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ANTIGENOTOXIC EFFECTS OF *BARLERIA CRISTATA* AND *B. PRIONITIS* ROOT EXTRACTS AGAINST METHYL PARATHION-INDUCED TOXICITY IN *ALLIUM* CELLS

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ABSTRACT Plant-derived compounds with therapeutic potential are attracting global attention, with growing interest in traditional and folk knowledge for novel drug discoveries. Free radicals generated during metabolism or environmental exposure damage DNA, proteins and lipids, contributing to genetic instability and disease. Natural antioxidants, particularly phenolics and flavonoids, are valued as safe and effective protective agents. This study investigated the antioxidant and antigenotoxic potential of root methanolic extracts of *Barleria cristata* and *B. prionitis* (Acanthaceae), both widely used in Ayurvedic and folk medicine. Antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays, where both extracts showed strong, dose-dependent activity, with *B. prionitis* being more effective. Antigenotoxic effects were assessed in vivo using the *Allium cepa* assay against methyl parathion (MP), an organophosphorus pesticide. MP exposure reduced mitotic index (MI) and induced chromosomal aberrations (CA) such as stickiness, bridges, and scattering. Pretreatment with the extracts reduced these cytogenotoxic effects in a dose-dependent manner, with *B. prionitis* offering maximum protection. Phenolic and flavonoid contents were quantified using Folin–Ciocalteu and Aluminium chloride colorimetric assays, confirming notable levels. The use of the *A. cepa* assay, a validated model correlating well with mammalian systems, provided reliable assessment of cytogenotoxicity. Overall, the findings highlight the strong antioxidant and antigenotoxic potential of *B. cristata* and *B. prionitis*, with *B. prionitis* emerging as a particularly promising candidate for further pharmacological exploration.

KEYWORDS : *Barleria* species, Antioxidant activity, Antigenotoxicity, *Allium cepa* assay.

INTRODUCTION

A variety of industrial, agricultural, and domestic effluents are discharged daily into the environment due to the alarming rise in population and industrial expansion, leading to environmental deterioration (Mattar et al., 2014). The increasing discharge of various genotoxic pollutants & other toxic chemicals has the potential to disrupt the balance of natural ecosystems and adversely impact the health of wildlife & human populations by altering their genetic material. Exposure to genotoxic substances can damage DNA in organisms and an increasing number of such chemicals in the aquatic environment lead to various deleterious effects including cancer (Guan et al., 2017).

Herbal remedies are natural products derived from plants and plant extracts that have been traditionally used to treat various diseases or to promote general health (Tapsel et al., 2006). The popularity and acceptability of herbal medicines are mainly due to the toxicity and undesirable side effects of the synthetic allopathic medicines. Natural products from plants have provided the pharmaceutical industry with one of its most important sources of lead compounds. Plant species belonging to the family Acanthaceae are globally known to possess various medicinal properties and have cultural and economic importance in traditional medicine (Gangaram et al., 2021). *Barleria* is the third largest genus in the family Acanthaceae. The flowers, leaves, stems, roots and seed extracts of plants belonging to this genus have exhibited significant medicinal potential for the treatment of various ailments and infections. This genus includes approximately 300 species of shrubs and herbs that are distributed in the subtropical and tropical regions of the world. Several species of *Barleria* are known for their medicinal or ornamental values. There have been studies reviewing the traditional, phytochemical and pharmacological properties of specific species of *Barleria* (Banerjee et al., 2021; Sudheer & Praveen, 2021), however studies on the anti-mutagenic properties of none have comprehensively been reported.

Mitotic cell division inhibition and CA induction have been widely used as indicators of cytotoxicity and genotoxicity. *Allium* chromosome aberration assay is a powerful tool to detect the cytotoxic potential of a sample by observing the clastogenic as well as the non-clastogenic CA and analysing the MI. It's a simple, easy and reliable in vivo assay. *A. cepa* assay is considered as one of the most efficient and cost-effective approaches to determine the toxic potential of chemical compounds because of its high sensitivity and correlation with the results of mammalian test systems (Fiskesjo & Levan, 1993). The similarity to the mammalian system makes it highly preferred by many researchers. Study of the effect of chemicals on plant mitosis may provide valuable information in relation to possible genotoxicity in mammals and especially in humans (Rank, 2003; Kuras et al., 2006) and so could be used as an alternative to laboratory animals in toxicological research. Therefore, this study is intended to evaluate the antioxidant and antigenotoxic property of the root extracts of two common species of the genus, *B. cristata* and *B. prionitis* using *Allium* model.

MATERIALS AND METHODS

The plant materials used for the current study are *B. cristata* L. and *B. prionitis* L. The plants were collected from wild populations, from different parts of Kozhikode districts of Kerala. Methanolic extracts of shade-dried roots were obtained using a Soxhlet apparatus, then concentrated and stored at 4 °C. Subsequently, it was suspended in 2% DMSO for further studies. Various dilutions of all the plant extracts were then prepared in distilled water.

Antioxidant Assays

The antioxidant activities of the root methanolic extracts were determined by two in vitro assays. Concentrations of the methanolic extracts ranging from 10 to 140 µg/ml were used for the in vitro antioxidant assays. Triplicate determinations were made for all the experiments. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activity was determined by the method proposed by Coruh et al. (2007). To assess antioxidant activity, 100 µl of the extract at different concentrations was mixed with 1 ml of 100 µM DPPH solution in methanol and incubated in the dark for 20 minutes at room temperature, after which absorbance was measured at 515 nm. In ABTS radical scavenging assay, the extract was allowed to react with $ABTS^{+}$, a stable free radical derived from 2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) or ABTS (Long & Halliwell, 2001). Various concentrations of the extract (10 µl) were added to 1 ml of ABTS radical solution, and the decrease in absorbance was measured at 6 minutes using PBS as the reference. Antioxidant activity in both the tests was expressed as percentage inhibition, which is given by the formula:

$$\text{Percentage inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the blank control (DPPH or ABTS radical solution without test sample) and A_{sample} is the absorbance of the test sample.

Allium Cepa Assay

Onion bulbs were purchased freshly from the local market. Old roots and dry scales were removed and placed on top of test tubes filled with distilled water and allowed to germinate by placing them upside down at the rim of bottles containing distilled water. The germinated onion bulbs were divided into 11 sets, each consisting of 5 bulbs. Eight sets of germinated onion bulbs were incubated for 12 h on top of test tubes filled with 0.05 % and 0.1 % of *B. cristata* and *B. prionitis* extracts (BCE 1, BCE 2 and BPE 1 & BPE 2 respectively). After incubation, four sets of germinated onion bulbs were exposed to 0.01% of MP solution prepared in distilled water at its peak mitotic time at 27±2°C for another 3 h period (Treated groups). The other four sets treated with

the four extracts was not exposed to MP and were allowed to produce new roots on top of the test tubes filled with 2 % DMSO only. The remaining 3 sets out of the 11 were also allowed to germinate in the same way in distilled water, but are not treated with the plant extracts. Among these, one set of bulbs was exposed to 0.01% and the other set to 0.1% MP respectively (positive control). The third set was exposed to 2 % DMSO and served as negative control.

Mitotic squash preparations of root tips were carried out using improved techniques (Sharma & Sharma, 1990). Two slides were made for each treatment and scoring was done from five sites that were randomly selected from these slides to determine the mitotic index (MI) and the percentage of chromosomal aberrations (CA). All the slides were scanned in Magnus Trinocular laboratory Microscope (Model: MLXi-Tr Pus) and the photographs were taken with MagCam Digital CMOS Camera (Model: DC-5) attached to the microscope. (Fig. 1).

Phytochemical Analysis

One gram of the dried powdered drug of the roots was extracted using 25 ml methanol for 6 h in a Soxhlet extractor. The extract was then cooled, filtered and concentrated to dryness in a vacuum evaporator. The extract was then dissolved in 10 ml methanol. It was then filtered through 0.20 mm membrane filter. This extract was used for the analysis.

Estimation of Total Phenolic Content

Total phenolic content (TPC) of the root extracts was determined using Folin–Ciocalteu reagent based assay as described by Oueslati et al. (2012) using gallic acid as a standard. Total phenolic content of the extracts was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) of the extract through the calibration curve with gallic acid. All samples were analysed in triplicates.

Estimation Of Total Flavonoid Content

The aluminium chloride colorimetric method with some modifications was used to measure the total flavonoid content (TFC) of all plant extracts (Oueslati et al., 2012). Quercetin was used as the standard for estimation of total flavonoids. Total flavonoid content was expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW), through the calibration curve of quercetin. All samples were analysed in triplicates.

Statistical Analysis

The data of MI and CA are represented in percentage mean ± SE of five scorings. For statistical analysis, one-way analysis of variance (ANOVA) and Tukey's HSD test (Tukey, 1949) were used. All statistical analyses were performed by using the computer software SPSS 20.0 for Windows. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS

The present investigation revealed that root methanolic extracts of *B. cristata* and *B. prionitis* possess significant antioxidant and antimutagenic properties, which can be attributed to their rich phytochemical composition.

Both extracts demonstrated potent free radical scavenging activity in DPPH and ABTS assays, with the activity increasing in a dose-dependent manner. The extracts showed strong antioxidant activity in DPPH assay at concentrations ranging from 20-140 µg/ml (Fig. 1; Table 1). Similarly, in the ABTS radical scavenging assay, significant activity was observed at concentrations between 10–70 µg/ml (Fig. 2; Table 1). Notably, *B. prionitis* showed greater efficiency in neutralizing DPPH radicals (IC_{50} = 31.06 ± 1.28 µg/ml) compared to *B. cristata* (IC_{50} = 34.46 ± 0.89 µg/ml) (Table 10) and exhibited slightly superior ABTS scavenging ability as well (IC_{50} = 12.89 ± 3.46 µg/ml for *B. prionitis* vs. 13.99 ± 2.91 µg/ml for *B. cristata*) (Figs 1, 2; Table 1).

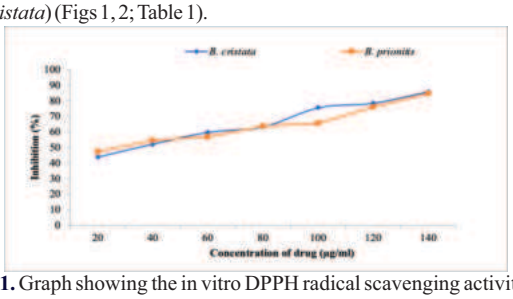

Fig. 1. Graph showing the in vitro DPPH radical scavenging activity of the extracts of *B. cristata* & *B. prionitis*

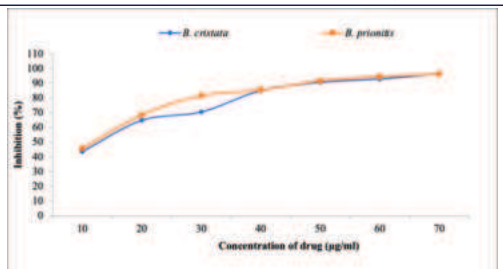

Fig. 2. Graph showing the in vitro ABTS radical scavenging activity of the extracts of *B. cristata* & *B. prionitis*

Table 1. In Vitro Antioxidant Activities Of Root Methanolic Extracts Of Root Methanolic Extracts Of *B. cristata* & *B. prionitis*

Extracts	IC_{50} (µg/ml)	
	DPPH radical scavenging assay	ABTS radical scavenging assay
B. cristata	34.46 ± 0.89	13.99 ± 2.91
B. prionitis	31.06 ± 1.28	12.89 ± 3.46

IC_{50} - Concentration of the samples causing 50% inhibition of radicals
Values are expressed as mean ± standard error (SE) (n=3)

Based on the promising antioxidant activity, both the extracts were evaluated for their antimutagenic potential by *A. cepa* assay. In the *A. cepa* assay, the extracts were found to be non-cytotoxic and displayed significant genoprotective potential against MP-induced damage. Cells treated with the extracts maintained mitotic indices comparable to the negative control (21.71–24.74%) and showed minimal CA (Table 2). Increasing the extract concentration did not reduce MI, whereas exposure to 0.01% MP led to approximately 50% reduction in MI (15.24%, $p < 0.05$) and induced a high frequency of mitotic abnormalities, the major abnormalities including chromosome stickiness, bridges, misorientation and fragmentation (Fig. 3; Table 2) whereas those treated with plant extracts had significantly lower levels of CA, comparable to the negative control ($p < 0.05$). The highest incidence of aberrant mitotic stages (93.68%) was observed in root tips treated with 0.1% MP (Table 2).

Table 2. Effect Of Methyl Parathion And *B. cristata* & *B. prionitis* Root Extracts On The Mitotic Behaviour Of *A. cepa* Root Tip Cells. Each Value Represents Mean Of Five Replicates.

Treatment	Mitotic index ± S. E.	Abnormality % ± S. E.
DMSO (2%) (Negative control)	23.88 ± 0.77 ^{a}	1.11 ± 0.24 ^{e}
Methyl parathion (0.01 %) (Positive control)	15.24 ± 0.86 ^{c}	54.80 ±2.70 ^{c}
Methyl parathion (0.1 %) (Positive control)	4.11 ± 0.36 ^{d}	93.68 ± 0.59 ^{a}
BC Extract 1	23.07 ± 0.50 ^{a}	0.72 ± 0.28 ^{e}
BC Extract 2	21.71 ± 0.92 ^{ab}	1.52 ± 0.37 ^{e}
BP Extract 1	24.74 ± 0.88 ^{a}	1.22 ± 0.20 ^{e}
BP Extract 2	22.12 ± 0.88 ^{ab}	0.93 ± 0.22 ^{e}
BC Extract 1 + Methyl parathion	16.16 ± 0.80 ^{bc}	64.57 ± 3.13 ^{b}
BC Extract 2 + Methyl parathion	17.85 ± 0.68 ^{b}	36.69 ± 2.57 ^{d}
BP Extract 1 + Methyl parathion	18.40 ± 0.94 ^{b}	56.11 ± 3.57 ^{c}
BP Extract 2 + Methyl parathion	22.24 ± 1.01 ^{ab}	27.77 ± 0.52 ^{d}

S.E. – Standard error. Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, Tukey–Kramer HSD test).

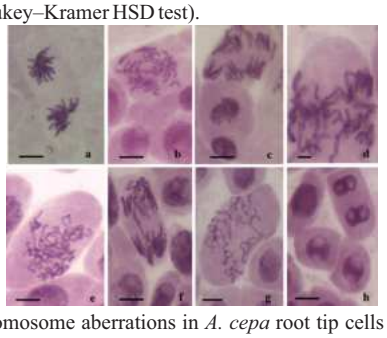

Fig. 3. Chromosome aberrations in *A. cepa* root tip cells induced by methyl parathion.

a. Diagonal anaphase b. Scattering c. Multipolar anaphase & Binucleate cell d. Scattering at metaphase e. Disturbed metaphase & ring chromosome f. Chromosome bridge g. Scattering with fragmentation h. Nuclear lesions Bar represents 10 µm.

Results showed that the effect of *B. cristata* was lesser compared to *B. prionitis* (Table 1) nevertheless it showed commendable inhibition of CA when compared with the positive control. Pre-treatment with the extracts, especially BPE 2, significantly reduced these effects, reducing chromosomal aberrations to 27.77% and restoring the mitotic index close to control levels. These results affirm the genoprotective and non-cytotoxic nature of the tested extracts.

The root methanolic extracts of *B. cristata* and *B. prionitis* were analyzed for TPC and TFC. TPC was quantified using the Folin–Ciocalteu method, expressed as mg gallic acid equivalents (GAE)/g dry weight (DW), based on a linear standard curve ($y = 0.03x - 0.08$, $R^2 = 1.00$). TFC was determined via the Aluminum chloride colorimetric assay, expressed as mg quercetin equivalents (QE)/g DW (standard curve: $y = 0.004x + 0.017$, $R^2 = 0.991$). Phytochemical analysis revealed substantial levels of total phenolics and flavonoids, with *B. prionitis* containing 22.64 ± 1.93 mg GAE/g DW and 5.84 ± 0.26 mg QE/g DW, respectively. *B. cristata* showed slightly lower values (19.34 ± 0.40 mg GAE/g DW and 3.80 ± 0.31 mg QE/g DW) (Table 3).

Table 3. Total Phenolic And Flavonoid Contents Of Root Methanolic Extracts Of *B. cristata* & *B. prionitis*

Extracts	Phenolic content (mg GAE/g DW)	Flavonoid content (mg QE/g DW)
B. cristata	19.34 ± 0.40 [a]	3.80 ± 0.31 [a]
B. prionitis	22.64 ± 1.93 [a]	5.84 ± 0.26 [a]

GAE – Gallic acid equivalents; QE – Quercetin equivalents
Values are expressed as mean ± standard deviation (n=3)

Means in a column followed by the same superscript letters are not significantly different ($P < 0.05$, one-way ANOVA, Tukey–Kramer HSD test)

DISCUSSION

In recent years, significant focus has been placed on exploring natural remedies for managing oxidative stress and mutation-related disorders. Natural products can be easily broken down in the body without causing harm, making them suitable candidates for plant-based therapies (Sangwan et al., 1998). Free radicals are generated in cells through normal metabolic processes as well as exposure to external agents. Various reactive oxygen intermediates such as superoxides, hydrogen peroxide and hydroxyl radicals can damage macromolecules by reacting with nucleic acids, proteins and other vital components, damaging them and thereby serving as both direct and indirect triggers of mutagenesis, carcinogenesis and aging (Dizdaroglu, 2002). The use and incorporation of antioxidants may be considered the safest approach for preventing processes that lead to mutagenesis.

DPPH and ABTS assays are widely used methods for evaluating antioxidant activity due to their sensitivity and reproducibility (Apak et al., 2007). The data of the present study shows that root methanolic extracts of the two taxa of Acanthaceae, displayed notable in vitro antioxidant activities as measured by their scavenging abilities against DPPH and ABTS radicals (Figs 1 & 2), which increased progressively with rising concentration. The extracts demonstrated strong and effective antimutagenic activity against MP as suggested by the findings of the *A. cepa* assay (Table 3). The *Allium* test has been found to show a strong correlation with other well-established test systems and in vivo studies on mice, rats and humans (Banti & Hadjikakou, 2019). Further, Onisan et al. (2025) demonstrated its effectiveness in detecting cytotoxic and genotoxic effects of heavy metals such as lead and copper, emphasizing its usefulness as a non-animal model for toxicological evaluation. Owing to this close concordance of results, the assay is recognized for its high reliability and validity. Therefore, *Allium* test represents a practical and ethical alternative to animal-based toxicological studies, minimizing animal use while providing dependable insights into cytogenotoxic responses (Fiskesjo, 1988, 1994).

MP is an organophosphorus insecticide whose insecticidal action stems from its inhibition of acetylcholinesterase (AChE), the same mechanism responsible for its toxicity in humans (Garcia et al., 2003). The mutagenic potential of organophosphates may be attributed to their capacity to interact with DNA through transalkylation (Epstein & Legator, 1971). Wild (1975) identified the electrophilic nature of organophosphates as the primary basis of their toxicity and proposed DNA alkylation as a contributing factor to chromosomal aberrations. Once inside an organism, MP can be oxidized into its more toxic metabolite, methyl paraoxon, which exhibits severe cytotoxicity due to its ability to generate superoxide radicals. These radicals trigger chain reactions that damage various biological molecules, including DNA. In the present study, the most frequently observed chromosomal abnormalities included bridges, misorientation, scattering, vagrant chromosomes, oblique metaphase, and stickiness (Fig. 3). The cytotoxic and genotoxic effects of MP on *A. cepa* root tip cells were confirmed under microscopic analysis by observing mitotic depressions and CA. Under these conditions, the plant extracts did not affect cell division or cause cytogenetic aberrations in root tip cells at either concentration tested. In fact, pretreatment with *B. cristata* and *B. prionitis* significantly inhibited MP–induced CA, with *B. prionitis* with the 0.1% extract, BPE 2 showing the strongest genoprotective effect.

Phytochemical analysis showed that the extracts are rich in phenolic compounds and flavonoids, which may be responsible for their diverse properties. Phenolics are gaining attention because they slow down the oxidative damage of biomolecules (Pandey & Rizvi, 2009 & Heim et al., 2002). Phenolic compounds are natural antioxidant metabolites found in plant foods, known for their ability to scavenge reactive oxygen species (ROS) and thus protect the cells against oxidative stress-related diseases. (Barraza-Garza, 2020). Their structure, a hydroxyl group attached to a benzene ring, allows them to act as free radical scavengers. When reactive oxygen species are present in sufficient amounts, the bond between oxygen and hydrogen in the hydroxyl group breaks. The released hydrogen ion then reacts with and neutralizes free radicals (Wojdyio, 2007).

The antioxidant compounds in the extracts help reduce oxidative stress, thereby protecting against mutations and DNA damage. They achieve this either directly via reacting with free radicals or indirectly inhibiting the expression of free radical generating enzymes or by boosting the activity of ROS-neutralizing enzymes. The observed antioxidant potential of the extracts can be attributed to their high phenolic and flavonoid contents, which are well-known for their DNA-protective properties. According to Schmalhausen (2007), these phytochemicals seem to act as mild prooxidants that can activate a series of biochemical pathways of the cellular antioxidant system. These compounds counteract oxidative stress, safeguard cells from damage, and reduce the risk of genetic alterations. (Lu et al., 2010).

According to Sawadogo et al. (2006), members of Acanthaceae are good sources of antioxidants and suggest their use in the treatment of several chronic ailments. The free radical scavenging potential of aerial parts of various acanthaceous plants has been widely investigated, with studies reporting a positive correlation between phenolic and flavonoid content and antioxidant activity (Udupa et al., 2025; Rattan, 2023; Chetan et al., 2011). The present findings align with earlier reports showing the strong antioxidant activity of *Barleria* species, underscoring their therapeutic value. (Rajurkar & Gaikwad, 2012; Bhatt & Dhyani, 2012). In most of the studies, alcoholic extracts showed better results than other solvent extracts.

Inhibition of mitotic cell division and the induction of CA are commonly used as markers of cytotoxicity and genotoxicity. The cytotoxicity of a substance can be assessed by changes in the MI. A significantly lower MI compared to the negative control suggests that the chemical exposure has affected the growth and development of the test organism. Conversely, a much higher MI indicates increased cell division, which may be harmful, leading to uncontrolled proliferation and potentially tumor formation (Leme & Marin-Morales, 2009). Such an increase may result from a shortened mitotic cycle, allowing interphase cells to enter the next division stage more quickly (Al-Ahmadi, 2013).

The observed protection against MP-induced damage suggests the extracts possess potent antimutagenic activity, likely mediated by antioxidant mechanisms that prevent oxidative DNA damage (Duthie, 1999). This is consistent with earlier reports where *Barleria* species showed protective effects against chemically induced genotoxicity in plant and mammalian systems (Sharma & Sahu, 2014; Sharma, &

Kansal, 2009). Maji et al. (2011) reported that the hydro-methanolic extract of *B. prionitis* contains glycosides, flavonoids, steroids, and tannins, while its crude ethanolic extract yields six natural compounds: balarenone, pipataline, lupeol, and prionisides A, B, and C (Ata et al., 2009). Lupeol is a strong chemopreventive and chemoprotective compound effective against various disorders, with studies by Ragasa et al. (2009) and Saleem (2009) confirming its antimutagenic effects.

Allium test is a well-established cytogenotoxic assay that shows strong correlation with mammalian systems (Fiskesjo, 1985; Fiskesjo & Levan, 1993), thereby enhancing the relevance of these results for potential pharmacological applications. The study supports the findings of Kehrer & Smith (1994), who reported that age-related oxidative damage to DNA and proteins contributes significantly to cellular degeneration and disease, whereas polyphenols, through free radical scavenging and stabilization of oxidative intermediates, exert a strong genoprotective effect. The minimal mitotic abnormalities observed in root tips treated with the root extracts of *B. cristata* and *B. prionitis* may be attributed to its constituents acting as nonspecific redox agents, free radical scavengers or toxin-binding ligands. Furthermore, the use of the *Allium* test, highlights its reliability as an ethical and effective alternative to animal-based toxicological assays.

CONCLUSION

The present study demonstrates that root methanolic extracts of *B. cristata* and *B. prionitis* are rich in bioactive phenolic and flavonoid compounds that confer strong antioxidant and antimutagenic effects. Their ability to scavenge free radicals and protect against pesticide-induced cytogenotoxicity highlight their potential utility as natural therapeutic agents or protective formulations against oxidative and genotoxic stress, reinforcing the traditional medicinal use of these species and laying the foundation for further pharmacological and toxicological studies. *B. prionitis* exhibited superior performance in all assays, indicating its promise as a candidate for further therapeutic research.

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