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"A Comprehensive Analysis of Radical Scavenging Potentials and Metal Ion Interactions"- Exploring the Potent Antioxidant Capacities of *Zea mays* (Maize) Leaves

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ABSTRACT
Introduction: *Reactive oxygen species (ROS) generated during biocellular reactions play a crucial role in cell growth and redox reactions. If the accumulation of free radicals exceeds the body's counteraction capacity, it leads to the creation of harmful ROS, damaging cellular components. Antioxidants mitigate the impact of ROS by converting them into harmless forms, yet oxidative damage accumulates throughout the life cycle, contributing to age-dependent diseases.* ***Objective****: This study investigates the antioxidant potential of Zea mays (maize) leaves, emphasizing their radical scavenging effects against various oxidant moieties. The research includes the evaluation of metal ion chelating and reducing properties.* ***Methodology:*** *In this study, Zea mays leaves from the 10th day of growth displayed the highest antioxidant content. Leaf extracts were prepared using water, methanol, and chloroform, and their antioxidant potential was assessed against radicals such as DPPH, ABTS, hydrogen peroxide, hydroxyl radicals, superoxide, and nitric oxide. Additionally, the study evaluated metal ion reducing and chelating properties, with the methanolic extract exhibiting the highest efficacy.* ***Results:*** *The findings highlight the robust antioxidant activity of Zea mays leaves, especially the methanolic extract consistently demonstrated the most significant scavenging effects, against a range of oxidant moieties. These results contribute valuable insights into the potential health benefits of Zea mays and support its traditional medicinal use.* ***Conclusion:*** *With the methanolic extract generally outperforming the aqueous and chloroform extracts. This solvent-dependent variation in antioxidant activity highlights the importance of solvent selection in extracting bioactive compounds from medicinal plants.*

Keywords: *Antioxidants, Free radicals, Zea mays, Radical Scavenging.*

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I. INTRODUCTION

Reactive oxygen species emerge as a by-product in biocellular reactions, playing a crucial role in the regulation of cell growth and redox reactions.[1,2] If the accumulation of free radicals exceeds the body's ability to counteract them, these radicals will target cellular components, leading to the creation of reactive oxygen species that can damage corresponding cellular elements.[3] Antioxidants counteract the harmful impact of reactive oxygen species by donating electrons, converting them into harmless forms. Whether originating externally or internally, antioxidants prevent damaging chain reactions that could affect various cellular components. Despite the body's antioxidant system, oxidative damage accumulates throughout the life cycle, potentially leading to cell death. Accumulated oxidative damage is proposed to be pivotal in age-dependent diseases like atherosclerosis, arthritis, neurodegenerative disorders, and cancer.[4] Growing evidence suggests that reactive oxygen species act as second messengers in intracellular signalling cascades, contributing to the oncogenic phenotype in cancer cells. ROS promotes tumorigenic properties by enhancing cell proliferation, survival, migration, and inducing DNA damage, initiating and sustaining tumor progression.[5] Medicinal plants are an expanding reservoir of remedies, known for their minimal or absent side effects. In India's rich biodiversity, identifying and scientifically validating sources of valuable pharmaco-phytochemicals can offer solutions to various serious diseases. Developing traditional medicinal systems with a commitment to safety, efficacy, and quality not only safeguards cultural heritage but also promotes the rational use of natural products in healthcare. *Zea mays* is commonly known as maize and belongs to the family Gramineae. This study aimed to investigate the radical scavenging effect of *Zea mays* against six distinct radical or oxidative species known to cause damage to cells and biomolecules. Additionally, assessments were conducted for metal ion chelating and reducing properties.

II. MATERIALS AND METHODS

The 10th-day *Zea mays* plant displayed the highest antioxidant content, leading to its selection for further investigation. In this study, leaf extracts were prepared using various solvents (water, methanol and chloroform) varying in their polarity, and the antioxidant potential was assessed through diverse radicals and oxidants. Molecular mechanisms were studied using reducing and chelating assays.

EVALUATION OF RADICAL SCAVENGING EFFECTS OF *Zea mays* LEAF EXTRACTS

Zea mays leaf extracts were examined for their capability to mitigate the impact of various oxidant moieties. The free radicals used for the study are DPPH, ABTS, Hydrogen peroxide, Hydroxyl radical, Superoxide, and Nitric oxide.

PREPARATION OF PLANT EXTRACTS: Fresh *Zea mays* leaves (1g) from the 10th-day plant were homogenized in 1 ml of methanol or chloroform. After centrifugation, the supernatant was dried at 60°C, yielding a residue. This residue was weighed and dissolved in DMSO to attain a final concentration of 20mg in 5µl. Aqueous extracts were prepared fresh for each experiment.

(i). DPPH SCAVENGING EFFECT: Plant extracts were assessed for antioxidant activity using the DPPH radical scavenging method following Mensor et al.'s (2001)[6] protocol, which gauges both electron or hydrogen atom-donating properties and the reaction rate of antioxidants with free radicals. The spectrophotometric method utilizes the 2,2-diphenyl-2-picrylhydrazyl free radical (DPPH•), showing peak absorbance at approximately 515 nm in methanol. The reduction in absorbance upon the addition of an antioxidant is directly proportional to the substance's concentration and antioxidant efficacy.

(ii). ABTS SCAVENGING EFFECT: Shirwaikar et al. (2006)[7] introduced an improved ABTS radical cation de-colourization assay method. This method entails the direct creation of the blue/green ABS●+ chromophore through the reaction between ABTS and ammonium persulfate, exhibiting absorption maxima at 745nm. The addition of antioxidants to the pre-formed radical cation leads to the reduction of ABTS, and the reaction time varies based on the antioxidant activity, concentration, and duration.

(iii). HYDROGEN PEROXIDE SCAVENGING EFFECT: The ability of *Zea mays* leaf extracts to scavenge H2O2 was determined by the method proposed by Ruch *et al.* (1989)[8]. The scavenging activity against H2O2 was quantified by spectrophotometrically measuring the reduction in absorbance at 230nm.

(iv). HYDROXYL RADICAL SCAVENGING EFFECT: The hydroxyl radical scavenging capacity was assessed based on the method of Elizabeth and Rao (1990)[9]. Hydroxyl radical attack on deoxyribose leads to TBARS formation, quantifiable through spectrophotometry. The assessment of hydroxyl radicals is achieved by oxidative degradation of 2'-deoxyribose, with malonaldehyde (MDA) quantified through condensation with TBA

(v). SUPEROXIDE SCAVENGING EFFECT: The extent of suppression of superoxide generation was evaluated using the Winterbourn et al. (1975)[10] method. The ability of leaf extracts to impede the formation of nitro blue tetrazolium formazon derived from the superoxide ion was measured spectrophotometrically.

(vi). NITRIC OXIDE SCAVENGING EFFECT: The efficiency of *Zea mays* leaf extracts in inhibiting the in vitro generation of nitric oxide was examined using the method outlined by Green et al. (1982)[11]. Nitric oxide was spontaneously generated from sodium nitroprusside in aqueous solution at physiological pH. The interaction with oxygen produced nitrite ions, which were quantified spectrophotometrically using Greiss reagent.

ASSAY OF METAL ION REDUCING and CHELATING PROPERTIES of *ZEA MAYS* LEAF EXTRACTS

The reducing property was assessed using the modified method of Oyaizu (1986)[12]. Potassium ferricyanide and ferric chloride, when reduced by an antioxidant, formed a colored complex that was measured spectrophotometrically. *Zea mays* leaf extracts' chelating ability was assessed by examining spectral changes following the method of Brown et al. (1998)[13]. Ferrous chloride formed a complex with potassium hexacyanoferrate (potassium ferricyanide), resulting in the colored complex ferrous ferricyanide, Turnbull's blue, with maximum absorption. The introduction of an antioxidant to the reaction mixture, under the same conditions, decreased the absorption.

III. RESULTS

The radical scavenging effects of *Zea mays* leaf extracts were assessed against various oxidant moieties, including DPPH, ABTS, H_2O_2, Hydroxyl radical, Superoxide, and Nitricoxide radicals. Results revealed significant DPPH radical scavenging activity, with the methanolic extract showing the highest effect, followed by the aqueous and chloroform extracts. The ABTS radical scavenging activity varied, with the methanolic extract being the most effective and the chloroform extract the least. All three extracts demonstrated strong scavenging effects on H2O2, with the methanolic extract exhibiting the highest efficacy. Results of the percent extent of DPPH, ABTS, and H_2O_2 scavenging by the *Zea mays* leaf extracts are presented in Figure 1.

Hydroxyl radical scavenging activity was measured using 2'-deoxyribose as a substrate, showing effective protection against damage, particularly with the methanolic extract. the values are presented in Figure 2. The extracts demonstrated good scavenging of superoxide radicals (SO·), with the methanolic extract exhibiting the highest inhibitory effect. Moderate reduction in nitric oxide (NO) generation was observed with all extracts. The percentage inhibition of SO• and NO generation in the presence of the leaf extracts is presented in Figure 3.

Further analyses revealed that the methanolic extract exhibited the highest reducing and chelating activities, as determined by spectrophotometric methods. Overall, *Zea mays* leaf extracts demonstrated potent antioxidant properties, with the methanolic extract consistently showing the most significant effects. The results of reducing and chelating activities of *Zea mays* leaf extracts are presented in Figure 4.

IV. DISCUSSION

Medicinal plants, rich in antioxidants, help neutralize free radicals, quench oxygen, and decompose peroxides. Antioxidant compounds can be isolated and used in the treatment of free radical-related disorders[14]. Naturally occurring dietary antioxidants found in medicinal plants can serve as alternatives to chemically designed anticancer agents[15]. Many researchers globally are seeing medicinal plants and their significant action against the damage caused by oxidative radicals. Many plants including *Zea mays* are yet to be brought into the limelight hence this study is a systematic approach to analyse the radical scavenging, reducing, and chelating properties of the *Zea mays* plant. The study revealed that the leaves reached their highest antioxidant levels on the 10th day of growth compared to the 5th, 15th, 20th, 25th, and 30th days of the growth period.

The radical scavengers are often associated with the termination of free radical propagation in biological systems[16]. In the present study, *Zea mays* leaves were extracted into three different solvents with different polarities and were tested for their ability to scavenge free radicals in H_2O_2-induced oxidative damaged systems. The solvents used were water, methanol, and chloroform. Numerous antioxidant methods have been developed to evaluate the antioxidant activity and to explain how antioxidants function, the different assays namely, DPPH scavenging, ABTS scavenging, deoxyribose degradation, reducing power, and chelating power, the most commonly accepted ones[17,18] have been used in the present investigation.

In the investigation of *Limoniastrum monopetalum*, the methanolic extract stood out with the highest DPPH scavenging activity among ten different extracts[19]. Similarly, the methanolic extract of *Tanacetum densum subsp*. Amani demonstrated superior DPPH free radical scavenging compared to other subspecies[20]. *Rubus ulmifolius*, highlighted in a study by Dall'Acqua et al. (2008)[21], exhibited notable antioxidant activity through effective DPPH scavenging. The recurring findings across these studies underscore the reliability of DPPH scavenging as a strong indicator for assessing the antioxidant potential of plant extracts. Our study contributes to this evidence by highlighting the robust antioxidant activity in *Zea mays* leaves, particularly with methanol extraction.

The results indicated that the methanolic extract exhibited more effective ABTS scavenging compared to the aqueous and chloroform extracts. This aligns with findings from other studies, such as the strong ABTS scavenging ability of the chloroform extract of *Chromolaena odorata* leaves[22] and the active ABTS scavenging effects of *Moldavian balm* extracts[23]. *Guava* leaf extracts demonstrated increased ABTS scavenging with higher concentrations, while *Annona muricuta* showed maximum quenching of ABTS among three Annona species[24,25].

The assessment of hydrogen peroxide scavenging activity is a valuable method for gauging the antioxidant capacity of substances in reducing pro-oxidants like hydrogen peroxide[26]. In our study, aqueous, methanolic, and chloroform extracts of *Zea mays* leaves were tested for their H_2O_2-scavenging potential, with the methanolic extract demonstrating the highest activity. This aligns with findings from studies on *Phyllanthus* plants[27], and *Pedilanthus tithymaloids*[28], all of which reported hydrogen peroxide scavenging effects. Deerberry (*Vaccinium stamineum L.*) exhibited potent scavenging activities against various radicals, including hydrogen peroxide, correlating with its antioxidant enzyme activities[29].

The hydroxyl radical is a highly reactive species in biological systems, implicated in causing extensive damage to molecules within living cells. In the present study, *Zea mays* leaf extracts demonstrated the ability to protect 2'-deoxy-D-ribose from oxidative degradation by scavenging hydroxyl radicals. The methanolic extract exhibited the highest scavenging effect, surpassing the aqueous and chloroform extracts. Numerous studies in the literature report on the hydroxyl radical scavenging properties of various plants. Methanolic extracts of *Camellia sinensis, Ficus bengalensis*, and *Ficus recemosa* showed notable hydroxyl radical scavenging activity[30]. Similarly, methanol extracts of aerial flowering parts of four endemic *Stachys taxa* exhibited significant scavenging of hydroxyl radicals[31].

Superoxide, a precursor of reactive oxygen species (ROS), is implicated in aging and tissue damage, as mentioned by Aruoma in 1998[32] and Wickens in 2001[33]. The ability of antioxidants to scavenge superoxide radicals holds physiological significance in preventing oxidation reactions associated with aging. In our study, *Zea mays* leaf extracts, especially the methanolic extract, effectively scavenged superoxide radicals, demonstrating their potential physiological impact. Various reports in the literature support the association

between the scavenging activity of superoxide radicals and strong antioxidant properties in plants. Awah et al. (2010)[34] found that the leaves of *Stachytarpheta angustifolia* exhibit antioxidant properties by effectively scavenging superoxide radicals. Similarly, Ardestani and Yazdanparast (2007)[35] demonstrated the potent superoxide scavenging ability of *Achillea santolina* extract in a dose-dependent manner.

Nitric oxide and reactive nitrogen species play crucial roles in physiological functions[36]. In our study, all three extracts of *Zea mays* leaves (aqueous, methanolic, and chloroform) demonstrated inhibition of nitric oxide, with the methanolic extract exhibiting superior performance. This observation finds support in the literature, where the ethyl acetate, petroleum ether, and methanolic extracts of *Aporosa lindleyana Baill* root were reported to inhibit nitric oxide radicals[37]. Similarly, the ethyl acetate extract of *Wikstroemia indica* root and *Amygdalus communis L*. hulls and shells showed inhibitory effects on nitric oxide production[38,39].

Metal ion chelating and reducing capacities are integral components of the antioxidant mechanism. Chelating agents, forming bonds with metals, act as secondary antioxidants, stabilizing oxidized metal ions[40]. The reducing capacity, attributed to hydrogen donation, involves reductones that counteract free radicals, terminating radical chain reactions[41]. *Zea mays* leaf extracts were examined for these capacities, with the methanolic extract exhibiting superior performance, followed by the aqueous and chloroform extracts. Supporting literature includes findings on *Toona sinensis* extracts and *Gallic acid* displaying antioxidant properties through reducing power[42]. *Celecoxib* and *Amtolmetin guacyl* demonstrated antioxidant and metal-chelating abilities, contributing to anti-inflammatory effects[43]. Mulberry extracts surpassed BHT in reducing power[44].

Antioxidant molecules reducing metal ions play a crucial role in limiting lipid peroxidation[45]. Various plant extracts, such as those from *Antrodia camphorata, Acacia auriculiformis, Leontice smirnowii, Hypsizigus marmoreus, Grifola frondosa, Morchella esculenta, Termitomyces albuminosus, Perilla pankinensis,* and *Pleurotus citrinopileatus,* have shown potent metal chelating and reducing powers[46-53].In the current study, *Zea mays* leaf extracts demonstrated significant metal chelating and reducing activities, reinforcing their antioxidant potential. The methanolic extract, in particular, exhibited maximum efficacy, highlighting the comprehensive antioxidant profile of *Zea mays* leaves.

V. CONCLUSION

The research on Zea mays leaf extracts presented a comprehensive evaluation of their radical scavenging, reducing, and chelating properties. The findings of this study contribute to the broader understanding of medicinal plants and their potential role in neutralizing free radicals, preventing oxidative damage, and combating various disorders associated with reactive oxygen species (ROS). The role of antioxidants in neutralizing free radicals and preventing oxidative damage is well-established, and this research delves into the specific antioxidant properties of Zea mays.

The choice of solvents for extraction played a crucial role in determining the antioxidant activity, with the methanolic extract generally outperforming the aqueous and chloroform extracts. This solvent-dependent variation in antioxidant activity highlights the importance of solvent selection in extracting bioactive compounds from medicinal plants. The results are consistent with existing literature on antioxidant activities of various medicinal plants, reinforcing the reliability of the chosen assays, particularly DPPH and ABTS scavenging, in assessing antioxidant potential.

In summary, the research on Zea mays leaf extracts provides valuable insights into the antioxidant properties of this medicinal plant. The findings underscore the importance of the 10th-day plant for optimal antioxidant content and suggest that Zea mays leaves, especially in methanolic extracts, could be explored further for their potential therapeutic applications in combating oxidative stress-related disorders. The systematic approach to evaluating radical scavenging, reducing, and chelating properties enhances our understanding of the plant's overall antioxidant profile, supporting the rational use of natural products in healthcare.

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Annexure

Figure 1: Graphical representation of the radical scavenging effect of *Zea mays* leaf extracts against DPPH, ABTS, and Hydrogen peroxide radicals

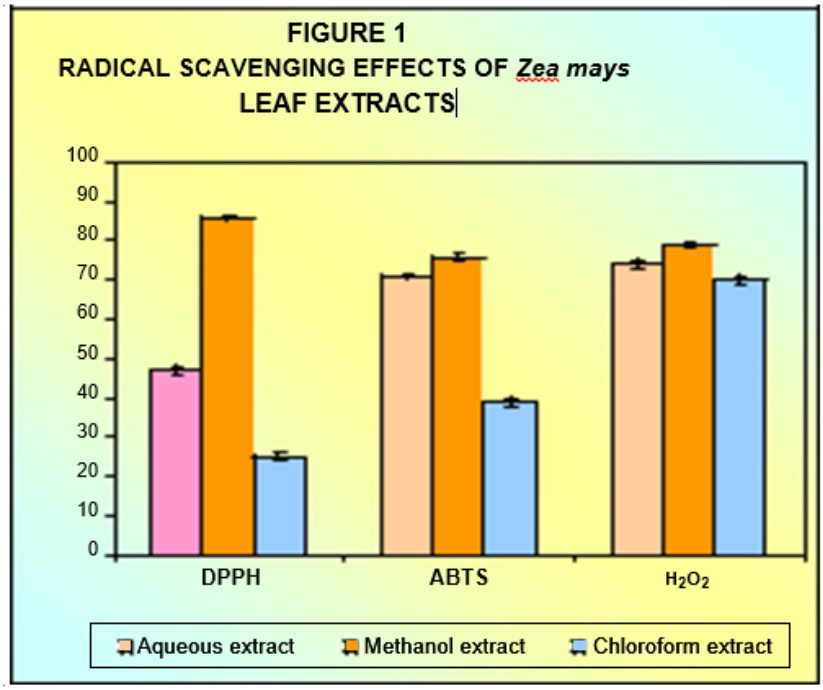

Figure 2: Graphical representation of the radical scavenging effect of *Zea mays* leaf extracts against Hydroxyl radical

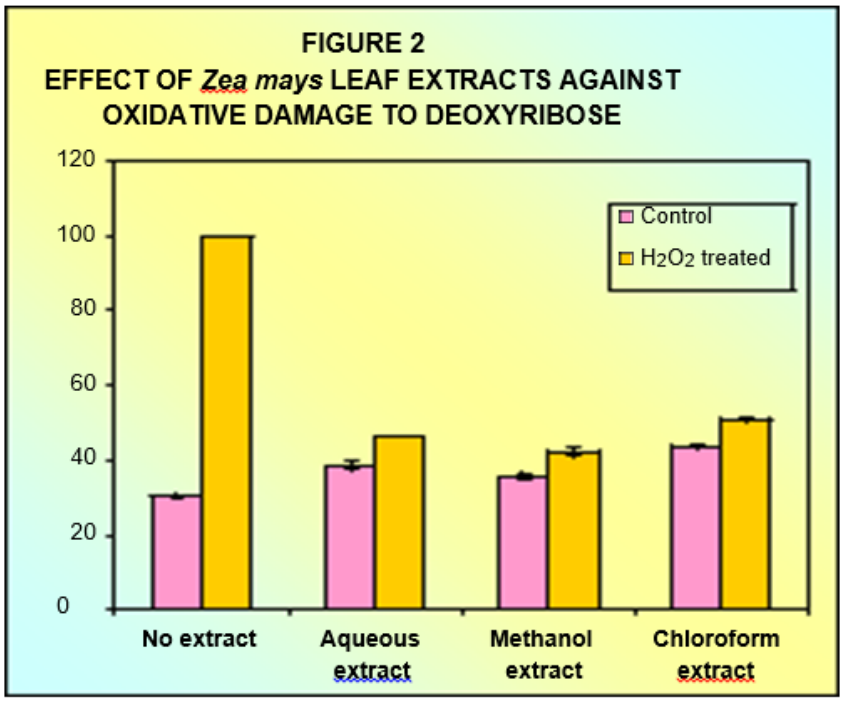

Figure 3: Graphical representation of the radical scavenging effect of *Zea mays* leaf extracts against Superoxide and Nitric Oxide free radicals

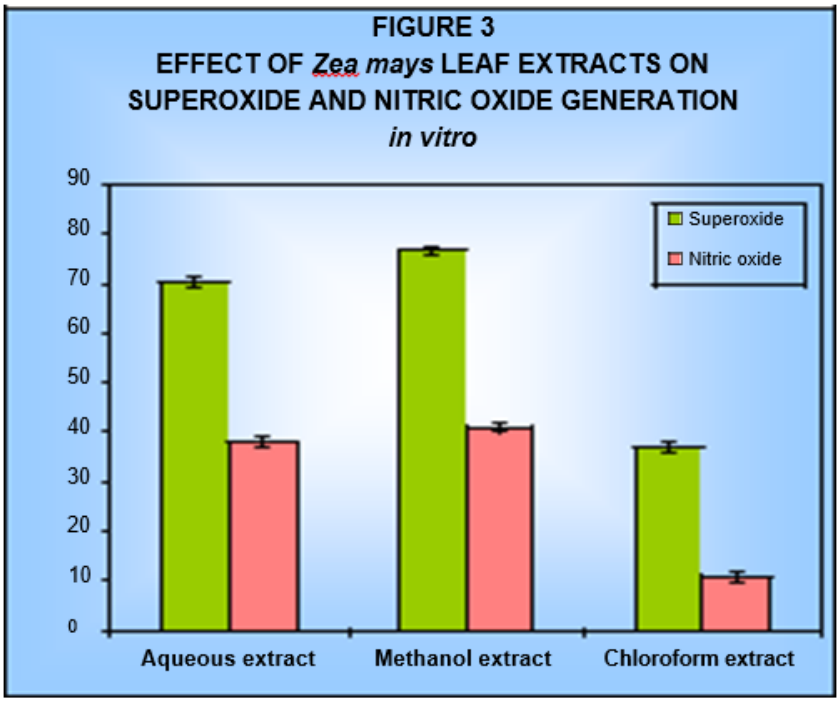

Figure 4: Graphical representation of the Metal ion reducing and chelating action of *Zea mays* Leaf extracts