and yellow varieties, which include 3-Tridecyl phenol with molecular formula C19H32O and molecular weight 276, which occurred at a retention time range of 41.703–41.712 min and had the lowest percentage compositions of 2.816 and 4.732% in red and yellow varieties, respectively. The compounds with the highest percentage compositions of 32.389 and 41.944% in red and yellow, respectively, were identified as (Z)-3-(Heptadec-10-en-1-yl) phenol (molecular formula C23H38O with molecular weight 330), occurring within the retention time range of 48.246 – 48.263 min. Other compounds with higher percentage compositions were identified in the samples, including (Z)-3-(pentadec-8-en-1-yl) phenol and 3-((4Z,7Z)-Heptadeca-4,7-dien-1-yl) phenol. However, 1,2,4,-Benzenetriol (molecular formula C6H6O with molecular weight 252) was only identified in the red variety, with a percentage composition of 8.883%, which occurred at a retention time of 22.040 min. The compounds identified in the bark were mostly phenols, whereas those in the inner roots were more fatty acids.

Keywords: Alleochemical; Alleopthathy; Quantitative Phytochrmicals; Anacardium occidental GCMS.

Abbreviations: Gas Chromatography Mass Spectroscopy (GC-MS)

INTRODUCTION

Allelochemicals are chemicals produced and released by plants that affect other plants and pests. These chemicals are provided by different parts of the plant or released through the natural decomposition of the associated plants. *Allelopathy* is the biological phenomenon by which an organism produces one or more biochemicals that influence the germination, growth, survival, and reproduction of other organisms. Allelopathy thus offers an attractive and environmentally friendly alternative to pesticides or herbicides in agricultural pest and weed management. Allelopathins are the secondary metabolites (Weir *et al.,* 2004; Iqbal & Fry, 2012). These compounds belong to different chemical groups, including triketones, terpenes, benzoquinones, coumarins, flavonoids, terpenoids, phenolic acids, tannins, lignin, fatty acids, and nonprotein amino acids. Allelochemicals can be classified into ten categories (Li *et al.,* 2010) based on their different structures and properties: 1. water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, ketones and 2. simple lactones; 3. long-chain fatty acids, polyacetylenes; and 4. quinines (benzoquinone, anthraquinone, and complex quinines) and 5. phenolics; 6. cinnamic acid and its derivatives; 7. coumarins; 8. flavonoids; 9. tannins; 10. Steroids and terpenoids (sesquiterpene lactones, diterpenes, and triterpenoids). Root exudates, including allelochemicals, are known to influence soil biota, inhibit the growth of competing plant species, and support beneficial symbioses (Bertin *et al.,* 2003). The allelochemical compositions of the roots of two varieties of *Anacardium occidentale* (cashew) have not been explicitly detailed in the provided papers. However,

relevant information can be inferred from the studies on the phytochemical and nutritional composition of different parts of the cashew plant. Nwosu *et al.* (2023) report the glycosides, flavonoids, phen, and proteins in extracts of the cashew plant, including the bark and leaves, which suggests that compounds may be present in the roots. Nwosu *et al.* (2024) identify active components such as alkaloids, tannins, flavonoids, coumarins, terpenoids, and saponins in the stem bark, root bark, and leaves, which could indicate the presence of these compounds in the roots as well (Nwosu *et al.,* 2023; Nwosu *et al.*, 2024).

Many chemical constituents may be found in a single plant (Okoronkwo & Echeme, 2015; Okoronkwo *et al.*, 2012a/b). Natural-product-based pesticides are generally safer than conventional synthetic pesticides. Furthermore, there is a strong rationale for examining natural products to identify novel compounds (Dayan *et al.,* 2012; Gerwick & Sparks, 2014). Some plants suppress the germination of understory plants despite the relative openness of the canopy and ample rainfall in the region where they are found. The inhibition of the growth of neighbouring plants by another plant can be explored for its possible use as a herbicide or pesticide by investigating the allelochemical compositions of the different parts of plants that may exhibit such allelopathy. The possible application of allelopathy in agriculture has become the subject of much research (Kong *et al.,* 2006) because the application of such allelopathic plant extracts can effectively control weeds and pests. Future research should focus on the non-destructive collection and identification of these allelochemicals to further elucidate their ecological functions (Liang *et al.,* 2005). Current research focuses on the effects of weeds on crops, crops on weeds, crops on crops (Kong *et al.,* 2008), and pests in general. Therefore, this study focused on the evaluation of allelochemical compositions of the root parts of two varieties of Anacardium occidentale, which have been observed to exhibit allelopathic properties.

MATERIALS AND METHODS

Preparation of Plant Extracts

The roots of the plants used in this study were collected from Abia State University Uturu, Nigeria. The samples were washed, air-dried, ground, and stored in tight containers until further use.

Allelochemical analysis for the presence of plant secondary metabolites, such as tannins, saponins, alkaloids, phenolics, flavonoids, benzoquinones, steroids, and terpenoids, was carried out using standard procedures (AOAC, 2015). The samples were then subjected to GC-MS analysis.

Qualitative Phytochemical Screening Test

Test for Carbohydrates (Molisch's test)

Three drops of Molisch's reagent were added to the 2 ml portion of the various extracts. This will followed by the addition of 2 ml of conc. H_2SO_4 to the bottom of the test tube. The mixture was then allowed to stand for two-to three minutes. The formation of a red or dull violet colour at the interphase of the two layers will be a positive test.

Test for Alkaloids

0.5 ml of each extract was dissolved in 1 % HCl and filtered the solution, and the filtrate was tested with Dragendroff's and Mayer's reagents separately. The appearance of turbidity is an indication of the presence of alkaloids.

Test for Cardiac glycosides (Keller Kelliani's test)

0.5 ml of each extract was treated with 2 ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This will be carefully added with 1 ml concentrated H_2SO_4. A brown ring at the interface indicated the presence of deoxy sugar characteristic of cardenolides. Below this layer greenish colour ring forms, which turns into violet after some*time.

Test for Flavonoids (Alkaline reagent test)

1 ml of each extract was treated with 3-4 drops of 20 % NaOH solution. The formation of an intense yellow colour, which becomes colourless with the addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Phenols (Ferric chloride test)

0.5 ml of each extract was treated with aqueous 5 % $FeCl_3$ 10 % Ferric chloride solution (light yellow).

Test for Quinines HCl Test

The 1ml of plant extract was taken. Added 2ml of concentrated hydrochloric acid (conc. HCL). The formation of a yellow colour indicated the presence of quinine.

Test for Proteins (1% ninhydrin solution in acetone)

2 ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Test for Terpenoids Salkowski Test

The 1 ml of plant extract in a test tube was taken. Added 2 ml chloroform in that extract. Along with that, added carefully 3 ml of sulfuric acid (conc. H_2SO_4) was for the formation of a layer. The formation of a reddish-brown colour indicated the presence of terpenoids.

Test for Saponins
1 ml of extract was added to 5 ml of water in a test tube. The mixture will be shaken vigorously and observed for the formation of persistent foam that will confirm the presence of saponins.

Test for Sterols (Liebermann-Burchard test)
1 ml of each extract was treated with 1-2 drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of deep pink or red colour.

Test for Tannins (Braymer's test)
1 ml of each extract was treated with 10 % alcoholic $FeCl_3$ solution and observed for the formation of a blue or greenish colour solution.

Quantitative Determination of Phytochemicals
Quantitative Estimation of Alkaloids
To 1 ml of test extract 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against the blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents.

Quantitative Estimation of flavonoids
Total flavonoid content was determined by the Aluminium chloride method using catechin as a standard. 1 ml of the test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min, 0.3 ml of 5 % Sodium nitrite, and 0.3 ml of 10% Aluminium chloride were added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Quantitative Estimation of Saponins
The test extract was dissolved in 80% methanol, 2 ml of Vanilin in ethanol was added, mixed well, and the 2 ml of 72% sulphuric acid solution was added, mixed well, and heated on a water bath at 600 c for 10 min. Absorbance was measured at 544 nm against the reagent blank. Diosgenin was used as a standard material and the assay was compared with Diosgenin equivalents.

Quantitative Estimation of Steroids
1 ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Quantitative Estimation of Phenolic Compounds
The total phenolic content in different solvent extracts was determined with the Folin- Ciocalteu's reagent (FCR). In the procedure, different concentrations of the extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min, 4 ml of sodium carbonate solution was added. The final volume of the tubes was made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. The absorbance of the sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard, and the total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight and the standard graph.

GC analysis
GC analysis was carried out on a GC102AF system comprising an Injector Port and Fid Detector auto sampler and GC instrument employing the following conditions: Column Elite - 1 fused silica capillary column (30 × 0.25 mm ID 1 × EM df, composed of 100% dimethylpolysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1 injector temperature 250° C; ion source temperature 280° C.

The oven temperature was programmed from 110 °C (isothermal for 2 min) with an increase of 10° C/min to 200°, then 5 EI/min to 280° C, ending with a 9 min isothermal at 280° C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 Da to 550 D

Data analysis
Data generated from all analysis were subjected to analyses of variance and means where significant ($p \leq 0.05$) were separated with Fisher's least significant difference using Statistical Package for Social Sciences (SPSS) version 13.0

RESULTS AND DISCUSSION

Results of Qualitative Phytochemical screening shown in table 1 shows the allelochemical compositions in the root parts of the red and yellow varieties of Anacardium occidentale. It shows the presence or absence of various plant secondary metabolites such as tannins, saponins, alkaloids, phenolics, flavonoids, benzoquinones, steroids, and terpenoids.

Table 1. Result of the Screening/Qualitative Compositions of the Inner and Bark of the Root of Two Varieties of the Plant.

Parameters	Root Bark				Inner Root			
	Red		Yellow		Red		Yellow	
	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
Alkaloids	+	+	+	+				
Flavonoids	+	+	+	+	+	-	+	-
Saponins	+	+	+	+	-	-	-	-
Tannins	+	+	+	+	+	-	+	-
Terpenoids	+	+	+	+	-	-	-	-
Phenols	+	+	+	+	+	+	+	+
Anthraquinone	-	-	-	-				
Cardiac Glycosides	-	-	-	-	-	-	-	-
Reducing Sugar	-	-	-	-				
Steroid	-	-	-	-	-	-	-	-

Result of the Quantitative Compositions of the inner and bark of the Roots of two plant varieties are shown in Table 2, which shows the percentage content of different compounds such as saponins, tannins, terpenoids, and phenols.

Table 2. Result of the Quantitative Compositions of the inner and bark of the Root of two varieties of the Plant.

Parameters	Samples			
	Root Bark		Inner Root	
	Red	Yellow	Red	Yellow
Alkaloids	9.67±4.04	7.83±3.03	-	-
Flavonoids	25.5±4.04	16.7±3.03	13.06±3.03	15.16±1.63
Saponins	55.5±2.05	85.33±3.03	-	-
Tannins	1.59±0.03	1.58±0.09	0.162±1.664	0.364±0.206
Terpenoids	8.50±1.73C	12.50±6.06	-	-
Phenols	1.78±0.04	1.87±0.40	1.72±0.123	1.347 ±0.07

Tables 3. Gc/Ms Result of The Root Bark of Red Variety *Anacadium Occidentale*.

Chromatogram Peak	Identified Compound Name	Molecular Formula	Molecular Weight	Retention Time (Mins)	Percentage Content
1	1,2,4,-Benzenetriol	C_6h_6o	252	22.040	8.883
2	3-Tridecyl Phenol	$C_{19}h_{32}o$	276	41.703	2.816
3	(Z)-3-(Pentadec-8-En-1-Yl) Phenol	$C_{21}h_{34}o$	302	44.924	17.663
4	(Z)-3-(Pentadec-8-En-1-Yl) Phenol	$C_{21}h_{34}o$	302	45.000	4.739
5	(Z)-3-(Pentadec-8-En-1-Yl) Phenol	$C_{21}h_{34}o$	302	45.101	7.915
6	Phenol,3-Pentadecyl-	$C_{21}h_{36}$	304	45.235	6.374
7	3-((4z,7z)-Heptadeca-4,7-Dien-1-Yl) Phenol	$C_{23}h_{36}o$	328	48.062	14.475
8	(Z)–3– (Heptadec-10-En-1-Yl) Phenol	$C_{23}h_{38}o$	330	48.246	32.389
9	(Z)–3– (Heptadec-10-En-1-Yl) Phenol	$C_{23}h_{38}o$	330	48.406	4.747

Tables 4. Gc-Ms Analysis Result of The Root Bark of *Yellow* Variety *Anacadium Occidentale*.

Chromatogram Peak	Compound Name	Molecular Formula	Molecular Weight	Retention Time (Mins)	Percentage Content
1	3-Tridecylphenol	$C_{19}h_{32}o$	276	41.712	4.732
2	(Z)-3-(Pentadec-8-En-1-Yl) Phenol	$C_{21}h_{34}o$	302	44.941	21.567
3	(Z)-3-(Pentadec-8-En-1-Yl) Phenol	$C_{21}h_{34}o$	302	45.017	7.123
4	Phenol,3-Pentadecyl-	$C_{21}h_{36}o$	304	45.252	9.300
5	3-((4z,7z)-Heptadeca-4,7-Dien-1-Yl) Phenol	$C_{23}h_{36}o$	328	48.070	15.334
6	(Z)-3-(Heptadec-10-En-1-Yl) Phenol	$C_{23}h_{38}o$	330	48.263	41.944

Table 5.a. GC/MS result of *Anacardium occidentale* inner root (yellow variety).

Chromatogram Peak	Compound Name	Molecular Formula	Molecular Weight	Retention Time (Mins)	Percentage Content
1	N-Hexadecanoic Acid	$C_{16}h_{32}o_2$	256	35.739	13.008%
2	9,12– Octadecadienoic Acid	$C_{18}h_{32}o_2$	280	39.304	74.841%
3	Octadecanoic Acid	$C_{18}h_{36}o2$	284	39.698	8.607%
4	Unidentified	-	-	59.135	-

Table 5.b. Gc/Ms Result of *Anacardium Occidentale* Inner Root (Red Variety).

Chromatogram Peak	Compound Name	Molecular Formula	Molecular Weight	Retention Time (Mins)	Percentage Content
1	N-Hexadecanoic Acid	$C_{16}h_{32}o_2$	256	35.739	13.008%
2	9,12– Octadecadienoic Acid	$C_{18}h_{32}o_2$	280	39.304	74.841%
3	Octadecanoic Acid	$C_{18}h_{36}o_2$	284	39.698	8.607%
4	Unidentified	-	-	59.135	-
5	P Hydroxybenzoic,	$C_7h_6o_3$	138.12	59.355	7.142%
6.	Vanillic	$C_8h_8o_4$	168.141	60.134	12.2%
7.	P-Coumaric	$C_9h_8o_3$	164.0473	61.234	11.234%

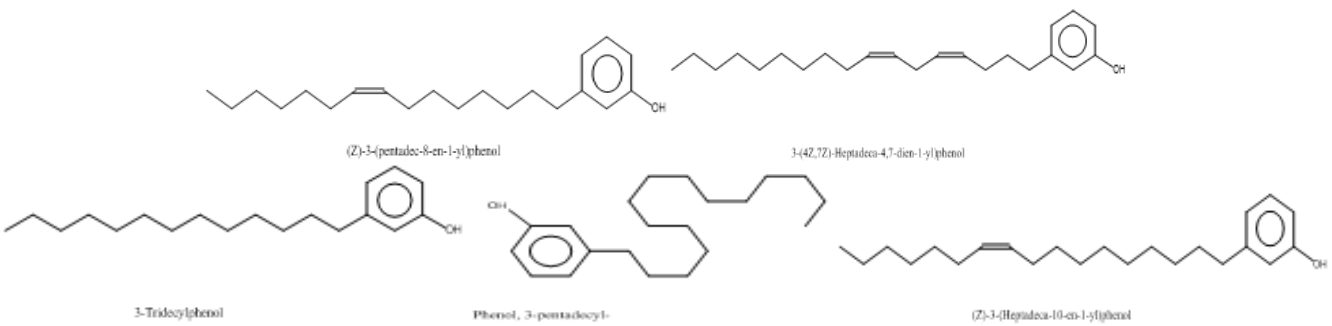

Figure 1. Structure of the compounds from GC-MS Analysis of the root bark.

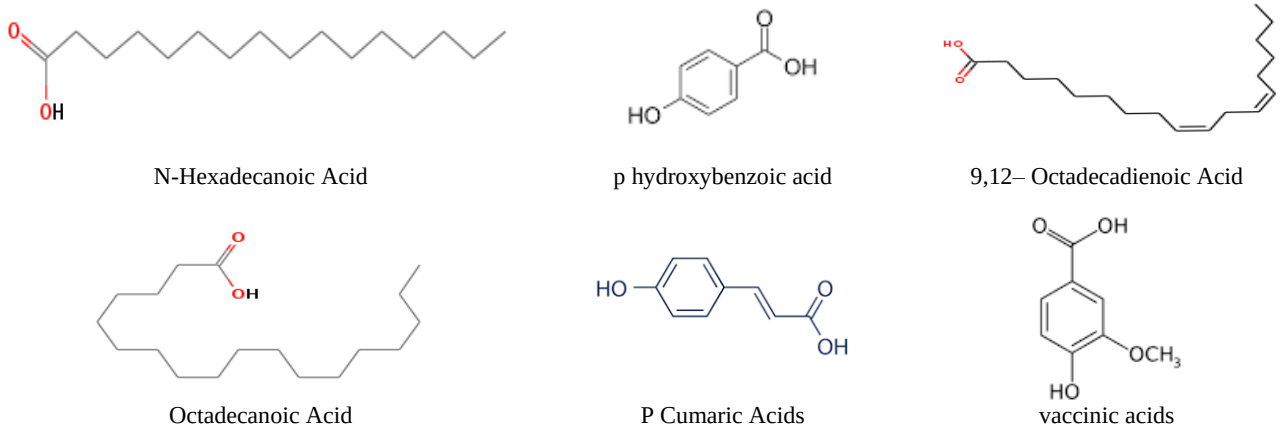

Figure 2. structures of some compounds identified from gc/ms analysis of the inner part of the root.

Discussion

The preliminary qualitative screening results shown in Table 1 show that alkaloids, saponins, and terpenoids were found exclusively in the root bark of both varieties, while tannins, flavonoids, and phenols were present in both parts of the plant. This initial screening is useful for identifying the types of allelochemicals that are present in the roots of the two varieties.

The results of the quantitative screening shown in Table 2 show the percentage content of different compounds such as saponins, tannins, terpenoids, and phenols. The table highlights that while the root bark of the red variety had the highest saponin content, the yellow variety exhibited phenol as its highest content. This quantitative analysis

Tables 3 and 4 show the results of GC/MS Analysis of the root bark of *Anacadium occidentale* (red and yellow plant varieties). The root bark of both the red and yellow varieties of Anacardium occidentale revealed a variety of chemical compounds with different structures and properties. These compounds play a significant role in the allelochemical profiles of plants, potentially affecting their interactions with other organisms in the ecosystem.

Researchers can investigate the bioactive properties and ecological roles of allelochemicals found in the root bark of each variety by identifying the specific compounds. The presence of compounds such as 1,2,4-Benzenetriol, 3-Tridecyl phenol, and (Z)-3-(pentadec-8-en-1-yl) phenol in the red variety and compounds like 3-Tridecylphenol, (Z)-3-(pentadec-8-en-1-yl) phenol, and 3-((4Z,7Z)-Heptadeca-4,7-dien-1-yl) phenol in the yellow variety highlights the chemical diversity of Anacardium occidentale.

Tables 5 and 6 show the GC/MS results of *Anacardium occidentale* inner root (yellow variety). The GC/MS results for both samples showed the presence of different compounds, and one of the compound peaks observed in the inner root was not identified (Table 5), which is the 4th peak; 9,12– octadecadienoic acid (fatty acid) was the compound with the highest composition identified in the yellow variety. Similar compounds were identified in the root bark of the red and yellow varieties. The compound with the highest percentage composition of 32.389 and 41.944% in the red and yellow varieties, respectively, was identified as (Z)-3-(Heptadec-10-en-1-yl) phenol (molecular formula $C_{23}H_{38}O$ with a molecular weight of 330) and occurred within the retention time range of 48.246 – 48.263 min.

The root bark of both varieties from the GC/MS results contained more phenolic compounds. The inner roots of the yellow variety contained more long-chain fatty acids. Figure 1 shows the structure of the compounds from the GC-MS Analysis of the root bark, while Figure 2 shows the structures of some compounds identified from the GC/MS Analysis of the inner part of the root.

CONCLUSIONS

The compounds in the bark were primarily phenols, whereas those in the inner roots were primarily fatty acids. Additionally, both parts of the plant contain terpenoids, tannins, and phenols, whereas flavonoids are only found in the bark. These allelopathies may be responsible for the observed allelopathic properties of the plants. The composition of allelochemicals in plant roots is diverse species-specific and has significant implications for plant interactions and soil ecology. The identification and study of these compounds are essential for understanding their roles in plant growth, defence, and potential for managing agricultural systems

Acknowledgements: Special thanks to TetFund, Nigeria and Abia State University management for sponsoring this research

Authors' Contributions: Nnenna Ejije, Okoronkwo designed the study, Nwosu Nkemakolam Bright conducted the laboratory. Jude Chibuzor Igwe analyzed the data. Nwosu Nkemakolam Bright & Precious O. Chukwu wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

REFERENCES

AOAC (2015). Official methods of analysis, 15th ed., Association of Official Chemists, Washington, D.C., USA.

Dayan F. E., Owens D. K. and Duke S. O. (2012). Rationale for a natural products approach to herbicide discovery. *Pest Mgt. Sci.* 68: 519–528

Gerwick, B. C., & Sparks, T. C. (2014). Natural products for pest control: an analysis of their role, value and future. *Pest Management Science*, *70*(8), 1169-1185. https://doi.org/10.1002/ps.3744

Iqbal, A., & Fry, S. C. (2012). Potent endogenous allelopathic compounds in Lepidium sativum seed exudate: effects on epidermal cell growth in Amaranthus caudatus seedlings. *Journal of Experimental Botany*, *63*(7), 2595-2604. https://doi.org/10.1093/jxb/err436

Kong, C. H., Hu, F., Wang, P., Wu, J. L., (2008). Effect of allelopathic rice varieties combined with cultural management options on paddy field weeds. *Pest Mgt. Sci.* 64: 276-282.

Kong, C. H., Li, H. B., Hu, F., Xu, X. H. and Wang, P., (2006). Allelochemicals released by rice roots and residues in soil. *Plant and Soil*, 288: 47-56.

Li, Z. H, Wang, Q, Ruan, X, Pan, C. D. & Jiang, D. A. (2010). Phenolics and Plant Allelopathy. *Molecules* 15(12), 8933 - 8952.

Nwosu, N. B., Okoronko, N. E., Njoku, D. K., & Emole, P. O. (2024). Extraction Characterization of Oils Extracted from different Parts of Red and Yellow Varieties of Anacardium occidentale (Lin). Journal of Applied Sciences and Environmental Management, 28(1), 61-68.https://doi.org/10.4314/jasem.v28i1.7

Nwosu, N. B., Okoronkwo, N. E., Onwuka, O. M., & Osuchukwu, T. U. (2023). Phytochemical And Nutritional Compositions of Two Varieties Of Anacardium Occidentale L. *World Journal Of Advanced Research And Reviews*, *19*(2), 966-977. https://doi.org/10.30574/wjarr.2023.19.2.1629

Okoronkwo N. E. and Echeme J. O. (2015). Isolation and characterisation of compound from *Stachytarpheta cayennensis* (Rich.) Vahl Leaves. *Chem. J.* 1 (3): 74 - 80.

Okoronkwo, N. E.; Echeme J. O. & Okwu, D. E. (2012a). Macrolide from *Tetrapluera tetraptera* root. *Acad. Research Intl*. *2(3*): 200 – 208.

Okoronkwo, N. E.; Echeme J. O. & Onwuchekwa, E. C. (2012b). Cholinesterase and Bacterial Inhibitory Activities of *Stachytapheta cayennensis*. *Acad. Research Intl*. *2(3*): 209 – 217.

Weir, T. L, Park, S-W, & Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Curr. Op. in Plant Biol*. 7(4), 472-479.