

Research Article

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Farmer's Awareness of the Antigenotoxicity Properties of *Vernonia amygdalina* Accessions in Nigeria

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Abstract

Keywords

Farmer's awareness, Antigenotoxic properties, *Vernonia amygdalina*, *Allium cepa* model, DNA fragmentation assay

The bitter leaf plant (*Vernonia amygdalina* Del.) holds a prominent position in traditional medicine across various cultures due to its potent therapeutic properties. This botanical marvel, native to tropical Africa, exhibits resilience to diverse ecological conditions and finds versatile applications beyond medicinal use. Given the role of oxidative stress in chronic diseases, particularly cancer, exploring compounds with genoprotective properties is crucial. *V. amygdalina* extracts have shown promise in suppressing cancerous cell growth and enhancing chemotherapy sensitivity. This study aimed to assess the antigenotoxic properties of commonly consumed *V. amygdalina* leaf varieties across Nigeria using the *Allium cepa* roots as an experimental model. In this study, an attempt to bring to the awareness of farmers, about the antigenotoxic properties of commonly ingested *V. amygdalina* accessions sourced from various regions of Nigeria was conducted. Fifty-two accessions of bitter leaf were collected from different regions in Nigeria, representing the country's six geopolitical zones. Ethidium bromide was used to induce genotoxicity in *A. cepa* roots, and the effects of *V. amygdalina* extracts on DNA fragmentation were assessed to ascertain which accession and from what location had the highest antigenotoxic properties. Finally, the significant disparities between the means were assessed at a 5% confidence level ($P < 0.05$) using the post hoc test known as the least significant difference. (LSD) to ascertain significant difference in means of the bitter leaves collected. Results revealed a significant percentage decrease in DNA fragmentation in *A. cepa* roots treated with *V. amygdalina* extracts, compared to ethidium bromide controls, indicating

antigenotoxic activity. Post-hoc analysis further confirms the antigenotoxic effects of *V. amygdalina*. Variations in antigenotoxicity among different accessions were attributed to differences in antioxidant activities. These findings shed light on the potential of *V. amygdalina* as a natural antigenotoxic agent, highlighting its importance in mitigating genotoxicity and warrant further exploration of its therapeutic applications.

Introduction

Bitter leaf (*Vernonia amygdalina*) stands out as a botanical marvel, deeply rooted in the traditional medicine of various cultures. Its historical and cultural significance underscores its therapeutic potential, making it a staple in traditional healing practices across generations (Ramawat et al., 2009). Indigenous to tropical Africa, notably Nigeria, Cameroon, and Zimbabwe, this perennial shrub thrives in humid environments and displays resilience to drought (Tekou et al., 2018).

Recognizable by its elliptic leaves and sturdy bark, *V. amygdalina* reaches heights of up to 3 meters (Ifedibalu Chukwu et al., 2020). It belongs to the Asteraceae family and is known by various cultural names, such as ewuro, etidot, onugbu, and ndole (Tonukari et al., 2015; Eraga et al., 2020). Beyond its medicinal uses, it serves as a hedge plant and flourishes in diverse ecological zones (Oyeyemi et al., 2018).

The plant's health benefits are notable, particularly its efficacy against nasopharynx-based human cancer cells found in its organic fraction extracts. Its biologically active components include saponins, alkaloids, terpenes, flavonoids, and phenolic acids, which contribute to its therapeutic properties (Alara et al., 2017; Alabi and Adeyemi, 2021). The characteristic bitterness is due to compounds like alkaloids, saponins, tannins, and glycosides (Ifedibalu Chukwu et al., 2020).

Oxidative stress from free radicals plays a crucial role in chronic diseases such as cancer, diabetes, and neurodegenerative disorders (Chen et al., 2018; Uttara et al., 2009). Protecting cells from DNA damage caused by genotoxic agents is essential to mitigate these diseases. Genotoxicity refers to the ability of agents to damage genetic

material, leading to various pathologies, including cancer (Bhattacharya, 2011; Nagarathna et al., 2013).

Given the potential of *V. amygdalina* to suppress, delay, or induce apoptosis in cancer cells, its extracts may enhance chemotherapy sensitivity and down-regulate transcription factors like NF- κ B, which are implicated in cancer metastasis (Farombi and Owoeye, 2011). The importance of discovering novel bioactive compounds that act as antigenotoxic agents is increasingly recognized, especially for reducing the mutagenic and carcinogenic effects of genotoxic agents (Bhattacharya, 2011; De Flora and Ferguson, 2005).

The World Health Organization reports that over 80% of the global population relies on traditional medicine for primary healthcare, highlighting the need for continued exploration of medicinal plants (Mendoza-Pérez et al., 2013). Natural antioxidants from plants have shown potential in managing conditions linked to free radicals, emphasizing the role of antigenotoxic agents in cancer prevention (López-Romero et al., 2018; Madrigal-Santillán et al., 2013; Egbune et al., 2022).

This study investigates the anti-genotoxic properties of commonly consumed *V. amygdalina* leaf varieties in Nigeria, focusing on their ability to mitigate ethidium bromide-induced genotoxicity. The primary goal is to create awareness among farmers and the public about these properties and to identify regions with the highest antigenotoxic potential, contributing to the broader understanding of *V. amygdalina*'s benefits.

Methodology

Collection of samples

Fifty two (52) accessions of bitter leaf (*V. amygdalina*) were collected from different parts of Nigeria representing the six (6) geopolitical zones. Dry healthy onion (*Allium cepa*) was purchased from Uselu market in Benin City, Edo State, Nigeria and the ethidium bromide was obtained from a laboratory shop in Onitsha market, Anambra State, Nigeria and brought to the laboratory. The outer papery brown layer of each onion was peeled away and the dried basal root plate was cleaned and weighed. Its roots were submerged in distilled water and allowed to stand for five (5) days. After five days, visible growth of the root was observed and measured. This was taken as the experimental control. The ethidium bromide was prepared according to the concentration used to induce the *A. cepa*. The ethidium bromide was handled with extreme care to avoid toxic effect by the use of hand gloves, mask, laboratory coats and the waste solutions was discarded immediately using appropriate methods.

Extraction of *V. amygdalina* using water

The fresh leaves underwent washing and air drying at room temperature, spread out on a laboratory table for 24 hours. About 50 grams of each sample were then collected, weighed, and homogenized by blending with 100 milliliters of water until a uniform mixture was achieved. The resulting mixtures were filtered using muslin cloth, and the filtrate was labelled based on the respective collection locations. These filtrates were stored in the refrigerator at 4°C until needed for further use.

Preparation/induction of ethidium bromide solution

About 0.03 g of ethidium bromide powder was weighed with weighing balance into a beaker and 2 ml of distilled water was added. The solution was stirred to dissolve the ethidium bromide.

Thereafter, 20 µl of the prepared ethidium bromide solution was injected (using a 5 ml injection syringe and needle for safety) into the *A. cepa* and then submerged on a 50 ml test tube containing water which was allowed to stand for five days. Root growth was observed for five days. The harvested roots were used for DNA fragmentation assay.

The experimental groupings were as follows:

Group A: Controls

Group B: *V. amygdalina* extract and *A. cepa*

Group C: Ethidium bromide and *A. cepa*

Group D: Ethidium bromide and *A. cepa* plus *V. amygdalina* extract.

Assay for DNA fragmentation

DNA fragmentation assessment was conducted using Wu et al. (2005) method with some modifications. The 50 mg of *Allium cepa* roots underwent homogenization in 10 ml of a TE solution at pH 8.0. The TE solution comprised 5 mmol Tris-hydrochloric acid, 20 mmol EDTA, and 0.2% Triton X-100. A 1 ml aliquot of the sample underwent centrifugation at high speed (27,000 × g for 20 min) to ensure proper separation of intact chromatin (pellet, B) from fragmented DNA (supernatant, T). The DNA content of both pellet and supernatant fractions was determined using a freshly prepared diphenylamine solution. Sample readings were taken at 620 nm using a spectrophotometer.

Calculation:

The amount of % fragmented DNA was calculated with the following formula;

$$\text{Fragmented DNA (\%)} = T \times 100 / (T + B),$$

Where, B = intact chromatin (pellets),

T = fragmented DNA (supernatant).

Statistical Analysis

All results were presented as means ± SD, and statistical analysis was conducted using analysis

of variance (ANOVA). Significant differences between means were determined at a 5% confidence level ($P < 0.05$) using the posthoc test known as least significant difference (LSD).

Results

The results of the antigenotoxicity properties of commonly consumed *V. amygdalina* (bitter leaf) accessions of *Allium cepa* roots growth in ethidium bromide solution collected from Northern, Southern, Eastern and Western Nigeria, are shown in Tables 1 to 4.

Table 1. Not genotoxic properties and Antigenotoxic properties (percentage increase of fragmented DNA) of commonly consumed *V. amygdalina* (bitter leaf) accessions on *Allium cepa* roots growth in ethidium bromide solution collected from Northern Nigeria.

Accession Number	<i>V. amygdalina</i> accessions from States/LGA	Percentage (%) of Fragmented DNA		% increase of fragmented DNA*
		<i>Allium cepa</i> roots growth in <i>V. amygdalina</i>	<i>Allium cepa</i> roots growth in ethidium bromide and <i>V. amygdalina</i>	
	Control (growth in water)	3.42 ± 0.46	3.46 ± 0.46	100
	Ethidium bromide control	45.05 ± 4.14	45.0 ± 4.14	92
	North Eastern States			
AG628002	Borno (Maiduguri)	1.30 ± 0.300	29.00 ± 4.00	55.17
AG628018	Gombe (Gombe)	1.20 ± 0.20	20.00 ± 5.00	125
AG627999	Taraba (Jalingo)	0.90 ± 0.40	23.00 ± 3.00	95.65
	North Western States			
AG628001	Kaduna (Kaduna)	2.00 ± 0.50	30.04 ± 5.00	49.8
AG628019	Kano (Kano)	1.20 ± 0.20	20.00 ± 2.00	125
AG628007	Kebbi (Benin Kebbi)	0.80 ± 0.31	31.00 ± 1.00	45.16
	North Central States			
AG627980	Kogi (Kogi)	0.63 ± 0.24	19.13 ± 4.00	135.2
AG628003	Nassarawa (Lafia)	1.50 ± 0.40	32.4 ± 2.40	38.88
AG628006	Niger (Minna)	1.50 ± 0.30	30.02 ± 5.00	49.9
AG627997	Abuja (FCT)	1.60 ± 0.29	30.00 ± 5.11	50
AG627982	Plateau (Jos)	0.90 ± 0.40	23.10 ± 3.05	94.8

*Percentage decrease = decrease/original number x 100. If the value is low, it is percentage increase

Table 2. Not genotoxic properties and Antigenotoxic properties (percentage increase of fragmented DNA) of commonly consumed *V. amygdalina* (bitter leaf) accessions on *Allium cepa* roots growth in ethidium bromide solution collected from Southern Nigeria.

Accession Number	<i>V. amygdalina</i> accessions from States/LGA	Percentage (%) of Fragmented DNA		% increase of fragmented DNA*
		<i>Allium cepa</i> roots growth in <i>V. amygdalina</i>	<i>Allium cepa</i> roots growth in ethidium bromide and <i>V. amygdalina</i>	
	Control (growth in water)	3.46 ± 0.46	3.46 ± 0.46	100
	Ethidium bromide control	45.05 ± 4.14	45.05 ± 4.14	92
	Southern States			
AG627998	Bayelsa (Yenagoa)	2.20± 0.20	30.16±3.75	49.2
AG627976	Rivers (Port harcourt)	0.70±0.20	28.28±2.95	59.12
AG628017	Cross Rivers (Calabar)	1.30±0.30	20.14±1.91	123.43
AG627983	Akwa Ibom (Utu)	1.46±0.32	27.0±7.10	66.67
	Delta			
AG628012	Warri South (Ogunu)	1.21±0.19	30.07±6.10	47.55
AG628011	Isoko North (Ozoro)	0.90±0.50	19.07±4.01	135.97
AG627986	Udu (Ayama)	1.14±0.05	26.07±1.00	72.6
AG628013	Ughelli North (Ogor)	1.70±0.20	35.07±5.11	28.31
AG627988	Bomadi (Kpakiamma)	1.62±0.02	34.10±4.05	31.96
AG627985	Isoko South (Oleh)	1.50±.50	35.08±3.05	28.27
AG627978	Ukwuani (Umubu)	1.60±0.40	33.04±2.95	36.19
AG627994	Burutu (Operemor)	1.27±0.21	22.03±2.05	104.21
AG628023	Ughelli South (Olomo)	1.40±0.31	30.30±1.56	48.5
AG627979	Ndokwa West (Ogume)	0.71±0.10	21.10±0.85	113.27
AG628022	Aniocha South (Igbudu)	0.40±0.20	11.07±1.00	306.5
AG628015	Anoicha North (Ubulubu)	0.41±0.21	10.10 ±4.95	345.54
AG628008	Warri North (Opuama)	0.31±0.20	8.07 ± 4.11	457.62
AG627995	Ethiope East (Abraka)	0.80±0.20	19.05 ±1.04	136.22
AG628016	Oshimili South (Okwe)	2.02 ±1.0	16.25 ±1.05	176.92
AG628024	Oshimili North (Okpanam)	0.70 ± 0. 11	9.09 ± 2.09	395.04
AG628014	Ethiope West (Oghara)	0.60 ±0.10	18.11±3.04	148.48
AG628009	Ikah North East (Owa)	2.51±0.42	35.07±.99	28.31
AG628021	Sapele (Sapele)	2.05±0.97	18.04±8.06	149.44
	Edo			
AG627993	Esan central (Opoji)	0.91±0.02	24.00±4.00	87.5
AG627996	Oredo (Iwegie)	0.80±0.11	24.33±1.52	84.95
AG627991	Orhionmwon (Ugboko)	0.80±0.40	25.11±1.17	79.21
AG628010	Akoko Edo (Igara)	0.11±0.02	5.41± 2.83	7.32
AG627989	Esan West (Ekpoma)	0.70±0.21	19.10 ± 2.0	135.6
AG627990	Esan South (Ohordua)	0.50±0.20	17.17±2.08	162.08
AG627987	Owan West (Ora)	0.60±0.31	17.54±3.64	156.55
AG627984	Ikpoba Okha (Agedo)	0.48±0.33	10.18±4.16	342.04
AG627992	Egor (Egor)	0.81±0.02	17.24±1.08	161.02

*Percentage decrease = decrease/original number x 100. If the value is low, it is percentage increase

Table 3. Not genotoxic properties and Antigenotoxic properties (percentage increase of fragmented DNA) of commonly consumed *V. amygdalina* (bitter leaf) accessions on *Allium cepa* roots growth in ethidium bromide solution collected from Eastern Nigeria.

Accession Number	<i>V. amygdalina</i> accessions from States/LGA	Percentage (%) of Fragmented DNA		% increase of fragmented DNA*
		<i>Allium cepa</i> roots growth in <i>V. amygdalina</i>	<i>Allium cepa</i> roots growth in ethidium bromide and <i>V. amygdalina</i>	
	Control (growth in water)	3.42 ± 0.46	3.46 ± 0.46	100
	Ethidium bromide control	45.05 ± 4.14	45.0 ± 4.14	92
	Eastern States			
AG628000	Abia (Umuahia)	1.13 ± 0.14	20.15 ± 4.89	123.33
AG628005	Anambra (Awka)	1.09 ± 0.01	22.73 ± 2.28	97.98
AG 628000	Abia (Umuahia)	1.13 ± 0.14	20.15 ± 4.89	123.33
AG 628005	Anambra (Awka)	1.09 ± 0.01	22.73 ± 2.28	97.98

*Percentage decrease = decrease/original number x 100. If the value is low, it is percentage increase

Table 4. Not genotoxic properties and Antigenotoxic properties (percentage increase of fragmented DNA) of commonly consumed *V. amygdalina* (bitter leaf) accessions on *Allium cepa* roots growth in ethidium bromide solution collected from Western Nigeria.

Accession Number	<i>V. amygdalina</i> accessions from States/LGA	Percentage (%) of Fragmented DNA		% increase of fragmented DNA*
		<i>Allium cepa</i> roots growth in <i>V. amygdalina</i>	<i>Allium cepa</i> roots growth in ethidium bromide and <i>V. amygdalina</i>	
	Control (growth in water)	3.42 ± 0.46	3.46 ± 0.46	100
	Ethidium bromide control	45.05 ± 4.14	45.0 ± 4.14	92
	Western States			
AG627981	Ondo (Akure)	0.94 ± .26	12.15 ± 2.22	270.37
AG627977	Oyo (Ibadan)	1.23 ± 0.01	36.12 ± 5.95	24.58
AG627973	Lagos (Ikeja)	0.30 ± 0.10	19.13 ± 0.17	135.23
AG627974	Ogun (Adeku)	0.910 ± 0.21	18.14 ± 1.89	148.07
AG628020	Ekiti (Iroko)	0.81 ± 0.32	19.05 ± 3.06	136.22

*Percentage decrease = decrease/original number x 100. If the value is low, it is percentage increase.

The pairwise differences in means of antigenotoxicity results were also analyzed (Table 5). At $P_{0.05}$; $LSD = 20.62$; there was significant difference (*) between the mean values of *Allium cepa* roots with ethidium bromide (EB) and *Allium cepa* roots with ethidium bromide (EB) treated with *Vernonia amygdalina* (VA) ($P < 0.05$).

Table 5. Pairwise differences in means of the antigenotoxic properties of *Vernonia amygdalina*.

	S₁ (EB) x₁ = 22.53	S₂ (EB + VA) x₂ = 1.905
S₁ (Growth in EB) x₁ = 22.53	-	* 20.62
S₁ (Growth in EB + VA) x₁ = 1.905		-

VA – *Vernonia amygdalina*; EB – ethidium bromide; * Significant at P_{0.05} (Significant).

The genotoxicity of ethidium bromide in experimental model have been evaluated (Ohta *et al.*, 2001). Ohta *et al.* (2001) also reported some work concerning the effects of ethidium bromide such as plant DNA strand breaks and chromosomal aberrations.

The significant increase in the percentage of fragmented DNA observed in the roots of *Allium cepa* grown in an ethidium bromide solution, as compared to the control group (*A. cepa* roots grown in water only), can be attributed to the genotoxic effect of ethidium bromide. This finding aligns with previous studies that have demonstrated ethidium bromide's capability to induce DNA fragmentation and chromosomal aberrations in various plant and animal models (Gunaseelan *et al.*, 2022; Zachariadis *et al.*, 2000). However, when the *A. cepa* roots were treated with commonly consumed *V. amygdalina* leaf extracts, a significant decrease in the percentage of fragmented DNA was observed compared to roots grown in the ethidium bromide solution alone. This reduction in DNA fragmentation suggests that *V. amygdalina* has potent antigenotoxic properties. Similar protective effects of *V. amygdalina* against genotoxic agents have been reported in other studies, where its extracts were shown to mitigate DNA damage and enhance DNA repair mechanisms (Saharan *et al.*, 2021; Hu *et al.*, 2009)).

These results are consistent with the findings of Kahaliw *et al.* (2018) and Josephet *al.* (2020), who reported that the bioactive compounds in medicinal plants, including *V. amygdalina*, could effectively reduce genotoxicity induced by various chemical agents. Additionally, the antioxidant properties of *V. amygdalina*, which

help in neutralizing reactive oxygen species, further support its role in protecting genetic material from damage (Saharan *et al.*, 2021).

This may be related to the antigenotoxic activities of *V. amygdalina* leaf extracts as shown in Tables 1 to 4. The highest percentage of fragmented DNA of *A. cepa* roots growth induced with ethidium bromide solution and treated with *V. amygdalina* leaf extracts that was observed, as shown in AG627977, AG627985, AG628009 and AG628013, collected from Oyo (Ibadan), Isoko South (Oleh), Ikah North East (Owa) and Ughelli North (Ogor); respectively, may be due to low antioxidant activities in these *V. amygdalina* accessions (Tables 2 and 4). The lowest percentage of fragmented DNA of *A. cepa* roots growth induced with ethidium bromide solution and treated with *V. amygdalina* accession AG628010 extracts (antigenotoxic), collected from Akoko Edo (Igara) (Table 2), that was observed could be due to the high antioxidant activities in the *V. amygdalina* accession. This is in accordance with Hu *et al.* (2009) who stated that antioxidant dietary supplement can reduce the level of DNA oxidative damage and protect normal cells against the adverse side-effects of some carcinogens.

Post Hoc Analysis with ANOVA on the Antigenotoxic Properties of *V. amygdalina* Accessions

The antigenotoxicity results were also analyzed (Table 5). At P_{0.05}; LSD = 20.62; there was significant difference (*) between the mean values of *Allium cepa* roots with ethidium bromide (EB) and *Allium cepa* roots with ethidium bromide (EB) treated with *Vernonia amygdalina* (VA) (P < 0.05).

The expression of antigenotoxic property of commonly consumed *V. amygdalina* accessions are shown in Tables 1 to 4. The control *Allium cepa* roots grown in water exhibited the lowest percentage of fragmented DNA, indicating minimal genotoxic stress under these conditions. Following this, the *A. cepa* roots treated with *Vernonia amygdalina* accession number AG628010, collected from Akoko Edo (Igara), also showed a relatively low percentage of fragmented DNA. This suggests that this particular accession of *V. amygdalina* possesses strong antigenotoxic properties.

In contrast, the highest percentage of fragmented DNA was observed in the *A. cepa* roots grown in ethidium bromide solution (genotoxic control), confirming the substantial genotoxic effect of ethidium bromide. Following this, the *A. cepa* roots treated with *V. amygdalina* accession AG627977, collected from Oyo (Ibadan), displayed a higher percentage of fragmented DNA compared to the Igara accession but still lower than the ethidium bromide control. This indicates that while both accessions of *V. amygdalina* have antigenotoxic effects, there is variability in their efficacy.

These findings are consistent with previous studies that have highlighted the variability in the antigenotoxic properties of different plant accessions and species. For instance, Bingham *et al.* (2014) reported significant reductions in DNA fragmentation with *V. amygdalina* extracts, attributing these effects to its rich phytochemical content. Similarly, research by Martinez-Aledo *et al.* (2020) and Rashwan *et al.* (2021) demonstrated that the bioactive compounds in medicinal plants could mitigate DNA damage caused by genotoxic agents.

Furthermore, the observed differences between the Igara and Ibadan accessions align with studies showing that environmental factors and genetic variability can influence the phytochemical composition and, consequently, the biological activity of medicinal plants (Fredotovi *et al.*, 2017; López-Romero *et al.*, 2018). Such variations can impact the effectiveness of these plants in reducing genotoxic stress.

Percentage increase of fragmented DNA of commonly consumed bitter leaf accessions on *A. cepa* roots grown in ethidium bromide solution are presented in Tables 1 to 4. Interestingly, the *V. amygdalina* accessions exhibited considerable variability in their effects on DNA fragmentation. The accession AG628008, collected from Warri North (Opuama), indicated the highest percentage increase of fragmented DNA ($457.62\% \pm 4.11$), followed by AG628024 from Oshimili North (Okpanam), AG628015 from Anoicha North (Ubulubu), and AG627984 from Ikpoba Okha (Agedo). In contrast, the accession AG628010 from Akoko Edo (Igara) had the lowest percentage increase in fragmented DNA ($7.32\% \pm 2.83$) (Table 2).

These results highlight the significant variability in the antigenotoxic potential of different *V. amygdalina* accessions. Such variability is consistent with findings from previous studies, which have shown that the geographical origin and environmental conditions of plant samples can significantly influence their phytochemical composition and biological activity (Fredotovi *et al.*, 2017). For example, Joseph *et al.* (2020) reported that different extracts of *V. amygdalina* could vary widely in their effectiveness at reducing DNA damage, depending on the specific bioactive compounds present.

Additionally, the high percentage of DNA fragmentation observed in the Opuama accession aligns with studies that have identified certain plant extracts as potentially pro-oxidant or less effective in mitigating genotoxic stress under specific conditions (Ramawat *et al.*, 2009). This underscores the importance of selecting the appropriate accession for therapeutic use, as not all accessions may provide the desired protective effects against DNA damage.

The notable efficacy of the Igara accession (AG628010) in minimizing DNA fragmentation is supported by research indicating that specific phytochemical profiles are more potent in antioxidant and antigenotoxic activities (López-Romero *et al.*, 2018). The low percentage of DNA fragmentation observed with this accession

suggests a higher concentration or more effective combination of protective compounds.

The significant increase observed in the percentage of fragmented DNA of *A. cepa* roots growth in ethidium bromide solution when compared with the control (*A. cepa* roots growth in water only) may be as a result of the genotoxic effect of ethidium bromide solution. However, the significant decrease observed in fragmented DNA percentage of *A. cepa* roots growth in ethidium bromide solution treated with the commonly consumed *V. amygdalina* accessions as compared with *Allium cepa* roots growth in ethidium bromide solution only may be related to the antigenotoxic activities of *V. amygdalina* accessions (Tables 1 to 4).

Conclusion

The findings of this study underscore the antigenotoxic properties of *Vernonia amygdalina* extracts, suggesting its potential as a natural remedy to counteract genotoxicity induced by ethidium bromide. The significant decrease in DNA fragmentation observed in *Allium cepa* roots treated with bitter leaf extracts indicates its ability to mitigate the damaging effects of genotoxic agents. Variability in antigenotoxic effects among different accessions of *V. amygdalina* suggests the importance of considering geographical factors in selecting plant sources for therapeutic use. Further research is warranted to elucidate the specific bioactive compounds responsible for the antigenotoxic properties of bitter leaf and to explore its potential applications in pharmaceuticals and healthcare. Overall, bitter leaf emerges as a promising candidate for the development of novel antigenotoxic agents, contributing to the growing body of evidence supporting the therapeutic value of traditional medicinal plants.

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