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ANTIGENOTOXICITY AND ANTIOXIDANT ACTIVITIES OF BITTER LEAF (*Vernonia amygdalina* Del.) ACCESSIONS FROM DIFFERENT PARTS OF NIGERIA

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ABSTRACT

Bitter leaf (*Vernonia amygdalina* Del.) plant is a tree species that is highly cultivated in Nigeria for its nutritive and therapeutic values. This study aimed to determine the antioxidant and antigenotoxicity effects (*in vitro)* of 52 accessions of *V. amygdalina* collected from six geopolitical zones of Nigeria (North East, North West, North Central, South South, East and West) by evaluating the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO^-) scavenging antioxidant activities, flavonoid and phenolic contents as well as ethidium bromide-induced DNA (deoxyribonucleic acid) damage of bitter leaf. The results showed that accessions MN628016 (Oshimili South, Delta State) and MN628010 (Akoko Edo, Edo State), respectively had higher amounts of flavonoid (287.19 mg/g/FW) and phenolic (307.90 mg/g/FW) contents. The accessions MN627984 (Ikpoba Okha, Edo State) and MN627975 (Owerri, Imo State) had the highest nitric oxide (85.73%) and DPPH radical scavenging inhibitory effect (98.92%). The highest percentage fragmented DNA (45.05%), was observed in *Allium cepa* roots homogenised and mixed with ethidium bromide followed by the *A. cepa* roots homogenised and mixed with the leaf extract of *V. amygdalina* accession MN627977 (36.12%). However, *V. amygdalina* accessions MN628008 (457.62%, Warri North, Delta State) had the highest percentage increase of fragmented DNA followed by MN628024 (395.04%, Oshimili North, Delta State), MN628015 (345.54%; Aniocha North, Delta State) and MN627984 (342.04%; Ikpoba Okha, Edo State) while accession MN628010 (7.32%; Akoko Edo, Edo State) had the lowest. Accession MN628010 which possessed the highest amount of phenolic content had the lowest percentage increase of fragmented DNA and accession MN627984 which possessed the highest nitric oxide radical scavenging inhibitory effect was among the accessions with the highest percentage increase of fragmented DNA. The findings of this study suggest that the observed lowest percentage of fragmented DNA of *A. cepa* roots growth induced with the solution of ethidium bromide and treated with *V. amygdalina* accessions MN628010 extracts (antigenotoxic) could be as a result of the high antioxidant activities in the *V. amygdalina* accessions. In summary, the findings of this study showed that the 52 *V. amygdalina* accessions obtained from different locations of Nigeria will help to combat ethidium bromide induced genoxicities and any other genotoxicant that may lead to different complications in plant (*A. cepa* roots), as all the accessions possessed antioxidant and antigenotoxic properties, as such, possessed comparable amount of natural antioxidant activities and antigenotoxicity.

Key words: Antioxidant, antigenotoxicity, bitter leaf, accessions, ethidium bromide, *Allium cepa*

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INTRODUCTION

Vernonia amygdalina Del. is a herb that belongs to the Asteraceae family. It mainly grows in tropical Africa. It is wide spread in West Africa and well distributed in Asia. In Africa, it is commonly called bitter leaf and different local names have been used by various local languages of the continent. It is called *ewuro* in Yoruba, '*etidot*' in Ibibio, *onugbu* in Igbo, *ityuna* in Tiv, *ilo* in Igala, *oriwo* in Edo, *grawa* in Amharic, *chusar-doki* and *shawaka* in Hausa [1] and it is called *origbo* in Urhobo language [2].

Bitter leaf is generally cultivated as annuals. However, a small number of it could be grown as perennials in different parts of the world [3]. As demonstrated in animal studies, its leaf extracts may also prevent, delay or destroy carcinogenic cells, prevent metastasis of cancerous cells in the body by inhibition of anti-apoptotic transcription factors [4, 5, 6].

Oxidation is a chemical reaction that can yield free radicals, resulting in series of reactions that may destroy the cells of organisms. Compounds that prevent oxidation are called antioxidants [7,8]. Plants contain high amounts of numerous redox-active antioxidants such as polyphenols, carotenoids, ascorbic acids, DPPH and flavonoids which combat against harmful oxidative cell damage of organisms [8].

Genotoxicity is the ability of different agents to cause damage to genetic material. Antigenotoxicity is the ability of different agents (generally called antigenotoxic agents) to decrease the DNA mutilation initiated by genotoxic agents. *V. amygdalina* extracts may suppress, delay, or kill cancerous cells which includes induction of apoptosis as determined in cell cultures and animal studies [9].

V. amygdalina has received substantial research attention on its nutritional status. A lot of work has been carried out on *V. amygdalina* to investigate the antioxidant activities such as total phenolic content, total flavonoid content, 2,2 diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity, ferric ion reducing antioxidant power (FRAP), reducing power as well as thiobarbituric acid reactive substances (TBARS) with substantial results [18]. However, little or no research work has been carried out to compare the antioxidant activities of *V. amygdalina* accessions obtained from different locations. Also, the determination of antigenotoxic properties of *V. amygdalina* from different locations have not been reported to the best of our knowledge.

This research will help to elucidate the antioxidant activities and antigenotoxic properties of *V. amygdalina* accessions collected from different parts of Nigeria. The findings of this study will help in combating ethidium bromide induced genotoxicities and any other genotoxicants that may lead to different complications (such as mutation, DNA fragmentation etc) in plant (*Allium cepa* roots). The findings of the study also have beneficial effect to humans in creating awareness on the accessions with the highest and lowest antioxidant activities of *V. amygdalina*.

The focus of this study was to determine the antioxidant and antigenotoxicity effects of accessions of *V. amygdalina* by evaluating the 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

and nitric oxide (NO^-) scavenging antioxidant activities, flavonoid and phenolic contents as well as ethidium bromide-induced DNA damage of bitter leaf.

MATERIALS AND METHODS

Collection of samples

Fifty two samples of bitter leaf (*V. amygdalina*) were collected from 6 geopolitical zones of Nigeria (North East, North West, North Central, South South, East, and West). They were identified, authenticated, and assigned voucher IDs and deposited as herbarium specimens (Appendix I).

The study involved the antioxidant activities and antigenotoxic properties assessment of the *V. amygdalina* accessions, to determine which accession has the most antioxidant activities and antigenotoxic properties. The antioxidant assays include: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, total flavonoid contents, total phenol contents and nitric oxide (NO-) scavenging activity. The antigenotoxic properties was ascertained using the DNA fragmentation assay and *Allium cepa*.

Aqueous extraction of *V. amygdalina*

The fresh leaves were rinsed with clean water and air dried at room temperature by spreading on the laboratory table for 24 h. The samples (50 g each per accession) were weighed, blended with 100 ml of water until a homogenous formulation was obtained. The formulation was filtered with a piece of muslin cloth and the filtrate was preserved in the refrigerator (4^{o}C) until ready for use.

Analysis of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of bitter leaf

The free radical scavenging ability of the sample extracts against DPPH free radical was estimated using the method described by Marcocci *et al.* [10]. Methanol solution (0.9 ml) and (0.1 ml) of the sample were mixed with 0.1 ml of 0.15% DPPH solution. The reaction mixture was incubated for 30 min in the dark at room temperature. The control contained all reagents without the samples and methanol was used as the blank. All reaction mixtures were done in triplicates and the DPPH radical scavenging activity of bitter leaf was determined at absorbance of 517 nm and expressed as the inhibition percentage of free radical of the sample after calculation using the following formula:

$$\text{RSA (\%)} = \frac{A_c - A_t}{A_c} \times 100$$

Where,
RSA (%) = Percentage of DPPH discoloration;
A_c = Absorbance of DPPH solution;
A_t = Absorbance of the solution when the sample was added at a particular level.

Analysis of Nitric Oxide radical scavenging activity of bitter leaf

The scavenging effect of bitter leaf on nitric oxide (NO^-) radical was measured according to the method of Marcocci *et al.* [10]. Organic sample homogenate (0.2 ml) was added in the test tubes of 1 ml sodium nitroprusside solution (25 mm). The test

tube was incubated at 37°C for 2 h. An aliquot (0.5 ml) of the incubated solution was removed and diluted with 0.3 ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthyl-ethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as the blank. Absorbance of reagent blank was read as control. Results were expressed as percentage radical scavenging activity (RSA).

$$RSA\ (\%) = \frac{1 - \Delta\ \text{Abs of sample}}{\Delta\ \text{Abs of control}}\ X\ 100$$

Determination of total flavonoid content of bitter leaf extract

Total flavonoid content of bitter leaf extract was determined by calorimetric method as described by Jia *et al.* [11]. The leaf extract (250 µl) was added to 1.25 ml distilled water and 75 µl of 5% $NaNO_2$. After 5 min, 150 µl of 10% $AlCl_3.H_2O$ was added. Then after 6 min, 500 µl of 1 M NaOH and 275 µl of distilled water were added. The solution was properly mixed and the colour intensity of the mixture was read at 510 nm. Ascorbic acid solution (1%, w/v) was used as the standard and the concentration of flavonoid expressed in mg ascorbic acid equivalent/ml of the extract. The total flavonoid content was calculated using a standard curve with rutin (0-100 mg/l) as the standard.

Determination of total phenol content of the leaf extract

This was carried out according to the method described by Singleton and Rossi [12]. About 5 ml of Folin Ciocalteu reagent (5 ml) (10-fold dilution with distilled water) was added to 0.5 ml of the sample. After 3 min, 0.5 ml of saturated Na_2CO_3 solution (16 g/100 ml) and 3.5 ml of distilled water were added. Then the reaction mixture was kept in the dark for 90 min. The absorbance was read at 725 nm. Ascorbic acid solution (1% w/v) was used as the standard and the concentration of phenol expressed in mg ascorbic acid equivalent/ml of extract. Gallic acid solution was used for the preparation of calibration curve. Total phenolic contents of samples were expressed as milligrams of gallic acid equivalent (mg GAE)/100 g of dry weight.

Antigenotoxicity of *V. amygdalina* and DNA fragmentation assay

The outer papery brown layers of 12 onion bulbs (5–10 g), were peeled away and the dried basal root plate were cleaned. About 20 µL of the prepared ethidium bromide (0.03 g/mL) solution was injected into the *Allium cepa.* Which was then submerged in a 50 mL test tube containing water (control) or extract of *V. amygdalina* and was kept for 5 days. The *Allium cepa* roots were then collected for DNA fragmentation assay.

Deoxyribonucleic acid (DNA) fragmentation assay was estimated using Wu *et al.* [13] method. The 50 mg of *Allium cepa* roots were homogenized in 10 ml of a TE solution, containing 5 mmol Tris–hydrochloric acid, 20 mmol EDTA and 0.2% triton X-100 at pH 8.0. Aliquot of sample (1 ml) was centrifuged at high speed (27,000 × *g* for 20 min) to allow proper separation of the intact chromatin (pellet, B) from fragmented DNA (supernatant, T). Freshly prepared diphenylamine solution was used to assay for DNA content of the pellet and the supernatant fractions. Sample readings were monitored at 620 nm with spectrophotometer.

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The percentage of fragmented DNA was calculated using the following formula;

Fragmented DNA (%) = T x 100 / (T + B); where, B = intact chromatin (pellets), T = fragmented DNA (supernatant)

Percentage decrease = decrease ÷ original number x 100. (If the answer is negative it is percentage increase).

Statistical Analysis

All results were represented as means ± SD and all data were analyzed using analysis of variance (ANOVA). Significant difference between means was determined at 5% ($P < 0.05$) confidence level using Posthoc test least significant difference (LSD).

RESULTS AND DISCUSSION

The analysis of antioxidant activities of bitter leaf (*V. amygdalina*) accessions showed that the accession MN628016 had the highest flavonoid content while MN627995 had the lowest flavonoid content (Fig. 1). The accession MN628010 had the highest phenolic content while the accession MN628009 had the lowest phenolic content (Fig. 2). Flavonoids are the primary category of phenolic compounds amongst the several categories, in which plants are grouped. They have strong antioxidant activities and are inherently occurring in plants. Flavonoids have positive effects on human health as studies on flavonoid by-products have shown a wide array of antibacterial, antiviral, anti-inflammatory, and anticancer activities [14,16]. *V. amygdalina* accession MN628016 had significantly highest flavonoid content when compared with the flavonoid contents of other *V. amygdalina* accessions while MN627995 had significantly the lowest flavonoid content (Fig. 1). *V. amygdalina* accessions MN628010 had significantly highest phenolic content while MN628009 had significantly the lowest phenolic content, when compared with the other *V. amygdalina* accessions (Fig. 2). The results of this study suggest that phenolic and flavonoid contents may be the major contributors for the antioxidant activity of the *V. amygdalina* accessions collected.

The mode of action of antioxidants is applied by preventing reactive oxygen species formation either by inhibition of enzymes or chelating trace elements which are involved in free radical generation, scavenging reactive species and augmenting the activity of the antioxidant enzymes [8,14,15]. MN627984 accession of *V. amygdalina* had the highest percentage inhibitory effect of nitric oxide while MN628021 had the lowest percentage inhibitory effect of nitric oxide (Fig. 3). Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide, which spontaneously interacts with oxygen to produce nitrite ions which are estimated by the use of Griess reagent. Nitric oxide has an unpaired electron that acts as a free radical that has a very important role in the pathogenesis of pain, inflammation, cancer, and so on [16].

The electron donation ability of natural products could be measured by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), purple-colored solution bleaching [17,18]. The method

is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution. The measure of color change is relative to the strength and potency of the antioxidants in the extracts. In the present study, all the different accessions of *V. amygdalina* collected showed significantly higher DPPH inhibition percentage. However, MN627975 accession of *V. amygdalina* had the highest percentage inhibitory effect of DPPH (98.92% ± 1.59) while MN627990 had the lowest percentage inhibitory effect of DPPH (46.38% ± 13.98) (Fig. 4).

The results of this study suggest that the different accessions of *V. amygdalina* contain phytochemical constituents that are capable of donating hydrogen to scavenge free radical and the phenolic components present in the different accessions of *V. amygdalina* collected, might be responsible for the nitric oxide scavenging effect. Studies have shown that scavengers of nitric oxide such as phenolic compounds compete with oxygen leading to the reduced production of free radicals [11,14].

Post Hoc Analysis with ANOVA on the antioxidant activities and antigenotoxic properties of *V. amygdalina* Accessions

The overall antioxidant results were analysed using the ANOVA with LSD as shown in Table 1. At $P_{0.05}$; LSD = 16.113; there was significant difference (*) between the mean values of phenol and flavonoid, phenol and DPPH as well as phenol and nitric oxide ($P < 0.05$). There was non-significant (ns) difference between DPPH and nitric oxide ($P > 0.05$) in the overall antioxidant results. However, when the specific ANOVA data for DPPH and nitric oxide was analysed at $P_{0.05}$; LSD = 1.9304; there was significant difference between the mean values of DPPH and nitric oxide ($P < 0.05$).

The antioxidant results that were significant at $P_{0.05}$ were further analysed at $P_{0.01}$; LSD = 21.4122; the results showed that there was highly significant difference (**) between the mean values of phenol and flavonoid, phenol and nitric oxide, phenol and DPPH, flavonoid and DPPH as well as flavonoid and nitric oxide ($P < 0.01$).

The antioxidant results that were significant at $P_{0.01}$ were further analysed at $P_{0.001}$; LSD = 27.4959; the results showed that there was very highly significant difference (***) between the mean values of phenol and flavonoid, phenol and nitric oxide, phenol and DPPH as well as flavonoid and nitric oxide ($P < 0.001$).

In the present study, all the different *V. amygdalina* accessions collected in Nigeria showed significantly higher inhibition percentage at $P<0.05$, $P<0.01$, and $P<0.001$ (Table 1). The results of this study suggest that the leaves of the different accessions of *V. amygdalina* contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

The antigenotoxicity results were also analysed (Table 1). At $P_{0.05}$; LSD = 1.5133; 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 genotoxicity of ethidium bromide in experimental model has been evaluated [20]. Ohta *et al.* [20] also reported some work concerning the effects of ethidium bromide on plant DNA strand breaks and chromosomal aberrations. The expression of antigenotoxic property of commonly consumed *V. amygdalina* accessions are shown in Fig. 5. The control *A. cepa* roots growth in water had the lowest percentage of fragmented DNA followed by *V. amygdalina* accession number MN628010. The highest percentage of fragmented DNA was observed in *Allium cepa* roots grown in ethidium bromide (control) followed by *V. amygdalina* accession MN627977. Percentage increase of fragmented DNA of commonly consumed bitter leaf accessions on *A. cepa* roots grown in ethidium bromide solution are presented in Fig. 6.

Interestingly, *V. amygdalina* accessions MN628008 indicated the highest percentage increase of fragmented DNA (457.62% ± 4.11) followed by MN628024, MN628015 and MN627984, while MN628010 had the lowest (7.32% ± 2.83) (Fig. 6). 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 (Fig. 5).

The highest percentage of fragmented DNA of *Allium cepa* roots growth induced with ethidium bromide solution and treated with *V. amygdalina* accessions extracts as shown in MN627977, MN627985, MN628009 and MN628013, may be as a result of the low phenolic contents, flavonoid contents, nitric oxide and DPPH radical scavenging activities in these *V. amygdalina* accessions. The lowest percentage of fragmented DNA of *Allium cepa* roots growth induced with ethidium bromide solution and treated with *V. amygdalina* accessions MN628010, MN628008, MN628024 MN628015 and MN627984 extracts (antigenotoxic) that were observed could be as a result of the high antioxidant activities in the *V. amygdalina* accession. This is in accordance with Hu *et al.* [21] 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.

The results of the correlation between the antioxidant activities and antigenotoxicity showed that no correlation was observed between the percentage of fragmented DNA of *Allium cepa* root induced with ethidium bromide and treated with *V. amygdalina* and flavonoid content ($r^2 = 0.1411$), nitric oxide radical scavenging activity ($r^2 = 0.0037$) and DPPH radical scavenging activity ($r^2 = 0.1539$). However, a strong positive correlation ($r^2 = 0.6708$) was observed between phenol content and percentage of fragmented DNA of *Allium cepa* root induced with ethidium bromide and treated with *V. amygdalina* leaf extracts (Fig. 7). This suggests that the high phenolic content antioxidant activity could be responsible for the treatment of the genotoxic effect of increased percentage of fragmented DNA of *Allium cepa* root by significantly ($P<0.05$) reducing the percentage of the fragmented DNA of *Allium cepa* root.

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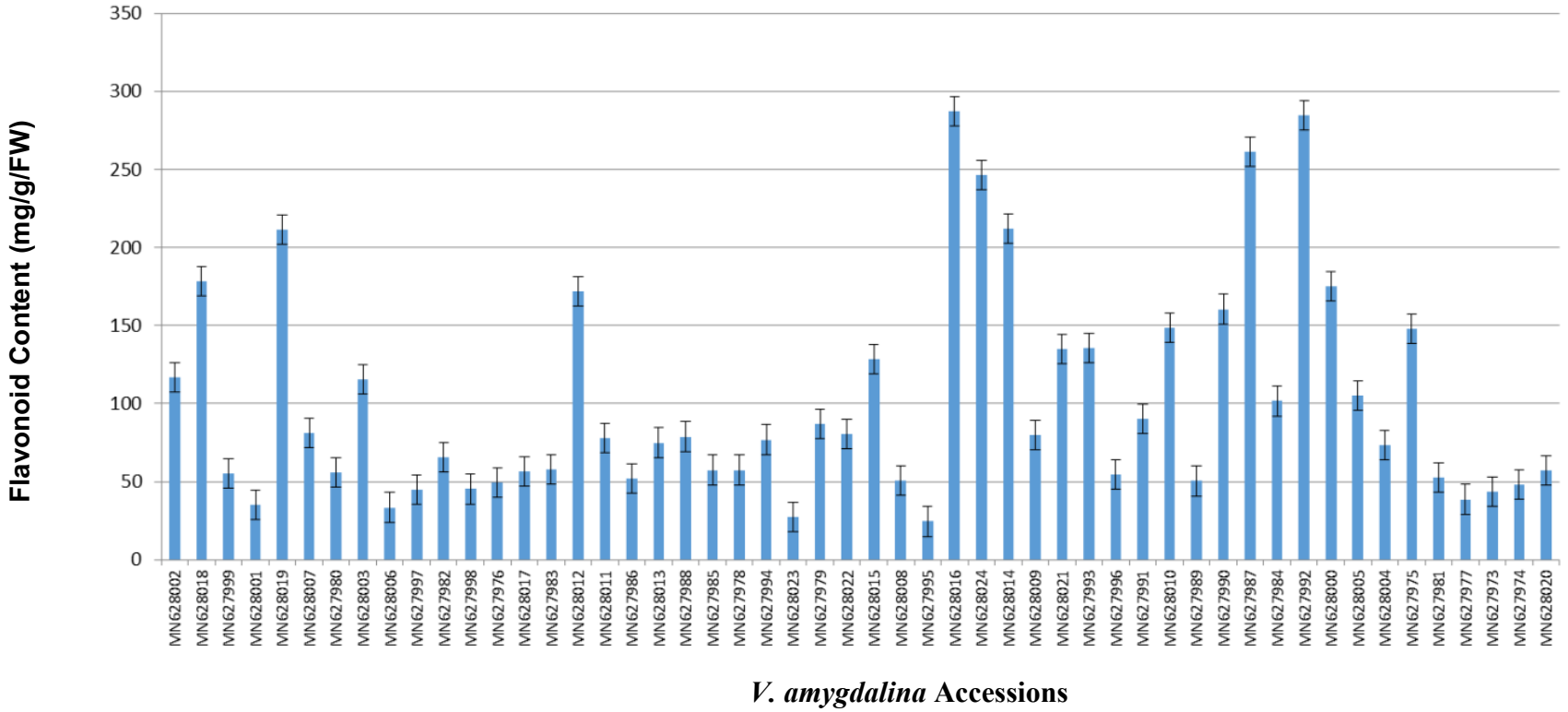

Figure 1: Expression of *V. amygdalina* accessions in relation to highest flavonoid content. Bars represent mean values (n=3)

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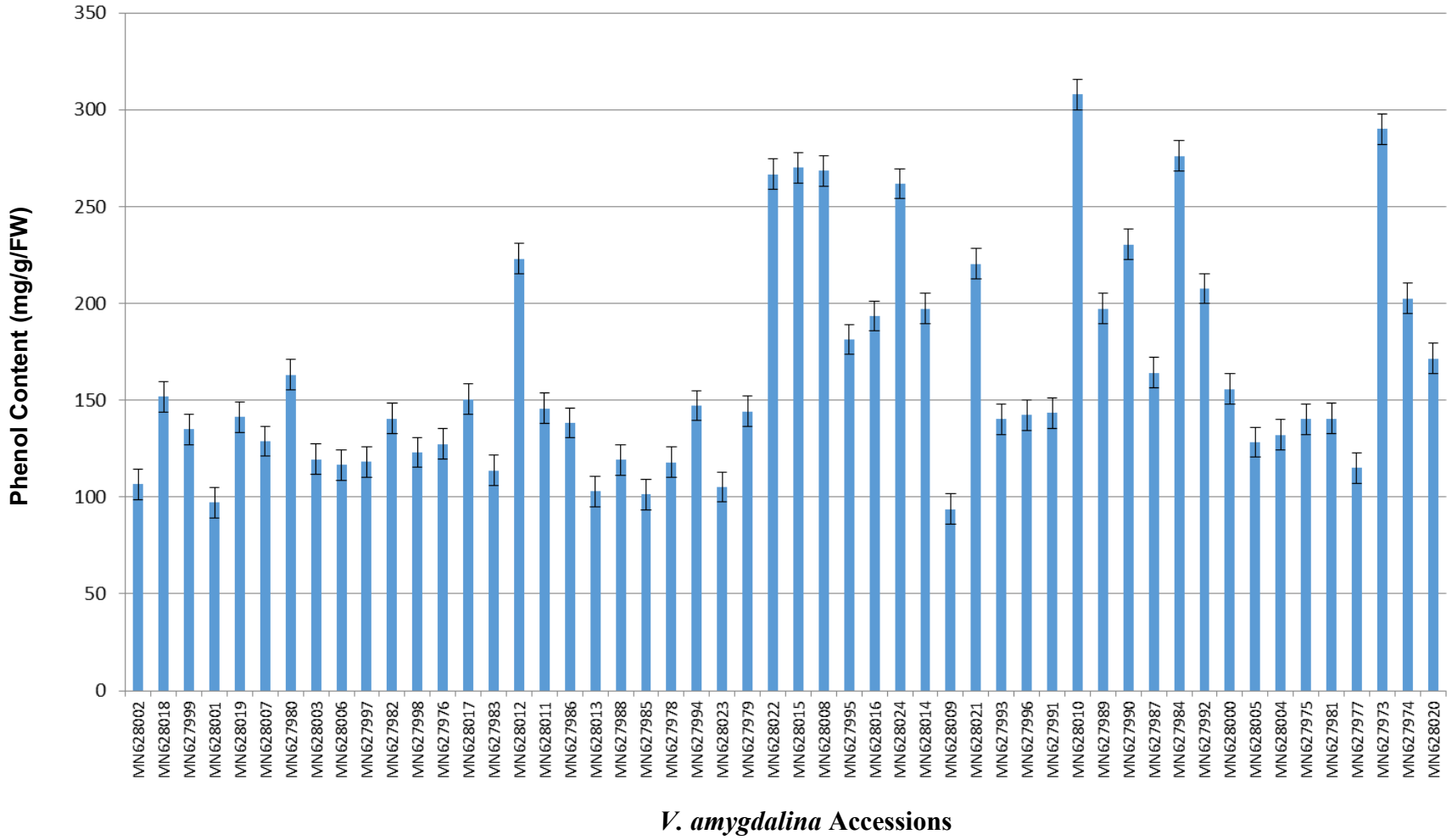

Figure 2: Expression of *V. amygdalina* accessions in relation to highest phenolic content. Bars represent mean values (n=3)

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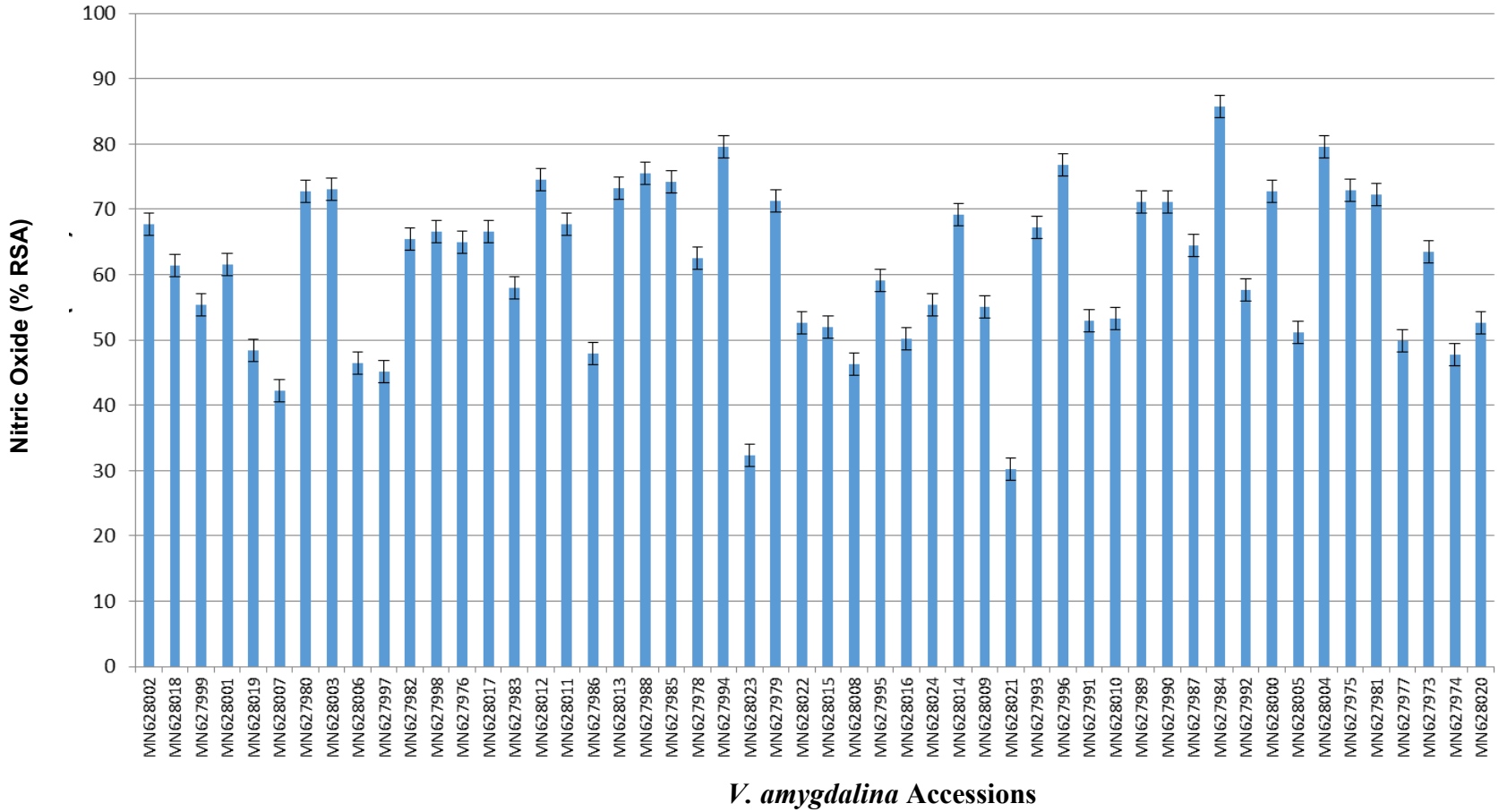

Figure 3: Expression of *V. amygdalina* accessions in relation with highest percentage inhibitory effect of nitric oxide (NO^-) RSA. Bars represent mean values (n=3). RSA = radical scavenging activity

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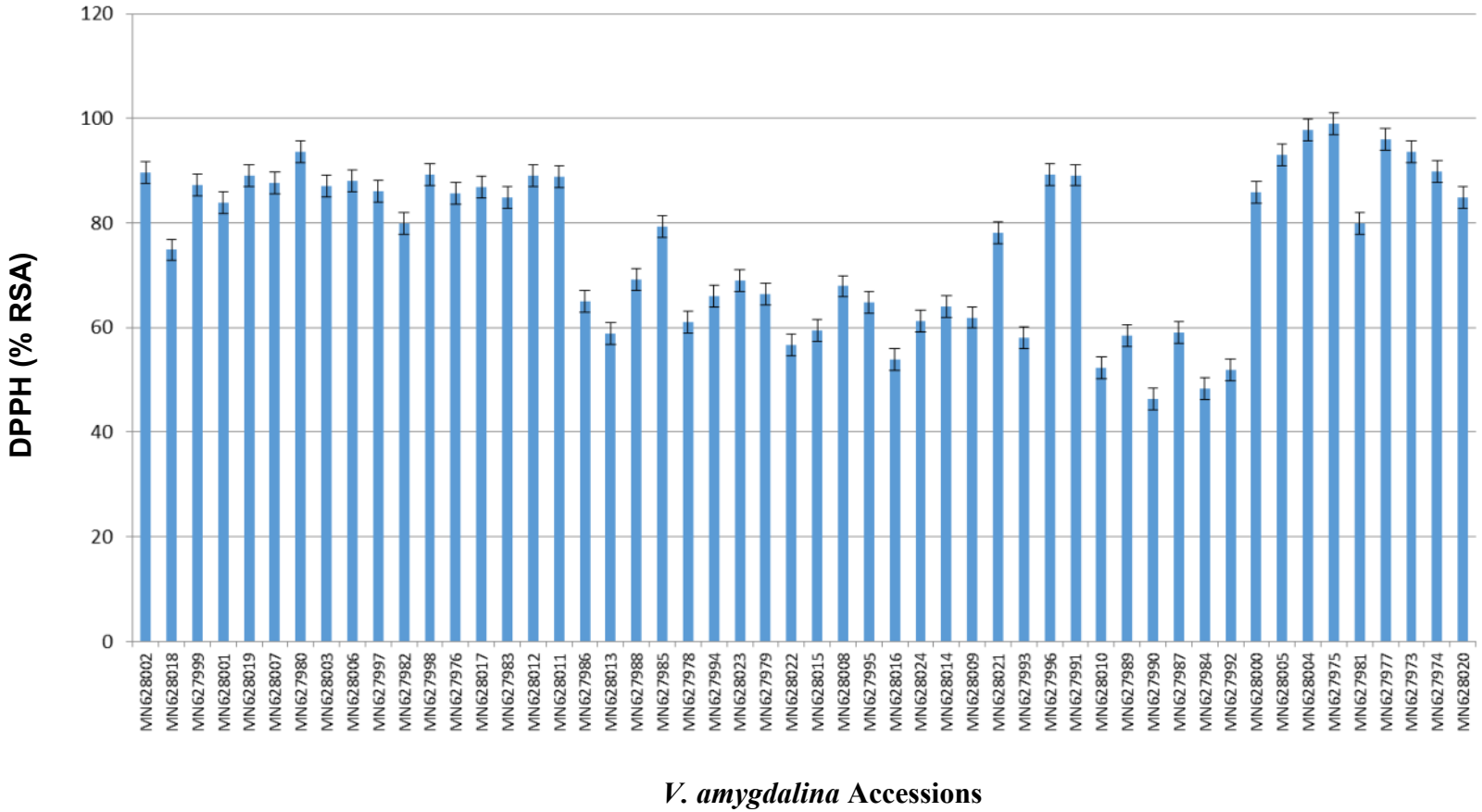

Figure 4: Expression of *V. amygdalina* accessions in relation with highest percentage inhibitory effect of DPPH RSA. Bars represent mean values (n=3). RSA = radical scavenging activity

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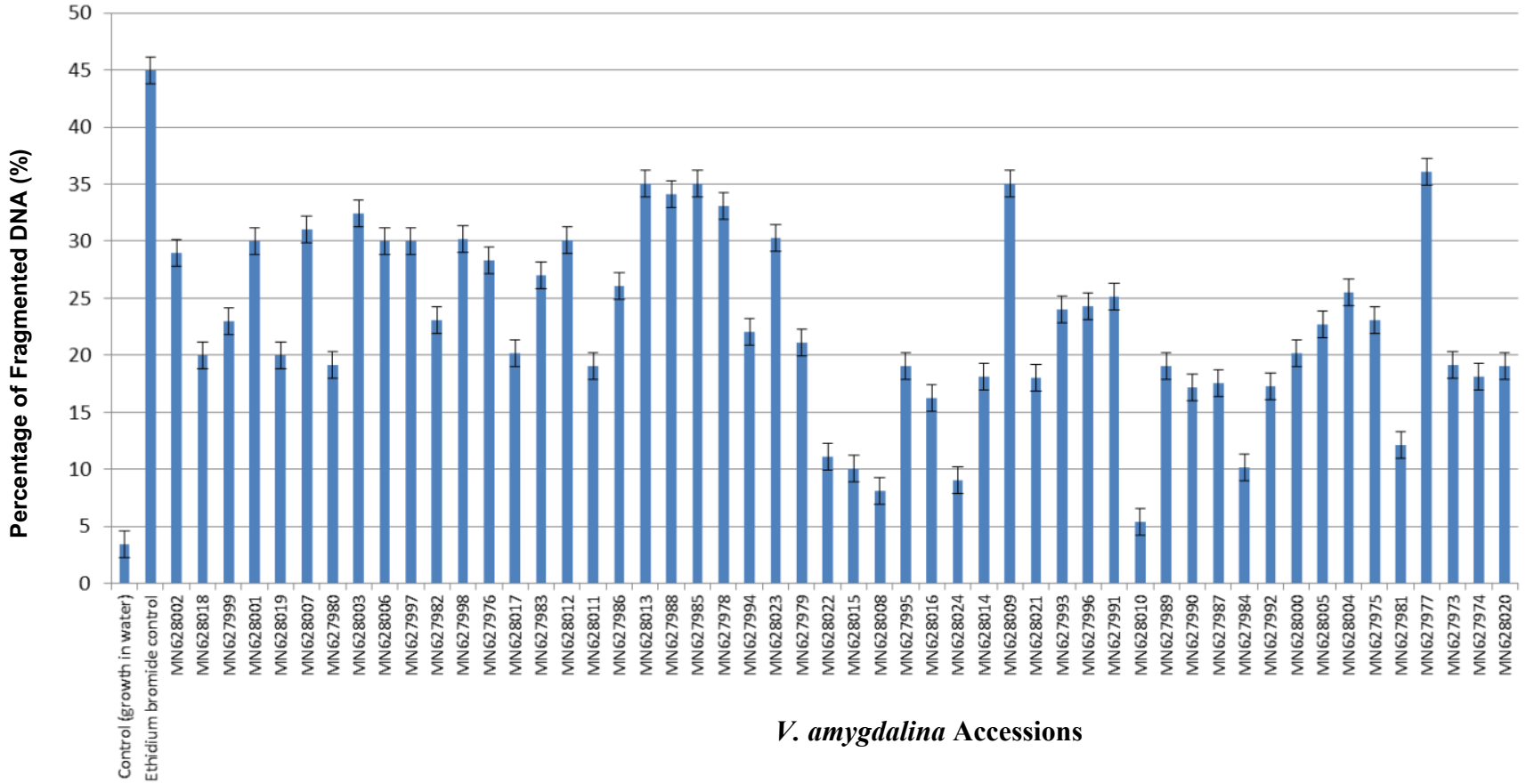

Figure 5: Expression of '*not genotoxic*' property of commonly consumed *V. amygdalina* (bitter leaf) accessions on percentage of fragmented DNA of *A. cepa* roots growth in ethidium bromide solution. Bars represent mean values of triplicates

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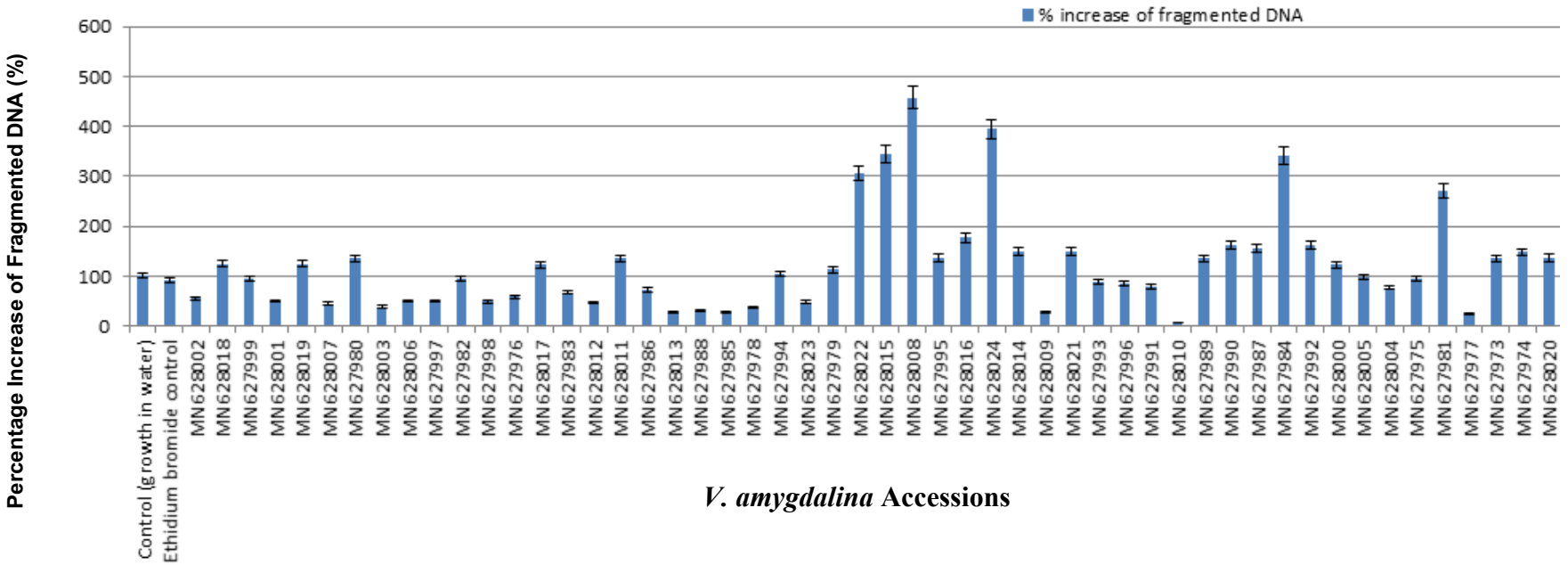

Figure 6: Expression of antigenotoxic property (percentage increase of fragmented DNA) of commonly consumed *V. amygdalina* (bitter leaf) accessions on *Allium cepa* roots growth in ethidium bromide solution. N/B: Percentage decrease = decrease/original number x 100. (If the answer is negative it is percentage increase)

Key (Fig. 1-6): MN628002 = Borno (Maiduguri); MN628018 = Gombe (Gombe); MN627999 = Taraba (Jalingo); MN628001 = Kaduna (Kaduna); MN628019 = Kano (Kano); MN628007 = Kebbi (Benin Kebbi); MN627980 = Kogi (Kogi); MN628003 = Nassarawa (Lafia); MN628006 = Niger (Minna); MN627997 = Abuja (FCT); MN627982 = Plateau (Jos); MN627998 = Bayelsa (Yenagoa); MN627976 = Rivers (Port harcourt); MN628017 = Cross Rivers (Calabar); MN627983 = Akwa Ibom (Utu); MN628012 = Warri South (Ogunu); MN628011 = Isoko North (Ozoro); MN627986 = Udu (Ayama); MN628013 = Ughelli North (Ogor); MN627988 = Bomadi (Kpakiama); MN627985 = Isoko South (Oleh); MN627978 = Ukwuani (Umubu); MN627994 = Burutu (Operemor); MN628023 = Ughelli South (Olomo); MN627979 = Ndokwa West (Ogume); MN628022 = Aniocha South (Igbudu); MN628015 = Anoicha North (Ubulubu); MN628008 = Warri North (Opuama); MN627995 = Ethiope East (Abraka); MN628016 = Oshimili South (Okwe); MN628024 = Oshimili North (Okpanam); MN628014 = Ethiope West (Oghara); MN628009 = Ikah North East (Owa); MN628021 = Sapele (Sapele); MN627993 = Esan central (Opoji); MN627996 = Oredo (Iwegie); MN627991 = Orhionmwon (Ugboko); MN628010 = Akoko Edo (Igara); MN627989 = Esan West (Ekpoma); MN627990 = Esan South (Ohordua); MN627987 = Owan West (Ora); MN627984 = Ikpoba Okha (Agedo); MN627992 = Egor (Egor); MN628000 = Abia (Umuahia); MN628005 = Anambra (Awka); MN628004 = Enugu (Enugu); MN627975 = Imo (Owerri); MN627981 = Ondo (Akure); MN627977 = Oyo (Ibadan); MN627973 = Lagos (Ikeja); MN627974 = Ogun (Adeku); MN628020 = Ekiti (Iroko)

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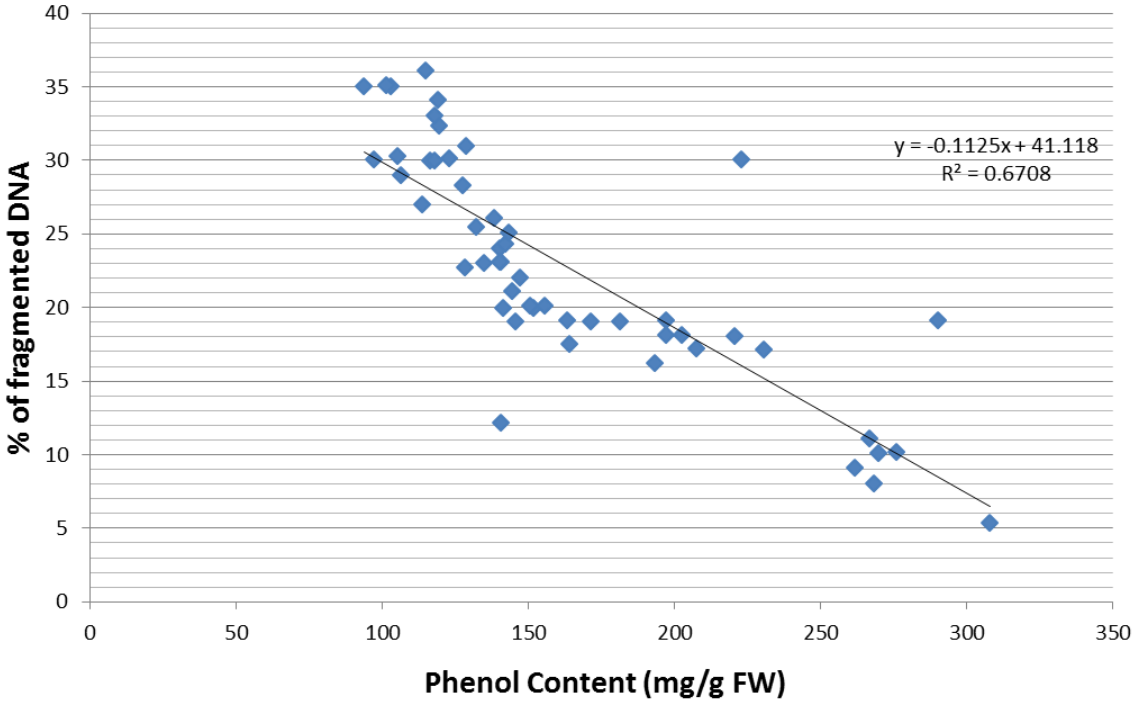

Figure 7: Correlation between phenol content and percentage of fragmented DNA of *Allium cepa* root induced with ethidium bromide and treated *V. amygdalina* leaf accessions collected

The change observed in the varying amount of antioxidant properties of *V. amygdalina* accessions in this study may be as a result of some environmental factors. Altitude, temperature, illumination, and moisture are important factors that could regulate the metabolism and accumulation of secondary metabolites [19]. Environmental differences such as: altitude, temperature, illumination, precipitation, humidity, soils in different production locations, could contribute to the differences in active ingredient contents and antioxidant activity of medicinal plants [19].

CONCLUSION

The results of this study showed that *V. amygdalina* accessions from different parts of Nigeria possesses flavonoids, phenolic content, natural nitric oxide and DPPH radical scavenging activities. Interestingly, the extracts of *V. amygdalina* significantly reduce the percentage of fragmented DNA of *A. cepa* roots growth induced with ethidium bromide. This suggests that the antigenotoxic property of *V. amygdalina* accessions could be because of its antioxidant activities. Generally, the lowest percentage of fragmented DNA of *A. cepa* roots growth was indicated in *V. amygdalina* accessions MN628010, MN628008, MN628024, MN628015 and MN627984. This could be because of the high antioxidant activities (phenolic content) in the *V. amygdalina* accessions as shown in the correlation results.

It is suggested that further studies be carried out to ascertain the impact of soil minerals and weather conditions in the investigated geographical regions on the morphological

characteristics of the *V. amygdalina* accessions collected as well as their antioxidants prowess.

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CONFLICT OF INTERESTS
The authors do not declare any conflict of interests.

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Table 1: Pairwise differences in means of antioxidant activities and antigenotoxic properties

	S_1 (Phenol) $\bar{x}_1 = 163.79$	S_2 (Flavonoid) $\bar{x}_2 = 100.74$	S_3 (DPPH) $\bar{x}_3 = 75.34$	S_4 (Nitric oxide) $\bar{x}_4 = 60.83$	S_5 (EB) $\bar{x}_5 = 22.53$	S_6 (EB + VA) $\bar{x}_6 = 1.905$
S_1 (Phenol) $\bar{x}_1 = 163.79$	-	*** 63.05	*** 88.45	*** 102.96		
S_2 (Flavonoid) $\bar{x}_2 = 100.74$		-	** 25.4	*** 39.91		
S_3 (DPPH) $\bar{x}_3 = 75.34$			-	* (ns) 14.51		
S_4 (Nitric oxide) $\bar{x}_4 = 60.83$				-		
S_5 (Growth in EB) $\bar{x}_5 = 22.53$					-	* 20.62
S_6 (Growth in EB + VA) $\bar{x}_6 = 1.905$						-

VA – *Vernonia amygdalina*; EB – ethidium bromide; * Significant at $P_{0.05}$ (Significant), ** Significant at $P_{0.01}$ (Highly significant), *** Significant at $P_{0.001}$ (Very highly significant) and ns = non-significant

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Table 2: **Bitter leaf (*V. amygdalina*) collection**

Voucher ID	Accession number	Date of collection	Town/City	LGA	State	Latitude	Longitude
PCG/UNN/03-01	MN627973	02-05-16	Ikeja	Ikeja	Lagos	6.519	3.384
PCG/UNN/03-02	MN628016	05-05-16	Okwe	Oshimili South	Delta	6.468	3.376
PCG/UNN/03-03	MN627974	09-05-16	Adeku	Adeku	Ogun	6.899	3.575
PCG/UNN/03-04	MN628017	11-05-16	Calabar	Calabar	Cross Rivers	6.224	8.601
PCG/UNN/03-05	MN627996	14-05-16	Iwegie	Oredo	Edo	6.413	5.571
PCG/UNN/03-06	MN627997	17-05-16	Abuja	Abuja	FCT	9.028	7.463
PCG/UNN/03-07	MN627975	20-05-16	Owerri	Owerri	Imo	5.620	7.012
PCG/UNN/03-08	MN627976	23-05-16	Port harcourt	Port harcourt	Rivers	4.862	6.929
PCG/UNN/03-09	MN627998	26-05-16	Yenagoa	Yenagoa	Bayelsa	4.805	5.806
PCG/UNN/03-10	MN627999	28-05-16	Jalingo	Jalingo	Taraba	8.848	11.401
PCG/UNN/03-11	MN627977	30-05-16	Ibadan	Ibadan	Oyo	7.400	3.865
PCG/UNN/03-12	MN628000	01-06-16	Umuahia	Umuahia	Abia	5.528	7.499
PCG/UNN/03-13	MN628018	04-06-16	Gombe	Gombe	Gombe	10.265	11.167
PCG/UNN/03-14	MN628019	06-06-16	Kano	Kano	Kano	12.010	8.526
PCG/UNN/03-15	MN628001	09-06-16	Kaduna	Kaduna	Kaduna	10.510	7.417
PCG/UNN/03-16	MN627978	13-06-16	Umubu	Ukwuani	Delta	9.200	12.493
PCG/UNN/03-17	MN628002	16-06-16	Maiduguri	Maiduguri	Bornu	10.328	12.343
PCG/UNN/03-18	MN627979	18-06-16	Ogume	Ndokwa West	Delta	6.412	8.226
PCG/UNN/03-19	MN628003	21-06-18	Lafia	Lafia	Nasarawa	8.512	8.517
PCG/UNN/03-20	MN627980	23-06-16	Kogi	Kogi	Kogi	7.815	6.639
PCG/UNN/03-21	MN628004	27-06-16	Enugu	Enugu	Enugu	6.435	7.532
PCG/UNN/03-22	MN627981	30-06-16	Akure	Akure	Ondo	7.097	4.836
PCG/UNN/03-23	MN628020	02-07-16	Iroko	Iroko	Ekiti	7.620	5.225
PCG/UNN/03-24	MN627982	07-07-16	Jos	Jos	Plateau	9.276	9.555
PCG/UNN/03-25	MN628005	13-07-16	Awka	Awka	Anambra	6.248	6.951
PCG/UNN/03-26	MN628006	18-07-16	Minna	Minna	Niger	10.142	5.394
PCG/UNN/03-27	MN628007	21-07-16	Benin Kebbi	Benin Kebbi	Kebbi	11.835	4.025
PCG/UNN/03-28	MN627983	25-07-16	Utu	Utu	Awka Ibom	4.929	7.868
PCG/UNN/03-29	MN627984	30-07-16	Agedo	Ikpoba Okha	Edo	6.469	5.292
PCG/UNN/03-30	MN628021	04-08-16	Sapele	Sapele	Delta	5.896	5.672
PCG/UNN/03-31	MN627985	09-08-16	Oleh	Isoko South	Delta	5.353	5.510
PCG/UNN/03-32	MN627986	15-08-16	Ayama	Udu	Delta	11.836	4.144
PCG/UNN/03-33	MN628008	18-08-16	Opuama	Warri North	Delta	5.738	6.186
PCG/UNN/03-34	MN627987	22-08-16	Ora	Owan West	Edo	5.656	6.110
PCG/UNN/03-35	MN627988	25-08-16	Kpakiama	Bomadi	Delta	5.565	5.783
PCG/UNN/03-36	MN628009	31-08-16	Owa	Ikah North East	Delta	5.467	5.832
PCG/UNN/03-37	MN628022	03-09-16	Igbudu	Aniocha South	Delta	5.681	5.947
PCG/UNN/03-38	MN627989	06-09-16	Ekpoma	Esan West	Edo	5.589	6.199
PCG/UNN/03-39	MN627990	10-09-16	Ohordua	Esan South	Edo	5.985	5.759
PCG/UNN/03-40	MN627991	14-09-16	Ugboko	Orhionmwon	Edo	5.627	5.651
PCG/UNN/03-41	MN628010	19-09-16	Igara	Akoko Edo	Edo	6.387	5.899
PCG/UNN/03-42	MN627992	23-09-16	Egor	Egor	Edo	6.388	5.565
PCG/UNN/03-43	MN628011	27-09-16	Ozoro	Isoko North	Delta	6.904	5.932
PCG/UNN/03-44	MN628012	30-09-16	Ogunu	Warri South	Delta	6.165	5.152
PCG/UNN/03-45	MN628023	04-10-16	Olomo	Ughelli South	Delta	7.352	6.079
PCG/UNN/03-46	MN628013	07-10-16	Ogor	Ughelli North	Delta	6.696	6.135
PCG/UNN/03-47	MN628024	08-10-16	Okpanam	Oshimili North	Delta	6.468	5.291
PCG/UNN/03-48	MN627993	21-10-16	Opoji	Esan central	Edo	4.930	7.873
PCG/UNN/03-49	MN627994	25-10-16	Operemor	Burutu	Delta	6.154	8.640
PCG/UNN/03-50	MN628014	28-10-16	Oghara	Ethiope West	Delta	5.478	6.030
PCG/UNN/03-51	MN627995	31-10-16	Abraka	Ethiope East	Delta	6.386	5.622
PCG/UNN/03-52	MN628015	04-11-16	Ululubu	Anoicha North	Delta	6.349	5.612

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