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Phytochemical Profiling and In Vitro–In Silico Assessment of Sprouted Sorghum Bicolor (L.) Moench as a Functional Food for Liver Health

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ABSTRACT: Oxidative-stress is a key factor in the commencement and development of liver diseases. Functional foods enriched with antioxidant phytochemicals offer a promising strategy for maintaining liver health. Sprouting enhances the nutritional quality and bioactive composition of cereals, making sprouted *Sorghum bicolor* a potential source of hepatoprotective compounds. This study aimed to illustrate the phytochemical profile of the ethanolic extract of sprouted *S. bicolor* grains (EESBG) and evaluate its hepatoprotective potential using integrated *in vitro* approaches to assess its suitability as a functional food for liver health. EESBG was subjected to phytochemical screening by GC–MS metabolite profiling. Antioxidant action was assessed using DPPH scavenging and CUPRAC assays. Hepatoprotective action was assessed by ethanol-induced HepG2 cells by measuring cell viability, lipid peroxidation (TBARS), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), reduced glutathione (GSH), and superoxide dismutase (SOD). Major metabolites, including maltol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), and 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP), were docked against the human Keap1 Kelch domain (PDB ID: 6TYM). Sprouting significantly enhanced the phytochemical and antioxidant profile of *S. bicolor*. EESBG improved HepG2 cell viability, TBARS, and LDH levels, and restored GSH, SOD, and ALP activities. Molecular docking demonstrated favourable interactions of the major phytoconstituents with Keap1, supporting activation of the Nrf2-mediated antioxidant defense pathway. EESBG possesses significant antioxidant and hepato-protective properties. The integrated phytochemical, biological, and computational findings support sprouted *S. bicolor* as a promising functional food for promoting liver health and preventing oxidative stress-associated liver disorders.

HIGHLIGHTS

1. Sprouting-associated phytochemical profiling elucidated the chemical composition of *Sorghum bicolor* and identified candidate bioactive constituents.
2. *In vitro* antioxidant and hepatoprotective assays evaluated the protective potential of sprouted sorghum grains extract against oxidative stress–associated hepatic injury.
3. Molecular docking provided predictive insights into the potential interactions of identified phytoconstituents with liver health-related molecular targets.

4. Integrated phytochemical, *in vitro,* and *in silico* findings offer complementary evidence supporting the potential hepatoprotective relevance of sprouted sorghum.

5. The findings establish a scientific basis for further validation of bioactive constituents, mechanistic pathways, and the functional food potential of sprouted sorghum for liver health.

INTRODUCTION

Liver disorders cause significant morbidity and mortality worldwide, making them a significant global health concern. Chronic liver disorders, including non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, viral hepatitis, and drug-induced liver injury, are primarily associated with oxidative stress, inflammation, mitochondrial dysfunction, and hepatocellular apoptosis (Chaudhary *et al.* 2025). Excessive formation of reactive oxygen species (ROS) alters the cellular redox equilibrium, causing lipid peroxidation, DNA damage, protein oxidation, and reduced liver function. Although there are various pharmaceutical therapies available, their long-term usage is frequently linked with low efficacy, side effects, and expensive treatment costs. Consequently, there is growing interest in identifying natural dietary interventions and functional foods capable of preventing or attenuating liver injury through multiple biological mechanisms (Zhu *et al.*2012).

Functional foods derived from cereals have concerned growing attention because of their rich nutritional composition and richness of bioactive phytochemicals. Among these, *Sorghum bicolor* (L.) Moench is the most vital cereal crop globally and assists as a staple food in many regions of Africa and Asia. Besides being an excellent source of dietary fiber, proteins, carbohydrates, vitamins, and vital minerals (Mukim *et al.* 2022). Sorghum contains a wide spectrum of phytochemicals, counting phenolic acids, flavonoids, anthocyanins, phytosterols, and antioxidant compounds. These secondary metabolites have been reported to exhibit antioxidant, anti-inflammatory, antidiabetic, cardioprotective, anticancer, and hepatoprotective activities, highlighting sorghum's potential as a functional food for promoting human health (Arif *et al.* 2026).

Sprouting is an economical, eco-friendly, and sustainable bioprocessing technique that significantly improves the nutritious and efficient quality of cereal grains. During germination, endogenous hydrolytic enzymes become activated, leading to the degradation of complex macromolecules and the biosynthesis of numerous bioactive metabolites. Sprouting enhances the bioavailability of amino acids, vitamins, minerals, and phenolic compounds while reducing antinutritional factors such as phytates and tannins (Mukim *et al.* 2023). Moreover, germination has been shown to increase the antioxidant capacity of cereals through the accumulation of free phenolics, flavonoids, and Maillard reaction-derived antioxidants. These biochemical changes contribute to improved biological activities, making sprouted cereals promising candidates for functional food development (Munoz *et al* .2022).

Recent advances in metabolomics have enabled comprehensive characterization of phytochemical constituents responsible for the biological activities of functional foods. Techniques such as gas chromatography-mass spectrometry (GC-MS) provide valuable information regarding volatile and semi-volatile metabolites generated during sprouting (Khalid *et al.* 2026). Several compounds identified in sprouted cereals, including maltol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP), and other antioxidant metabolites, have been linked with free radical scavenging, cytoprotective, and anti-inflammatory activities. Comprehensive phytochemical profiling therefore provides an important foundation for understanding the mechanisms underlying the health-promoting effects of sprouted grains (Varunesh. *et al.* 2026).

In vitro cell-based assays using human hepatocellular carcinoma (HepG2) cells provide an effective platform for assessing cytoprotective effects against chemically induced oxidative stress by monitoring cell viability, intracellular antioxidant status, lipid peroxidation, membrane integrity, and liver-specific biochemical markers. Complementarily, molecular docking offers a rapid and cost-effective strategy to predict interactions between phytoconstituents and proteins involved in oxidative stress regulation and hepatocellular defense, thereby providing mechanistic insights into their potential modes of action. Although computational predictions require biological validation, they serve as valuable supportive evidence for identifying promising bioactive compounds (Singh *et al.* 2024).

Despite increasing evidence supporting the nutritional benefits of sorghum, comprehensive investigations integrating phytochemical reporting, *in vitro* hepatoprotective evaluation, and *in silico* molecular interaction studies of sprouted *S. bicolor* remain limited. Furthermore, the phytochemical transformations induced by sprouting and their relationship with liver-protective activity have not been fully elucidated (Mukim *et al.*2022). Therefore, the current study intended to

comprehensively characterize the phytochemical composition of the ethanolic extract of sprouted *Sorghum bicolor* grains using GC-MS, and evaluate its hepatoprotective potential through *in vitro* assays in HepG2 cells, and investigate the interaction of major phytoconstituents with key molecular targets associated with oxidative stress and hepatocellular protection using molecular docking. The integration of phytochemical, biological, and computational evidence is expected to provide scientific support for the development of sprouted sorghum as a functional food with potential applications in promoting liver health and preventing oxidative stress-mediated hepatic disorders.

METHODOLOGY

Procurement and certification of plant material

S. bicolor grains were purchased in October 2022 from Bakshipur market of district Gorakhpur (India) and authenticated by the All-India Institute of Ayurveda (AIIA), New Delhi to find out the reference number (RRDR/AIIA/phg. /132).

Drug, Chemicals and Instruments

HepG2 Cell lines were attained from National Centre for Cell Science, Pune, India. Trolox (Otto Kemi - Cat no.–T7723), Cupric Chloride (SD fine – Cat no. 37834K05), Ammonium acetate (Fischer scientific – Cat no.- 11145), Neocuproine (SRL chem – Cat no. 93007), DPPH solution (SRL Chem Cat no.– SR-29128), Acetic acid, Methanol (SD fine- Cat no.- 10930lC250), Ascorbic Acid (SRL, Cat no- 23006), Para-nitrophenol, Tris HCl/NaCl, 5,5'-Dithiobis (2-Nitro benzoic acid), Phenazonium methosulphate (HiMedia-GRM1677), Nitro blue tetrazolium (SRL-Chem-11207), Potassium pyro-phosphate buffer (Sigma -Adrich-322431), Riboflavin (SRL-Chem – 34392), GSH (SD-fine – F14A/2214/0304/62), Sodium Lactate Lactic acid (HI media Cat. No. GRM243), Potassium phosphate buffer, Gelatin (Fisher Scientific, Cat. No-900-70-8), NAD+ Enzyme- 400U/ml, TBARS assay kit (Cat No. OPKA003), Ecoline diagnostic kits, Glutathione (GSH) assay kit, Super oxide dismutase (SOD) activity assay kit, Alkaline phosphatase (ALP) assay kit (Cat No. KEA017) and microplate reader (iMark Biorad USA). The GC-MS device is a QP-2010 Ultra, capillary nonpolar 60 M TRX 5-MS column, 30 m Dimension; 0.25 mm ID and 0.25 mm film.

Sprouting of S. bicolor grains and extract preparation

To ensure optimal sprouting, 250 g of *S. bicolor* grains were divided into four portions and thoroughly rinsed with distilled water to eliminate dust, debris, and broken pieces. To decrease microbial contamination, the grains were superficial sterilized by soaking in a 0.1% sodium hypochlorite for five minutes. The grains were then rinsed three or four times with sterile distilled water to eliminate any remaining disinfectant. To enhance hydration and germination, the sterilized grains were drenched in sterile distilled water at room temperature (32-40°C) for 10-12 hours. After soaking, excess water was drained and the grains were spread evenly on sterile germination trays lined with sterile moist muslin cloth. The trays were covered with sterile cloth and incubated under controlled conditions at 34–42°C. To maintain adequate moisture and prevent microbial growth, the grains were rinsed with sterile distilled water every second day and excess water was removed to avoid water stagnation. The grains were permitted to germinate for 12 days, under regular observation of the germination process (Fig.1). Any grains showing visible fungal or bacterial contamination were immediately discarded. After completion of sprouting, the germinated grains were collected and dried in a hot air oven at 38–44°C to stop further metabolic activity. The dried sprouted grains were ground into coarse powder using a mechanical grinder (Borgonovi *et at.* 2025). Following a 72-hour cold maceration process, 100 g of the powdered sprouted grains and 25g fresh unsprouted grains were extracted with 250 and 100 mL of 90% v/v ethanol. The extract was passed through muslin cloth for filtration, after which the filtrate was concentrated under reduced pressure and subsequently vacuum dried (Kumar *et at.* 2023). The yield of ethanol extract of sprouted and unsprouted of *S. bicolor* grains (EESBG) and (EEUSBG) was 11.24% and 8.16% w/w. EESBG was subjected to preliminary phytochemical investigation and GC–MS analysis to identify various phytoconstituents. (Singh *et al.* 2023) Both EESBG and EEUSBG was *in vitro* evaluated for hepatoprotective and antioxidant activities.



Figure 1. Days 12 sprouted grains of *Sorghum bicolor*

Determination of *in vitro* hepatoprotective effect of EESBG and EEUSBG

In vitro cytotoxic activity

Mitochondrial synthesis was estimated using the tetrazolium assay to determine the 50% cytotoxic concentration (CTC_{50}). At the concentrations of 50, 100, 150, 200, 250 and 300 µg/mL EESBG and EEUSBG, $HepG_2$ cells (5.0 × 103 cells/well) were preserved in 96 well culture plates for 72 h in the existence of 100 µL. After the incubation time, the test solutions were withdrawn from the wells, and 60 µL of MTT solution made in improved Eagle's medium was poured to all well. The plates were slightly agitated and incubated up to the 2.5 h at 35 ± 3 °C in a 5% CO_2 environment. During the gestation, the supernatant was rejected, and 50 µL of propanol was presented to dissolve the formazan crystals, with constant shaking up to the 40 min at room temperature. Absorbance of the sample was taken at the wavelength of 540 nm by microplate finder from BioTek Instruments, Inc., (Winooski, VT) USA (Ogbole *et at.* 2017). The growth embarrassment was measured as:

(%) Growth inhibition = 100 − (Mean of OD for specific test /Mean of OD for control) × 100

A dose response curve was plotted with % growth inhibition on the Y-axis and concentrations of extract (µg/mL) on the X-axis, from which the CTC_{50} value was derived.

Measurement of cell viability, ALT, and AST activities

The cell viability activity of EESBG and EEUSBG was assessed in HepG2 cells, with ethanol (80 mM) as the hepatotoxic agent and silymarin as the positive control (Rai *et al.* 2022). The experimental study was divided into four groups, group I cultured cells considered as normal control (NC) and 100 µL of silymarin, EESBG, and EEUSBG as per se controls. The group II toxic group (TC) cells were exposed to 100 µL of 80 mM ethanol for 24 h. The group III cells were treated with 100 µL Silymarin and EEUSBG comprising 80 mM ethanol up to 24 h. The group IV cells were conserved with EESBG and EEUSBG (50, 100, and 200 µg/mL) holding 80 mM ethanol for 24 h. Further, cell viability, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) assays were conducted following standard protocols with Ecoline diagnostic kits (Pareek *et at.* 2023).

Measurement of LDH release in HepG2 cells

When cells are damaged, they release LDH into the extracellular space; under normal conditions, LDH remains a stable enzyme within the cytoplasm of most cells. The LDH assay is a widely used procedure for assessing cell viability and cytotoxicity. For the evaluation of cytoprotective activity of EESBG and EEUSBG four sets of well selected, in which 50 µL stock solution (1M Acetic acid), 10 µL buffer solution Tris. HCl-NaCl at (pH 8.0) and 80 µL enzyme reaction mixture containing Solution A (5% Gelatin), Solution B (10mM Phenazine methosulfate), and Solution C, (100 mM Nitro blue Tetrazolium) was added. HepG2 cells were pre-treated with 100 µL of 80 mM ethanol for 24 h to induce hepatotoxicity. Cells were then treated with tested sample EESBG, EEUSBG and silymarin in Solution D (NAD+ and Enzyme 400U/mL) and

incubated at 37±4 °C for 24 h. Finally, 10µL substrate mixture was added to each well of all four groups and the absorbance was taken at 570 nm (Al-Qubaisi *et al.* 2011).

LDH release activity = (A_{Test} - $A_{Control}$ /$A_{Control}$) ×100

(A_{Test} = Absorbance of test sample)

($A_{Control}$ = Absorbance of control)

Estimation of MDA content (TBARS Assay)

The TBARS assay is a common technique for estimating MDA content, a product of lipid peroxidation, in plant extracts. In this method, lipid peroxidation is induced in a 0.5 mL of linoleic acid solution (0.2%) prepared in ethanol and the inhibitory effect of the EESPG and EEUSBG are evaluated. The assay involves reacting MDA with thiobarbituric acid (TBA) in acidic environments and at high temperature, creating a pink colored adduct that can be checked UV spectrophotometer. In a clean test tube 100 µL of EESBG and EEUSBG at (50, 100, and 200 µ/mL) concentrations were mixed thoroughly to 500 µL of Solution A (TCA-Trichloroacetic acid). The contents were centrifugated at 2500 rpm for 5 minutes, and the supernatant was separated for future experiments. In a tube, 100 µL of tested sample was mixed with 1800 µL of TBA reagent. The solution was incubated at 80 °C for 15 minutes. Then the absorbance was taken at 532 nm with spectrophotometer (De Leon & Borges 2020). Malondialdehyde levels were determined by means of an extinction coefficient of 1.56×10^5 $mM^{-1}cm^{-1}$.

MDA concentration (M) = (Absorbance 532 nm / 1.56×10^5) × dilution factor

Alkaline phosphatase assay

Alkaline phosphatase (ALP) is an isoenzyme found throughout the body, with higher concentrations in the liver, bile ducts, and bones. It plays a role in various biological processes, like bone formation, liver function, and kidney function. The liver is the primary source in most patients with elevated enzyme levels. The ALP activity assay was performed with alkaline phosphate assay kit (Cat no. KEA017). 10µL of sample (cell lysate) and standard was occupied in the defined wells of 96 well plate. 50 µL of AMP buffer containing 50 µL PNP substrate is added to the each well of the defined set. Then, it was incubated at 37°C for 15 minutes. Then, 10 µL of NaOH was supplemented to break the reaction. Then, the absorbance was taken at 405 nm in a microplate reader (Tang & Ma 2019).

ALP Activity (U/mg of protein) = (A/B)

A [ALP activity in test (moL/min.)] = (Test absorbance × standard amount)/Standard absorbance ×15

B [Protein quantity (mg)] = {Test absorbance × Standard amount (g) × 210}/ (Standard absorbance × 10 × 1000)

Estimation of reduced glutathione

A 140 µL enzyme solution (Glutathione reductase + Potassium phosphate buffer) was added in reaction mixture (Potassium phosphate buffer and enzyme) to each well of 96 well plate in all the four sets and after that 10 µL of each sample was supplemented to all well of set two and three, and 10 µL standard solution was added to all well of set four and extra buffer was added to make up the volume 100 µL in each well of set 1-4. To test EESBG and EEUSBG concentrations of 50, 100, and 200 µ/mL, standard, blank, and control tubes were produced and added into a 96-well plate. After incubating the plate, 50 µL of the substrate solution 5,5'-Dithiobis (2-Nitro benzoic acid) was added to each well of the set 1-4. The absorbance was immediately checked at 415 nm using a microplate reader. Total GSH concentration was estimated in µM with the help of standard curve of Glutathione (Alisik & Erel 2019).

Estimation of SOD activity

The SOD evaluate relies on the ability of superoxide dismutase to suppress the production of blue-colored formazan crystals generated from the reaction of GSH, phenazine methosulfate, and nitro blue tetrazolium. The absorbance of color product is noted at 560 nm using a spectrophotometer. During bacterial killing, white blood cells employ enzymes like NADPH oxidase to yield superoxide and other reactive oxygen species (ROS). Under stressful conditions, the cellular concentration of SOD generally rises, as these enzymes function as antioxidants that safeguard cellular components from ROS-induced oxidation. All reagents and the homogenate were adjusted to a temperature of 37°C. The test samples EESBG and EEUSBG were ready at concentrations of 50, 100, and 200 µ/mL, along with blank, standard, and control then transferred into a 96 well

plate. The SOD enzyme was prepared in concentrations ranging from 6.25 to 100 U/mL to create the standard curve plot. In a cuvette, 1.2 mL of buffer potassium pyrophosphate (pH 8.3, 0.025M) was added, followed by 1 mL of PBS at 186 µM and 0.3 mL of Nitro blue tetrazolium at 300 µM, and the mixture was thoroughly combined. Subsequently, 0.5 mL of PBS and 0.05 mL of different concentrations of enzyme were added to sample cuvettes, with proper mixing. To make the volume of reaction up to 8mL, 1.15 mL deionized water was added and then, the reaction was initiated by adding 0.2mL GSH (780µM) and the reading was recorded at absorbance of 560 nm (Vives-Bauza & Garcia 2007).

In vitro antioxidant activity

CUPRAC assay

Mixed (10Mm) Cupric chloride solution, 1.0M Ammonium acetate in buffer at pH 7.0 and Nc in equal proportion to make the desired quantity of reagent mixture. 10 µL of different concentration of the EESBG, EEUSBG and Trolox (50, 100, 150, 200, 250, & 300 µg/mL) were poured in defined wells of 96 well plates. Then, 200 µL of reagent mixture was added in a 96 well plates. The reaction mixture in quadruplicate form and blank was arranged in methanol and 10 µL of different concentrations of tested sample and standard incubated for 30 minutes in dark. The wells without any accomplishment served as the control group. Following incubation, the decrease in absorbance was restrained at 512 nm with a microplate reader. For the control reaction, 20 µL of deionized water was added in the place of the tested sample. The scavenging effect was stated as percentage inhibition relative to the control (Ozyurek & Apak 2011). The IC_{50} rate was determined and graph was plotted with sample concentration on the X-axis and percentage inhibition (compared to control) on the Y-axis.

DPPH scavenging assay

In a 96 well plates, 10 µL of different concentration of test samples EESBG, EEUSBG, and standard Ascorbic acid (50, 100, 150, 200, 250, & 300 µg/mL) were added to 0.2 mL of 0.1mM DPPH dissolved in methanol. Wells that did not contain the sample were used as controls, while those lacking DPPH were treated as blanks. The experiment was conducted in quadruplicate, and all plates were reserved in the dark for 30 minutes. After incubation, absorbance was recorded at 540 nm using a microplate reader. A reaction mixture containing 20 µL of distilled water was utilized as the control. The scavenging capacity was articulated as % inhibition relative to control, sample concentration (X-axis) against % inhibition (Y-axis) (Gulcin & Alwasel 2023).

GC-MS examination of EESSBG

The GC-MS investigation of EESSBG was directed using a GCMS-QP2010 Ultra instrument. The flow rate of the carrier gas, helium, was maintained at 1.21 mL min^{-1}. The oven temperature of the GC instrument was increased from 100 °C to 250 °C. EESSBG, dissolved in methanol, was analyzed over a mass range of 10–500 m/z, and the results were related utilizing the Wiley spectral library. The mass spectra were detected within 0–25 min. The identification of compounds present in EESBG was carried out by comparing the obtained mass spectra with those stored in standard spectral libraries such as the NIST (National Institute of Standards and Technology) and Wiley libraries. The retention time (RT), molecular weight, and fragmentation patterns were considered for confirmation of compounds. The comparative percentage of individual phytoconstituents was intended using the peak area normalization method. The chromatographic peak areas were expressed as percentages without applying correction factors. The GC–MS chromatogram was analyzed to recognize major and minor phytoconstituents present in EESBG. The identified compounds were further correlated with their reported pharmacological activities, particularly antioxidant and hepatoprotective properties, based on available scientific literature (Qadir *et at.* 2022).

Molecular docking study

Molecular docking was achieved using the LibDock algorithm in Discovery Studio to evaluate the binding potential of selected bioactive compounds, including 4,5-diamino-6-hydroxypyrimidine, 3,4-Altrosan, Isosorbide, Maltol, and DDMP. These compounds were chosen based on their reported antioxidant and hepatoprotective activities. The crystal structures of target proteins-Keap1, IKKβ, TGFBR1, NF-ϰB (p65), TNF-α, IL-6, and Bcl-xL were recovered from the Protein Data Bank. Ligands were geometrically optimized prior to docking. Active sites were defined based on co-crystallized ligand coordinates, and docking was carried out to identify favorable binding conformations. The best poses were selected based on LibDock scores and interaction profiles, including hydrogen bonding, hydrophobic interactions, and van der Waals forces (Khalid *et at.* 2021).

Statistical analysis

Results were articulated as mean ± standard deviation (S.D) and mean ± standard error of the mean (SEM). Statistical significance was assessed using one-way ANOVA following Dunnett's t-test. in GraphPad Prism 5.0 (San Diego, CA, USA), with $P < 0.05$ and < 0.01 considered as significant. IC_{50} values were obtained through linear regression analysis (Mukim *et at.* 2021).

RESULTS

The sprouting process of *S. bicolor* grains breaks down complex starches, making the grains easier to digest and allows better absorption of minerals like iron, calcium, magnesium, and zinc. The different amino acids present in EESSB have antioxidant, anti-inflammatory, and regenerative properties, offer promising therapeutic potential for liver diseases.

***In vitro* hepatoprotective activity of the EESBG and EEUSBG**

In vitro cytotoxic activity

The results of cytotoxicity reveal that EESBG and EEUSBG exhibited low level of toxicity to HepG2 cells. At the highest dosage (300 µg/mL) EESBG and EEUSBG exhibited 18.24 and 26.42% cell death whereas standard drug Paclitaxel showed 94.64%. However, at the highest dosage (300 µg/mL) both tested EESBG and EEUSBG, showed less than 50% cell death (Fig. 2).

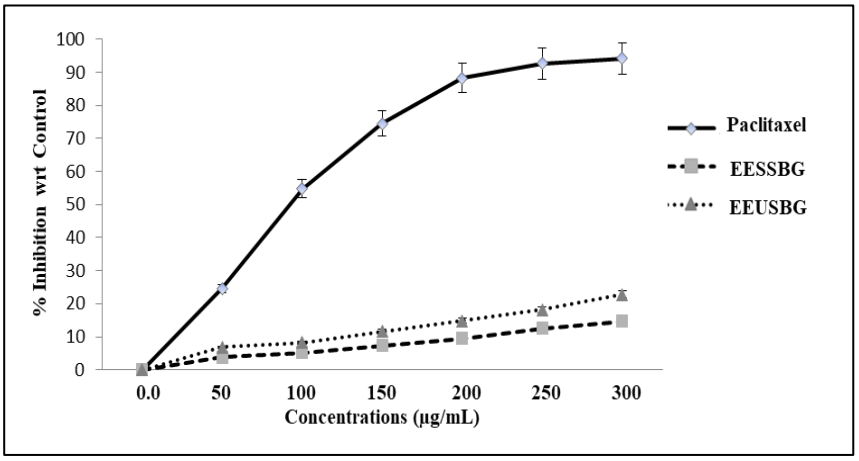


Figure 2: MTT cytotoxicity effect of the ethanol extract of sprouted and unsprouted *Sorghum bicolor* grains (EESSBG & EEUSBG) in HepG2 liver cells. Data are stated as percentage mean ± SD (n = 4).

Measurement of cell viability, ALT, and AST activities

As shown in the figure 3, group-I (NC) HepG2 cells showed 93.51 ± 1.32% and per se control group of Silymarin, EESSBG, and EEUSBG treated HepG2 cells showed 92.86 ± 1.38, 92.94 ± 1.62 and 92.56 ± 1.43 %. The group-II, treated with 80 mM ethanol showed significant ($^{\#\#}p < 0.01$) reduction in cell viability as compared to NC group-I. The HepG2 cells treated with EESSBG (50, 100, and 200 µg/mL) exhibited a significant renovation of HepG2 cell viability. All the tested doses (50, 100 and 200 µg/mL) of EESBG exhibited the cytoprotective activity and dose 200 µg/mL showed more significant effect. The outcomes of the study AST and ALT levels represented as (Table 1), group-II, treated with 80 mM ethanol showed significant ($^{\#\#}p < 0.01$) rise in the level of AST and ALT as related with normal control group-I and HepG2 cells treated with EESSBG (50, 100, and 200 µg/mL) exhibited a significant demolish of hepatic enzyme levels (ALT and AST). EEUSBG did not show significant effects in compared to EESPG and silymarin.

In vitro cytotoxicity evaluation by LDH release assay in HepG2 cells

At the concentrations of 50, 100 and 200 µg/mL, EESPG confirmed protective effects on HepG2 cells by maintaining membrane integrity (Table 1). The highest concentration 200 µg/mL presented the significant ($^{**}p < 0.01$) decrease in LDH levels, which was equivalent to the standard hepatoprotective drug Silymarin whereas EEUSPG showed non-significant effect. The reduction in LDH release indicates stabilization of the cellular membrane and prevention of enzyme leakage.

These results indicate that the extracts were non-cytotoxic at the tested concentrations and were able to protect the hepatocytes against toxicant-induced cellular damage.

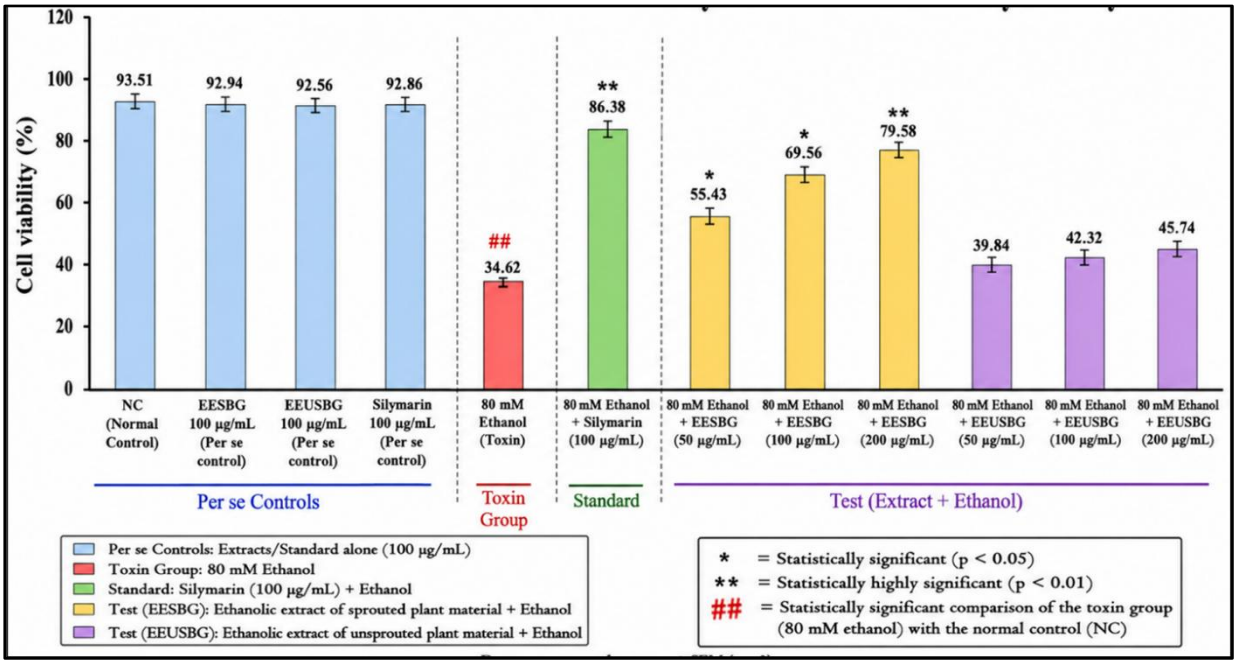


Figure 3: Effect of EESBG and EEUSBG on Cell viability (%) in ethanol-induced cytotoxicity. Cells treated with 80 mM ethanol (toxin control) exhibited a significant drop in cell viability compared with the normal control. Treatment with silymarin (100 µg/mL) and EESPG at 50, 100, and 200 µg/mL produced a concentration-dependent renovation of HepG2 cell viability, whereas EEUSPG produced comparatively non-significant renovation of HepG2 cell viability. The per se treatments with EESBG, EEUSBG, and silymarin at 100 µg/mL did not markedly alteration of HepG2 cell viability compared with the normal control. Data are articulated as mean ± SEM.

Table 1: *In vitro* hepatoprotective activity of ethanolic extract of sprouted and unsprouted *Sorghum bicolor* grains against damage induced by 80 mM ethanol in HepG2 cells towards AST, ALT and LDH production.

Group	**AST (IU/L)**	**ALT (IU/L)**	**LDH (IU/L)**
Group I Normal control (NC)	12.62 ± 3.11	9.43 ± 4.12	136.29 ± 1.51
EESBG 100 µg/mL (Per se control)	13.08 ± 3.64	10.14 ± 4.28	139.62 ± 1.42
EEUSBG 100 µg/mL (Per se control)	13.35 ± 3.18	11.21 ± 4.31	137.85 ± 1.54
Silymarin 100 µg/mL (Per se control)	13.76 ± 2.92	11.42 ± 3.62	138.26 ± 1.72
Group II 80 mM ethanol (Toxin)	61.61 ± 3.85$^{\#\#}$	47.23 ± 4.21$^{\#\#}$	246.26 ± 2.81$^{\#\#}$
Group III (Standard) 80 mM ethanol + silymarin (100 µg/mL)	16.32 ± 3.22**	14.28 ± 4.54**	145.48 ± 1.28**
Group IV (Test) 80 mM ethanol + EESBG (50 µg/mL)	35.54 ± 3.92*	28.43 ± 2.52*	210.13 ± 1.32*
80 mM ethanol + EESBG (100 µg/mL)	29.42 ± 3.63*	19.85 ± 4.84*	178.28 ± 1.53*
80 mM ethanol + EESBG (200 µg/mL)	18.65 ± 2.54**	15.64 ± 3.82**	152.36 ± 1.42**
80 mM ethanol + EEUSBG (50 µg/mL)	56.58 ± 3.75	44.56 ± 3.76	242.55 ± 1.63

80 mM ethanol + EEUSBG (100 µg/mL	53.24 ± 2.52	41.81 ± 3.48	238.25 ± 1.73
80 mM ethanol + EEUSBG (200 µg/mL	49.84 ± 2.85	38.97 ± 3.72	235.62 ± 1.51

Data represent mean ± SEM (n = 6), Analysis of variance (One-way ANOVA) was performed surveyed by Dunnett's t-test. $^{\#\#}p < 0.01$ Group-I (Normal control) versus Group-II (Toxin treatment 80 mM ethanol). $^{*}p < 0.05$ & $^{**}p < 0.01$ Group-II (Toxin treatment 80 mM ethanol) versus test Group-III & Group-IV (Different concentrations of EESBG and EEUSBG).

Estimation of MDA content (TBARS Assay)

The TBARS assay can be susceptible to interference from other compounds in plant extracts that also react with TBA, potentially leading to overestimation of MDA (Figure 4). MDA is a well-known marker of lipid peroxidation, and it elevated points indicate increased damage to cellular membranes due to reactive oxygen species (ROS). Therefore, the dose-dependent rise in MDA levels reflects a progressive intensification of lipid peroxidation, implying that the sample is inducing oxidative damage in concentration dependent means.

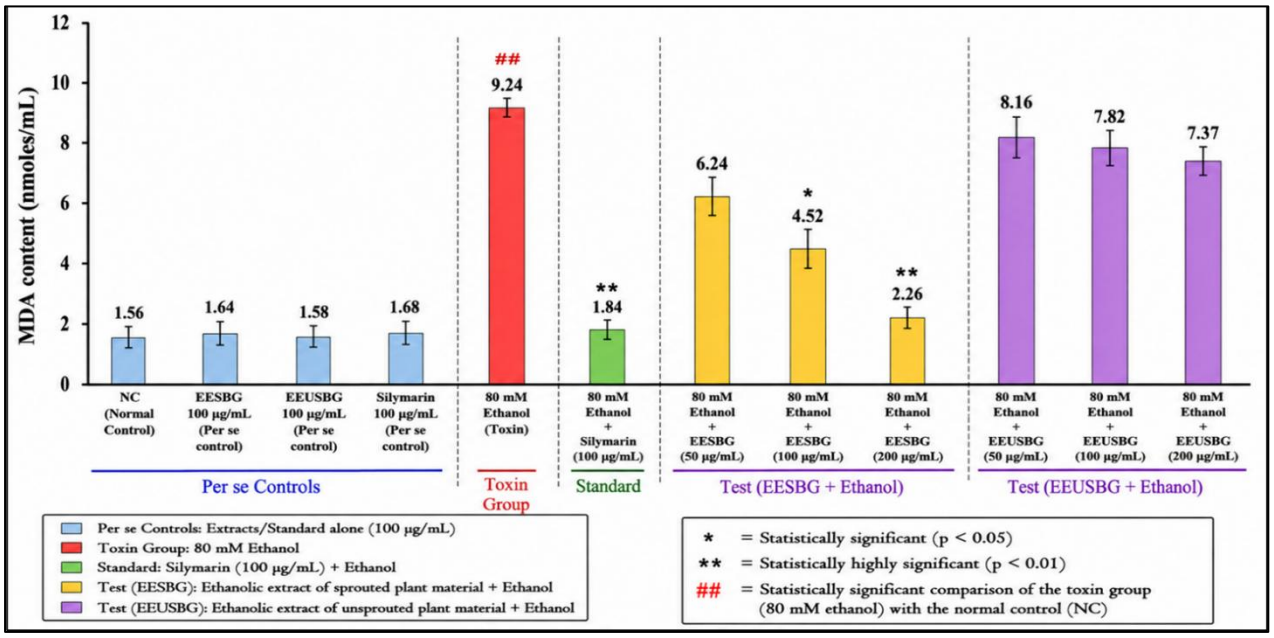


Figure 4: Effect of EESBG and EEUSBG on malondialdehyde (MDA) content in ethanol-induced cytotoxicity. MDA content was measured as a pointer of lipid peroxidation. Cells treated with 80 mM ethanol (toxin control) showed a marked rise in MDA content related with the normal control. Treatment with silymarin (100 µg/mL) and EESPG at 50, 100, and 200 µg/mL produced a concentration-dependent reduction in MDA levels, whereas EEUSPG produced comparatively limited reduction. The per se treatments with EESPG, EEUSPG, and silymarin at 100 µg/mL did not markedly alter MDA levels related with the normal control. Data are expressed as mean ± SEM.

Alkaline phosphatase assay

The activity of Alkaline Phosphatase was estimated in HepG2 cell lysates to evaluate hepatocellular damage and the protective effect of the EESBG. The level of the ALP enzyme action seems to be measured by the extent of EESBG (Table 2). In the present study, the toxic control group-II exhibited a significant elevation ($^{\#\#}p < 0.01$) in ALP activity related with the normal control group-I. Treatment with EESBG shows a dose-dependent significant ($^{**}p < 0.01$) restored in enzyme activity in the given concentrations (200 µg/mL) as compare to toxic group. The standard Silymarin also significantly ($^{**}p < 0.01$) restored ALP levels toward normal values, whereas EEUSBG showed nonsignificant restoration of ALP levels.

Estimation of reduced glutathione

GSH in plant extracts can be valuable for understanding a plant's antioxidant capacity and potential health benefits. GSH is a vital antioxidant that supports to defend the cells from damage triggered by free radicals. The toxic control group-II presented a significant depletion in the intracellular GSH level associated with the normal control group-I, indicating the induction of oxidative stress in HepG2 cells. Similarly, treatment with EESBG at higher concentrations (200 µg/mL) caused in a significant (**$p < 0.01$) increase in GSH levels likened to the toxic control group. Among the tested samples, EESBG presented a more pronounced elevation of GSH levels than EEUSBG, suggesting enhanced antioxidant activity due to sprouting (Table 2).

Estimation of SOD activity

Measuring SOD action in plant extracts provides valuable insights into their antioxidant potential and potential health benefits. In contrast, the toxic control group-II exhibited a significant reduction in SOD activity linked to the normal control group-I, suggesting the initiation of oxidative stress and extreme liberation of reactive oxygen species (ROS) in HepG2 cells. Treatment with the standard drug silymarin and EESBG significantly restored SOD activity toward normal levels, and EESBG demonstrated a greater enhancement of SOD activity than EEUSBG (Table 2).

***In vitro* antioxidant activity**

The CUPRAC and DPPH radical sequencing test was performed to evaluate the total antioxidant potential of EESBG and EEUSBG. As shown in (Fig. 5 and 6) the EESBG sample, showed a dose-dependent antioxidant activity, by means of the CUPRAC and DPPH radical procedures. Based on the outcomes found from the investigation, antioxidant property CUPRAC test was observed in models and 50% inhibitory concentration of EESBG was 148.4 ± 0.84 µg/mL and equivalent to 65.8 ± 0.68 µg/mL of Trolox. The results gotten from the investigational work, antioxidant activity (DPPH assay) assessed in EESBG sample, IC50 of tested sample was found to be 180.65 ± 0.24 µg/mL and equivalent to 87.64 ± 0.82 µg/mL Ascorbic acid.

Table 2: *In vitro* effects of ethanolic extract of sprouted and unsprouted *Sorghum bicolor* grains against damage induced by 80 mM ethanol towards ALP, GSH and SOD.

Groups	**ALP (U/mg Protein)**	**GSH (U/mg Protein)**	**SOD (U/mg Protein)**
Group I Normal control (NC)	52.42 ± 2.82	9.24 ± 3.52	27.48 ± 2.26
EESBG 100 µg/mL (Per se control)	54.76 ± 3.71	9.34 ± 3.54	29.12 ± 2.42
EEUSBG 100 µg/mL (Per se control)	54.84 ± 2.94	9.32 ± 3.62	29.43 ± 2.68
Silymarin 100 µg/mL (Per se control)	56.23 ± 3.12	9.56 ± 3.42	31.76 ± 2.84
Group II 80 mM ethanol (Toxin)	186.24 ± 3.84##	2.48 ± 3.54##	13.64 ± 2.92##
Group III (Standard) 80 mM ethanol + silymarin (100 µg/mL)	76.45 ± 3.28**	7.826 ± 3.44**	26.48 ± 2.48**
Group IV (Test)			
EESBG (50µg/mL)	144.42 ± 3.26	3.95 ± 3.39	17.42 ± 2.28
EESBG (100 µg/mL)	128.64 ± 3.38*	4.92 ± 3.68*	21.84 ± 2.26*
EESBG (200 µg/mL)	98.28 ± 3.62**	6.84 ± 3.72**	24.84 ± 2.32**
EEUSBG (50 µg/mL)	174.35 ± 3.82	3.62 ± 3.72	13.88 ± 2.62
EEUSBG (100 µg/mL)	168.82 ± 3.73	3.74 ± 3.26	14.23 ± 2.83
EEUSBG (200 µg/mL)	162.73 ± 3.74	3.89 ± 3.82	15.64 ± 2.81

Data represent mean ± SEM (n = 6), one-way (ANOVA) analysis of variance was performed followed by Dunnett's t-test. ##p<0.01 Group-I (Normal control) versus Group-II (Toxin treatment 80 mM ethanol). *p<0.05 & **p<0.01 Group-II (Toxin treatment 80 mM ethanol) versus test Group-III & Group-IV (Different concentrations of EESBG and EEUSBG).

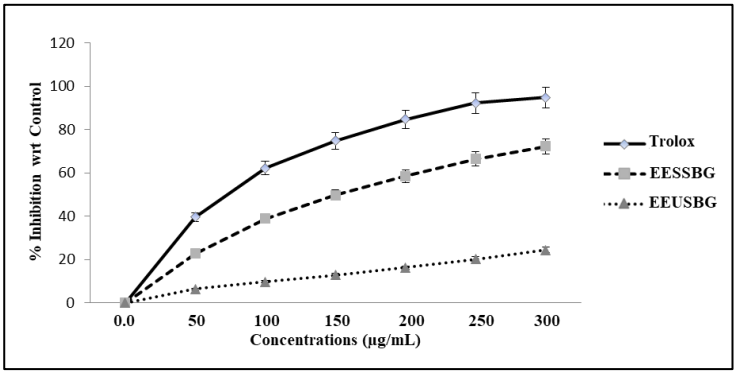


Fig. 5: Dose-dependent antioxidant activity, by the ethanol extract of sprouted and unsprouted *Sorghum bicolor* grains (EESSBG & EEUSBG) using the CUPRAC. The data represent the percentage reduction of Cu (II) to Cu (I) by the tested sample. Each value signifies mean ± S.D., (n = 4). IC_{50} of tested sample EESBG and Trolox was found to be 148.4 ± 0.84 and 65.8 ± 0.68 µg/mL. EEUSBG was found to be very low active as compared to standard – Trolox and 50% inhibitory concentration was observed above dose limit.

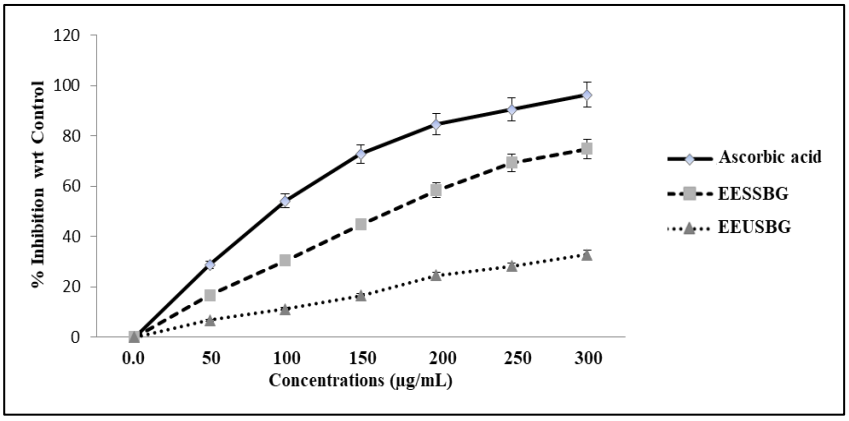


Fig. 6: Dose dependent scavenging of DPPH radicals by the ethanol extract of sprouted and unsprouted *Sorghum bicolor* grains (EESSBG and EEUSBG). The data signify the percentage inhibition of DPPH radicals. Each value signifies mean ± S.D., (n = 4). IC_{50} of tested sample EESBG and Ascorbic acid was found to be 180.65 ± 0.24 and 87.64 ± 0.82 µg/mL. EEUSBG was found to be very low active as compared to standard Ascorbic acid and 50% inhibitory concentration was observed above dose limit.

GC-MS data of EESSBG

The GC-MS examination results enabled the documentation of several bioactive phyto-compounds, which were determined using MS integrated with GC-MS examination. Figure 7 presents a characteristic GC chromatogram of EESSB, while Table 3 provides the corresponding retention times, peak areas, chemical names, molecular weights, and molecular formulas of the compounds. The comparative percentage of respective component was intended by linking its middling peak area to the total peak areas. A total of 15 major phytoconstituents were observed in EESSB which molecular structures are given in figure 3. The observed phyto components found in EESSB are α-Furfuryl alcohol (0.78 %), Filicinic acid (0.25 %), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (1.63 %), Glycerol (2.34 %), Butylene glycol diacetate (1.94 %), 2,5-Dimethyl-4-hydroxy- 3(2H)-furanone (0.50 %), 4,5-Diamino-6-hydroxypyrimidine (1.74 %), Maltol (1.49 %), Acetyl-2-hydroxy-γ-butyrolactone (1.59 %), 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (22.27 %), 1,4:3,6-Dianhydro-α-d-glucopyranose (0.85 %), Glyceryl diacetate (0.99 %), 5-Methyl-2-isopropyl-1-phenol (0.17 %), 3,4-Altrosan (0.85 %), and α-D-Methylglucopyranoside (58.71 %). Based on the GC–MS chromatogram of *S. bicolor*, the major identified compounds include 4,5-Diamino-6-

hydroxypyrimidine, Maltol, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP), 1,4:3,6-Dianhydro-α-D-glucopyranose, 3,4-Altrosan, and α-D-Methylglucopyranoside (Figure 8).

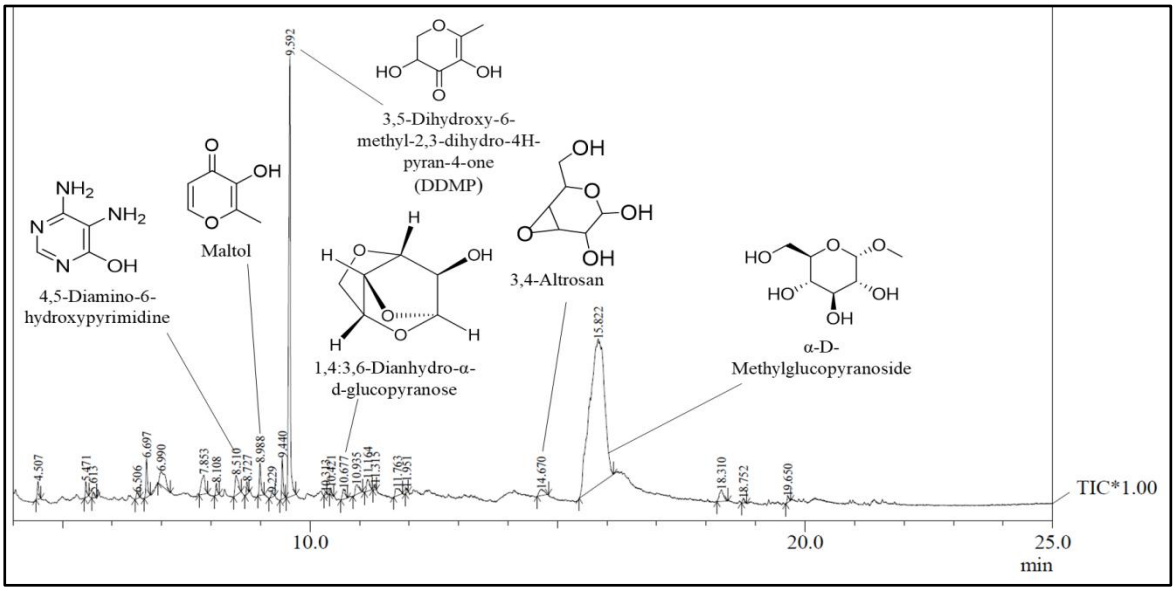


Figure 7. GC-MS chromatogram of methanol extract of sprouted *Sorghum bicolor* grains with probable marker compounds for hepatoprotective activity

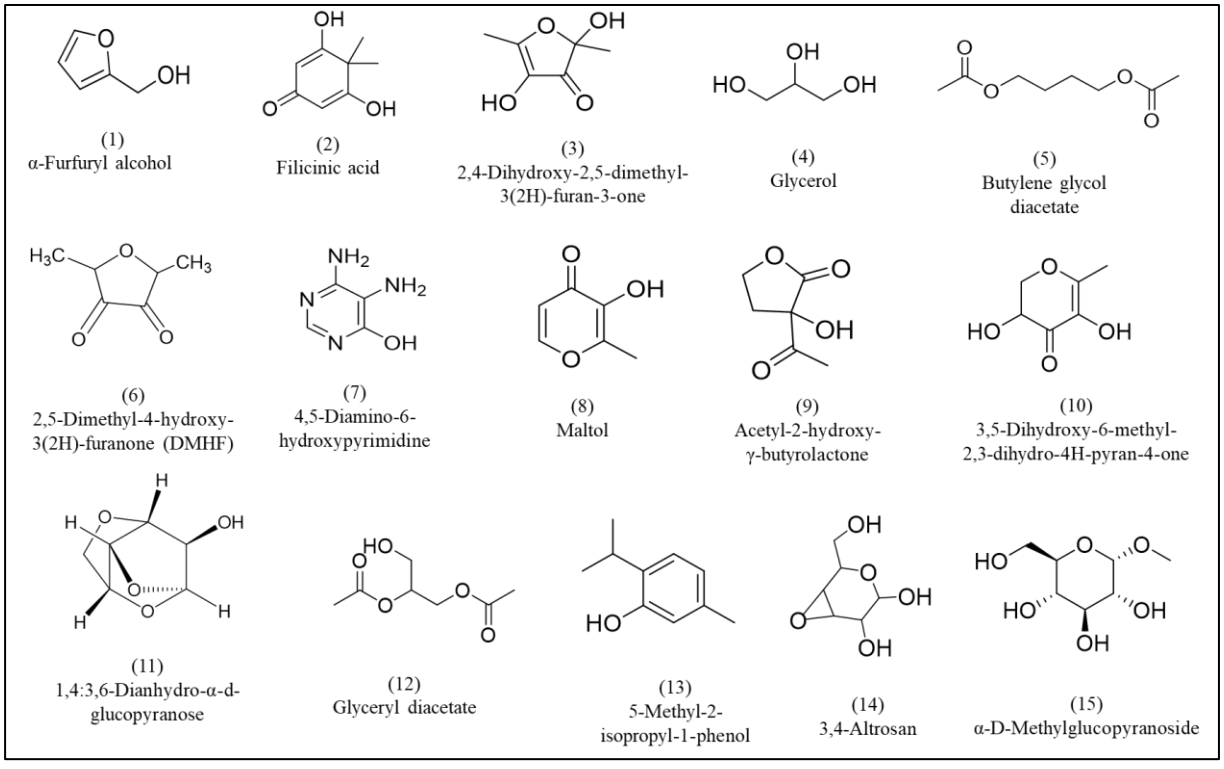


Figure 8. Phytocomponents hits upon the GC-MS chromatogram of the methanol extract of sprouted *Sorghum bicolor* grains.

The prominent peaks corresponding to DDMP (RT 9.592 min) and 3,4-Altrosan (RT 15.822 min) indicate that these compounds are major constituents of the extract. The GC–MS examination of methanol extract of sprouted *S. bicolor* grains revealed a diverse array of bioactive phytoconstituents belonging to pyrimidine derivatives, pyranones, sugar derivatives, and carbohydrate degradation products. The presence of these compounds provides valuable insight into the nutritional and

pharmacological potential of sorghum, particularly its antioxidant and health-promoting properties.

Table 3. Bioactive compounds found in the ethanol extract of sprouted *Sorghum bicolor* grains using GC-MS analysis

Peak No.	R. Time	Area%	Molecular Formula	m/z (g/mol)	Compounds Name
1	4.507	0.78	$C_5H_6O_2$	98	α-Furfuryl alcohol
2	5.613	0.25	$C_8H_{10}O_3$	154	Filicinic acid
3	6.697	1.63	$C_4H_8O_4$	144	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4	6.990	2.34	$C_3H_8O_3$	92	Glycerol
5	7.853	1.94	$C_8H_{14}O_4$	174	Butylene glycol diacetate
6	8.108	0.50	$C_6H_8O_3$	128	2,5-Dimethyl-4-hydroxy- 3(2H)-furanone (DMHF)
7	8.510	1.74	$C_4H_6N_4O$	126	4,5-Diamino-6-hydroxypyrimidine
8	8.988	1.49	$C_6H_6O_3$	126	Maltol
9	9.440	1.59	$C_6H_8O_4$	144	Acetyl-2-hydroxy-γ-butyrolactone
10	9.592	22.27	$C_6H_8O_4$	144	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP)
11	10.677	0.85	$C_6H_8O_4$	144	1,4:3,6-Dianhydro-α-d-glucopyranose
12	11.164	0.99	$C_7H_{12}O_5$	176	Glyceryl diacetate
13	11.951	0.17	$C_{10}H_{14}O_1$	150	5-Methyl-2-isopropyl-1-phenol
14	14.670	0.85	$C_6H_{10}O_5$	162	3,4-Altrosan
15	15.822	58.71	$C_7H_{14}O_6$	194	α-D-Methylglucopyranoside

Among the recognized compounds, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (DDMP) was one of the predominant constituents. DDMP is a well-known Maillard reaction product reported to possess strong antioxidant action through free radical scavenging and reserve of lipid peroxidation. The abundance of this compound suggests that *S. bicolor* may exhibit significant antioxidant potential, supporting previous reports that sorghum is rich in bioactive compounds capable of protecting biological systems from oxidative stress.

The detection of Maltol, a naturally occurring pyrone derivative, further supports the antioxidant capacity of the extract. Maltol has been stated to show antioxidant, metal-chelating, and cytoprotective effects. It can reduce oxidative damage by stabilizing reactive oxygen species and enhancing the bioavailability of essential minerals. The presence of maltol therefore contributes to the functional and nutraceutical value of sorghum. Another important constituent identified was 4,5-Diamino-6-hydroxypyrimidine, a nitrogen-containing heterocyclic compound associated with purine and pyrimidine metabolism. Pyrimidine derivatives have concerned consideration due to their antimicrobial, antioxidant, and biological regulatory activities. Its occurrence may indicate the incidence of metabolically active nitrogenous compounds that play to the physiological functions of the grain. The chromatogram revealed several carbohydrate-derived compounds, including 1,4:3,6-Dianhydro-α-D-glucopyranose, 3,4-Altrosan, and α-D-Methylglucopyranoside. These compounds are commonly formed through the transformation of polysaccharides and monosaccharides and reflect the carbohydrate-rich nature of sorghum grains. Such sugar derivatives are often associated with antioxidant activity and may serve as intermediates in various metabolic pathways (**Figure** 9**)**.

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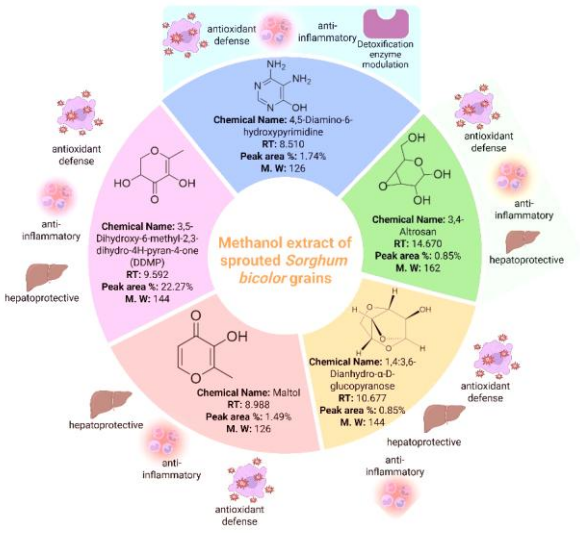


Figure 9. Reported pharmacological activities of phytoconstituents identified in the methanolic sprouted *Sorghum bicolor* grains (EESBG).

The prominent peak corresponding to 3,4-Altrosan suggests a substantial contribution of carbohydrate-derived constituents to the phytochemical profile of the extract. The occurrence of these carbohydrate derivatives is particularly relevant in sprouted sorghum, where enzymatic hydrolysis during germination enhances the release of soluble sugars and bioactive metabolites. Germination is known to expand the nutritional excellence and antioxidant capacity of sorghum by enhancing the availability of phytochemicals and reducing antinutritional factors.

Molecular docking study

Molecular docking studies results indicate moderate binding affinity and suggest potential for structural optimization to enhance multi-target activity. 4,5-Diamino-6-hydroxypyrimidine: Multi-target Docking (Keap1, IKKβ, TGFBR1).

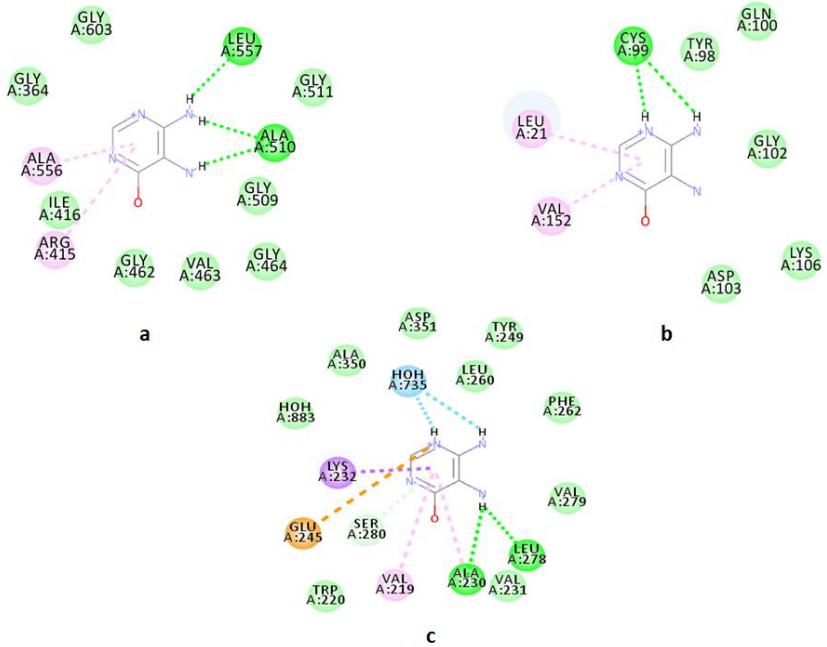


Figure 10. 2D interaction diagrams of 4,5-diamino-6-hydroxypyrimidine with (a) Keap1 (PDB ID: 4IQK), (b) IKKβ (PDB ID: 4KIK), and (c) TGFBR1 (PDB ID: 6B8Y), illustrating hydrogen bonding, hydrophobic (π–alkyl), and electrostatic interactions contributing to ligand stabilization within the respective active sites.

Docking analysis revealed that 4,5-diamino-6-hydroxypyrimidine exhibited stable binding within the active sites of Keap1, IKKβ, and TGFBR1. The compound formed key hydrogen bonds with ALA510 and LEU557 in Keap1, CYS99 in IKKβ, and LEU278 and ALA230 in TGFBR1, along with supporting π–alkyl and electrostatic interactions. Although consistent binding was observed across all targets, the interaction network was simple with limited hydrophobic and π–π interactions compared observed (Figure 10).

3,4-Altrosan: Docking with NF-ϰB (p65)

3,4-Altrosan demonstrated moderate binding affinity within the NF-ϰB (p65) active site. The interaction was mainly stabilized by hydrogen bonds with SER238, ARG253, and GLU211, along with van der Waals contacts involving surrounding residues. An unfavorable donor–donor interaction with ARG255 was also observed (Figure 11).

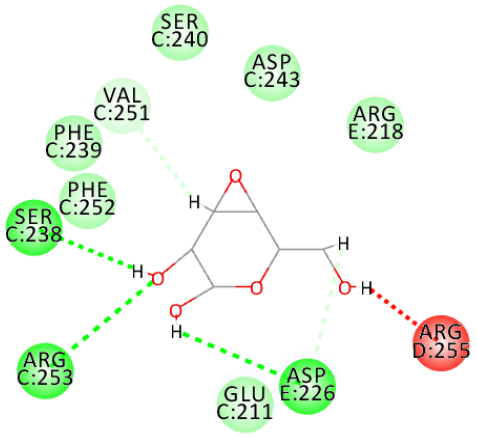


Figure 11: 2D interaction diagram of 3,4-Altrosan with NF-ϰB (p65 subunit) (1NFI), highlighting hydrogen bonding, van der Waals, and unfavourable interactions within the binding site.

1,4:3,6-Dianhydro-α-D-glucopyranose: Docking with IL-6 and TNF-α

Docking studies showed that 1,4:3,6-dianhydro-α-D-glucopyranose interacts effectively with IL-6 and TNF-α, demonstrating multi-target potential. Strong hydrogen bonding interactions were observed with IL-6, indicating better binding stability, while comparatively weaker and water-mediated interactions were noted with TNF-α (Figure 12).

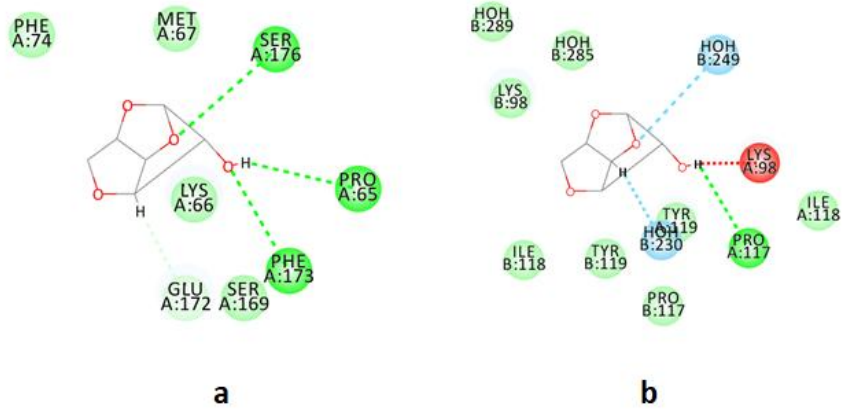


Figure 12: 2D interaction diagrams of 1,4:3,6-Dianhydro-α-D-glucopyranose with (a) IL-6 complex (1ALU) and (b) protein 2AZ5, illustrating hydrogen bonding, water-mediated interactions, van der Waals contacts, and unfavourable interactions contributing to ligand stabilization.

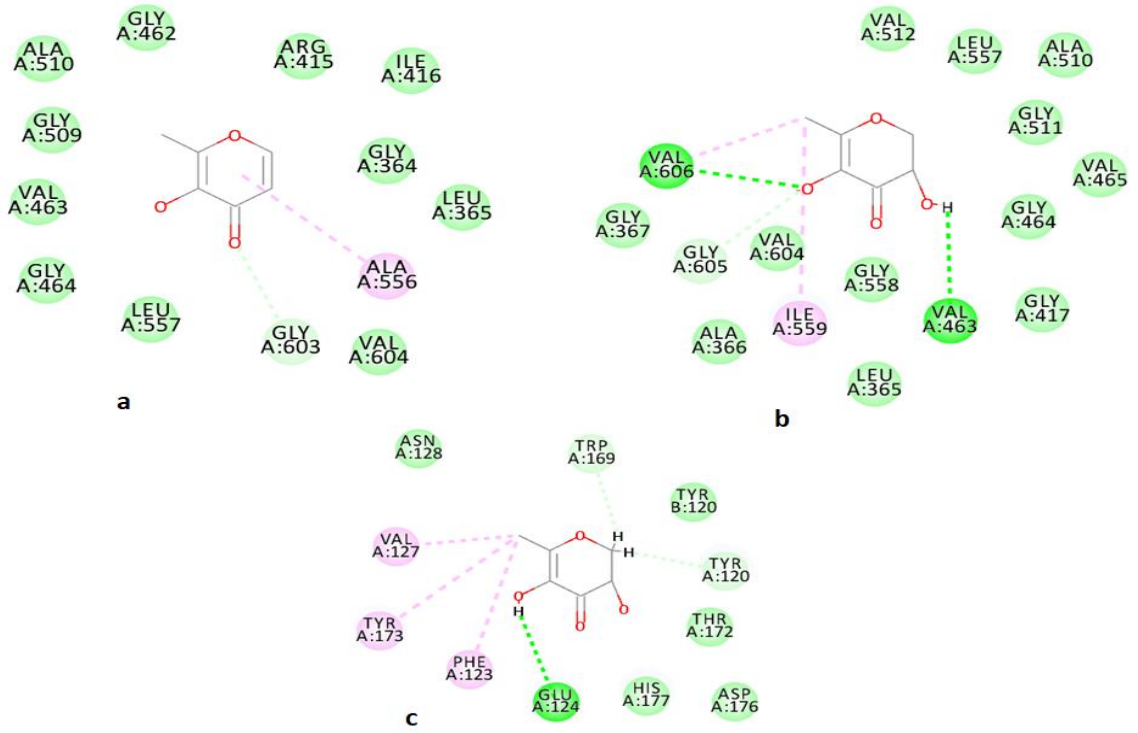


Figure 13: 2D interaction diagrams of the docked ligands: (a) Maltol with the Keap1 Kelch domain (PDB ID: 6TYM), (b) DDMP with the Keap1 Kelch domain (PDB ID: 6TYM), and (c) DDMP with the anti-apoptotic protein Bcl-xL (PDB ID: 2YXJ). The interactions highlight hydrogen bonding, hydrophobic, and van der Waals contacts that contribute to ligand stabilization within the respective protein binding pockets.

Maltol and DDMP: Docking with Keap1 (6TYM)

Both Maltol and DDMP successfully occupied the Keap1 binding pocket; however, DDMP exhibited stronger interactions. Maltol showed mainly van der Waals interactions with limited hydrophobic contacts, whereas DDMP formed a conventional hydrogen bond with VAL463 along with multiple hydrophobic interactions involving VAL604, VAL606, and ILE559. These results suggest that DDMP has higher binding affinity and may more effectively modulate the Keap1–Nrf2 pathway compared to Maltol. Further docking analysis revealed that DDMP binds effectively to the anti-apoptotic protein Bcl-xL (Figure 13).

DISCUSSION

Preliminary phytochemical test of the EESBG discovered the existence of carbohydrates, reducing sugar, phenolic, flavonoids compounds, and amino acids as major phytoconstituents. The *in vitro* cytotoxic activity of EESBG and EEUSBG is a colorimetric test for measuring the cell metabolic action and measures mitochondrial dehydrogenase activity. In which viable cells reduce yellow MTT into purple formazan crystals.

AST is an enzyme found in various tissues, but high levels in the blood often indicate liver damage. When cells are damaged, AST leaks into the bloodstream, causing elevated levels in a blood test. The liver is a primary source of AST, so elevated levels are often a sign of the diseased liver. ALT and AST levels are a key indicator of liver health, with elevated levels often suggesting liver damage from various causes, including hepatitis, alcohol abuse, or other liver diseases and this assay can be utilized to screen the progression of liver disease. Levels of the AST and ALT enzyme action looks to be precise by the extent of EESBG. Interestingly, EESBG showed comparatively higher cytoprotective effects than EEUSBG, indicating that the sprouting process might enhance the beneficial phytochemicals such as phenolic compounds, flavonoids, and antioxidants, which may contribute to improved cellular tolerance and protective effects (Oyewole & Atoyebi 2012).

LDH is a stable enzyme located in the cytosol, which is discharged into the culture medium upon impairment to the plasma membrane. The extracellular activity of LDH serves as a reliable indicator of cell membrane integrity and cytotoxicity.

Treatment of HepG2 cells with the toxic control 80 mM ethanol caused a marked elevation in LDH release related with the NC group. In contrast, treatment with EESBG showed a concentration-dependent reduction in LDH leakage and it is significantly lower LDH release when compared with the toxic control (Mukim *et al.* 2021).

The TBARS assay is widely used, to acknowledge that the TBA reacts with other aldehydes, present in the tested sample not just MDA. Based on the outcomes attained from the lipid peroxidation test (TBARS assay), the sample EESBG shows an increase in malondialdehyde (MDA) content in a dose dependent method suggests that higher doses of the treatment lead to greater oxidative stress in the system.

Liver diseases cause a huge upsurge in the action of liver ALP that takes place entirely in the plasma membrane. The action of the membrane phosphatase may be elevated in the livers by permeating the toxic drugs. The results indicate that the EESBG exhibits notable hepatoprotective potential by mitigating toxin-induced elevation of ALP in HepG2 cells, likely due to its antioxidant phytochemicals found in the EESBG (Iluz-Freundlich & Minuk 2020).

Analyzing GSH levels in plant extracts reveal their potential as natural antioxidants neutralizing harmful ROS. Plant extracts rich in glutathione (GSH) can be integrated into functional foods and supplements to enhance human health.

The restoration of SOD activity in HepG2 cells treated with EESBG suggests that these extracts possess considerable antioxidant and hepatoprotective properties. The improvement in SOD activity may be ascribed to the presence of phenolic compounds, flavonoids and other antioxidant phytoconstituents in *S. bicolor* which can neutralize reactive oxygen species and enhance the cellular antioxidant defines mechanism. Measuring SOD activity helps determine the plant's overall antioxidant capacity and its potential for protecting against oxidative stress-related damage.

The assessment CUPRAC is a method used to estimate the antioxidant power of a sample by measuring the reduction of Cu (II) to Cu (I) in the incidence of antioxidants compounds. This process is based on the formation of a colored Cu (I) neocuproine chelate, which is then measured spectrophotometrically. The antioxidant activity of the EESBG was found to be 76.54% by CUPRAC assay and 88.84% DPPH assay at 300 µg/mL). The percentage antioxidant activity increased with increasing concentration, at 300 µg/mL, EESBG showed the maximum cupric ion reducing activity, whereas EEUSBG exhibited comparatively lower activity.

The GC–MS profile demonstrates that *S. bicolor* contains a complex mixture of antioxidant-rich compounds, including pyranones, pyrimidine derivatives, and sugar based metabolites. The findings support the growing recognition of *S. bicolor* as a functional food and a promising foundation of natural bioactive compounds for pharmaceutical and nutraceutical purpose. The GC–MS investigation confirmed the occurrence of several biologically important composites in *S. bicolor*. The predominance of DDMP, maltol, and carbohydrate-derived metabolites highlights the grains as a source of natural antioxidants and health-promoting phytochemicals. These outcomes offer a scientific basis for the traditional use of sorghum and justify further studies aimed at isolating and evaluating the pharmacological activities of the identified constituents (Uttam *et. al.* 2020).

The binding profile of 3,4-Altrosan suggests that the ligand interaction is predominantly hydrogen bond-driven with limited hydrophobic contributions, indicating scope for further optimization. The interaction of 1,4:3,6-Dianhydro-α-D-glucopyranose: Docking with IL-6 and TNF-α suggest that the compound has promising anti-inflammatory potential, particularly through IL-6 modulation, though further optimization may improve its overall binding affinity. The interaction of Maltol and DDMP with Keap1 (6TYM) stabilized by a hydrogen bond with GLU124, along with multiple van der Waals and hydrophobic interactions involving residues such as TYR120, TRP169, and PHE123. This stable binding suggests that DDMP may interfere with Bcl-2 family proteins and promote apoptotic signaling, highlighting its dual role in antioxidant defense and apoptosis regulation.

CONCLUSION

The current study demonstrates that the ethanolic extract of sprouted *Sorghum bicolor* grains possesses significant potential as a functional food for supporting liver health. Phytochemical profiling exposed the existence of bioactive constituents, reckoning phenolic compounds, flavonoids, and Maillard reaction-derived metabolites such as maltol and DDMP, which are recognized for their antioxidant and hepatoprotective possessions. The *in vitro* investigations showed that the sprouted sorghum extract exhibited notable free-radical scavenging activity and improved cellular antioxidant status, suggesting its ability to mitigate oxidative stress-associated hepatic injury. Furthermore, the *in silico* molecular docking analysis indicated

favorable interactions of major phytoconstituents with liver-related antioxidant and stress-response targets, supporting their possible role in modulating oxidative defense mechanisms. These computational findings complement the experimental observations and provide mechanistic insight into the hepatoprotective potential of the sprouted grains. Overall, the collective phytochemical, *in vitro*, and *in silico* evidence suggests that sprouting enhances the functional value of *S. bicolor*, making it a promising dietary source of hepatoprotective phytochemicals.

FUTURE PERSPECTIVE

Nevertheless, further investigations involving detailed molecular studies, bioavailability assessment, and well-designed in vivo and clinical evaluations are necessary to confirm its therapeutic efficacy and establish its application as a scientifically validated functional food for liver health management.

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REFERENCES

[1] Alisik, M., Neselioglu, S., & Erel, O. (2019). A colorimetric method to measure oxidized, reduced and total glutathione levels in erythrocytes. *Journal of Laboratory Medicine*, *43*(5), 269-277.

[2] Chaturvedi V, Goyal S, Jyoti Sharma M, Sanjeevani, Sharma A, Verma I, et al. Comprehensive analysis of the genus Plumeria: Phytochemistry, traditional uses, and therapeutic potential. Yemen J Med. 2026;5(2):341-347.

[3] Al-Qubaisi, M., Rozita, R., Yeap, S. K., Omar, A. R., Ali, A. M., & Alitheen, N. B. (2011). Selective cytotoxicity of goniothalamin against hepatoblastoma HepG2 cells. *Molecules*, *16*(4), 2944-2959.

[4] Arif, M., Chaudhary, N., Panda, P. D., & Islam, A. (2026). Pharmacognostical and Nutritional Assessment of Sprouted Pennisetum glaucum and Sorghum bicolor as Body-Sustaining (Dhatu-Poshaka) Functional Foods in Ayurveda: Pharmacognostical Standardization and Nutritional Evaluation of Sprouted Millets as Ayurvedic Functional Foods for Tissue Nourishment. *International Journal of Ayurveda360*, *2*(6), 1185-1198.

[5] M. Mukim, M. Chaturvedi, and R. Patel (2022), titled *"Qualitative Phytochemical Investigation and Antioxidant Activity of Chlorophytum Borivilianum Santapau & R.R.Fern. Leaves"* Volume 11, Issue 1 (pages 611–617).

[6] Borgonovi, S. M., Marzocchi, S., Pasini, F., Bordoni, A., Barbiroli, A., Marti, A., ... & Di Nunzio, M. (2025). Exploring Germination to Unlock the Nutritional Potential of Sorghum (Sorghum bicolor). *Molecules*, *30*(17), 3622.

[7] Chaudhary, N., Arif, M., Shafi, S., Kushwaha, S. P., & Soni, P. (2025). Emerging role of natural bioactive compounds in navigating the future of liver disease. *Iliver*, *4*(1), 100140.

[8] De Leon, J. A. D., & Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *Journal of visualized experiments: JoVE*, (159), 10-3791.

[9] Mohammad Mukim, Mohit Chaturvedi, Rakesh Patel.Pharmacognostical Standardization and Phytochemical Analysisof Chlorophytum borivilianum Santapau and R.R. Fern. Leaves.Research Journal of Pharmacy and Technology. 2022; 15(6):2402-6. doi: 10.52711/0974-360X.2022.00399

[10] Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes*, *11*(8), 2248.

[11] Iluz-Freundlich, D., Zhang, M., Uhanova, J., & Minuk, G. Y. (2020). The relative expression of hepatocellular and cholestatic liver enzymes in adult patients with liver disease. *Annals of hepatology*, *19*(2), 204-208.

[12] Khalid, M., Alqarni, M. H., Shoaib, A., Arif, M., Foudah, A. I., Afzal, O., ... & Altamimi, A. S. (2021). Anti-arthritic and anti-inflammatory potential of Spondias mangifera extract fractions: An in silico, in vitro and in vivo approach. *Plants*, *10*(5), 825.

[13] Singh, P. K.;Tripathi, S.; Goyal, S.; Chaturvedi,V.; Jeelani, U.; Khan, S. K.; Mukim, N.; Rawat,A. K. S. Pharmacognostical Standardizationand Phytochemical Character izationof Ipomea Eriocarpa Leaves., Journalof Chemical Health Risks., 2023, 13(3),952–959.

[14] Khalid, M., Shoaib, A., Singh, S., Alqarni, M. H., Foudah, A. I., Khan, S., ... & Arif, M. (2026). In silico molecular docking analysis of potential antilipidemic bioactive constituents of Coriandrum sativum root extract using GC-MS. *Arabian Journal of Chemistry*, *19*, 1-7.

[15] Kumar, S., Arif, M., Jawaid, T., Al-Khamees, O. A., Anjum, A., Shafi, S., ... & Qadir, A. (2023). Antiplatelet and thrombolytic activity of phenolic-insistent fractions from the new-fangled stem of Ficus benghalensis with concurrent GC-MS analysis. *Intelligent Pharmacy*, *1*(4), 224-231.

[16] Munoz, T. A. Q., Rodriguez, S. J. V., & Uco, J. G. T. (2022). Nutraceutical Properties of Medicago sativa L., Agave Spp., Zea mays L. and Avena sativa L.: A Review of Metabolites and Mechanisms. *Metabolites 2022, 12, 806*.

[17] Mukim M, Kabra A, Hano C, Drouet S, Tungmunnithum D, ChaturvediM, et al. Rivea hypocrateriformis (Desr.) choisy: An overview of itsethnomedicinal uses, phytochemistry, and biological activities andprospective research directions. J Chem. 2022;2022:9099672.

[18] Ogbole, O. O., Segun, P. A., & Adeniji, A. J. (2017). In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. *BMC complementary and alternative medicine*, *17*(1), 494.

[19] Oyewole, O. I., Oladipupo, O. T., & Atoyebi, B. V. (2012). Assessment of renal and hepatic functions in rats administered methanolic leaf extract of Jatropha tanjorensis. *Annals of Biological Research*, *3*(2), 837-841.

[20] Mohammad Mukim, M.C., Rakesh Patel, Supriya Roy, Pratishtha Sharma,Varunesh Chaturvedie, Saloni Goyale and Mohsina F. Patewkarf, Crohn's disease:A review on epidemiology, diagnosis and therapeutic management. INDIAN DRUGS,2021. 59(09): p. 16-28.

[21] Özyürek, M., Güçlü, K., & Apak, R. (2011). The main and modified CUPRAC methods of antioxidant measurement. TrAC Trends in Analytical Chemistry, 30(4), 652-664.

[22] Pareek, A., Godavarthi, A., Issarani, R., & Nagori, B. P. (2013). Antioxidant and hepatoprotective activity of Fagonia schweinfurthii (Hadidi) Hadidi extract in carbon tetrachloride induced hepatotoxicity in HepG2 cell line and rats. *Journal of ethnopharmacology*, *150*(3), 973-981.

[23] Qadir, A., Khan, N., Arif, M., Warsi, M. H., Ullah, S. N. M. N., & Yusuf, M. (2022). GC–MS analysis of phytoconstituents present in Trigonella foenumgraecum L. seeds extract and its antioxidant activity. *Journal of the Indian Chemical Society*, *99*(6), 100503.

[24] Singh, S., Habib, M., McClements, D. J., Bashir, K., Jan, S., & Jan, K. (2024). Exploring the potential of sorghum with reference to its bioactivities, physicochemical properties and potential health benefits. *Food & Function*, *15*(24), 11847-11864.

[25] Rai S, Siddiqui M, Nagar J. Pharmacological and medicinal importance of Pas-siflora edulis: a review. Int J Res Rev. 2022;9:341–9.

[26] Tang, Z., Chen, H., He, H., & Ma, C. (2019). Assays for alkaline phosphatase activity: Progress and prospects. *TrAC Trends in Analytical Chemistry*, *113*, 32-43.

[27] Vives-Bauza, C., Starkov, A., & Garcia-Arumi, E. (2007). Measurements of the antioxidant enzyme activities of superoxide dismutase, catalase, and glutathione peroxidase. *Methods in cell biology*, *80*, 379-393.

[28] Mukim M, Sharma P, Mohsina FP, Faheem IP, Kukkar R, Patel R, et al. (2021). Multiple Used Medicinal Plant: Glycyrrhiza Glabra – Review. SunText Rev Pharm Sci 2(1): 111.

[29] Zhu, R., Wang, Y., Zhang, L., & Guo, Q. (2012). Oxidative stress and liver disease. *Hepatology Research*, *42*(8), 741-749.

[30] Uttam Singh Baghel, Himani Sharma, Anamika Chouhan, Abhay Sharma, Mohammad Mukim, Deeksha Singh. Gradient RP-HPLC Method development for simultaneous estimation of Dextromethorphan hydrobromide, Phenylephrine hydrochloride, and Triprolidine hydrochloride in Liquid Dosage Form. 2020-13-2-14