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Original Article

Levels and Cytogenotoxicity of Phytochemicals and Heavy Metals in Guava (*Psidium guajava* L.) Leaves Obtained From Birnin Kebbi, Nigeria

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ABSTRACT

Background: *Psidium guajava* (guava tree) is widely used in Nigeria to treat diseases. However, a paucity of information exists on the safety of the plant.

Objectives: This study determined the safety of *P. guajava* leaves collected in Birnin Kebbi, Nigeria.

Methods: The methanolic extract of the plant's leaves was subjected to phytochemical and heavy metal screening using standard protocols, and thereafter, subjected to a cytogenetoxicity test using the *Allium cepa* toxicity assay. Twenty-one *A. cepa* bulbs divided equally into seven groups were grown over beakers containing distilled water (negative control), formaldehyde (positive control), as well as 0.25, 0.5, 1, 2, and 4 g of the extract, respectively, for five days. The root-tip cells of the *A. cepa* bulbs were treated and then examined for chromosomal aberrations.

Results: The phytochemical screening revealed high levels of saponins, and moderate levels of phenols, tannins, and flavonoids, while quinones and terpenoids were sparingly available. The heavy metal analysis showed non-permissible levels of cadmium and zinc, while two other tested heavy metals (lead and copper) were undetected. Except for the *A. cepa* treated with 0.25 and 0.5 g, the extract induced dose-dependent root growth and mitotic index inhibition ($P<0.05$). The extract also induced cytogenetic effects, mainly sticky, vagrant, and fragmented chromosomes as well as anaphase bridges.

Conclusions: It can be inferred from the results that low to medium doses of the extract are safe but may elicit harmful effects at high doses. Advice from a phytomedicine or phytotherapy expert should be sought before using it.

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Introduction

Guava (*Psidium guajava*) is a tropical tree known for its succulent fruits. It is a member of the Magnoliophyta phylum, Magnoliopsida class, and Myrtaceae family, with roughly 133 genera and 3800 species [1, 2]. *P. guajava* is cultivated in many tropical or subtropical climates, including West Africa [3, 4]. The plant is rich in phytochemicals, such as glycosides, terpenoids, tannins, alkaloids, steroids, saponins, amino acids, anthraquinones, proteins, flavonoids, and phenols [5]. Furthermore, *P. guajava* is rich in minerals, such as iron, phosphorus, and calcium, as well as vitamins A, B, and C [6, 7]. Because of these phytochemicals, the plant exhibits antibacterial, antioxidant, and antifungal activities, among others [8]. It is also used in traditional medicine to treat various diseases, such as diabetes, diarrhea, gastroenteritis, caries, stomachaches, hypertension, toothaches, vomiting, ulcers, inflamed gums, coughs, and malaria [9, 10].

In Nigeria, the leaves of *P. guajava* are used for treating malaria, typhoid fever, gastroenteritis, hypertension, vomiting, diarrhea, wounds, ulcers, toothaches, coughs, sore throats, inflamed gums, reproductive disorders, and a variety of other conditions [11-13]. Unfortunately, there is a dearth of information on the toxicity of the plant in Nigeria, especially in the northwest of the country. This information has become necessary because studies conducted by Manekeng et al. [14] in Cameroun and Abwage et al. [13] in Calabar (Nigeria) indicated that at certain doses, the plant can cause weight and sperm abnormalities as well as hematological, biochemical, hormonal, and histological changes. This information is even more important in light of the fact that environmental pollutants caused by anthropogenic activities may contaminate plants or increase the concentration of natural compounds in plants.

Some of the techniques used to test plant toxicity include phytochemical screening, heavy metal analysis, and cytogenotoxicity tests using the Allium cepa. The *A. cepa* toxicity test has been used consistently in environmental monitoring since the middle of the 19th century because it is inexpensive and gives accurate results in both plant and animal test subjects. It is easy to carry out, takes a little time, and does not require many reagents and laborious preparations of test substances [15]. This study, therefore, employed phytochemical screening, heavy metal analysis, and *A. cepa* genotoxicity testing to determine the safety of *P. guajava* leaves obtained from Birnin Kebbi, Nigeria.

Materials and Methods

Sample collection

Some leaves of *P. guajava* were obtained from Kalgo town in Birnin Kebbi, Kebbi State, Nigeria, in October 2021. The plant was identified by a plant taxonomist at the Department of Biological Sciences, Federal University Birnin Kebbi, Kebbi State, Nigeria. A sample of the authenticated plant with voucher number FUBK-H_74 was deposited in the herbarium section of the department. At the same time, 80 purple varieties of onion (Allium cepa) bulbs with a mean weight of 32±3 g were obtained from Birnin Kebbi central market.

Preparation of the extract

Fresh leaves of the plant were dried at room temperature until a constant dry weight was obtained (after seven days). The leaves were crushed into a fine powder using a TENCAN laboratory grinder (Model Number XQM-20-100). Exactly 200 g of the powder was weighed using a KERN precision electronic weighing balance (model: KB-2000-2N) into a rubber container and soaked with 1100 mL of methanol (98%) for 72 hours. The resulting mixture was filtered with a muslin cloth, and the filtrate (about 650 ml) was evaporated with a BUCHI vacuum rotary evaporator (model: N-1001) at 45°C to remove the solvent from the crude extract in the flask. After that, the crude extract was poured into two Petri dishes to allow the remaining solvent to evaporate. In the end, about 6 g of solid extract from the leaves of the plant was made and kept in a desiccator prior to use.

Qualitative and quantitative phytochemical screening

The qualitative and quantitative analyses of the plant were done following the procedures of Yahaya et al. [16]. The stock solution of the extract was prepared by measuring 0.06 g into a 50-mL beaker and dissolving it with distilled water. More distilled water was added to the solution to top it off at the meniscus of the beaker. The solution was then tested for the presence of alkaloids, tannins, flavonoids, saponies, terpinoids, phenols, quinone, and cardiac glycosides. The phytochemicals that were detected in abundance and moderate abundance (tannins, flavonoids, phenols, and saponins) in the qualitative analysis were then subjected to quantitative analysis following the method by Yahaya et al. [16].

Heavy metal analysis

The heavy metal profiles of the extract were determined following the procedures of Yahaya et al. [17]. The extract was digested by placing 1 g in a 100-mL beaker and an analytical grade of 25 mL of aqua-regia and 5 mL of 30% H_2O_2 were added. The digestion was carried out at 80°C until the mixture became a homogeneous solution. The content was filtered into a 50-mL volumetric flask, left to cool, and then filled to the meniscus with distilled water. A microplate spectrophotometer (model: VM1208PTS2) was used to determine the levels of copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn) in the extract.

Cytogenotoxicity test

The cytogenotoxicity of the *P. guajava* leaf extract was evaluated using the *A. cepa* toxicity test as conducted by Yahaya et al. [15]. The viability of the *A. cepa* bulbs was tested before commencing the test by growing 70 bulbs in the dark for 96 hours in beakers containing 100 ml of tap water at room temperature. Twenty-one of them were chosen for this study. The outer scales and the dead, dried roots of the onion bulbs were removed and scrapped aseptically to expose the apices of the root primordia. The bulbs were divided into seven groups of three each. Groups 1 and 2 served as negative and positive controls, respectively, and were grown in beakers containing 100 mL of distilled water and 70% formaldehyde for seven days at room temperature and with humidity. Groups 3–7 (test groups) were grown under the same conditions and duration as the controls over beakers containing 0.25, 0.5, 1.0, 2.0, and 4 g of the solutions of the plant extract, respectively. The root growth of the *A. cepa* bulbs was recorded during the experiment, after which they were cut and fixed instantly in Aceto-alcohol in the ratio of 1:3. Each bulb's root tips were cut and macerated in drops of 1 N HCl at 60°C for 3 minutes before staining in Carbol Fuchsin. The mixture was then squashed in a 45% acetic acid solution containing 2% aceto-orcein. Permanent slides were made and mounted on Canadian turpentine where chromosomal abnormalities were examined and photographed under a light microscope (X 100). The chromosomal abnormalities were determined and calculated by examining 1000 cells in each slide and characterized and classified as bridges, c-mitoses, vagrants, fragments, stickiness, bi-nucleus, and multi-polar.

Quality control and assurance

All of the chemicals used in the current study were of high purity. Each container was scrubbed with soap before being rinsed with water and chemicals to be put in. Background contamination of the samples was checked to ensure the accuracy of the heavy metal analysis. This was accomplished by inspecting blank samples intermittently. In addition, each sample analysis was reproduced three times with almost 100% reproducibility. Thus, the average of the three values for each heavy metal was used for further analysis.

Statistical analysis

The Statistical Package for Social Sciences: SPSS software, version 23 for Windows and Microsoft Office Excel 13 were used for data analysis, values are presented as Mean±SD. The *f*-test (ANOVA) was used to compare values between the control and test groups. Statistical significance was defined as $P \leq 0.05$.

Results

Phytochemicals in the extract

Table 1 shows the phytochemicals detected in the extract of *P. guajava* leaves obtained from Birnin Kebbi, Nigeria. Saponins were highly available, flavonoids, tannins, and phenols were moderately available, terpenoids and quinones were sparingly available, and alkaloids were not detected.

Quantification of moderate to high levels of phytochemicals in the extract

The levels of phytochemicals that were moderately or highly available in the extract as indicated in Table 1 above are presented in Table 2. Saponins were the most abundant, followed by tannins, phenols, and flavonoids.

Concentrations of heavy metals in the extract

Table 3 shows the levels of Cd, Zn, Pb, and Cu in the leaf extract of *P. guajava* obtained from Birnin Kebbi. Cd and Zn were present in the extract above the World Health Organization's permissible limits, while Cu and Pb were not present.

Root growth of the *A. cepa*

Table 4 reveals the daily root growth of the controls and *A. cepa* bulb treated with leaf extract of *P. guajava*. The negative control and the bulbs treated with 0.25 and 0.50

Table 1. Quality of phytochemicals detected in the extract of *P. guajav* leaves obtained from Birnin Kebbi, Nigeria

Phytochemicals	Inference
Flavonoids	++
Tannins	++
Cardiac glycosides	+
Saponins	+++
Terpenoids	+
Alkaloids	-
Phenol	++
Quinones	+

Keys: (-): Not available; (+): Sparingly available; (++): Moderately available; (+++): Highly available

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Table 2. Mean levels of phytochemicals in *P. guajava* leaves obtained from Birnin Kebbi, Nigeria

Compounds	Concentrations (mg/mL)
Flavonoids	0.13±0.04
Tannins	0.17±0.03
Phenols	0.14±0.02
Saponins	0.39±0.06

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Table 3. Mean levels of heavy metals in *P. guajava* obtained from Birnin Kebbi, Nigeria

Heavy Metals	Level (mg/kg)	Recommended [18]
Zn	3.9	≤5
Cd	0.05	≤0.01
Cu	BDL	≤0.2
Pb	BDL	≤0.01

BDL: Below detection levels

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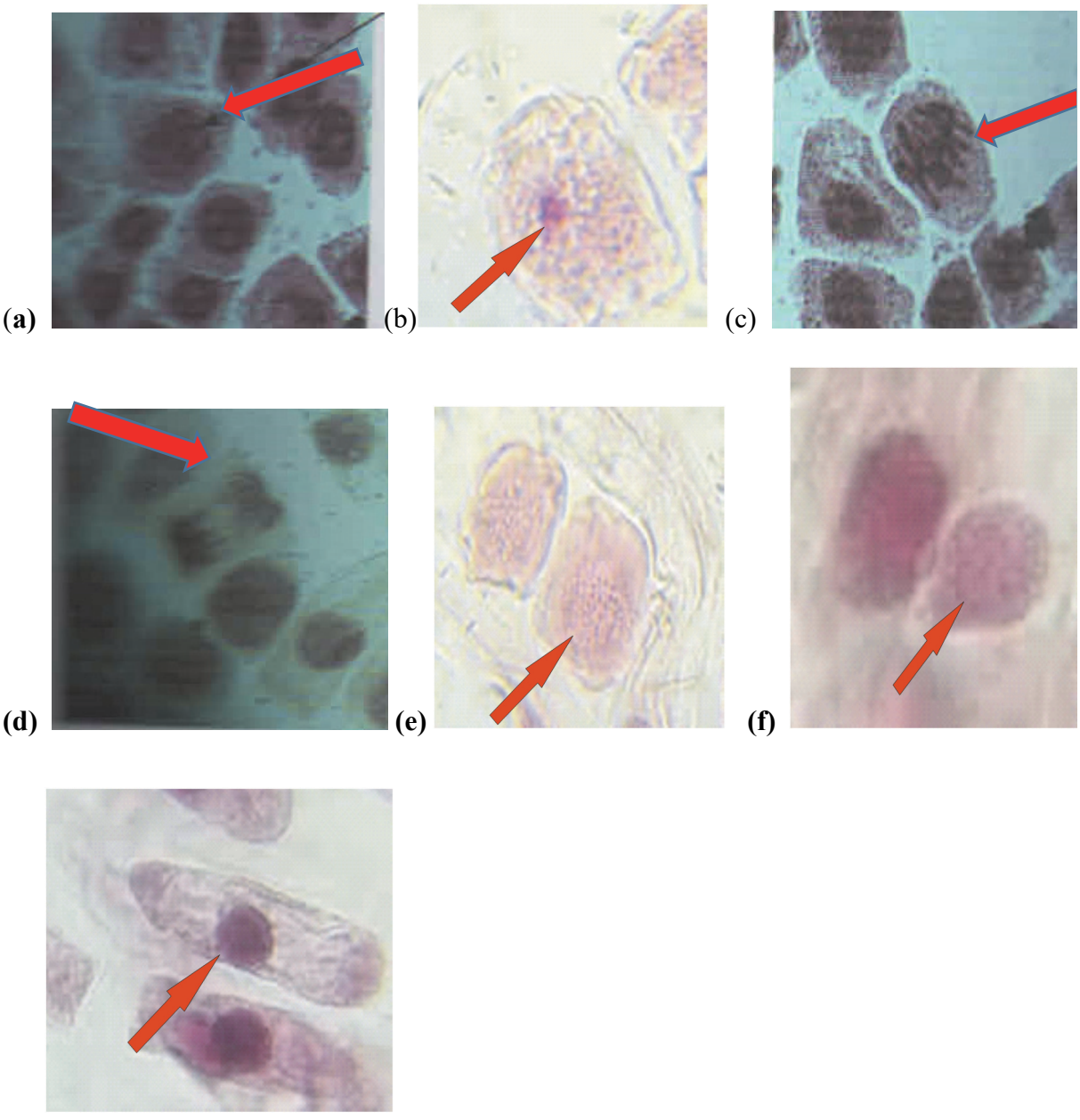
g of the extract showed a significant ($P<0.05$) growth increase, while other test groups showed an insignificant growth increase.

Chromosomal aberrations in the *A. cepa*

Table 5 reveals the number of dividing cells, mitotic index (MI), and chromosomal aberrations in the treated and control *A. cepa*. The negative and positive control had 40 and 15 dividing cells, respectively, while the bulbs treated with 0.25, 0.5, 1, 2, and 4 g had 35, 31, 28, 25, and 18 dividing cells, respectively. Compared to the negative control, the MI of the treated onions were reduced in the order of 4<2<1<0.5<0.25, respectively. However, the reduction was insignificant ($P\geq0.05$) in the bulbs treated with 0.25 and 0.5 g of the extract. Similar trends were also observed in the number of chromosomal abnormalities induced.



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Figures 1 A-G. Chromosomal aberrations detected in the root-tip cells of the control and *A. cepa* bulbs treated with the extract of *P. guajava* leaves obtained from Birnin Kebbi, Nigeria. the negative control (treated with distilled water) showing normal prophase; b the positive control (treated with formaldehyde) showing vagrant chromosome; c *A. cepa* treated with 0.25 g of the extract showing normal metaphase; d the *A. cepa* treated with 0.5 g of the extract showing normal anaphase; e *A. cepa* treated with 1 g of the extract showing sticky chromosome; f *A. cepa* treated with 2 g of the extract showing bridge fragment; and g the *A. cepa* treated with 4 g of the extract showing vagrant chromosome.

Figures 1 A-G show the chromosomal abnormalities observed in the root-tip cells of the control and treated *A. cepa* bulbs. The cells of the negative control and bulbs treated with 0.25 and 0.5 g of the extract had normal chromosomes (Figure 1a, c, and d). The positive control had sticky chromosomes (Figure 1b), while the bulbs treated with 1, 2, and 4 g had stickiness observed at anaphase, sticky chromosomes observed at metaphase, and sticky chromosomes seen at metaphase (Figures 1e-g), respectively.

Discussion

This study was conceived to determine the safety of the *P. guajava* leaves obtained from Birnin Kebbi, Nigeria. Compared with the negative control, the extract

Table 4. Mean values of the root growth of the controls and *A. cepa* bulbs treated with the extract of *P. guajava* leaves obtained from Birnin Kebbi, Nigeria

Treatment Concentrations (g)	Mean±SD					Growth Increase	P
	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4		
Negative control	2.92±0.12^{a}	3.51±0.17^{b}	4.30±0.06^{c}	5.00±0.23^{d}	5.51±0.12^{e}	2.60	0.0020*
Positive control	2.21±0.12^{a}	2.21±0.12^{a}	2.21±0.12^{a}	2.21±0.12^{a}	2.21±0.12^{a}	0.00	-
0.25	1.92±0.23^{a}	2.03±0.21^{a}	2.13±0.20^{a}	2.31±0.23^{a}	3.30±0.23^{b}	2.38	0.0042*
0.50	2.01±0.19^{a}	2.47±0.19^{a}	3.57±0.13^{b}	3.77±0.13^{a}	4.01±0.13^{a}	2.01	0.0048*
1.0	1.91±0.15^{a}	1.97±0.18^{a}	1.97±0.18^{a}	2.17±0.37^{a}	2.17±0.37^{a}	0.34	0.877
2.0	1.83±0.15^{a}	1.97±0.09^{a}	1.97±0.09^{a}	2.17±0.37^{a}	2.17±0.37^{a}	0.34	0.764
4.0	1.93±0.07^{a}	2.00±0.12^{a}	2.17±0.12^{a}	2.20±0.26^{a}	2.23±0.31^{a}	0.30	0.32

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Note: a, b, c, d, e: Are significantly different at P≤0.05; *: are significantly (P≤0.05) different from the negative control (ANOVA); Negative: *A. cepa* treated with distilled water; Positive: *A. cepa* treated with formaldehyde.

Table 5. Chromosomal aberrations in the root tips of control and *A. cepa* treated with extract of *P. guajava* leaves obtained from Birnin Kebbi, Nigeria

Treatment Concentrations (g)	TCN	ND	ST	CM	BF	VG	LG	TA (%)	MI	MI±SEM
Negative control	1000	40 (P_8 M_{12} A_{12} T_3)	0	0	0	0	0	0.00	4.0	4.0±0.35
Positive control	1000	15 (P_4 M_5 A_4 T_2)	4	0	2	0	4	40.00	1.5	1.5±0.17*
0.25	1000	35 (P_9 M_{11} A_{10} T_5)	0	0	0	0	0	0.00	3.5	3.5±0.17
0.5	1000	31 (P_9 M_{10} A_6 T_6)	0	0	0	0	0	0.00	3.1	3.1±0.35
1	1000	28 (P_8 M_6 A_7 T_7)	6	0	1	1	2	35.71	2.8	2.8±0.12*
2	1000	25 (P_5 M_6 A_6 T_8)	8	0	0	2	4	56.00	2.5	2.5±0.17*
4	1000	18 (P_6 M_5 A_3 T_4)	8	0	2	1	6	94.44	1.8	1.8±0.06*

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Note: Values were expressed as Mean±SD, (n=3); TCN: total cell number; ND: Number of dividing cells; ST: Stickiness; CM: C-mitosis; BF: Bridge fragrant; VG: Vagrant; LG: Lagged; TA: Total aberration; MI: Mitotic index; SEM: Standard error of the mean; P: Prophase; M: Metaphase; A: Anaphase; T: Telophase.

of the plant induced a concentration-dependent reduction in the root growth (Table 4) and mitotic index (Table 5) of the *A. cepa* bulbs used in the cytogenotoxicity testing of the plant's extract. Moreover, cytogenetic effects, such as stickiness, anaphase bridge, as well as a vagrant and fragmented chromosomes were detected in the root cells of the A. *cepa* bulbs. However, the A. *cepa* bulbs treated with 0.25 and 0.5 g of the extract had an insignificant root-growth reduction and no chromosomal aberrations and thus can be considered safe levels.These findings are consistent with those of Luber et al. [19], who observed that *P. guajava* infusion induced some cytogenetic effects, mainly altered cell cycles, in the root and meristematic cells of *Latuca sativa.* In the same study, seed germination and root growth decreased with increasing infusion concentration. The findings of the current study are also in line with those of Ofodile et al. [20], who reported cytogenetic effects, such as c-mitosis, vagrant chromosomes, chromosome bridges, and binucleate cells in the root cells of onion bulbs treated with *P. guajava* leaves, even at a low concentration of 0.02 g/mL. Furthermore, Fadipe et al. [21] observed cytogenetic effects as well as a dose-dependent decrease in growth and mitotic index of root cells of some onion bulbs grown in *P. guajava* aqueous extract. However, the findings contradict those of Andrade-Vieira et al. [22],

who demonstrated in an experiment that *P. guajava* infusions produced antigenotoxic effects on the root and meristematic cells of *L. sativa* used in genotoxicity testing. The findings also contrast with those of Cesar et al. [23], who demonstrated that *P. guajava* infusion induced antigenotoxic effects in some treated rats.

Some phytochemicals detected in high concentrations in the plant under the present study, particularly flavonoids, phenols, tannins, and saponins, could be responsible for the cytogenetic effects observed. In a plant extract with a high concentration of flavonoids, cytotoxic and genotoxic effects were observed [24]. Liu et al. [25] demonstrated that saponins can induce DNA damage and chromosome mutations in mammalian cells *in vitro* by reducing cell defense against oxidative stress through the inhibition of glutathione-S-transferase activity. Jiang et al. [26] also showed high saponins to be toxic to the cells. High phenol content (40 to 160 µg/mL) was shown by Mendoza-Meza et al. [27] to induce cytotoxic and genotoxic effects on immortalized human keratinocyte cell lines. Phenol was shown to have the highest concentration in the extract of *Jatropha mollissima*, which exhibited chromosomal abnormalities in the root cells of onion bulbs [28]. Tannic acid has been shown to promote chromosomal aberrations in human leucocyte cells [29]. A review by Islam [30] shows that many substances rich in terpenoids are cytotoxic, genotoxic, and mutagenic. The concentration of phytochemicals in plants is generally influenced by their environmental and climatic conditions [31]. When plants are stressed by their environment, like when they are exposed to chemicals or pests, they make more phytochemicals [31].

The high heavy metal content of the extract, particularly Cd and Zn, could also be responsible for the observed cytogenetic effects in the exposed *A. cepa* bulbs. Zn is an essential component of Zn-finger proteins and acts as a cofactor for enzymes required for cellular metabolism and the maintenance of DNA integrity. However, excess Zn exposure increases genome instability and hence DNA damage [32]. Excess Zn exposure has been shown to reduce sugarcane root growth and mitotic efficiency, enhancing chromosomal aberrations [33]. Cadmium was found to have a high cytotoxic potential even at low concentrations, as low as 2.5 µM [34]. Wang et al. [35] also demonstrated the cytogenotoxicity of a cadmium compound. Other heavy metals not tested in the plant under the current study could have also contributed to the observed cytogenetic effects. Most heavy metals do not work in isolation; thus, the observed genetic abnormalities could have synergetic effects among the heavy metals. Heavy metals are found naturally in minute quantities in plants. However, plants can extract heavy metals from the environment, raising the levels of their natural deposits. Birnin Kebbi is not an industrialized town; thus, the possible sources of Cd and Zn in the plants are the soil. High levels of heavy metals in a plant can raise its stress hormones and thus its phytochemicals and could be responsible for the high concentrations of some phytochemicals observed in the current study.

Conclusion

The results demonstrated that the leaf extract of *P. guajava* obtained from Birnin Kebbi is rich in health-boosting phytochemicals, such as saponins, terpenoids, tannins, flavonoids, phenols, and quinones. However, it contains high levels of saponins, suggesting that constantly taking the plant for an extended period of time can be injurious. The extract also contained non-tolerable levels of Cd and Zn, which adds to the proof that the plant, can elicit side effects after prolonged high-dose use. This is confirmed in the cytogenotoxicity study of the extract, in which low to moderate doses (0.25 to 0.5 g) caused non-toxic effects on treated *A. cepa* bulbs. However, high doses (1, 2, and 4 g) retarded the root growth of the treated *A. cepa* and caused a reduced mitotic index as well as chromosomal aberrations, such as sticky, vagrant, and fragmented chromosomes.

Based on the results obtained, the leaves of the plant should be used with the assistance of a phytomedicine or phytotherapy expert. High doses and prolonged consumption of the plant should be avoided. It is safer to cultivate *P. guajava* trees meant for herbal medicine to prevent heavy metal and other chemical contamination. More studies should be conducted to verify these claims and evaluate more phytochemicals, heavy metals, and other parts of the tree.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Tajudeen Yahaya; Methodology: Tajudeen Yahaya and Mohammed Musa; Investigation, writing, original draft, review and editing: All authors; Data collection: Musa Mohammed, Bala Abdulgafar; Data analysis: Israel Obaroh, Mutiu Sifau, and Titilola Salisu.

Conflict of interest

The authors declared no conflict of interest.

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