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RESEARCH ARTICLE

Genotoxic and antioxidant potential of methanolic and chloroformic extract of *Schweinfurthia papilionacea* (L.) Boiss

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Abstract

The genotoxic and antioxidant activities of methanolic and chloroform extracts of *Schweinfurthia papilionacea* (L.) Boiss was assessed using a comet assay on human lymphocyte genomic DNA and a 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical-scavenging assay. The genotoxic activity of *S. papilionacea* showed that the methanolic extract exhibited dose-dependent genoprotection, i.e., 60.5 at 75 mg 100mL^{-1} and 103.6 at 100 mg 100mL^{-1}, while the chloroform extract exhibited dose-dependent genoprotection, 92.2 at 75 mg/100mL and 109.7 at 100 mg 100mL^{-1}. The antioxidant activity revealed that the methanolic extract of *S. papilionacea* showed the highest antioxidant activity (63.36 %) at 150 mg mL^{-1}, while the chloroform extract of *S. papilionacea* exhibited the highest (%) antioxidant activity (58.42 %) at 150 µg mL^{-1}, but less than the standard drug ascorbic acid (AA) (78.35 %). The present results showed that *S. papilionacea* possess genoprotective and antioxidant properties and can be used to treat several diseases. However, further studies are also needed to understand the radical-scavenging and metal-chelating activities of plants in a concentration-dependent manner.

Keywords: antioxidants; DPPH; genotoxic; geno-protective; scavenging

Introduction

The drugs or therapeutics being produced are either out of reach of common people or are unaffordable due to their costs. These drugs also produce adverse effects and over time, they lose efficacy because the disease-causing pathogens increasingly develop resistance. Medicinal plants have long been a source of treatment for various diseases throughout the world for ages. Over the past few years, scientific progress has led to the development of drugs that treat a wide range of diseases and health conditions. However, current treatments are not fully effective, as they primarily alleviate symptoms rather than fully cure the disease (1).

Consequently, researchers are actively exploring natural compounds to find therapies that can effectively treat various diseases while being more affordable and causing fewer side effects than traditional synthetic drugs. Plants are among the main sources of therapeutics. For centuries, plants have been used to treat a wide range of diseases. Quinine, derived from the bark of Cinchona, has long been used to treat malaria (2). It has also been applied to certain oral and throat infections. Atropine, obtained from *Atropa belladonna*, is commonly used to dilate the pupil (3). Aspirin was originally isolated from the bark of *Salix alba* (4). It is now widely used as an analgesic and anti-inflammatory agent. Despite these well-known examples, the world's vast plant diversity remains largely unexplored for therapeutic potential. Along with identifying new potential therapeutics from plant sources, the search for these plants' potential negative effects must also be carried out in parallel. It is of utmost importance to know that the plants used for nutritional purpose or therapeutic potential properties are not posing any danger to health in one or another way (5).

Schweinfurthia papilionacea (L.) *Boiss* is from the Scrophulariaceae family. It is commonly called *Sanipat* or *Nepalnimbo* (Hindi). It has been found in very dry atmospheric regions of Baluchistan and Karak in Pakistan. It is one of the rarest and threatened species of plants according to (6). It has been used in the past traditionally as a diuretic-promoting agent, in the treatment of typhoid and as a tonic as well (7).

Genotoxicity refers to the ability of substances, such as chemicals and environmental pollutants, to damage genetic material within cells, leading to mutations that can result in infertility or cancer (8). Oxidative stress can negatively affect DNA, causing instability in genome structure, leading to aging, alterations in gene expression, abnormal cell division and other multiple therapeutic abnormalities (9). Plants used in traditional medicine, such as Ayurveda, possess genotoxic potential that can induce DNA damage and mutations (10).

Various environmental agents and biological processes going on in the human body is one of the major causes of producing free radicals in the body. These extra radicals are the source of damage to the body's biomolecules (9). The antioxidant activity of plant extracts is a vital area of research with significant implications for health and disease prevention. The diverse phytochemical profiles of these extracts, along with the varying extraction methods used, contribute to their antioxidant potential.

Continued exploration of these natural products is essential for understanding their mechanisms of action and for harnessing their benefits in combating oxidative stress-related diseases (11).

The present research is the first one that explores the genotoxic and antioxidant properties of the methanol and chloroform extracts of *Schweinfurthia papilionacea* (L.) Boiss. This study has shown a previously undescribed bioactivity phenotype of the plant by demonstrating that the methanolic extracts not only have DNA-protective activity in human lymphocytes but also exhibit strong radical-scavenging activity, indicating their potential as sources of novel therapeutic and nutraceutical products.

Materials and Methods

Chemicals and instruments

Acetic acid, carrageenan, atropine sulphate, diclofenac sodium, methanol, chloroform, distilled water, normal saline, Whatman No.1 paper filter paper (supplied by Qurtuba pharmacy), castor oil, activated charcoal (Karachi pharmaceuticals), plythesmometer, Gas chromatography-mass spectrometry (GC-MS) instrument (University of Peshawar, Chemistry Department), gum acacia. beakers, hot plate, balance, tubes, pipette, petri dishes, aluminium foil, magnet stirrer (Qurtuba Botanical Lab).

Plant collection and identification

Schweinfurthia papilionacea plant was collected from the village Thoot Shams area of district Karak (33°10'57" N 70°57'15" E), Khyber Pakhtunkhwa, Pakistan. It was identified with the help of the Flora of Pakistan and was given Voucher No. Atifa Quddoos 01. It was preserved at the Herbarium of Qurtuba University of Science and Technology, Peshawar, for future use.

Preparation of crude extract and solvent fractions

Plant material was thoroughly washed after collection to remove any excess dirt. After washing, the plant sample was kept in shade for 26 days to completely dry it without losing its essential active components. It was then ground into a fine powder using an electric grinder. A crude extract was obtained by cold maceration. 200 g of powder was macerated in 50 mL of methanol and chloroform and left at rest for about 2-3 days. It was then filtered using Whatman filter paper No. 1. The filtrate obtained was further concentrated using a rotary evaporator by reducing the pressure to about 40 °C. Prior to GC–MS analysis, the concentrated extracts were diluted with their respective solvents at a ratio of 1:10 (v/v) to obtain an appropriate concentration for injection and to avoid column overloading.

Determination of active constituents of plant extracts

The bioactive chemical constituents in the plant extracts were identified using GC–MS with a Shimadzu QP2010 spectrometer (Japan). The gas used to carry the sample was Helium. Using acetone, sample of extract was diluted. The GC-MS analysis procedure that was used in the current research was based on the already published and well-tested GC-MS protocols of analyzing plant extracts with slight modification to facilitate better separation and identification of the compounds (12). The instrumental conditions, including column type, injection volume, split ratio, oven temperature programming and MS detection parameters, were selected according to standard procedures described in the literature. The chemical constituents were identified by comparing the acquired mass spectra and the retention times with those in the NIST mass spectral library, as is generally known.

Genotoxic activity potential using comet assay

Genotoxic activity potential was analyzed using a standardized protocol with slight modifications (13). Through the comet assay, the value of damage caused in DNA (breakage in strands of DNA) per cell is measured. The movement of the DNA strand is directly proportional to the damage caused to it. So, the greater the damage in DNA, the greater the movement in DNA strands will be observed. It was performed in an alkaline sodium hydroxide (NaOH) base. Lymphocyte cells were submerged in agar gel followed by their arrangement on a microscopic slide. To eliminate histone (proteins), the cells were treated with sodium chloride. DNA fragments were stained with propidium iodide to obtain more explicit images of the strands migrating away from the nucleus. Using a fluorescence microscope equipped with a CCD-300E camera and a 40× objective lens, the extent of DNA damage was measured. ImageJ software was applied to quantify the lengths of the DNA head and tail. Lymphocytes were provided at concentrations of 100, 200, and 300. A total of 100 cells on each slide were analyzed using Comet Assay IV software (Haverhill, UK).

Comet assay was performed under diffused light conditions. For quantifying the DNA damage induced, zero-to-four classes were allocated, i.e., from undamaged to least damage to extremely damaged strands of DNA.

By considering the length of the tail, the total comet score was measured as,

$$DI = \frac{0 \times \text{cells in C0} + 1 \times \text{cells in C1} + 2 \times \text{cells in C2} + 3 \times \text{cells in C3} + 4 \times \text{cells in C4} \div}{\text{Total number of cells observed}} \quad \text{(Eqn. 1)}$$

Where,

DI= Damage Index

C0= Class of undamaged cells

C1= class1, C2= class 2, C3= class 3 and C4= class 4

Antioxidant activity

The antioxidant potential of the methanolic and chloroform extracts of *S. papilionacea* was studied using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay, following standardized protocols with minor modifications (9, 14). A solution was prepared by mixing 0.145 mM DPPH in methanol. Taking one mL of this solution in one mL of methanol and chloroform extract of plant in concentrations of 100, 200 and 300 μg mL^{-1} each. It was then kept at rest for about 30 min in the dark at 25 °C. Ascorbic acid (AA) was taken as a standard drug for reference. For control reference, 1 mL DPPH + 1 mL DPPH + 1 mL methanol mixture was taken. After a time span of 30 min, 60 min and 90 min, the rate of absorbance was measured at 517 nm with the assistance of UV-VIS spectrophotometry. The observations were carried out in triplicate.

The inhibition % was measured using the following formula.

$$\text{Inhibition \%} = \text{Ac-As} \div \text{Ac} \times 100 \quad \text{(Eqn. 2)}$$

Where 'Ac' is the absorbance of the control and 'As' is the absorbance of the sample.

Statistical analysis

The data were analyzed using Dunnett's t-test. For the statistical tests a p< 0.001, 0.01 and 0.05 was considered as significant. Results were shown as mean ± SEM (standard error of the mean). One-way ANOVA was applied for comparison between the control groups and the test groups' mean values. The statistical data was obtained by utilizing software (IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA)

Results

The comet assay was used to evaluate the genotoxic effects of the methanol extract of *S. papilionacea* on human lymphocytes. The study included negative control (only lymphocytes), a positive control (lymphocytes treated with hydrogen peroxide (H_2O_2)) and two treatment groups with methanolic extract at concentrations of 75 mg 100 mL^{-1} and 100 mg 100 mL^{-1}. The results were categorized into five classes based on the extent of DNA damage (Table 1).

In the negative control group, the majority of cells (93.4± 19.3 %) were in Class 0, indicating minimal DNA damage. Classes 1, 2, 3 and 4 had 5.0 ± 3.6 %, 3.0 ± 1.6 %, 2.4 ± 1.09 % and 1.3 ± 0.7 % of cells, respectively, showing low levels of DNA damage.

The positive control group, treated with H_2O_2, exhibited significant DNA damage with only 13.4 ± 7.5 % of cells in Class 0. The percentages of cells showing DNA damage in Classes 1, 2, 3 and 4 were 48.3 ± 9.5 %, 57.3 ± 9.08 %, 27.6 ± 10.5 % and 13.5 ± 7.3 %, respectively. This indicates a high level of genotoxicity induced by H_2O_2.

% In the group treated with the methanolic extract at 75 mg 100 mL^{-1}, 84.3 ± 15.3 % of cells were in Class 0, indicating reduced DNA damage compared to the positive control. Classes 1, 2, 3 and 4 had 30.0 ± 13.4 %, 7.3 ± 3.5 %, 5.3 ± 2.7 % and 0.00 ± 0.00 % of cells, respectively. The total comet score (TCS) for this group was 60.5, indicating a significant reduction in DNA damage relative to the positive control (TCS = 299.7).

At the higher concentration of 100 mg 100 mL^{-1}, *S. papilionacea* treatment resulted in 72.5 ± 10.4 % of cells in Class 0. Classes 1, 2, 3 and 4 had 41.4 ± 12.3 %, 14.5 ± 8.6 %, 10.4 ± 5.2 % and 2.0 ± 1.03 % of cells, respectively. The TCS for this group was 103.6, showing a dose-dependent increase in DNA protection compared to the positive control.

The results (Fig.1) demonstrate that the methanol extract of *S. papilionacea* significantly reduces DNA damage in human lymphocytes, as evidenced by the lower TCS values and higher percentages of cells in Class 0 compared to the positive control. These findings suggest that methanolic extract of *S. papilionacea* possesses protective effects against DNA damage, which could be attributed to its antioxidant properties. The statistical analysis showed significant differences relative to the positive control at *p < 0.002 and **p < 0.0001, using one-way ANOVA and Tukey test.

The comet assay was employed to assess the genotoxic effects of the chloroform extract of *S. papilionacea* on human lymphocytes. The experiment included a negative control group (only lymphocytes), a positive control group (lymphocytes treated with H_2O_2) and two treatment groups with chloroform extracts of *S. papilionacea* at concentrations of 75 mg 100 mL^{-1}and 100 mg 100 mL^{-1}. The results were categorized into five classes based on the extent of DNA damage (Table 2).

In the negative control group, the majority of cells (90.3±17.5 %) were in Class 0, indicating minimal DNA damage. Classes 1, 2, 3 and 4 had 4.2 ± 1.8 %, 2.4 ± 1.07 %, 1.7 ± 0.4 % and 0.00 ± 0.00 % of cells, respectively, showing low levels of DNA damage.

The positive control group, treated with H_2O_2, exhibited significant DNA damage with only 7.3 ± 4.6 % of cells in Class 0. The percentages of cells showing DNA damage in Classes 1, 2, 3 and 4 were 23.4 ± 11.5 %, 60.4 ± 12.7 %, 46.6 ± 8.6 % and 26.5 ± 10.5 %, respectively. This indicates a high level of genotoxicity induced by H_2O_2.

% In the group treated with the chloroform extract of *S. papilionacea* at 75 mg 100 mL^{-1}, 87.2±13.4 % of cells were in Class 0, indicating reduced DNA damage compared to the positive control. Classes 1, 2, 3 and 4 had 36.4±14.3 %, 14.0±7.7 %, 6.2±4.5 % and 2.3±0.8 % of cells, respectively. The total comet score (TCS) for this group was 92.2, indicating a significant reduction in DNA damage relative to the positive control (TCS = 390).

At the higher concentration of 100 mg 100 mL^{-1}, the chloroform extract of *S. papilionacea* treatment resulted in 67.3 ± 12.7 % of cells in Class 0. Classes 1, 2, 3 and 4 had 28.3 ± 11.6

Table 1. Comet assay of genomic DNA of human lymphocytes exposed to methanol extract of *S. papilionacea*

Class	Negative control (Only lymphocytes)	Positive control (Lymphocytes + H_2O_2)	75mg 100 mL^{-1}	100mg 100mL^{-1}
0	93.4± 19.3	13.4± 7.5	84.3 ± 15.3	72.5 ± 10.4
1	5.0± 3.6	48.3 ± 9.5	30.0 ± 13.4	41.4 ± 12.3
2	3.0 ± 1.6	57.3 ± 9.08	7.3± 3.5	14.5 ± 8.6
3	2.4 ± 1.09	27.6 ± 10.5	5.3 ± 2.7	10.4 ± 5.2
4	1.3 ± 0.7	13.5± 7.3	0.00 ± 0.00	2.0 ± 1.03
TCS	23.4	299.7	60.5	103.6

Total comet score (TCS). Values are expressed as mean ± standard deviation (SD). Differences were significant relative to the positive control at *p <0.002, **p <0.0001 (One-way ANOVA, Tukey Test).

Table 2. Comet assay of genomic DNA of human lymphocytes exposed to the chloroform extract of *S. papilionacea*

Class	Negative control (Only lymphocytes)	Positive control (Lymphocytes + H_2O_2)	75mg 100mL^{-1}	100mg 100mL^{-1}
0	90.3± 17.5	7.3± 4.6	87.2 ± 13.4	67.3 ± 12.7
1	4.2± 1.8	23.4 ± 11.5	36.4 ± 14.3	28.3 ± 11.6
2	2.4 ± 1.07	60.4 ± 12.7	14.0± 7.7	8.3 ± 5.6
3	1.7 ± 0.4	46.6 ± 8.6	6.2 ± 4.5	12.0 ± 8.5
4	0.00 ± 0.00	26.5± 10.5	2.3 ± 0.8	7.2 ± 3.2
TCS	14.1	390	92.2	109.7

Total comet score (TCS). Values are expressed as mean ± standard deviation (SD). Differences were significant relative to the positive control at *p <0.002, **p <0.0001 (One-way ANOVA, Tukey Test).

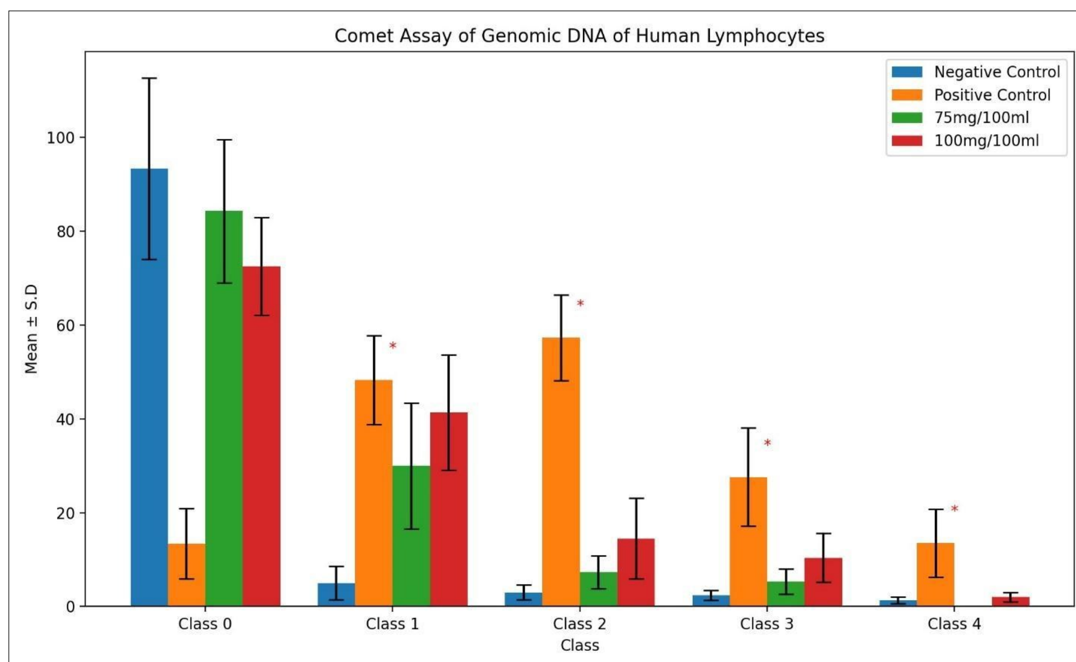

Fig. 1. The graph shows the distribution of DNA damage across different classes exposed to methanol extract of *S. papilionacea.* Significant differences relative to the positive control are marked with red asterisks (*) for Classes 1-4.

%, 8.3 ± 5.6 %, 12.0 ± 8.5 % and 7.2 ± 3.2 % of cells, respectively. The TCS for this group was 109.7, showing a dose-dependent increase in DNA protection compared to the positive control.

The results demonstrate that the chloroform extract of *S. papilionacea* significantly reduces DNA damage in human lymphocytes, as evidenced by lower TCS values and a higher percentage of cells in Class 0 compared to the positive control. These findings (Fig. 2) suggest that chloroform extract as *S. papilionacea* possesses protective effects against DNA damage, potentially due to its antioxidant properties. The statistical analysis showed significant differences relative to the positive control at $*p < 0.002$ and $**p < 0.0001$, using one-way ANOVA and the Tukey Test.

The study evaluated the antioxidant activity of AA using methanol and chloroform and the DPPH radical scavenging assay (Table 3). Ascorbic acid demonstrated significant antioxidant activity, with a % DPPH radical scavenging activity of 63.28 ± 1.36 at 50 µg mL^{-1}, 68.41 ± 1.52 at 100 µg mL^{-1} and 78.3 5 ± 2.08 at 150 µg mL^{-1}. The IC_{50} value for AA was determined to be 50 µg/mL, indicating its high potency. Methanolic extract of *S. papilionacea* showed moderate antioxidant activity with % DPPH radical scavenging activities of 46.06 ± 1.27 at 50 µg mL^{-1}, 52.51 ± 2.16 at 100 µg mL^{-1} and 63.36 ± 1.57 at 150 µg mL^{-1}, resulting in an IC_{50} value of 77.05 µg mL^{-1}. Chloroform extract of *S. papilionacea* exhibited the lowest antioxidant activity among the samples, with % DPPH radical scavenging activities of 42.52 ± 0.73 at 50 µg mL^{-1}, 48.26 ± 1.43 at 100 µg/mL and 58.42 ± 1.61 at 150 µg mL^{-1} and an IC_{50} value of 114.67 µg mL^{-1}. These results (Fig. 3) indicate that AA is the most effective antioxidant among the tested samples, followed by the methanolic and chloroform extracts of *S. papilionacea*.

Discussion

The comet assay remains a pivotal method for genotoxicity testing due to its sensitivity in detecting DNA damage and its ability to assess DNA repair (15). The comet assay results showed that the methanol extract of *S. papilionacea* has significant protective effects on the genomic DNA of human lymphocytes. These findings align with earlier research demonstrating that rosemary extracts protect lymphocytes from H_2O_2-induced oxidative DNA damage (16). Methanol extracts from various plants could mitigate DNA damage induced by genotoxic agents similar to the protective effects observed with *S. papilionacea* methanol extract at 75mg $100mL^{-1}$ and 100mg $100mL^{-1}$ concentrations (17). The chloroform extract of *S. papilionacea* also demonstrated a significant reduction in DNA damage when compared to controls treated with genotoxic agents. Comparative studies using the comet assay have shown that chloroform extracts can effectively reduce DNA

Table 3. The antioxidant activity of the methanolic and chloroform extract of *S. papilionacea*

Sample	Concentration (µg mL^{-1})	% DPPH Radical scavenging activity (Mean ± SD)	IC_{50} (µg mL^{-1})
Ascorbic acid	50	63.28 ± 1.36[a]	50
	100	68.41 ± 1.52[b]	
	150	78.35 ± 2.08[c]	
Methanolic Extract	50	46.06 ± 1.27[d]	77.05
	100	52.51 ± 2.16[e]	
	150	63.36 ± 1.57[f]	
Chloroform Extract	50	42.52 ± 0.73[g]	114.67
	100	48.26 ± 1.43[h]	
	150	58.42 ± 1.61[i]	

Values represent mean ± standard deviation (SD) of three independent experiments.

IC_{50}: Concentration of sample required to scavenge 50 % of DPPH free radicals.

DPPH: 2,2-diphenyl-1-picrylhydrazyl radical.

(a–i) indicate significant differences between concentrations within the same sample group ($p \le 0.05$, Tukey's HSD test).

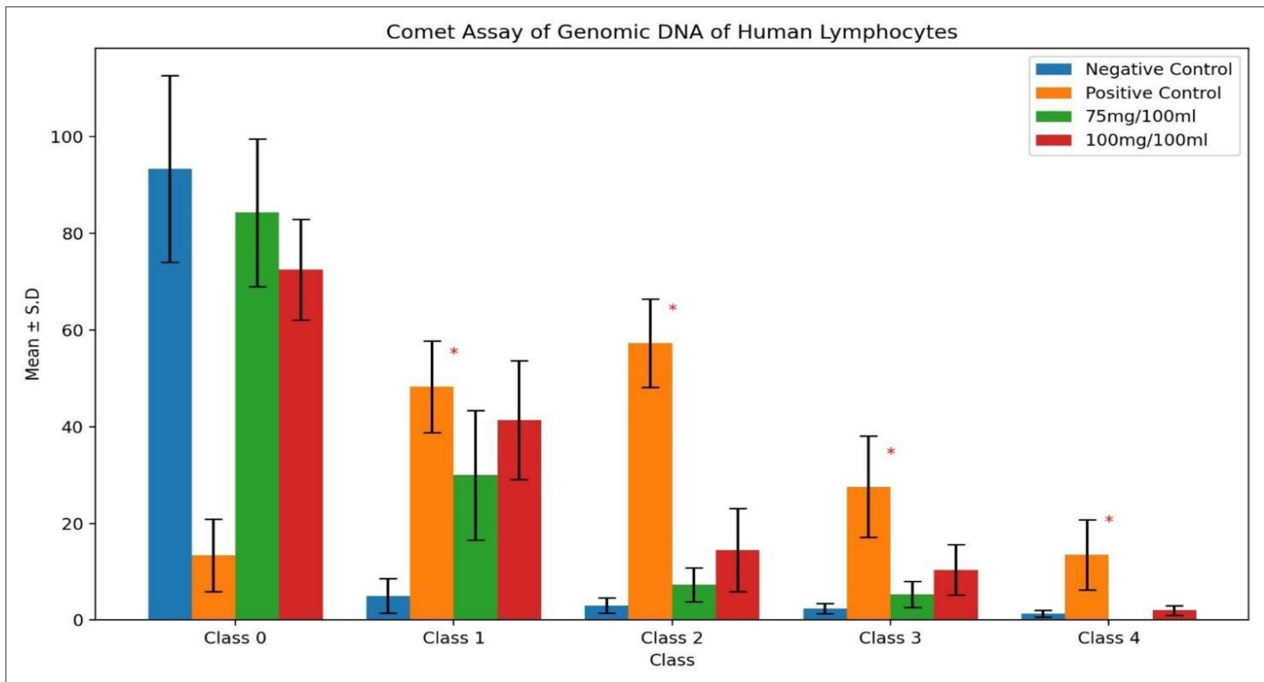

Fig. 2. Distribution of DNA damage across different classes exposed to the chloroform extract of *S. papilionacea.* Significant differences relative to the positive control are marked with red asterisks (*) for classes 1-4.

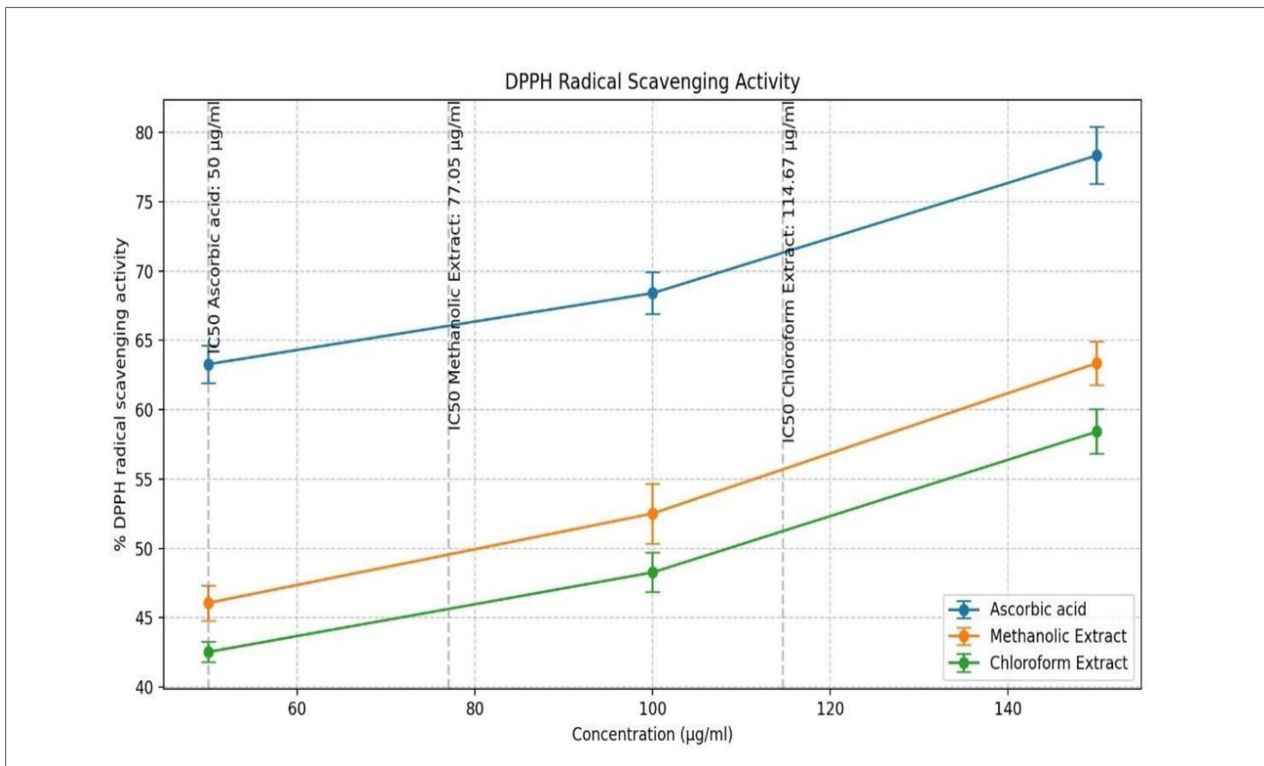

Fig. 3. Scavenging activity of ascorbic acid, methanolic extract and chloroform extract of *S. papilionacea* at different concentrations. IC_{50} values are indicated by vertical dashed lines.

damage induced by various genotoxic agents. The protective effects of chloroform extracts are often attributed to their antioxidant properties (18).

The antioxidant properties of plant extracts often contribute to their protective effects against DNA damage. For example, the antioxidant activity of *L. cubeba* demonstrated significant DNA protection, similar to the genoprotective effects observed with the methanolic extract of *S. papilionacea*. Ascorbic acid, a well-known antioxidant, demonstrated strong DPPH radical-scavenging activity that increased with concentration (19). Its IC_{50} value was 50 µg/mL, confirming its high antioxidant potential. Methanolic extract showed moderate DPPH radical scavenging activity, with 46.06 ± 1.27 % at 50 µg mL^{-1}, 52.51 ± 2.16 % at 100 µg mL^{-1} and 63.36 ± 1.57 % at 150 µg mL^{-1}. This level of activity is comparable to findings of previous studies investigating the antioxidant properties of *Mimosa pudica* and *Blume* leaf extract, respectively. Chloroform extract exhibited the lowest DPPH radical scavenging activity, with 42.52 ± 0.73 % at 50 µg mL^{-1}, 48.26 ± 1.43 % at 100 µg mL^{-1} and 58.42 ± 1.61 % at 150 µg mL^{-1} (19, 20). The lower antioxidant activity of the chloroform extract might be attributed to a lesser concentration of active phenolic compounds compared to AA and the methanolic extract of *S. papilionacea* (21). Ascorbic acid consistently shows high antioxidant activity across various assays, reinforcing its

effectiveness as a benchmark antioxidant. However, the methanolic and chloroform extracts of *S. papilionacea* exhibit moderate antioxidant potential, indicating them as natural sources of antioxidants

The results of our study on the genotoxic and antioxidant properties of methanol and chloroform extracts of *S. papilionacea* are consistent with the growing literature on the dual biologic actions of plant extracts. Recent reports have pointed out that plant phytochemicals may have either genotoxic or anti-genotoxic action, depending on dose and conditions, which is also seen in our observations in which methanolic extracts showed significant DNA protection at certain doses, but higher doses showed genotoxic activity. A recent wide-ranging evaluation of the genotoxic and antigenotoxic properties of medicinal plant extracts highlights that numerous extracts exhibit a continuum of effects between DNA protection and genotoxicity, dependent on the composition of compounds and the conditions under which the extracts were employed and suggests the need for mechanistic studies (22).

Regarding antioxidant profiling and biological activity, in-depth GC-MS research on diverse plant species has consistently provided rich phytochemical profiles and high antioxidant activity, which supports our finding that *S. papilionacea* extracts exhibit radical-scavenging activity. As an illustration, GC–MS and complementary bioactivity assay studies conducted on *Beta vulgaris* extracts have reported comparable antioxidant activities, whether in *Beta vulgaris* extracts or in *Chlorophytum comosum*, indicating that a meticulous phytochemical fingerprinting analysis, together with a biological assessment, can be used to identify bioactive compounds associated with antioxidation (23, 24).

Conclusion

This research paper presents a pioneer assessment of both toxic and antioxidant properties of the methanol and chloroform extracts of *Schweinfurthia papilionacea* (L.) Boiss. Contrary to previous literature, which has predominantly investigated antioxidant properties of plant extracts, our study shows that besides having strong DNA-protective abilities on human lymphocytes, methanolic extracts of *S. papilionacea* have dose-dependent radical-scavenging properties, whereas chloroform extracts showed moderate antioxidant activity. These two evaluation points indicate that this plant has a distinct bioactivity profile and it can serve as a source of new natural compounds that have therapeutic uses.

Although these are encouraging findings, the study has its limitations because it was designed in vitro and its results may not necessarily translate to in vivo efficacy or safety. Further mechanistic investigations, active constituent isolation and *in vivo* validation are needed to ascertain the pharmacological potential of *S. papilionacea*. In general, the article contributes to the existing body of knowledge by establishing unreported genoprotective activity and antioxidant capabilities and by providing a basis for further phytochemical and pharmacological studies.

Authors' contributions

AQ and MA conceived the project and designed the experiments. AQ and MN conducted the experiments. AQ and MA analyzed the data, prepared the figures and tables and drafted the manuscript. AQ, MA and MN revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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