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Original Article

Hypoglycaemia and Antioxidants Potential of *Sorghum Bicolor* Seeds

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Abstract

One of the most significant seeds crops in the world, *Sorghum bicolor*, is recognized for its vibrant phytochemicals, which may have medicinal uses. The aim of this study is to determine the antioxidant and hypoglycemic properties of *Sorghum bicolor* seeds. The ethanol and methanol extracts of these plants were subjected to antidiabetic conditioning using methods such as glucose adsorption capacity, muscle glucose uptake, and yeast glucose uptake, while iron-sulphate and sodium nitroprusside-

induced lipid peroxidation were used to analyse the antioxidant capabilities. The results demonstrate that *Sorghum bicolor*'s ethanol and methanol extracts adsorbed glucose, with the concentration of glucose adsorption rising as extract concentrations did. The muscle glucose uptake levels and glucose adsorption capabilities differed significantly ($p \leq 0.05$). The yeast cells' ability to absorb glucose was likewise enhanced by the plant extracts, and glucose uptake was measured. In the brain and liver of iron-sulfurate-induced lipid peroxidation, the MDA (malondialdehyde) product was considerably ($p \leq 0.05$) elevated. However, there was no significant change in the MDA product of sodium nitroprusside-induced lipid peroxidation. The ethanol and methanol extracts of *Sorghum bicolor* were found to have further potential as antioxidant and anti-glycemic agents, respectively, in this study.

Keywords: *Sorghum bicolor*, Antioxidant, Hypoglycemic, Lipid peroxidation.

Introduction

Diabetes mellitus (DM) is a worldwide public health issue that is currently spreading like wildfire. An extensively recognized estimate states that in 2000, the prevalence of diabetes in all age groups was 2.8%, and by 2030, there will be 366 million cases of diabetes, or 4.4% of the total population [1]. With an estimated 35 million individuals out of a billion people worldwide, India has the biggest number of diabetics worldwide. The situation is especially dire in emerging nations like India. 79 million additional persons suffer from bloodied glucose forbearance. The nation will have 200 million individuals (about 15% of the population) afflicted with diabetes or a precursor in just over 20 times or 2025 [2].

Chronic hyperglycemia is a hallmark of diabetes mellitus, a metabolic condition that is brought on by either aberrant skeletal muscle glucose uptake or an increase in the liver's glucose product [3]. An essential gluconeogenic enzyme called phosphoenolpyruvate carboxykinase (PEPCK) is controlled by the p38 or adenosine monophosphate-activated protein kinase (AMPK) pathways. In addition, the rate-limiting component for glucose uptake in skeletal muscle is the glucose transporter (GLUT4), and protein kinase B, or Akt, is a key mediator of insulin-induced GLUT4 translocation from cytosol to membrane. In streptozotocin (STZ)-induced diabetic rats, these protein expressions are linked to pathophysiology [2]. After wheat, rice, corn and barley, sorghum is the world's fifth-most important cereal crop. Due to its superior performance under various environmental stressors compared to other cereals, sorghum is typically less expensive to produce. More than 35 percent of sorghum is farmed specifically for human use. The remainder is mostly utilized to make industrial goods, alcohol, and animal feed. With 20% of the global product and approximately 80% of the world's sorghum exports in 2001-2002, the United States is the leading producer and exporter of sorghum [4]. During this time, 57 million metric tons of sorghum were produced worldwide. Numerous phytochemicals, including policosanols, industrial sterols, and phenolic composites, are present in sorghum that are essential cellular components or secondary plants byproducts. While policosanols and industrial sterols play a significant role in the production of wax and plant oils, phenols aid in the natural defense of plants against pests and

illnesses. Owing to their possible health advantages, cholesterol-lowering qualities, and antioxidant activity, phytochemicals have garnered considerable attention. Phenolic acids and flavonoids are the two main groups of phenols found in sorghum. Benzoic or cinnamic acid derivatives are the phenolic acids [4,5], whereas tannins and anthocyanins are the most significant components of flavonoids that have been isolated from sorghum to date [6-10]. Similar in makeup to those derived from sorghum, sorghum phytosterols mostly consist of free sterols, also known as stanols, and their adipose acid/ferulate esters [11,12].

Cereal has components that have been linked to human health advantages, such as antioxidants, anti-diabetic agents, and anti-disease factors, according to recent studies. Oats, rye, barley, buckwheat, sorghum, and millet are minor grains, whereas wheat, rice, and maize are the main cereals [13,14]. Three cereals account for over 50% of the calories consumed by people worldwide: rice (23%), wheat (17%), and sludge (10%) [15].

As an example, phytic acid was found to be crucial in the management of hypercholesterolemia, hypercalciuria, cancer, and order monuments [16]. Further research has also shown that diets high in carbs, high in dietary fibre, and mostly derived from cereals allowed diabetic participants to stop taking oral hypoglycemic medications or cut back on their insulin dosage [17].

Methodology

Grain Extraction Preparation for Sorghum Bicolor

The Botany Garden at Lagos State University Faculty of Science identified and verified *Sorghum bicolor* seeds that were gathered in the Alabarago neighborhood. The seeds of *Sorghum bicolor* were pulverized and split into two sections. A separate measurement of 125 g of sorghum was made, and the seeds were extracted using ethanol and methanol in separate solvents. The tincture process is the extraction procedure that is applied. 125 g of *Sorghum bicolor* were dissolved in 200 ml of each solution. The solution was permitted to remain undisturbed for a period of 48 hours before filtering it and concentrating the filtrate by oven-drying it for a few hours. After that, the concentrated extract was put in a glass beaker that was sealed with aluminum foil and kept in a refrigerator at 4 °C to avoid damage.

Chemicals and Reagents

The chemicals and reagents used in this study are as follow: Glucose oxidase peroxidase kit, Baker's yeast, Glucose, metronidazole, dialysis bags, tris HCl buffer, $FeSO_4$, sodium dodecyl sulfate, acetic acid, thiobarbituric acid, Glucometer, and Glucose strips.

Assessment of the Hypoglycemic Effects of Plant Extracts Through Diverse In Vitro Methods

Assessing the Capacity for Glucose Adsorption

150 mL of a glucose solution with different concentrations (5, 10, 20, 50, and 100 mM), plant extract samples (1%) were added. Following thorough stirring, the mixture was incubated for 6 hours at 37 °C in a shaker water bath. After centrifuging at 4,000 ×g for 20 minutes, the amount of glucose in the supernatant was measured. At 505 nm, absorbance was measured.

After six hours of incubation at 37 °C, 10 µL of the supernatant was removed and put into each test tube. After mixing the reaction mixture and adding 1

milliliter of glucose reagents, it was incubated for 10 minutes at 37 °C. At 505 nm, the absorbance was measured and confirmed as satisfactory.

Standard: 10 µL of the metronidazole standard was obtained and 1 ml of glucose reagents was added in a reaction mixture to get the absorbance.

The calculation of bound glucose concentration was performed using the formula outlined by [18]:

$$Glucose\ Bound = \frac{G1-G0}{Weight\ of\ the\ sample}\ x\ Volume\ of\ solution \quad (1)$$

Where, G1 represents the initial glucose concentration in the solution and G6 denotes the glucose concentration after 6 hours.

Uptake of Glucose in Yeast Cells

After subjecting commercial baker's yeast to repeat centrifugation in distilled water (3,000×g for 5 minutes) until the supernatant became clear, a 10% (v/v) suspension was prepared. A mixture of 1 milliliter glucose solution (5-25 mM) and various extract concentrations (1-5 mg) was incubated for 10 minutes at 37 °C. Following the addition of 100 µL of yeast suspension and vortexing, the reaction continued incubating for 60 minutes at 37 °C. After this period, tubes were centrifuged (2,500 × g, 5 min), and the glucose amount in the supernatant was measured. The percentage of the rise in glucose uptake by yeast cells was determined using the formula below [19].

$$Increase\ in\ glucose\ uptake = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance\ _{control}}\ x\ 100 \quad (2)$$

In this context, Abs control refers to the absorbance of the control reaction, which includes all reagents except the test sample, while Abs sample represents the absorbance of the actual test sample.

Lipid Peroxidation

Preparation of Tissue Homogenates

The animal was decapitated during a light diethyl ether anesthesia. Subsequently, the liver and the entire brain (cerebral tissue) were swiftly extracted, placed on ice, and weighed. Following this, the tissues were subjected to homogenization using a laboratory mortar and pestle in a cold saline solution (1/10 w/v). After centrifugation of the homogenate for 10 minutes at 3000×g, a pellet was formed, which was discarded, and the low-speed supernatant (S1) was preserved for the lipid peroxidation assay.

Reactions of lipid peroxidation and thiobarbibutric acid

A pro-oxidant solution was prepared using 70 µM sodium nitroprusside and freshly made 10 µM $FeSO_4$. To achieve a total volume of 100 µL, 100 µL of supernatant was added to a mixture containing 30 µL of 0.1M pH 7.4 Tris-HCl buffer, pro-oxidants (freshly prepared $FeSO_4$ and 70 µM sodium nitroprusside), and *Sorghum bicolor* seed extract at varying concentrations (50, 100, 200, and 400 g/ml). Water was added to increase the volume to 300 µL, followed by incubation for one hour at 37 °C.

Afterwards, the reaction mixture was mixed with 600µl of acetic acid/HCl (pH 3.4) and 600 µL of 0.8% TBA (thiobarbituric acid) to initiate the color reaction. Following this, 100 µL of sodium

dodecyl sulphate (8.1%) was introduced, and the mixture was incubated at 100 °C for one hour. The amount of thiobarbituric acid reactive species (TBARS) produced was measured at 532 nm, and the absorbance was compared to a reference curve utilizing malondialdehyde (MDA).

Muscle Glucose Uptake

After removing the Psoas muscle from the animal sample, it was rinsed with Kreb's buffer and sliced into little pieces weighing 500 mg each. The muscle was then weighed and added to Krebs' buffer along with 1 millilitre of extract at several concentrations (50, 100, 200, 400, and 800 µg/ml) into test tubes. Following the addition of 1 millilitre of the muscle combination, 3 millilitres of distilled water were added to the reaction mixture.

Prior to incubation, 1 ml of aliquot was collected, and 1 ml of glucose reagent was then added to the reaction mixture.

The uptake of glucose by muscles was determined by calculating the amount of glucose (in mg) absorbed per gram of muscle tissue, employing the following formula:

$$\text{Muscle glucose uptake} = \frac{G_{c1} - G_{c2}}{0.5 \text{ muscle tissue}} \quad (3)$$

Where, Gc1 represents the glucose concentration before incubation, and Gc2 denotes the glucose concentration after incubation.

Statistical Analysis

Measurements were performed twice, and the data underwent analysis using two-way ANOVA, followed by Sidak's multiple comparison tests to detect significant differences. Statistical significance was set at $p < 0.05$. Graphs were created using Graph Pad Prism 8 software.

Results and Discussion

Antioxidant compounds including procyanidins, flavonoids, and phenolic acid are among the polyphenols found in sorghum seeds [20]. Worldwide, sorghum is a crop that is growing in importance. New methods of incorporating it into human diets have emerged, promoting consumption through items like pasta, bread, and cereal alternatives [21]. This is particularly beneficial for individuals with celiac disease, as sorghum lacks the gluten proteins triggering immune responses present in wheat, oats, barley, and rye [22]. Sorghum, rich in polyphenolic compounds like procyanidins, 3-deoxy anthocyanidins, and phenolic acids, demonstrates strong antioxidant properties that combat diseases associated with oxidative stress, anti-proliferative qualities linked to cancer prevention, antimicrobial attributes, and improvement in glucose metabolism associated with diabetes [22]. Consequently, sorghum emerges as a valuable source of active components with biological effects, suitable for incorporation into foods with specific health benefits and various pharmaceutical operations aiming to isolate these beneficial compounds [23].

According to Figure 1, when the concentration of *Sorghum bicolor* ethanol and *Sorghum bicolor* methanol extracts grew concurrently at different concentrations of the extracts, glucose concentration also increased. However, as the concentration increased further, SBE showed a substantial rise in comparison to SBM. Similarly, stimulated cells may not absorb glucose in the same way as cells in eukaryotes or the human body. A phosphotransferase enzyme system may not cooperate with one another to enhance the movement of glucose through the yeast membrane. As the concentration of glucose immersion was raised in Figure 2, SBE showed a

significantly greater increase than SBM. When the extracts were at their greatest concentration, the SBM had a relatively low concentration of muscle glucose absorption, as demonstrated in Figure 3.

The capacity of certain pro-oxidants, such as sodium nitroprusside and $FeSO_4$, to cause lipid peroxidation in the liver and brains of cows is described in this study. The aforementioned finding illustrates the protective potential of the *Sorghum bicolor* methanol and ethanol extracts. Figure 4 shows that ethanol and methanol extracts generate a decrease in MDA product for Iron-Sulfurate-Confirmed Lipid Peroxidation. There was no notable distinction noted between SBE and SBM in the liver or brain.

Figure 5a depicts a reduction in MDA product levels with SBE extracts, exhibiting a significant ($p \leq 0.05$) decrease compared to low-concentration SBE in the brain during sodium nitroprusside-induced lipid peroxidation. In Figure 5b, while there was no notable difference with increasing extract concentration in the liver, MDA product concentration decreased at the maximum extract concentration, with SBM showing a significant ($p \leq 0.05$) drop compared to SBE. Various health issues, such as insulin resistance, glucose intolerance, type II diabetes mellitus, atherosclerosis, chronic low-grade inflammation, and non-alcoholic fatty liver disease, are influenced by oxidative stress and elevated body weight [24].

Sorghum bran contains numerous phenolic compounds and antioxidants, with low glycemic index beneficial fiber [25]. Diabetes-related hyperglycemia can lead to complications such as retinopathy and nephropathy [26]. Certain medications inhibit digestive enzymes like α-amylase and α-glucosidase to lower hyperglycemia. The tannin-rich polyphenolic extracts Sorghum seed have demonstrated efficacy in inhibiting these enzymes both *in vitro* and *in vivo* [27].

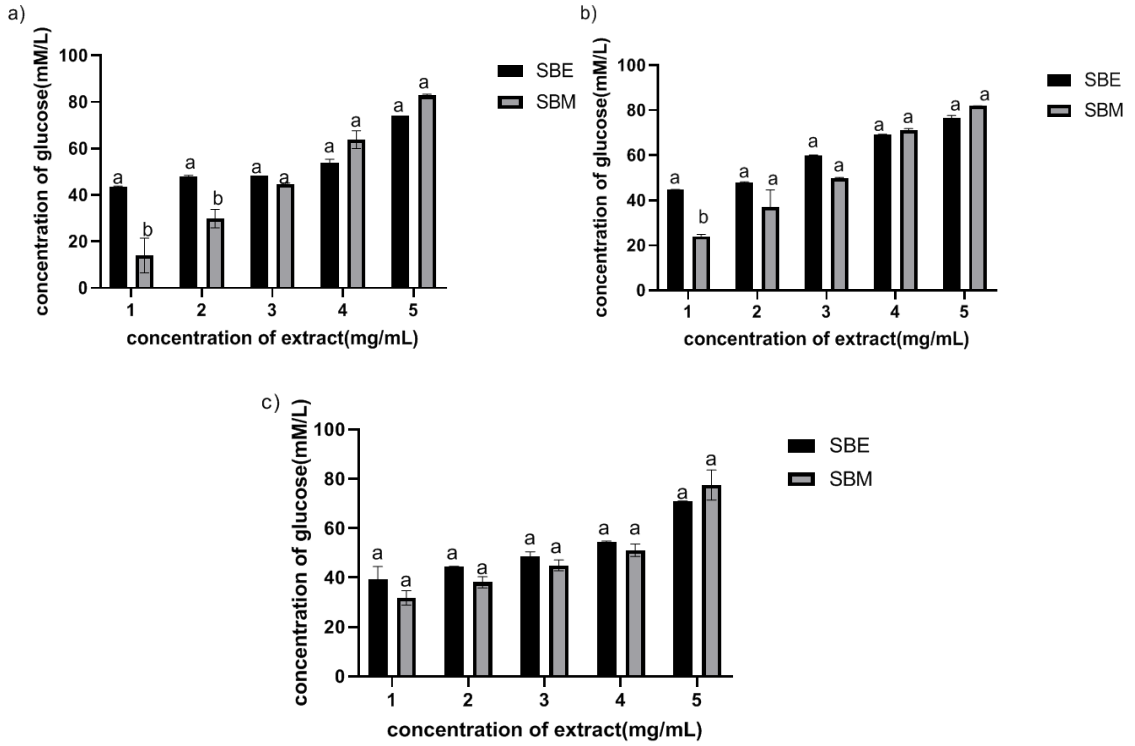

Figure 1 Glucose uptake by yeast cells at (a) 5 mM, (b) 10 mM, and (c) 25 mM in the presence of ethanol and methanol extract of *Sorghum bicolor* seeds. SBM: Methanol extract of *Sorghum bicolor* seeds and SBE: Ethanol extract of *Sorghum bicolor* seeds. The error bars depict the standard error (SE) of duplicated data, showing the variability within the dataset

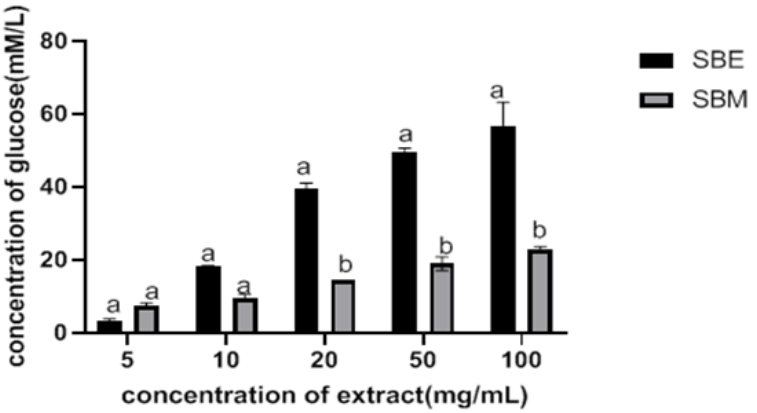

Figure 2 Glucose adsorption of the ethanol and methanol extract of *Sorghum bicolor* seeds at different concentrations. The error bars depict the standard error (SE) of duplicated data, showing the variability within the dataset. SBM: Methanol extract of *Sorghum bicolor* seeds and SBE: Ethanol extract of *Sorghum bicolor* seeds

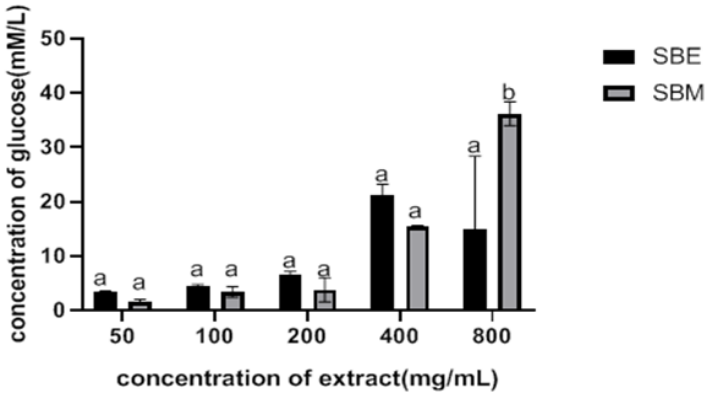

Figure 3 Effect of methanol and ethanol extract of *Sorghum bicolor* seeds on the muscle glucose uptake at different concentrations. The error bars depict the standard error (SE) of duplicated data, showing the variability within the dataset. SBM: Methanol extract of *Sorghum bicolor* seeds and SBE: Ethanol extract of *Sorghum bicolor* seeds

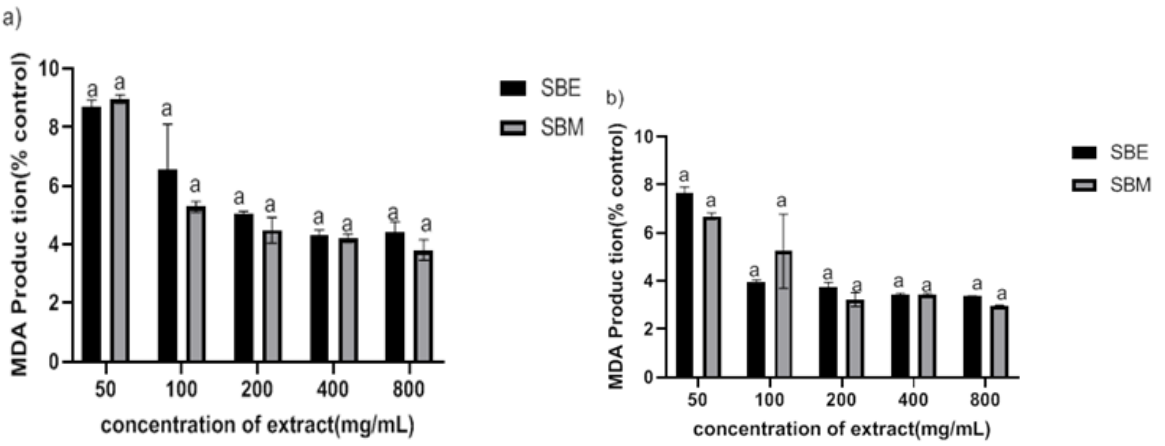

Figure 4 Effect of methanol and ethanol extracts of *Sorghum bicolor* seeds on Fe (II) - induced lipid peroxidation in cow's (a) liver and (b) brain. The error bars depict the standard error (SE) of duplicated data, showing the variability within the dataset. SBM: Methanol extract of *Sorghum bicolor* seeds. SBE: Ethanol extract of *Sorghum bicolor* seeds

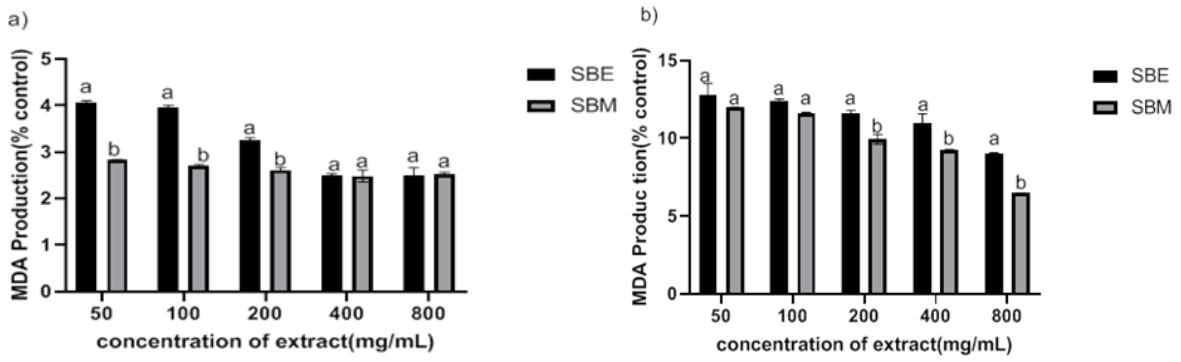

Figure 5 Effect of methanol and ethanol extracts of *Sorghum bicolor* on sodium-nitroprusside induced lipid peroxidation in cow's (a) brain and (b) liver. The error bars depict the standard error (SE) of duplicated data, showing the variability within the dataset. SBM: Methanol extract of *Sorghum bicolor seeds* and SBE: Ethanol extract of *Sorghum bicolor seeds*. Bars labeled with significant differences at a significance level of $P \leq 0.05$ are denoted by different letters

Conclusion

Sorghum bicolor's ethanol and methanol extracts show promise in the management of oxidative stress and hyperglycemia. In comparison to the methanol extract, which has similarly been shown to have an antioxidant impact, the ethanol extract of *Sorghum bicolor* has a stronger hypoglycemic effect. The plant's bioactive components give both *Sorghum bicolor* extracts their hypoglycemic and antioxidant properties. The study's variety of animal systems shed light on potential processes through which *Sorghum bicolor*'s methanol and ethanol extract may help to reduce the postprandial rise in blood glucose levels. Using a basic *in vitro* model of incentive cells, it was observed that the hypoglycemic effect of the methanol and ethanol extract of *Sorghum bicolor* seeds was moderated by increasing adsorption of glucose, promoting the facilitation of glucose movement across the cell membrane was observed. In addition, a dose-dependent reduction in MDA levels in the brain and liver tissues, induced by $FeSO_4$ and sodium nitroprusside, was noted.

Conflict of Interest

The authors declared that they have no financial interest in the subject matter or materials discussed in it, nor any affiliations or involvement with any entity that may have one.

Consent for Publication

None.

Availability of Data and Materials

All the data are available on request.

Authors' Contributions

All co-authors participated in all stages of this study while preparing the final version.

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Ethical Consent

The study's authors state that since no animals were utilized in the investigation, ethics committee permission was not

necessary. The research of plants in the paper was conducted using data gathered from publicly accessible web sources that do not gather or retain personally identifiable information. Every relevant legislation, rule, and regulation required for the study's implementation has been complied with.

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