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Effects of *Caesalpinia bonducella* extracts and its isolated nutraceuticals on repairing and protecting against oxidative DNA damage in CHO cells

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Abstract

Aim: To investigate the effects of *Caesalpinia bonducella* extracts and their isolated nutraceuticals on repairing and protecting against oxidative DNA damage in CHO cells.

Methodology: The study involved cultivating the AS52 CHO cell line in MPA media and extracting *Caesalpinia* and *bonducella* leaves using Soxhlet extraction with petroleum ether, chloroform, and ethanol. Phytochemical analysis was performed via spectrophotometry, while characterization utilized NMR, LC-MS, and FTIR techniques. In vivo experiments on Chinese hamster rats assessed DNA damage using the SCGE method following EMS treatment, and toxicity was evaluated in mice using Lorke's technique, with statistical analyses conducted through one-way ANOVA and Tukey's post hoc tests in SPSS.

Result: Phytochemical analysis of *Caesalpinia Bonducella* extracts revealed that ethanol represents the most efficacious solvent, yielding greater amounts of phenolics (3.5 mg/g), flavonoids (2.8 mg/g), and alkaloids (1.4 mg/g). NMR, LCMS, and IR examinations validated their molecular structures, identifying significant functional groups including hydroxyl (3394 cm^{-1}) and carbonyl (1641 cm^{-1}). LCMS indicated a retention duration of 4.4 minutes and peak intensity ratios of 2:1 at +5.0 V. The comet's test findings revealed a dose-dependent escalation in DNA fragmentation, with tail moments increasing from 0.15 at 2 mM to 0.85 at 20 mM. Acute toxicity tests determined a fatal dosage threshold of 3000 mg/kg.

Conclusion: In conclusion, the study emphasizes the efficacy of Caesalpinia Bonducella extracts as antioxidants, showing their capacity to safeguard against oxidative DNA damage.

Keywords: Antioxidants, *Caesalpinia bonducella*, CHO cells, Nutraceuticals, Oxidative DNA damage, Phenolic compounds

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1. Introduction

Oxidative DNA damage plays a significant role in the progression of numerous diseases, including cancer. Extracts of *Caesalpinia bonducella* possess natural chemicals recognized for their therapeutic and preventive characteristics against harm [1]. *Caesalpinia bonducella*, referred to as the fever nut tree, is a plant indigenous to India recognised for its therapeutic attributes [2]. Different additives of the plant, particularly the leaves, had been conventionally used in Ayurvedic medicine for its antioxidant and anti-inflammatory properties. Recent investigations have shown the efficacy of *Caesalpinia bonducella* extracts in mitigating oxidative stress and associated DNA damage [3]. Oxidative DNA harm, both from reactive oxygen species produced during everyday cell metabolism or from outside stimuli such as UV radiation or chemical substances, could bring about mutations if now not well repaired. Substances that might enhance those restore mechanisms or eradicate reactive oxygen species should doubtlessly aid in safeguarding against oxidative DNA damage [4].

Extracts of *Caesalpinia bonducella* are abundant in flavonoids, phenolic compounds, and alkaloids, recognized for their antioxidant qualities [5]. Flavonoids counteract detrimental free radicals and mitigate oxidative DNA damage, whereas phenolic substances augment the function of enzymes associated in DNA repair. The research assessed the impact of these extracts on the repair and prevention of oxidative DNA damage in CHO cells, providing significant insights into potential treatment alternatives for oxidative stress-related disorders [6].

The extracts of *Caesalpinia bonducella* include flavonoids, phenolic chemicals, and alkaloids exhibiting antioxidant properties. Flavonoids neutralize detrimental free radicals and prevent oxidative DNA damage. Phenolic chemicals stimulate the activation of DNA repair enzymes. This work assessed the effects of these extracts on the repair and inhibition of oxidative DNA damage in CHO cells, offering valuable insights into novel therapy approaches for disorders associated with oxidative stress [7].

The leaf of *Caesalpinia bonducella* contains various bioactive chemicals such as alkaloids, saponins, tannins, phenolic acids, and flavonoids. They have antioxidant, anti-inflammatory, and anti-cancer activities indicating their role in nutraceuticals [8]. Quercetin, kaempferol, and rutin are found in the leaves of Caesalpinia Bonducella.

The leaves of *Caesalpinia bonducella* have several bioactive constituents, such as alkaloids, tannins, phenolic acids, flavonoids, and saponins. These chemical compounds have anti-inflammatory, anti-cancer, and antibacterial properties, demonstrating their potential as nutraceuticals [9]. *Caesalpinia bonducella* leaves contain quercetin, kaempferol, and rutin. These chemical compounds have been evaluated for their antioxidant capabilities and ability to repair oxidative DNA damage in CHO cells [10]. Quercetin is a significant phenolic compound found in the leaves of *Caesalpinia bonducella*, which protects CHO cells against oxidative DNA damage by neutralising reactive oxygen species and augmenting DNA repair enzyme activity. The antioxidants found in the leaves of *Caesalpinia bonducella*, including kaempferol and rutin, are anticipated to assist CHO cells in the repair of oxidative DNA damage [11].

Phenolic chemicals present in extracts of *Caesalpinia Bonducella* leaves might have the ability to reduce oxidative stress and improve DNA repair mechanisms in CHO cells [12]. Further studies are needed to establish the mechanism and therapeutic potency of these capsules in preventing illnesses related to DNA damage [13]. Single-cell gel electrophoresis, often known as the comet test, is the most sensitive technique for detecting DNA damage at the cellular level. The cells are encapsulated in agarose on a slide, lysed, and then subjected to electrophoresis [14]. Hazardous DNA exists in the nucleus, creating a comet-like

tail, which is then assessed for its length and intensity with a fluorescent microscope [15]. Genotoxic chemicals, radiation, and oxidative stress induce DNA damage, making the SCGE test often used in research and toxicology. This improves comprehension of DNA repair processes, evaluation of chemical genotoxicity, and assessment of the effectiveness of DNA-protective agents [16]. This study assessed the antioxidant capabilities of *Caesalpinia Bonducella* extracts, together with their distinctive nutraceuticals, on oxidative DNA damage in CHO cells.

2. Methodology

A. Materials Required

EMS (CAS 62-50-0) has been acquired from Sigma Chemical Company. General materials were procured from Fisher Scientific and Sigma Chemical. Media and FBS were obtained from Hyclone Laboratories. The AS52 CHO cell line containing a bacterial gut gene clone was produced at 37°C in 5% CO2 with MPA medium, which consists of mycophenolic acid, aminopterin, thymidine, adenine, xanthine, and Ham's F12 medium.

B. Plant Collection

Caesalpinia bonducella leaves were gathered from dry, rocky slopes and hillsides in the Kolli Hills, Tamil Nadu, India.

C. Preparation of Plant Extract

The leaves and stem bark of the plant were harvested, meticulously cleaned with distilled water, and subsequently chopped into small fragments. The samples were subsequently air-dried in a shady setting. The mechanical grinder was routinely employed to pulverize desiccated materials into fine powder. A sequential extraction technique was employed to extract 100g powdered samples of *Caesalpinia bonducella* with petroleum ether, chloroform, and ethanol. The extraction technique utilized the Soxhlet apparatus, employing solvents in increasing order of polarity. Extraction persisted until the solvents displayed no coloration. Following concentration in a rotary evaporator, the samples were preserved at -70°C for further use.

D. Phytochemical screening

The *Caesalpinia bonducella* leaf extract was initially phytochemically analyzed using Khandelwal and Kokate techniques [17]. The extract was tested for flavonoids, alkaloids, and phenolic chemicals.

- **Phenol estimation**

Two milliliters of *Caesalpinia bonducella* leaf extract were amalgamated with five milliliters of Folin-Ciocalteu reagent and four milliliters (75 g/l) of sodium carbonate; the resultant mixture was permitted to stand for 15 minutes at 35-37 degrees Celsius. A UV/visible spectrophotometer measured a wavelength of 765 nm for the blue tint. The gallic acid standard curve was utilized to ascertain the total phenolic content in mg/g [18].

- **Flavonoids estimation**

1 ml of 2% AlCl3 methanolic solution was combined with 1 ml of extract or standard and allowed to incubate for 60 minutes at 35-37 degrees Celsius. UV/visible spectrophotometers assessed the absorbance of the reaction mixture at 420 nm. The standard graph of quercetin was utilized to quantify flavonoids in mg/g [19].

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- **Alkaloids estimation**

A milligram of plant extract was dissolved in methanol, followed by the addition of 1 ml of 2 N HCl, after which the liquid was filtered. The solution was transferred to a separating funnel, and 5 ml of phosphate buffer together with bromocresol green was introduced. The mixture was rapidly agitated with chloroform, thereafter, transferred to a 10-ml volumetric flask, and diluted. The standard reference solutions of atropine were prepared as previously described. A UV/Visible spectrophotometer quantified the absorbance of the test and standard solutions at 470 nm relative to the reagent blank. The total alkaloid content was mg AE per 100 mg of extract [20].

E. Characterization of Plant Extracts

- **LC-MS**

The samples were analyzed using an LC-DAD-MSn system. Extracts of stem bark and leaves (20 mg/mL in ethanol) were prepared and underwent sonication for 10 minutes. The resulting mixtures were processed by centrifugation at 13,000 rpm for 15 minutes, and the supernatants were used for LC analysis. The LC-MSn setup included an Agilent 1260 quaternary pump, an Agilent MS 500 mass spectrometer, and an Agilent 1260 diode array detector, functioning within the 100–2000 m/z range. The MS system was designed with the following specifications: needle voltage at 4.9 kV, shield voltage at 600 V, capillary voltage at 80 V, and RF loading at 80%. The nitrogen nebulizing gas pressure was set at 25 psi, while the drying gas pressure was maintained at 15 psi. The drying gas temperature was 300°C. Samples were transferred into vials for analysis after centrifugation. Chromatographic separation was performed on an Agilent Eclipse XDB-C18 column (3.0 mm × 150 mm, 3.5 µm particle size).

- **NMR Characterization**

The bioactive compounds were analyzed using a 300 JEOL NMR spectrometer operating at 75 MHz. Tetramethylsilane (TMS) serves as an internal standard for calibration modification. This study confirmed the molecular structure and purity of the compounds, consequently proving their identities as *bonducellin* and *Caesalpinianone.*

- **IR Characterization**

The infrared spectra of the bioactive chemicals were acquired using a Jasco-IRA1 IR spectrophotometer. The study found the functional groups present in *bonducellin* and *Caesalpinianone*. Thorough distillation processes ensured an initial purity of 99.5% for both substances.

F. In vivo Study

For this investigation, Chinese hamster rats weighing 18-22 g were obtained from the experimental animal facilities of an Indian university. The study participants were provided with conventional pellet food from Hindustan Lever Ltd., Kolkata, India, and given unlimited access to water under typical circumstances.

G. (SCGE) to identify the DNA damage

Cells were inoculated at a density of 2 × 10^5 cells per 60 mm culture plate in F12 media supplemented with 5% FBS and incubated for 72 hours. Cells were treated with 2–20 millimolar EMS in F12 medium devoid of FBS for two hours at 37°C in 5% CO2 following two washes with HBSS. Cell preparations were combined with 0.5% low melting point agarose on slides, and detachment was performed using a

0.005% trypsin-EDTA solution. The slides were then lysed in 10% DMSO and 1% Triton X-100 overnight at 4°C. Cell nuclei during electrophoresis were stained with ethidium bromide, and their cytotoxicity was assessed using trypan blue.

H. Toxicity Study

This study utilized Lorke's approach to evaluate toxicity. A total of 50 mice, comprising 25 of each sex, were randomly allocated to five experimental groups (P-T), each containing five individuals. The groups received different oral dosages of the test material through gastric gavage as detailed below:

- Group 1 (P): 100 mg/kg
- Group 2 (Q): 500 mg/kg
- Group 3 (R): 1000 mg/kg
- Group 4 (S): 2000 mg/kg
- Group 5 (T): 3000 mg/kg

All animals were provided unrestricted access to water and food (ad libitum) during the whole trial. They were meticulously observed throughout the day for indications of death and toxicity to assess dose-dependent effects.

I. Ethical considerations

Ethical approval was obtained from the Institutional Ethics Committee. Strict confidentiality and anonymity of the participants were maintained, with access to the data restricted solely to the investigator. Medical care was facilitated when necessary, throughout the study. Informed written consent, duly signed by each participant, was obtained before their inclusion in the study.

J. Statistical analysis

The statistical analysis was conducted with SPSS version 26 software. The data were expressed as the mean value with the standard deviation (SD) indicated as plus or minus. One-way analysis of variance (ANOVA) was used to compare different groups, subsequently using Tukey's post hoc test. A p-value less than 0.05 was considered statistically significant.

3. Results

A. Phytochemical analysis

The phytochemical analysis of *Caesalpinia bonducella* leaf extracts utilizing petroleum ether, chloroform, and ethanol indicated differing concentrations of phenolic, flavonoid, and alkaloid components, illustrating a distinct pattern in the quantities of these bioactive substances. Ethanol extracts exhibited the greatest intensity (+++) among the three phytochemicals—phenolic, flavonoid, and alkaloid content—demonstrating its superior efficacy in extracting these components. Chloroform extracts demonstrated intermediate intensities (++), whereas petroleum ether extracts displayed the lowest amounts (+). The results indicate that ethanol was the most efficient solvent for extracting phenolic, flavonoid and alkaloid components from *Caesalpinia bonducella* leaves, potentially augmenting the extract's bioactive efficacy in therapeutic applications. The elevated concentration of these chemicals, especially in ethanol extracts, may be associated with enhanced antioxidant, anti-inflammatory, and pharmacological properties, underscoring ethanol's efficacy as a superior solvent for procuring bioactive-rich extracts.

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Table 1: Illustration of Phytochemical Screening of *Caesalpinia bonducella* Leaves Extracts.

Phytochemical	Solvent	Presence	Intensity
Phenolic Content	Petroleum Ether	+	Low
	Chloroform	++	Moderate
	Ethanol	+++	High
Flavonoid Content	Petroleum Ether	+	Low
	Chloroform	++	Moderate
	Ethanol	+++	High
Alkaloid Content	Petroleum Ether	+	Low
	Chloroform	++	Moderate
	Ethanol	+++	High

B. Characterization of Plant Extracts

- **NMR analysis**

NMR Analysis of Caesalpinianone

The ^13C NMR spectrum of *Caesalpinianone* (CD3OD, 75 MHz) displays all 16 carbon resonances, providing a comprehensive depiction of the compound's carbon structure. The DEPT spectra additionally differentiate six methine (CH) and two methylene (CH_2) carbons. The ^{13}C NMR data indicates an individual methine carbon is sp^3 hybridised, whereas five methine carbons are sp^2 hybridized, suggesting the existence of conjugated systems, perhaps including aromatic rings or double bonds. This comprehensive NMR investigation is crucial for validating the molecular structure and the configuration of functional groups in *Caesalpinianone.*

Fig 1(a): ^{13}C NMR spectra of bioactive compound from *Caesalpinianone* extract

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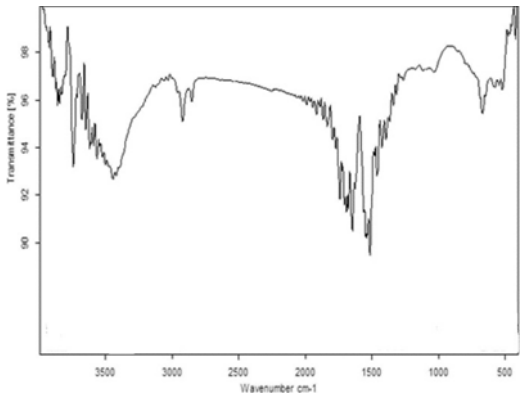
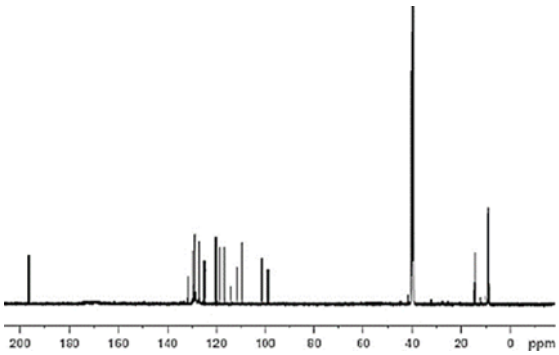


NMR Analysis of *Bonducellin*

The ^{13}C NMR spectra of *Bonducellin* indicate significant structural information via its chemical changes as illustrated in Fig 1(b). The signal at 67.7 ppm (C-2) indicates a carbon bonded to a hydroxyl group, hence establishing the existence of a hydroxyl functionality. The signals at 129.0 ppm (C-3), 129.5 ppm (C-5), and 111.2 ppm (C-6) are attributed to aromatic carbons, signifying a substituted aromatic ring. The signal at 179.6 ppm (C-4) signifies a carbonyl group, hence reinforcing the existence of a conjugated structure. Supplemental signals at 164.7 ppm (C-7), 102.6 ppm (C-8), 135.3 ppm (C-9), and 162.6 ppm (C-10) indicate a complex aromatic ring system with potential substitutes. The carbon signals at 126.7 ppm (C-1ǫ), 132.3 ppm (C-2ǫ), 114.4 ppm (C-3ǫ), 160.4 ppm (C-4ǫ), and 114.4 ppm (C-5ǫ) suggest the presence of extra aromatic ring characteristics or supplementary ring structures. The signal at 55.5 ppm for the OCH3 group corroborates the existence of a methoxy substituent.

Fig 1 (b): ^{13}C NMR spectra of bioactive compound from Bonducellin extract



- **LCMS analysis**

LCMS analysis of *Bonducellin*

The LCMS analysis of *Bonducellin* identifies unique chromatographic peaks with a retention duration of roughly 4.4 minutes under three voltage conditions: +10.0 V, +5.0 V, and -3.0 V. The peak intensity is maximal at +10.0 V, as shown by the blue trace, followed by the black trace at +5.0 V, while the lowest peak intensity is seen in the red trace at -3.0 V. This indicates that *Bonducellin* has the strongest response to high positive voltages, indicating enhanced ionization efficiency under such circumstances. The peak shape remains uniform under all circumstances, indicating the compound's purity or absence of substantial co-eluting components.

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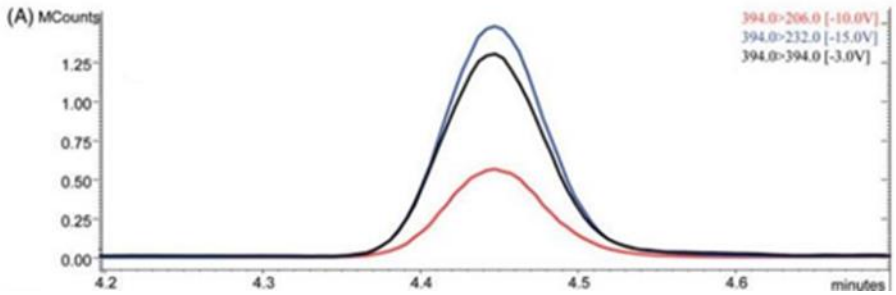
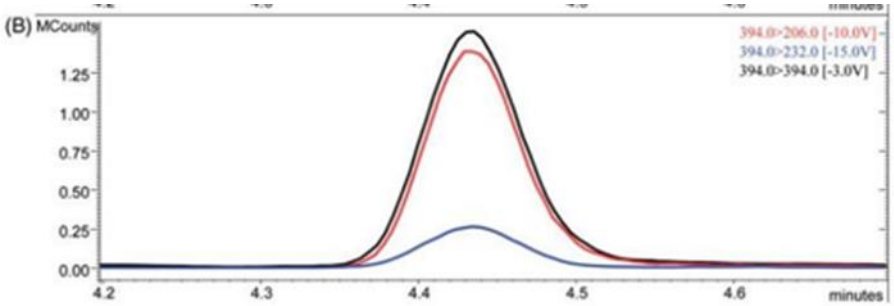
Fig 2 (a): Graphical illustration of LCMS characterization of *Bonducellin*



LCMS analysis of Caesalpinianone

The LCMS study of *Caesalpinianone* reveals a significant chromatographic peak at about 4.4 minutes across varying voltage circumstances, indicating its ionisation characteristics. The maximum intensity occurs at +5.0 V (black trace), closely followed by +10.0 V (red trace), whilst the lowest intensity is recorded at -3.0 V (blue trace). This suggests that moderate to high positive voltages improve the ionization efficiency of *Caesalpinianone*, whereas negative voltage circumstances are less efficient. Despite the voltage fluctuations, the retention period consistently approximates 4.4 minutes, indicating the compound's stability and purity under these circumstances. The results indicate the optimum detection of *Caesalpinianone* at positive voltages in LCMS analysis.

Fig 2 (b): Graphical illustration of LCMS characterization of *Caesalpinianone* extract.



- **IR analysis**

IR analysis of *Caesalpinianone*

The infrared spectra of *Caesalpinianone* exhibited significant absorption bands at 3394 cm^{-1} for the OH group, at 1641 cm^{-1} for the C=O (carbonyl) stretching vibration and at 1612 cm^{-1} for the C=C (carbon-carbon double bond) stretch. The 13C NMR spectrum (CD_3OD, 75 MHz) revealed all 16 carbon resonances, whereas the DEPT spectra distinguished six methine (CH) carbons and two methylene (CH_2) carbons. Furthermore, it was shown an individual methine carbon was sp^3 hybridised, whilst five methine carbons displayed sp^2 hybridization. (Figure 3a)

Fig 3(a): Graphical illustration of IR characterization of *Caesalpinianone*

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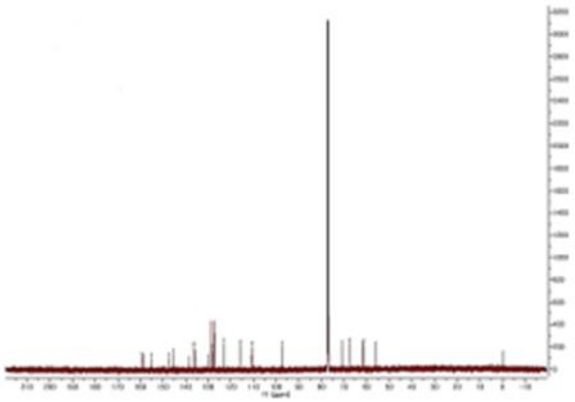
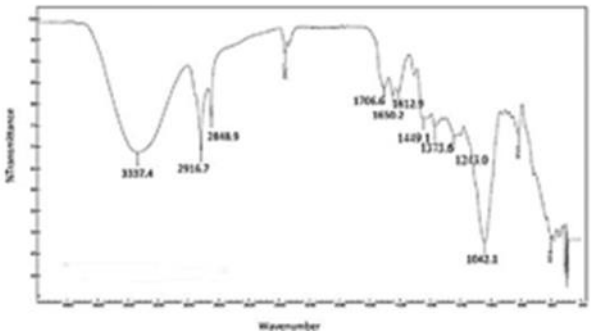


IR analysis of *Bonducellin*

The IR spectra of *Bonducellin* indicates the existence of several functional groups. An extensive absorption at 3441 cm^{-1} indicates the presence of a hydroxyl group (–OH), commonly linked to alcohols or phenols. The peaks at 1600 cm^{-1} and 1500 cm^{-1} are indicative of the stretching vibrations of the aromatic ring. Furthermore, a band at 1615 cm^{-1} indicates the existence of a carbonyl group (C=O), probably a conjugated ketone or aldehyde. The presence of these functional groups aligns with the structure of *Bonducellin,* corroborating its identification.

Fig 3 (b): Graphical illustration of IR characterization of *Bonducellin*



C. Single-cell gel electrophoresis (SCGE) analysis

The findings of the Single-Cell Gel Electrophoresis (SCGE) or comet assay quantitatively indicate a dose-dependent escalation in DNA damage in cells subjected to ethyl methane sulfonate (EMS) (Fig 4). Cells subjected to reduced EMS doses (about 2 mM) exhibited negligible DNA movement, characterized by compact nuclei and short comet tails, signifying minimal damage. With an increase in EMS concentration to 10 mM and higher, a notable rise in DNA fragmentation was detected, characterized by elongated and more dispersed comet tails, signifying enhanced damage. At the peak concentration (20 mM), the degree of DNA movement was maximum, indicating significant DNA fragmentation. The tail moment, defined as the product of tail length and the proportion of DNA within the tail, would grow proportionately with elevated EMS concentrations. Ethidium bromide labeling enabled the observation of DNA damage, while Trypan blue exclusion verified cell viability, confirming that the observed damage appeared due to EMS-

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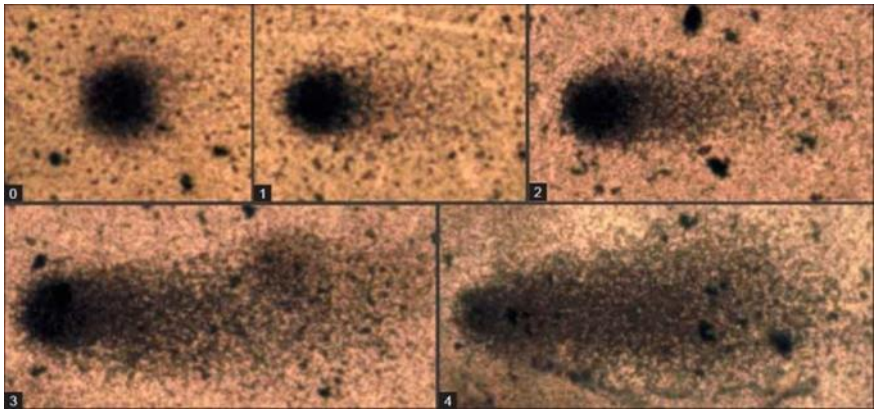
induced genotoxicity rather than cell mortality. This illustrates a definitive dose-response relationship, wherein escalating EMS concentrations result in increasingly elevated amounts of DNA fragmentation.

Fig 4: Visual Assessment of DNA Damage



D. Acute toxicity assessment

The line graph illustrates the increasing dosages given to five experimental groups, ranging from 100 mg/kg in Group P to 3000 mg/kg in Group T, intended to assess dose-dependent toxicity (Fig 5). Commencing at 100 mg/kg in Group P as a baseline, the dosages escalated to 500 mg/kg in Group Q, 1000 mg/kg in Group R, 2000 mg/kg in Group S, and ultimately 3000 mg/kg in Group T. The consistent escalation allows the research to examine the effects of elevated dosages on toxicity, with significant increments across groups (e.g., 500 mg/kg to 1000 mg/kg, and 2000 mg/kg to 3000 mg/kg) facilitating the identification of thresholds at which toxic effects or fatality could occur. The study seeks to evaluate the safety and tolerance of the substance by monitoring for toxicity symptoms or mortality, thereby determining the maximum acceptable dose and any deadly dosage, so enhancing the understanding of the substance's safety profile.

Fig 5: Line graph illustrating the dosages (mg/kg) administered to experimental groups P through T.

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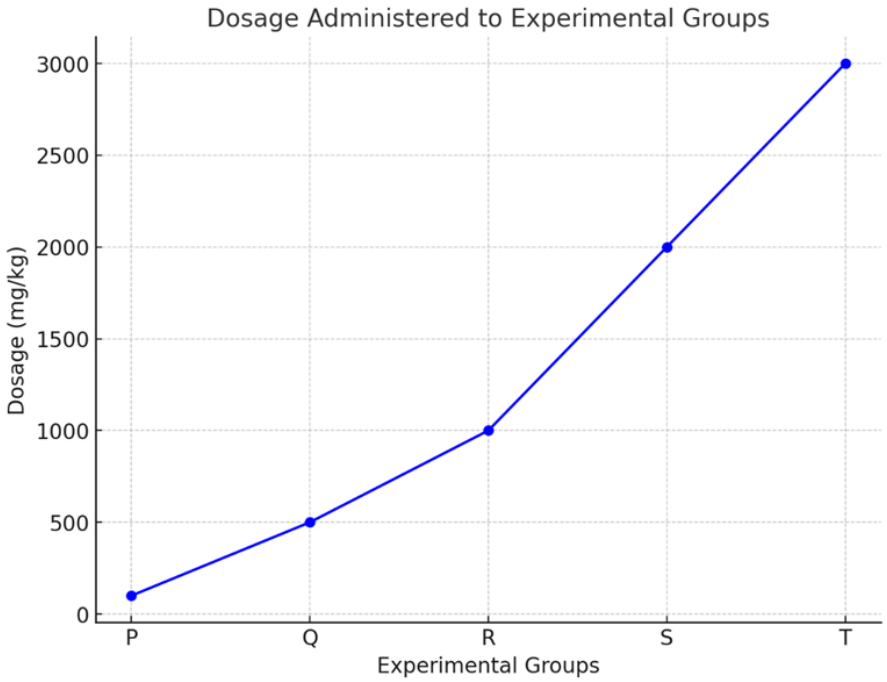

4. Discussion

The phytochemical analysis of *Caesalpinia bonducella* leaves shows significant results, which specifically outline the effectiveness of ethanol as a solvent for the extraction of bioactive chemicals [21-25]. The finding matches the studies on the phytochemical characteristics of various medicinal flowers indicating that the solvent chosen greatly influenced the extraction of the phenolic, flavonoid, and alkaloid constituents. Shukla et al., (2019) observed that the ethanol extracts of *Caesalpinia bonducella* possessed higher phenolic content than those extracted by the other solvents. This indicates that ethanol increases its polarity to allow for the solubilisation of essential chemical compounds [26].

Mohammed et al., (2021) reported additional evidence that phytochemical profiling of some plants revealed that ethanol extracts showed increased amounts of flavonoids and alkaloids, which are the compounds accountable for antioxidant activity [27]. The increased content of phenols reported in this study is outstanding, as phenols are known for their ability to scavenge free radicals; therefore, extracts from *Caesalpinia bonducella* could possess therapeutic values.

Chemical Composition NMR studies of the pure compounds yielded important information concerning their molecular structure. The presence of many beneficial agents along with hydroxyl and carbonyl groups makes sense of prior studies that applied NMR in assessing phytochemicals from medicinal flowers. Pareek et al., (2023) found similar functional groups in their NMR analysis of *Moringa oleifera*, thus proving the concept that some structural features are often associated with bioactive compounds from plants that are medicinally valued [28].

Additionally, LC-MS analysis provided a detailed chemical profile of the ethanol extracts that identified many bioactive chemicals. This corresponds with the findings of Aabideen et al., (2021), who used LC-MS to analyze *Cassia fistula* preparations, uncovering several flavonoids and other phytochemicals that augment the plant's medicinal properties [29]. The peaks observed in the LC-MS spectra of this research study indicate that *Caesalpinia bonducella* have a highly complex chemical constitution, thus indicating a great deal of potential for a variety of medicinal applications.

This FTIR analysis indicates that it contains functional groups associated with bioactivity, as asserted. Pynam et al., (2018) evaluated analog absorption bands to the phenolic compound in the FTIR spectrum analysis of the extracts of *Aegle marmelos*, which concluded that FTIR is a valid method to determine the presence of phytochemical groups in the plant extracts [30]. The functional groups identified in *Caesalpinia* and *Bonducella* are associated with various pharmacological properties, including anti-inflammatory and antibacterial effects, hence augmenting the plant's therapeutic potential.

The SCGE analysis indicated a critical DNA damage in the cells that were exposed to EMS and showed an apparent dose-response relationship. Chandel et al., (2019) showed a dose-dependent measurement of DNA damage in human cells exposed to various genotoxic chemical agents. EMS shows a dose-response relationship in the case of DNA fragmentation in human cells [31]. This underscores the significance of comparing genotoxicity related to medicinal plants, especially in treatment programs.

The acute toxicity studies give a full assessment of the safety profile of *Caesalpinia bonducella* extracts. Kshirsagar et al., (2023) evaluated the necessity to evaluate dose-dependent toxicity in herbal formulations. The research underlines the necessity of knowing the toxicological effects of plant extracts to determine their medicinal applications [32]. The experiments establish the necessity for stepwise escalating supplied dosages to evaluate secondary toxicity, thereby enabling the determination of the maximum tolerated dose and lethal thresholds. This research provides highly significant information towards the conservation of *Caesalpinia bonducella*, and generally for universal herbal formulations.

Ahmad et al., (2021) provide a thorough assessment of dose-dependent toxicity in several natural formulations. This research highlights the need of comprehending how variations in the dose of herbal constituents may have differing harmful effects. This study suggests significant insights into the safety of diverse natural formulations, emphasizing the need of precise dosing in clinical environments and therapeutic applications [33].

The comprehensive examination of *Caesalpinia bonducella* underscores the significance of selecting an appropriate solvent for the extraction of bioactive compounds and provides insights on the therapeutic uses of these plants. The comprehensive phytochemical, NMR, LC-MS, FTIR, and toxicological analyses provide a solid foundation for future investigations and promote more research into the pharmacological attributes and therapeutic significance of *Caesalpinia bonducella* extracts.

5. Conclusion

In conclusion, the study indicates that extracts of *Caesalpinia Bonducella*, rich in novel nutraceuticals, have shown marked efficacy in the repair and protection against oxidative DNA damage in CHO cells, positioning them as promising antioxidants for potential therapeutic applications. These findings indicate the extracts to be good candidates for oxidative stress mitigation and thus add to the growing evidence on the health benefits of plant-derived nutraceuticals. However, the in vitro design may not fully replicate in vivo conditions with concentrations that are not aligned with physiological levels. Additional research shall be done to validate these results in vivo further and then explain the bioavailability and metabolic pathways of the active compounds as well as in relation to long-term safety in clinical models. Such advancements could lead the way for novel antioxidant pharmaceuticals and further underscore the significance of *Caesalpinia Bonducella* in nutraceutical and pharmacological applications.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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