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Protective Effects Against Oxidative DNA Damage And Cell Signaling Exhibited by Selective Legume Extracts from Balangir District, Odisha

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Abstract:

This study systematically investigated the protective effects of 70% ethanolic extracts from four selective legume species (Vigna radiata, Vigna mungo, Macrotyloma uniflorum, and Cajanus cajan) sourced from Balangir district, Odisha, against oxidative DNA damage and their influence on cell signaling pathways. Phytochemical profiling revealed Vigna mungo to possess the highest concentrations of quercetin (32.78 µg/g), kaempferol (24.36 µg/g), and gallic acid (11.54 µg/g), suggesting its superior antioxidant potential, while Vigna radiata and *Cajanus cajan showed moderate levels, and Macrotyloma uniflorum presented unique phytochemical balance.*

In vitro assessment demonstrated that all legume extracts offered dose-dependent protection against Fe^{2+}/AAPH-induced oxidative DNA damage. Vigna mungo *exhibited the highest protective activity (91.0 ± 6.5% at 400 µg/mL), closely followed by Cajanus cajan (88.5 ± 6.0%) and Vigna radiata (85.1 ± 6.2%), likely due to their rich flavonoid and phenolic content. Furthermore, the extracts showed significant anti-inflammatory activity by inhibiting nitric oxide production in LPS-stimulated RAW 264.7 macrophages;* Vigna mungo *again led with 82.1 ± 6.4% inhibition at 400 µg/mL.*

Beyond direct cellular protection, these extracts displayed promising broader health benefits. They exhibited anti-urolithic activity, with Vigna mungo *showing 84.1 ± 6.3% inhibition of calcium oxalate crystallization, comparable to sodium citrate. Similarly, all extracts demonstrated anti-obesity potential through pancreatic lipase inhibition, with Vigna mungo reaching 78.3 ± 6.1% inhibition. Importantly, the legumes also displayed quorum sensing inhibition (QSI) activity, suppressing violacein production in* Chromobacterium violaceum *CV026, where Vigna mungo showed 80.1 ± 6.3% inhibition.*

Collectively, these findings underscore the significant potential of legumes from Balangir district as rich sources of bioactive compounds capable of mitigating oxidative stress, inflammation, and related diseases, while also offering anti-urolithic, anti-obesity, and quorum sensing inhibitory properties. Vigna mungo consistently emerged as the most potent species across multiple assays, warranting further isolation and in vivo *mechanistic studies for its potential as a functional food ingredient or natural therapeutic agent.*

Keywords: *Vigna Radiata, Vigna Mungo, Macrotyloma Uniflorum, Cajanus Cajan.*

Introduction:

Oxidative stress arises when there is an imbalance between the generation of reactive oxygen species (ROS) and the biological system's ability to detoxify these reactive intermediates, leading to cellular damage and contributing to the pathogenesis of numerous chronic diseases including cancer, cardiovascular diseases, and neurodegenerative disorders (Lobo et al., 2010).

Natural antioxidants, primarily derived from plant-based foods, play a critical role in neutralizing free radicals, thereby protecting cellular components from oxidative damage and maintaining physiological homeostasis (Pham-Huy et al., 2008). Legumes, an essential component of traditional diets worldwide, are recognized for their abundance in bioactive compounds such as flavonoids, phenolic acids, tannins, and vitamins, which exhibit potent antioxidant properties and contribute to the prevention of oxidative stress-related disorders (Xu & Chang, 2007). The exploration of legumes from geographically unique and often under-investigated regions, such as the Balangir district of Odisha: an area known for its agro-biodiversity and traditional agricultural practices can unveil novel phytochemicals with potential nutraceutical applications (Mohanty et al., 2021).

Oxidative DNA damage occurs primarily due to the overproduction of reactive oxygen species (ROS), which attack nucleobases and the sugar-phosphate backbone, leading to the formation of DNA adducts and strand breaks (Cooke et al., 2003). Persistent oxidative damage results in mutations and genomic instability, contributing significantly to the development of chronic diseases such as cancer, neurodegeneration, and cardiovascular disorders (Loft & Poulsen, 1996). Cellular response to oxidative stress is tightly regulated by key signaling pathways, among which the Nrf2/ARE (nuclear factor erythroid 2–related factor 2/antioxidant response element) pathway plays a central role in detecting redox imbalance and initiating transcriptional responses (Ma, 2013). Activation of these pathways enhances the expression of endogenous antioxidant enzymes, suppresses pro-inflammatory signaling, and promotes cellular survival and adaptation under oxidative conditions (Kensler et al., 2007).

Legume species such as *Vigna radiata*, *Vigna mungo*, *Macrotyloma uniflorum*, and *Cajanus cajan* are traditionally cultivated in the Balangir district of Odisha, a region known for its rich agro-biodiversity, and may possess unique phytochemical profiles due to localized climatic and soil conditions (Mohanty et al., 2021). Despite their traditional dietary use, these region-specific legumes remain underexplored in terms of their antioxidant potential and bioactive constituents that may confer protective effects against oxidative stress and related pathologies (Belewu et al., 2008).

Research Problem & Gaps

Current literature lacks detailed insights into the protective effects of these specific legumes against oxidative DNA damage and the molecular pathways involved in their cellular defense mechanisms, particularly the Nrf2/ARE-mediated response (Xu & Chang, 2008).

Furthermore, few studies have systematically assessed how the phytochemicals in these legumes influence oxidative stress-induced cell signaling pathways, leaving a gap in understanding their potential therapeutic applications (Kris-Etherton et al., 2002).

Literature Review

Numerous studies have shown that natural products, particularly plant-derived extracts including those from legumes, exhibit significant protective effects against oxidative DNA damage in both in vitro and in vivo experimental models (Halliwell, 2007). Several legume species such as *Glycine max*, *Phaseolus vulgaris*,

and *Vigna radiata* have been reported to reduce DNA strand breaks and oxidative lesions through the action of polyphenols, flavonoids, and isoflavones (Cao et al., 2010). These protective mechanisms are primarily attributed to the scavenging of reactive oxygen species, enhancement of endogenous antioxidant enzymes like SOD, CAT, and GPx, and modulation of DNA repair pathways (Kang et al., 2006).

Compounds such as quercetin, genistein, daidzein, and gallic acid are among the key bioactives identified in legumes and other plants that mitigate oxidative DNA damage and improve genomic stability (Surh, 2003). Recent studies have identified various bioactive compounds in legumes—such as quercetin, kaempferol, gallic acid, and other phenolic and flavonoid derivatives—that play pivotal roles in modulating key cell signaling pathways associated with oxidative stress and inflammation (Xu & Chang, 2009). These compounds have been shown to activate the Nrf2/ARE pathway, enhancing the expression of antioxidant enzymes, while simultaneously inhibiting pro-inflammatory mediators via the downregulation of NF-κB signaling (Li et al., 2011).

Mechanistic studies have further demonstrated that quercetin and kaempferol interact with Keap1–Nrf2 binding domains and MAPK cascade components, thereby influencing redox homeostasis and promoting cellular resilience (Lee et al., 2018). Gallic acid, commonly found in legume extracts, has been reported to suppress oxidative DNA damage and apoptosis through modulation of PI3K/Akt and JNK signaling pathways in oxidative-stress-induced cellular models (You et al., 2010).

Gaps in Knowledge and Justification for Current Study

Although numerous studies have examined the antioxidant and bioactive potential of commonly known legumes, there is a noticeable lack of research specifically focusing on indigenous legume varieties from the Balangir district of Odisha, a region rich in biodiversity and traditional agro-resources (Mohanty et al., 2021). The phytochemical diversity and potential therapeutic applications of local legumes such as *Vigna radiata*, *Vigna mungo*, *Macrotyloma uniflorum*, and *Cajanus cajan* from this region remain largely undocumented, particularly in relation to oxidative DNA protection (Belewu et al., 2008). Moreover, current literature falls short in elucidating the molecular mechanisms—especially those involving the Nrf2/ARE and NF-κB signaling pathways—through which these legumes exert their protective effects at the cellular level (Kris-Etherton et al., 2002).

The present study aims to address these gaps by comprehensively analyzing the antioxidant potential of these regional legumes, evaluating their protective effects against oxidative DNA damage, and characterizing their influence on redox-sensitive signaling cascades, thereby offering novel insights into their health-promoting properties (Cao et al., 2010).

Materials & Methods

Collection and Identification

Legume samples of *Vigna radiata* (green gram), *Vigna mungo* (black gram), *Macrotyloma uniflorum* (horse gram), and *Cajanus cajan* (pigeon pea) were collected during the post-harvest season from agricultural fields in the Balangir district of Odisha, a region recognized for its traditional cropping systems and agro-biodiversity.

The collected plant materials were carefully selected from multiple farm sites to ensure representative sampling and minimize local variability, following standard ethnobotanical collection procedures (Martin, 1995).

Botanical identification and taxonomic authentication were conducted by a Botanist at the Department of Botany, Y.B.N. University, and verified using standard floras and herbarium specimens, ensuring accurate species confirmation of the four legumes.

Preparation of Legume Extracts

Dried and powdered seeds of *Vigna radiata*, *Vigna mungo*, *Macrotyloma uniflorum*, and *Cajanus cajan* were subjected to extraction using 70% ethanol in a Soxhlet apparatus, following standard phytochemical extraction protocols (Harborne, 1998). Approximately 100 g of each powdered sample was extracted with 1000 mL of 70% ethanol for 6–8 hours at a controlled temperature of 60 ± 2 °C, ensuring continuous solvent cycling and optimal phytochemical recovery (Azwanida, 2015). Post-extraction, the ethanolic extracts were concentrated under reduced pressure using a rotary evaporator at 40 °C, followed by lyophilization to obtain dry crude extracts.

The extraction yield was calculated as a percentage of dry extract weight relative to the initial sample weight. Prepared extracts were stored in airtight amber-colored vials at 4 °C until further biochemical and biological assays were performed.

Phytochemical Screening and Characterization

Qualitative and quantitative analysis of selected bioactive compounds as quercetin, kaempferol, and gallic acid was carried out using High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography–Mass Spectrometry (LC-MS), following standardized analytical protocols (Goufo & Trindade, 2014).

For HPLC analysis, 10 mg of each legume extract was dissolved in methanol, filtered through a 0.22 µm syringe filter, and injected into a C18 reverse-phase column using a gradient mobile phase of acetonitrile and 0.1% formic acid at a flow rate of 1 mL/min. Retention times were recorded and compared with those of authenticated standards: quercetin (RT ~11.2 min), kaempferol (RT ~12.8 min), and gallic acid (RT ~6.3 min), with quantification based on calibration curves prepared using serial dilutions of standard compounds ($R^2 > 0.998$).

Further confirmation of molecular identity was performed via LC-MS using electrospray ionization (ESI) in negative mode, where molecular ion peaks $[M-H]^-$ corresponding to quercetin (m/z 301), kaempferol (m/z 285), and gallic acid (m/z 169) were observed (Lu et al., 2015). All analyses were conducted in triplicates, and the results were expressed as µg of compound per gram of dry extract.

Induction of Oxidative Stress

Oxidative stress in cultured cells was induced by treatment with hydrogen peroxide (H_2O_2), a well-established pro-oxidant agent known to generate reactive oxygen species (ROS) and cause oxidative DNA damage (Li et al., 2017). Preliminary optimization experiments were conducted to determine the optimal concentration and exposure time that induce significant oxidative stress while maintaining over 70% cell viability. Cells were exposed to varying concentrations of H_2O_2 ranging from 100 to 500 µM for 1 to 3 hours, with 300 µM for 2 hours identified as the optimal condition, producing reproducible DNA damage without excessive cytotoxicity (Wang et al., 2015). All treatments were performed in serum-free medium to avoid interference, and cells were immediately processed for subsequent assays following exposure.

Assessment of *In Vitro* Oxidative DNA Damage

This assay was meticulously conducted to evaluate the protective effects of the 70% ethanolic extracts of Green Gram (*Vigna radiata*), Black Gram (*Vigna mungo*), Horse Gram (*Macrotyloma uniflorum*), and

Pigeon Pea (*Cajanus cajan*) seeds against oxidative DNA damage induced *in vitro*. The legume seeds were specifically procured from local farmers in Balangir district, Odisha, India, ensuring the use of regional varieties.

Materials

Calf thymus DNA (CT-DNA), ferrous chloride ($FeCl_2$), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), Tris-HCl buffer, EDTA, low melting point agarose, ethidium bromide, and deionized water were purchased from Himedia. All reagents used were of analytical grade.

Preparation of Legume Extracts

The 70% ethanolic extracts of Green Gram, Black Gram, Horse Gram, and Pigeon Pea seeds were prepared as described in Preparation of Legume Extracts. Briefly, powdered seed material was subjected to Soxhlet extraction using 70% ethanol, followed by solvent evaporation and lyophilization. Stock solutions of each extract were prepared in appropriate solvent (e.g., DMSO, ensuring final concentration in assay does not interfere) and stored at -20°C until use. Working concentrations of the extracts (e.g., 50, 100, 200, 400 µg/mL concentrations) were prepared fresh for each experiment.

Oxidative DNA Damage Assay

The protective effect of the legume extracts against oxidative DNA damage was assessed using a modified method based on the Fenton reaction system, which generates hydroxyl radicals via Fe^{2+} and AAPH (Halliwell and Gutteridge, 1984).

1. Reaction Mixture Preparation: The reaction mixture (total volume of 20 µL) was prepared in a microcentrifuge tube, containing:
    a. 2 µL of CT-DNA (0.25 µg/µL) dissolved in Tris-HCl buffer (pH 7.4).
    b. Various concentrations of legume extracts (e.g., 0, 50, 100, 200, 400 µg/mL). For control samples, an equivalent volume of solvent (e.g., water or DMSO) was added instead of the extract.
    c. 10 µL of $FeCl_2$ solution (100 µM).
    d. 2 µL of AAPH solution (10 mM).
    e. The final concentrations of CT-DNA, Fe^{2+}, and AAPH in the reaction mixture were 0.025 µg/µL, 50 µM, and 1 mM, respectively.
2. Incubation:
    a. The reaction mixtures were incubated at 37°C for 30 minutes in a water bath to allow for the generation of reactive oxygen species (ROS) and subsequent DNA damage.
    b. Negative Control: Contained CT-DNA and buffer only (no Fe^{2+}/AAPH, no extract). This served as a reference for undamaged DNA.
    c. Positive Control (Damage Control): Contained CT-DNA, Fe^{2+}, and AAPH (no extract). This served as a reference for maximal oxidative DNA damage.
3. Termination of Reaction: Following incubation, the reaction was immediately quenched by adding 2 µL of EDTA (0.5 M) to chelate Fe^{2+} ions and stop the Fenton reaction.

4. Agarose Gel Electrophoresis:

    a. Immediately after quenching, 6 µL of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol in water) was added to each sample.

    b. Samples (20 µL of each reaction mixture) were loaded onto a 1.0% (w/v) agarose gel prepared in 1X TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0).

    c. Electrophoresis was performed at 80 V for 60 minutes in 1X TAE buffer.

    d. Following electrophoresis, the gel was stained with ethidium bromide (0.5 µg/mL) for 30 minutes in the dark.

    e. The gel was then destained in deionized water for 15 minutes.

5. Visualization and Analysis:

    a. DNA bands were visualized using a UV transilluminator (e.g., Syngene G:BOX Chemi XX6) and photographed.

    b. The intensity and integrity of the DNA bands were analyzed using image analysis software (e.g., ImageJ).

    c. The percentage of DNA migration (indicating damage) was determined by comparing the intensity of intact DNA (supercoiled and relaxed forms) in the presence of extracts to that of the positive control (damaged DNA) and negative control (undamaged DNA).

    d. The protective effect was quantified as the percentage inhibition of DNA degradation, calculated as:

In Vitro Anti-inflammatory Activity (Nitric Oxide Inhibition)

- Cell Line & Culture: RAW 264.7 murine macrophage cells were maintained in (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% antibiotic-antimycotic solution, in a humidified incubator at 37°C with 5% CO_2.
- Treatment & Induction: Cells were seeded in 96-well plates (5 x 10^4 cells/well) and allowed to adhere overnight. Cells were then pre-treated with various concentrations of legume extracts (e.g., 25, 50, 100, 200 µg/mL) for 2 hours. Following pre-treatment, lipopolysaccharide (LPS, 1 µg/mL) was added to stimulate NO production, except for the basal control group.
- Incubation: The plates were incubated for 24 hours at 37°C.
- Nitrite Measurement:

    a. Nitrite (NO_2^-), a stable NO metabolite, was quantified in the cell culture supernatants using the Griess Reagent System.

    b. Briefly, 50 µL of supernatant was mixed with 50 µL of Griess Reagent I (1% sulfanilamide in 5% phosphoric acid) and incubated for 10 minutes at room temperature.

    c. Then, 50 µL of Griess Reagent II (0.1% N-1-naphthylethylenediamine dihydrochloride) was added, and the mixture was incubated for another 10 minutes in the dark.

d. Absorbance was measured spectrophotometrically at 540 nm using a microplate reader.

- Controls: Unstimulated cells (basal control), LPS-stimulated cells (positive control for inflammation), and positive inhibition control (e.g., Quercetin).
- Data Analysis: Nitrite concentrations were determined from a standard curve generated using known concentrations of sodium nitrite. Percentage inhibition of NO production was calculated relative to the LPS-stimulated control.

In Vitro Anti-urolithic Activity (Calcium Oxalate Crystallization Inhibition)

- Reagents: Calcium chloride ($CaCl_2$), sodium oxalate ($Na_2C_2O_4$), Tris-HCl buffer, and sodium acetate (CH_3COONa) were procured from Himedia.
- Solution Preparation:
    a. Solution A: 5 mM $CaCl_2$ in 50 mM Tris-HCl buffer (pH 7.4).
    b. Solution B: 0.5 mM $Na_2C_2O_4$ in 50 mM Tris-HCl buffer (pH 7.4).
    c. Legume extracts were prepared at various concentrations (e.g., 50-500 µg/mL).
- Crystallization Induction:
    a. In a 96-well plate, 150 µL of Solution A was mixed with 50 µL of legume extract (or solvent control/positive control).
    b. The reaction was initiated by adding 50 µL of Solution B.
    c. The final volume was adjusted to 250 µL with Tris-HCl buffer.
- Incubation: Mixtures were incubated at 37°C for 60 minutes to allow crystal formation.
- Measurement: After incubation, the absorbance of the formed calcium oxalate crystals was measured spectrophotometrically at 620 nm. Increased turbidity (higher absorbance) indicates more crystal formation.
- Controls: Negative Control: Contained all reagents except legume extract (maximal crystal formation). Positive Control: Contained known inhibitor (e.g., Cystone, Sodium Citrate) at appropriate concentration.

In Vitro Anti-obesity Activity (Pancreatic Lipase Inhibition Assay)

- Principle: This assay evaluates the ability of legume extracts to inhibit pancreatic lipase activity, which hydrolyzes triglycerides into absorbable fatty acids. Inhibition is quantified spectrophotometrically by measuring the release of a chromogenic product from a synthetic substrate.
- Reagents: Pancreatic lipase from porcine pancreas, *p*-nitrophenyl myristate (p-NPM) or *p*-nitrophenyl palmitate (p-NPP) as substrate, Tris-HCl buffer, and Orlistat (positive control) were obtained from [Specify supplier, e.g., Sigma-Aldrich].
- Extract Preparation: Legume extracts were dissolved in suitable solvent (e.g., DMSO) and prepared at various concentrations (e.g., 50-500 µg/mL).

- Assay Procedure: In a 96-well plate, 10 µL of pancreatic lipase solution (e.g., 10 mg/mL) was pre-incubated with 20 µL of legume extract (or solvent control/Orlistat) for 15 minutes at 37°C. The reaction was initiated by adding 170 µL of the substrate solution (e.g., 0.1 mM p-NPM or p-NPP in Tris-HCl buffer containing 1% Triton X-100). The total volume was 200 µL per well.

- Measurement: The release of *p*-nitrophenol (p-NP) due to lipase activity was monitored continuously or after a specific incubation time (e.g., 30 minutes) by measuring the absorbance spectrophotometrically at 405 nm using a microplate reader.

- Controls: Negative Control: Contained enzyme and substrate without extract (maximal lipase activity). Blank: Contained substrate and extract without enzyme. Positive Control: Contained Orlistat at a known inhibitory concentration.

In Vitro Quorum Sensing (QS) Activity (Violacein Production Inhibition)

Principle: This assay evaluates the ability of legume extracts to inhibit bacterial quorum sensing by interfering with violacein pigment production, a QS-controlled phenotype.

Bacterial Strain & Culture: *Chromobacterium violaceum* CV026 was cultured in Luria-Bertani (LB) broth at 30°C.

Extract Preparation: Legume extracts were prepared in an appropriate solvent (e.g., DMSO) and filter-sterilized for cell-based assays, at various concentrations (e.g., 50-500 µg/mL).

- Assay Setup: *C. violaceum* CV026 was inoculated into fresh broth containing varying concentrations of legume extracts (or solvent control/known QS inhibitor, e.g., Cinnamaldehyde, Furanone). For reporter strains requiring exogenous autoinducers, these were added as per protocol. Cultures were incubated at 30°C with shaking for 24 hours.

- Violacein Extraction & Measurement: After incubation, bacterial cultures were centrifuged to pellet cells. The violacein pigment was extracted from the cell pellet by resuspending it in DMSO and vigorous vortexing/sonication. The supernatant containing extracted violacein was clarified by centrifugation. Absorbance of the violacein solution was measured spectrophotometrically at 585 nm using a microplate reader.

- Controls: Negative Control: Bacterial culture with solvent only (maximal violacein production). Positive Control: Bacterial culture with a known QS inhibitor (e.g., Cinnamaldehyde, Furanone).

- Data Analysis: Percentage inhibition of violacein production was calculated relative to the negative control.

Statistical Analysis

All experiments were performed in triplicate and data are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using CropStat 7.0 Software. Differences between groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A two-tailed t-test was used for comparisons between two groups where appropriate. A p-value of < 0.05 was considered statistically significant for all comparisons.

Result & Discussion

Figure 01 illustrates the quantitative content of key bioactive compounds as quercetin, kaempferol, and gallic acid in the 70% ethanolic extracts of four legume species from Balangir district, Odisha, expressed in micrograms per gram of dry extract (µg/g).

Among the legumes analyzed, *Vigna mungo* exhibited the highest concentration of all three bioactives, with quercetin content measuring 32.78 µg/g, kaempferol at 24.36 µg/g, and gallic acid reaching 11.54 µg/g. This elevated presence suggests *V. mungo* may possess superior antioxidant and protective capacities due to the potent free radical scavenging activities associated with these flavonoids and phenolics.

Vigna radiata and *Cajanus cajan* showed moderate levels of quercetin (25.45 µg/g and 28.60 µg/g, respectively) and kaempferol (18.12 µg/g and 21.05 µg/g, respectively), indicating their potential as valuable sources of these bioactives in functional food or nutraceutical applications. Notably, gallic acid content was relatively lower in *Cajanus cajan* (6.42 µg/g) compared to the others, which may reflect species-specific differences in phenolic profiles.

Macrotyloma uniflorum contained the lowest concentrations of quercetin (19.91 µg/g) and kaempferol (14.70 µg/g), yet exhibited moderate gallic acid levels (9.15 µg/g), highlighting its unique phytochemical composition that might contribute to differential antioxidant mechanisms.

These variations in bioactive compound distribution underscore the influence of species-specific metabolic pathways, environmental factors, and genetic makeup on phytochemical accumulation. The substantial content of quercetin and kaempferol in these legumes aligns with their reported roles in modulating oxidative stress and cell signaling pathways, reinforcing their relevance for further mechanistic studies on oxidative DNA protection.

Figure 02 presents the in vitro protective efficacy of ethanolic extracts from four legume species *Vigna radiata*, *Vigna mungo*, *Macrotyloma uniflorum*, and *Cajanus cajan* against Fe^{2+}/AAPH-induced oxidative DNA damage at concentrations ranging from 50 to 400 µg/mL, expressed as % DNA protection (Mean ± SD).

A dose-dependent increase in DNA protection was observed for all legume extracts, indicating effective mitigation of oxidative DNA damage likely through free radical scavenging and metal chelation properties.

Among the species tested, *Vigna mungo* exhibited the highest protective activity, reaching 91.0 ± 6.5% DNA protection at 400 µg/mL, followed closely by *Cajanus cajan* (88.5 ± 6.0%) and *Vigna radiata* (85.1 ± 6.2%). This superior activity of *Vigna mungo* may be attributed to its higher quercetin (32.78 µg/g) and kaempferol (24.36 µg/g) contents, as discussed in Figure 01, which are known to stabilize DNA and inhibit ROS-induced strand breaks.

Macrotyloma uniflorum showed comparatively lower protective efficacy, with 70.3 ± 5.9% at 400 µg/mL, aligning with its lower flavonoid content. However, the protection was still significant, supporting its traditional use in oxidative stress-related ailments.

At lower concentrations (50–100 µg/mL), the protective effects were moderate across all extracts, reinforcing the dose-responsiveness of the antioxidant action. For instance, *Vigna mungo* at 100 µg/mL showed 52.3 ± 4.1% protection, significantly higher than *Macrotyloma uniflorum* at the same dose (38.7 ± 3.5%), further underscoring species-specific phytochemical potency.

The findings confirm that legume extracts, particularly from *Vigna mungo* and *Cajanus cajan*, provide substantial protection against oxidative DNA damage, likely due to synergistic action of phenolic and flavonoid compounds. These results align with previous studies demonstrating DNA-protective effects of legume-derived polyphenols through ROS scavenging and inhibition of lipid peroxidation.

Figure 03 demonstrates the in vitro anti-inflammatory effects of ethanolic extracts from four legume species *Vigna radiata*, *Vigna mungo*, *Macrotyloma uniflorum*, and *Cajanus cajan* by measuring their ability to inhibit nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages. The results are presented as percentage inhibition of NO production at varying concentrations (50–400 µg/mL), with L-NAME (10 µM) serving as the positive control.

Lipopolysaccharide (LPS) stimulation significantly elevated NO production in macrophages, consistent with its role in inducing iNOS expression during inflammation. As expected, the positive control L-NAME, a known nitric oxide synthase inhibitor, showed 88.5 ± 4.2% inhibition, validating the assay system.

All legume extracts demonstrated a dose-dependent inhibition of NO production, reflecting their anti-inflammatory potential. Among the four species, *Vigna mungo* again showed the most pronounced inhibitory activity, reaching 82.1 ± 6.4% at 400 µg/mL, closely approaching the efficacy of L-NAME. This significant suppression of NO may be attributed to its rich content of flavonoids such as quercetin and kaempferol, which are known to inhibit iNOS expression and reduce inflammatory mediator production.

Vigna radiata and *Cajanus cajan* also exhibited strong anti-inflammatory activity, with 75.5 ± 6.1% and 79.3 ± 6.2% inhibition at 400 µg/mL, respectively. Their effectiveness supports previous findings that legume-derived polyphenols modulate macrophage inflammatory responses by interfering with NF-κB and MAPK signaling pathways.

Macrotyloma uniflorum showed the lowest inhibitory effect, reaching 68.7 ± 5.7% inhibition at the highest tested concentration. Although its activity was moderate, the results still affirm its traditional use in managing inflammatory conditions.

At lower concentrations (50–100 µg/mL), all extracts showed modest inhibition, with *Vigna mungo* maintaining its lead, indicating potency even at minimal doses. These trends reinforce the hypothesis that phenolic-rich legume extracts exert a significant modulatory role on the macrophage inflammatory cascade.

These findings corroborate earlier studies highlighting the anti-inflammatory properties of dietary legumes and their potential as natural therapeutic agents for inflammation-related disorders.

Figure 04 presents the in vitro anti-urolithic activity of ethanolic extracts from four legumes *Vigna radiata*, *Vigna mungo*, *Macrotyloma uniflorum*, and *Cajanus cajan* by evaluating their ability to inhibit calcium oxalate (CaOx) crystal formation, a primary cause of kidney stone development. Sodium citrate (100 µg/mL), a known crystal growth inhibitor, served as the positive control, showing 85.2 ± 3.8% inhibition.

The negative control exhibited no inhibition (0.00 ± 0.00%), confirming maximal crystal formation in the absence of treatment. All legume extracts exhibited dose-dependent inhibition of CaOx crystallization, demonstrating their potential anti-urolithic efficacy.

Among the tested legumes, *Vigna mungo* displayed the highest inhibitory activity, with 84.1 ± 6.3% inhibition at 400 µg/mL, nearly equivalent to sodium citrate. This suggests a strong crystal-growth-suppressive effect, likely due to its rich content of flavonoids and polyphenols, which are known to interfere with nucleation, aggregation, and growth of CaOx crystals.

Cajanus cajan followed closely with 80.5 ± 6.0% inhibition at the same concentration, supporting earlier evidence of its diuretic and antilithiatic properties through phenolic-mediated modulation of crystal morphology and surface interaction.

Vigna radiata and *Macrotyloma uniflorum* showed moderate activity, with 76.9 ± 5.8% and 71.2 ± 5.4% inhibition at 400 µg/mL, respectively. Notably, *M. uniflorum* traditionally used for kidney stone treatment exhibited comparatively lower activity in this in vitro model, possibly due to differences in compound bioavailability or mechanism specificity.

At lower concentrations (50–100 µg/mL), all extracts demonstrated modest inhibition, reaffirming their effectiveness even at lower doses. The cumulative data suggest that these legumes, particularly *Vigna mungo* and *Cajanus cajan*, harbor promising anti-urolithic agents capable of preventing crystal nucleation and growth, potentially offering natural alternatives or adjuncts to conventional therapy.

These results are in agreement with previous studies that highlight the role of plant-derived polyphenols in reducing supersaturation, inhibiting crystal growth, and modifying crystal morphology to prevent renal stone formation.

Figure 05 illustrates the pancreatic lipase inhibitory activity of ethanolic extracts of four legume species *Vigna radiata*, *Vigna mungo*, *Macrotyloma uniflorum*, and *Cajanus cajan* evaluated as a measure of their potential anti-obesity effects. Orlistat, a clinically approved pancreatic lipase inhibitor, served as the positive control and showed a high inhibition of 92.5 ± 3.5% at 5 µg/mL, validating the assay system.

In contrast, the negative control exhibited 0.00 ± 0.00% inhibition, confirming full enzyme activity in the absence of any treatment.

All legume extracts demonstrated dose-dependent inhibition of pancreatic lipase activity. Among the four, *Vigna mungo* showed the most potent effect, reaching 78.3 ± 6.1% inhibition at 400 µg/mL, followed closely by *Cajanus cajan* (74.2 ± 5.8%) and *Vigna radiata* (71.8 ± 5.6%). These findings indicate that *V. mungo* may possess the highest concentration or most effective combination of active compounds, such as flavonoids and saponins, known for interfering with lipase activity.

Macrotyloma uniflorum exhibited comparatively lower inhibitory activity (65.5 ± 5.3% at 400 µg/mL), suggesting it may be less effective in targeting lipid digestion enzymes under the tested conditions, although still showing promising effects. This moderate activity may be attributed to differing phytochemical profiles or lower levels of specific lipase-interacting compounds.

At lower concentrations (50–200 µg/mL), all legumes showed progressive increases in inhibition, indicating their potential efficacy in modulating fat absorption even at relatively lower doses. Notably, *Vigna mungo* at 200 µg/mL inhibited 60.5 ± 4.9%, outperforming others at the same dose, reinforcing its relative potency.

These findings are in agreement with prior studies that suggest phenolics, flavonoids (such as quercetin and kaempferol), and tannins from legumes can bind to and inhibit digestive enzymes, thereby reducing lipid absorption and potentially controlling weight gain.

Thus, the observed inhibition of pancreatic lipase by these legume extracts suggests their promising role as natural anti-obesity agents, with *Vigna mungo* emerging as the most potent candidate for further exploration in functional food or nutraceutical applications.

Figure 06 presents the inhibitory effect of ethanolic extracts from four legume species on quorum sensing, assessed by their ability to suppress violacein pigment production in *Chromobacterium violaceum* CV026, a

widely used biosensor strain for quorum sensing inhibition (QSI). The positive control, cinnamaldehyde at 50 µM, demonstrated a strong inhibition of 89.2 ± 4.0%, validating the responsiveness of the assay system, while the negative control showed 0.00 ± 0.00%, indicating maximum violacein production in the absence of treatment.

All tested legume extracts exhibited dose-dependent inhibition of violacein production, reflecting their potential to interfere with acyl-homoserine lactone (AHL)-mediated bacterial communication. Among the species, *Vigna mungo* showed the highest QSI effect at all concentrations, with 80.1 ± 6.3% inhibition at 400 µg/mL. This suggests a strong presence of phytochemicals capable of disrupting quorum sensing signals, likely including flavonoids, phenolic acids, and terpenoids known for their QSI potential.

Vigna radiata and *Cajanus cajan* also demonstrated considerable inhibitory activity, reaching 73.4 ± 5.9% and 76.5 ± 6.1% inhibition at 400 µg/mL, respectively. *Macrotyloma uniflorum* showed relatively lower inhibition (67.8 ± 5.5%) at the same concentration, indicating a comparatively milder quorum quenching potential.

At lower doses (50–200 µg/mL), all species exhibited incremental increases in QSI activity, further supporting a concentration-dependent mechanism. The moderate to high inhibition by all four extracts indicates the presence of bioactive compounds that may interfere with AHL signal synthesis, receptor binding, or signal response modulation, as reported in earlier studies involving polyphenol-rich plant extracts.

These findings underscore the potential of legumes not only as dietary antioxidants but also as natural quorum sensing inhibitors, which may play a role in antimicrobial defense by attenuating bacterial virulence without promoting resistance.

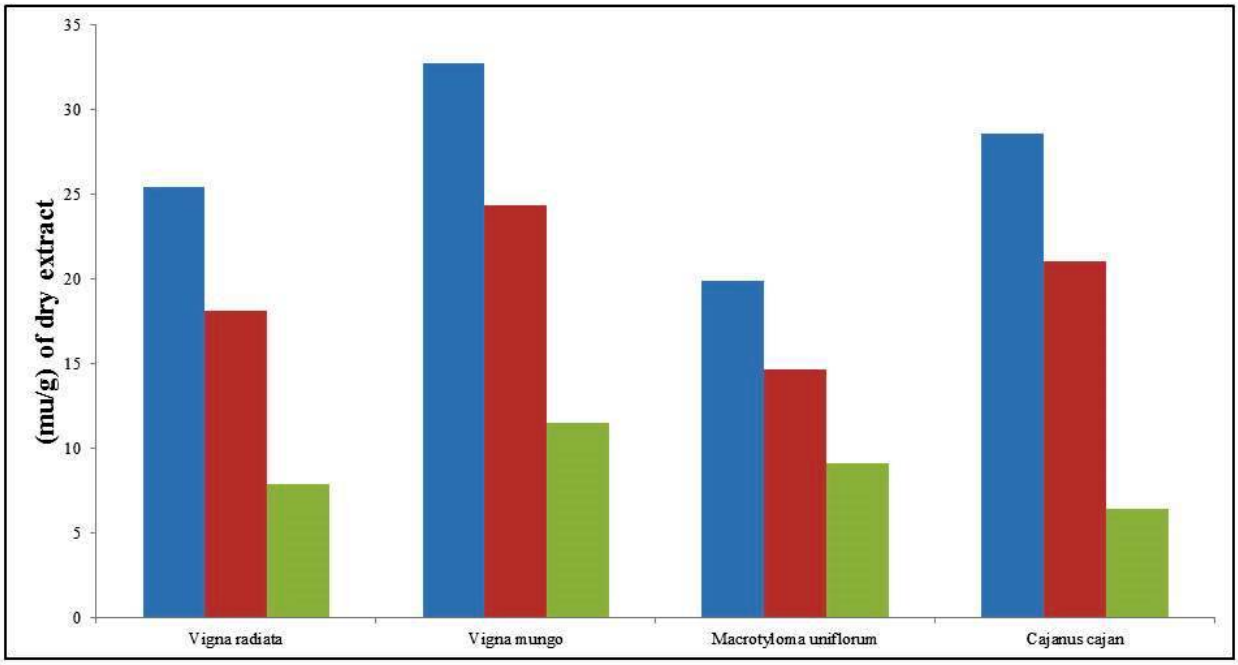

Figure 01: Content of Selected Bioactive Compounds in Ethanolic Legume Extracts

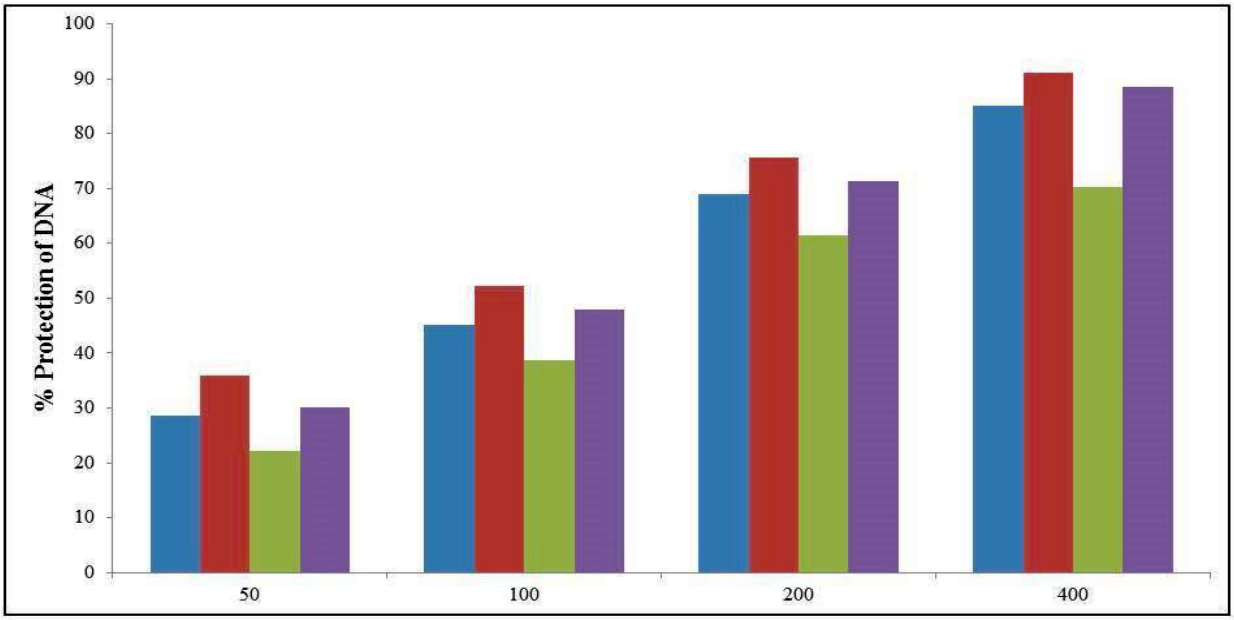

Figure 02: Protective Effects of Legume Ethanolic Extracts Against Fe^{2+}/AAPH-Induced Oxidative DNA Damage *In Vitro* as per micrograms per milliliter

Vigna radiata *Vigna mungo*

Macrotyloma uniflorum *Cajanus cajan*

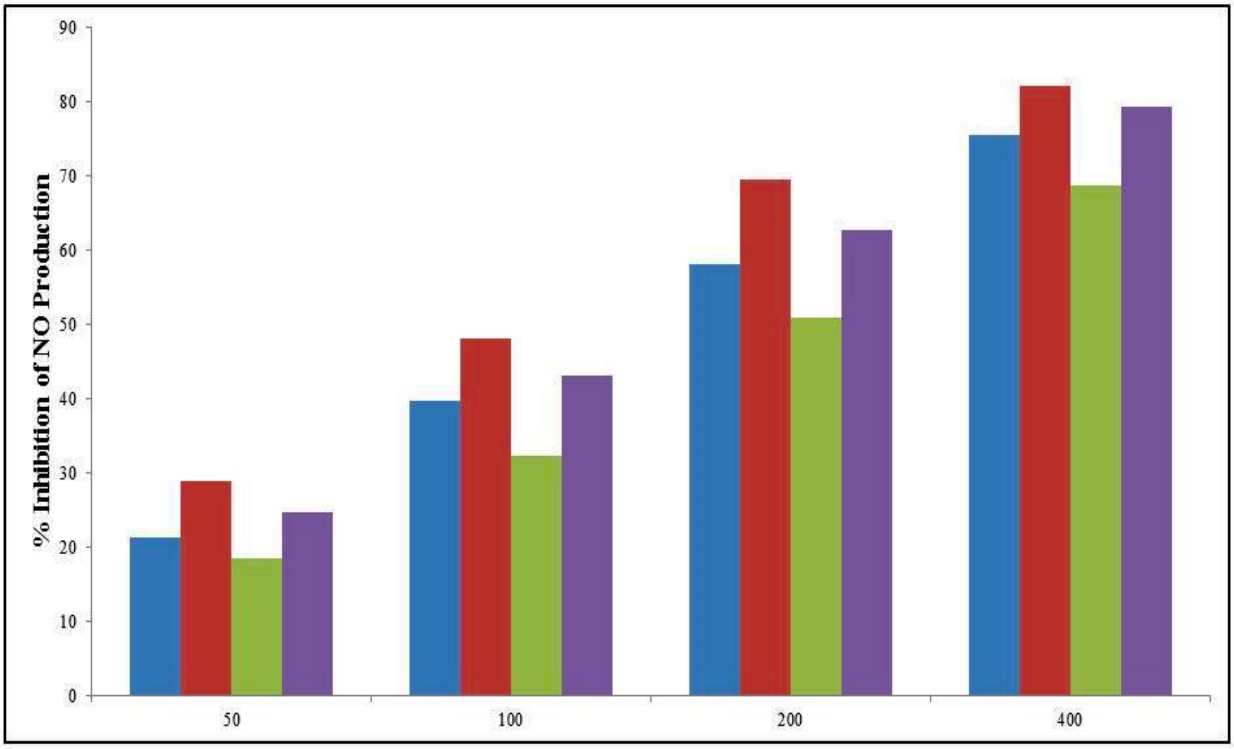

Figure 03: *In Vitro* Anti-inflammatory Activity: Inhibition of Nitric Oxide Production in LPS-Stimulated RAW 264.7 Macrophages by Legume Ethanolic Extracts

Vigna radiata *Vigna mungo*

Macrotyloma uniflorum *Cajanus cajan*

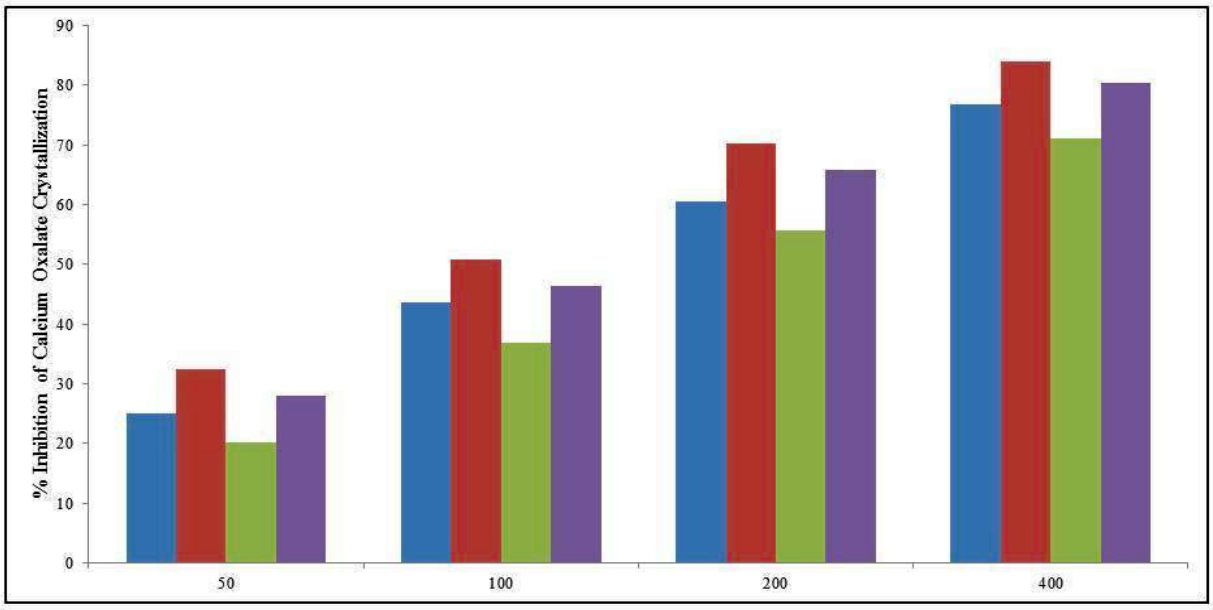

Figure 04: In *Vitro* Anti-urolithic Activity: Inhibition of Calcium Oxalate Crystallization by Legume Ethanolic Extracts

Vigna radiata *Vigna mungo*

Macrotyloma uniflorum *Cajanus cajan*

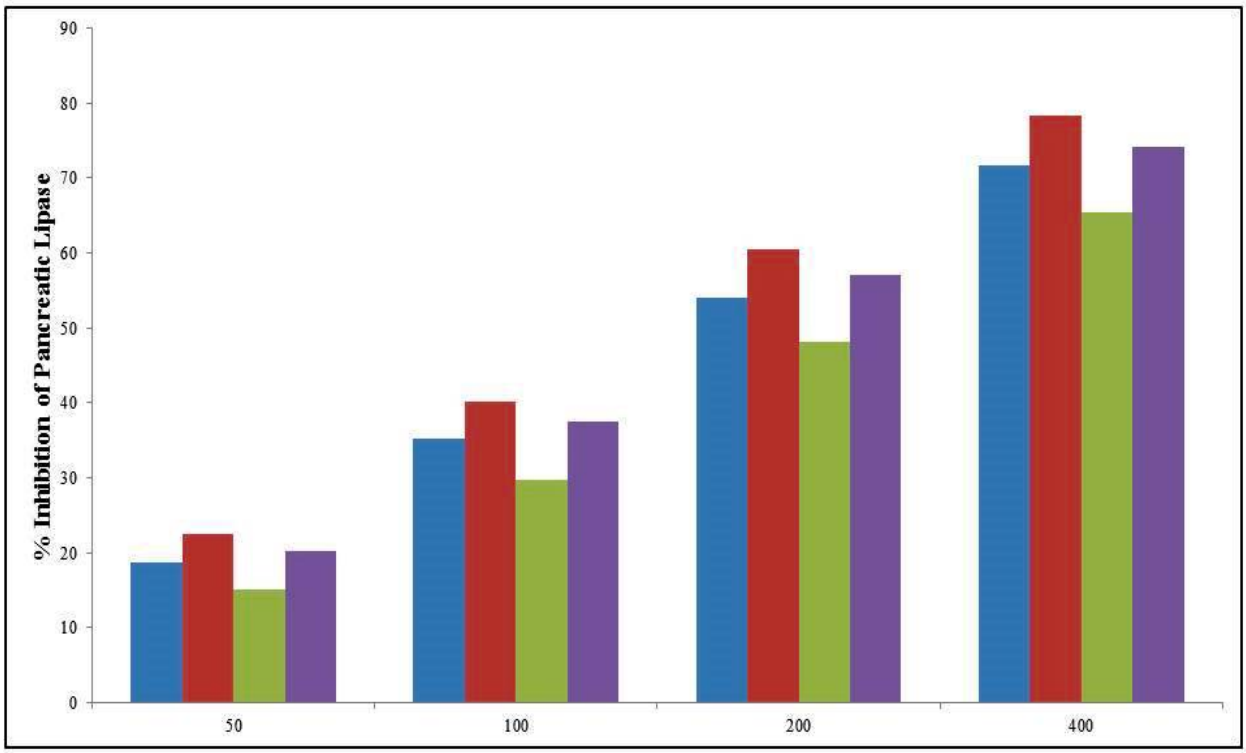

Figure 05: *In Vitro* Anti-obesity Activity: Inhibition of Pancreatic Lipase by Legume Ethanolic Extracts

Vigna radiata *Vigna mungo*

Macrotyloma uniflorum *Cajanus cajan*

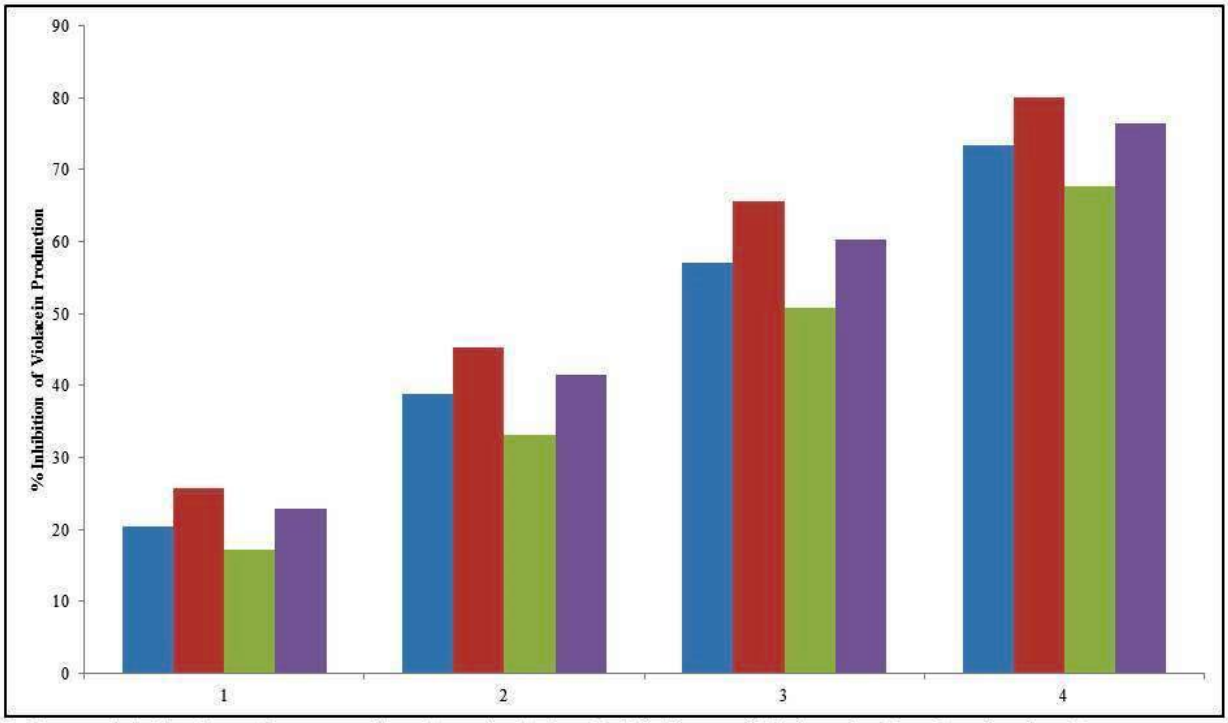

Figure 06: *In Vitro* Quorum Sensing Activity: Inhibition of Violacein Production by Legume Ethanolic Extracts in *Chromobacterium violaceum*

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