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Scientific Research

Assessment of the antioxidant and cytotoxic properties of 1,2,3-Benzenetriol and 1,5-Anhydro-6-deoxyhexo-2,3-diulose extracted from *Camellia sinensis*.

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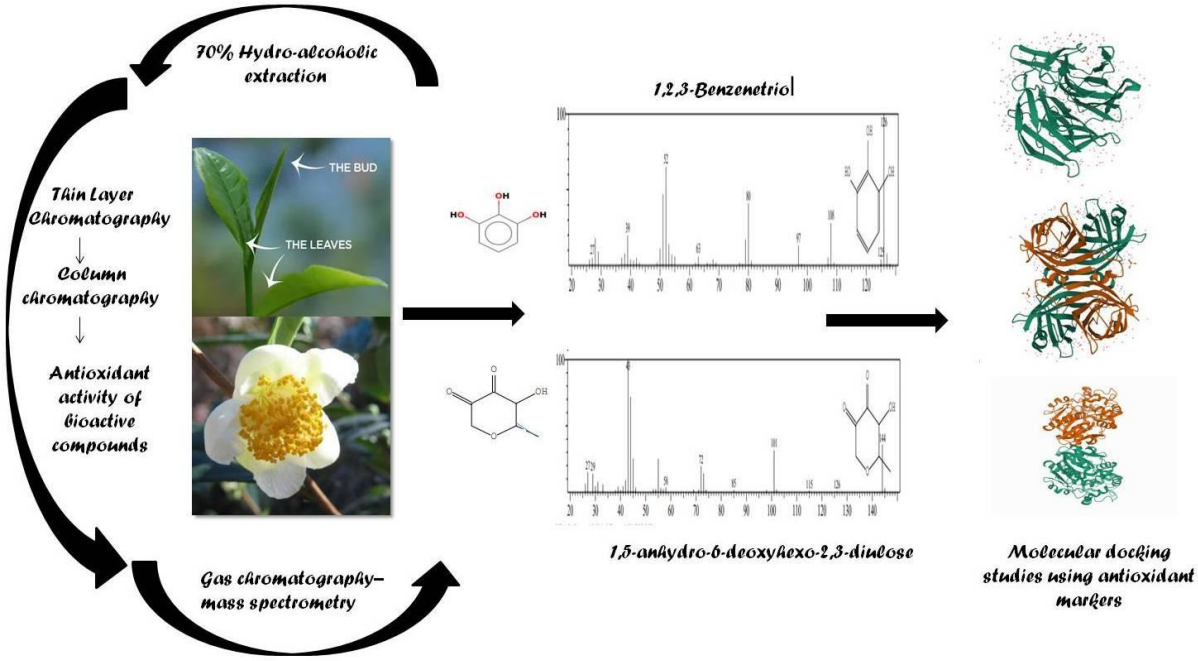
ABSTRACT

Antioxidant qualities of natural substances hold promise for combating oxidative stress-related disorders. Two such substances, 1,2,3-Benzenetriol and 1,5-anhydro-6-deoxyhexo-2,3-diulose (ADD), derived from *Camellia sinensis*, were investigated for their antioxidant potential. The study aimed to evaluate the ability of 1,2,3-Benzenetriol and ADD to neutralize free radicals and regulate biomarkers of oxidative stress. Active compounds were obtained by isolating them from leaves and flowers of Camellia sinensis using column chromatography with a mixture of ethyl acetate and hexane. Their antioxidant properties were assessed using GC-MS techniques. Bioactive fractions, namely CSLBF-2 and CSFBF-2, were identified as having the highest inhibition percentages of DPPH free radicals. Inhibitory concentrations were determined at 132 and 147 µg mL-1 for CSLBF-2 and CSFBF-2, respectively. Molecular docking studies were conducted using GOLD software to assess binding interactions with NFE2L2, a gene implicated in defending against oxidative stress. CSLBF-2 and CSFBF-2 demonstrated significant inhibition percentages of DPPH free radicals, with values of 92.05% and 88.33%, respectively. Molecular docking studies revealed beneficial binding interactions with NFE2L2, suggesting potential antioxidant mechanisms. Notably, 1,2,3-Benzenetriol exhibited the strongest binding energy among the studied compounds. Additionally, purified compounds displayed considerable cytotoxicity against the Vero cell line and exhibited favorable drug bioavailability properties. The study concludes that 1,2,3-Benzenetriol and ADD exhibit promising antioxidant properties and potential for development into novel pharmaceutical agents. Their effectiveness in neutralizing free radicals and regulating oxidative stress biomarkers suggests a promising avenue for further research and pharmaceutical development.

Abbreviations

ADMET - Absorption, Distribution, Metabolism, Excretion, and Toxicity; BBB: Blood-Brain Barrier; CNS - Central Nervous System; DPPH - 2,2-Diphenyl-1-picrylhydrazyl; EA - Ethyl Acetate; GC-MS - Gas Chromatography-Mass Spectrometry; HPLC - High-Performance Liquid Chromatography; IC50 - Half maximal inhibitory concentration; MTT - 3-(4,5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide; NFE2L2 - Nuclear factor erythroid 2-related factor 2; NF-κB - Nuclear Factor-kappa B;TLC - Thin Layer Chromatography; UV – Ultraviolet; WHO - World Health Organization.

Graphical Abstract



Highlights

- 1,2,3-Benzenetriol and 1,5-anhydro-6-deoxyhexo-2,3-diulose from Camellia sinensis demonstrated significant inhibition of DPPH free radicals, indicating strong antioxidant potential.
- Molecular docking studies showed favorable binding interactions between the compounds and the NFE2L2 gene, suggesting potential antioxidant mechanisms.
- In silico assessment revealed good drug bioavailability and non-toxic nature, supporting their potential for further drug development as antioxidant agents.

1. Introduction

Alternative therapies are more desirable because current drug research has a limited and unfavorable therapeutic index caused by the harmful effects of drugs, which can be reduced without compromising their therapeutic benefits (Koch et al., 2019; Li et al., 2019; Prasanth et al., 2019; Anderson et al., 2020). According to WHO report states that more than 80% of the world's population relies on traditional medicine as their main source of healthcare. The utilization of phytochemicals extracted from plants has considerably enhanced human health and is acknowledged for producing efficacious medicines (Sasidharan et al., 2011; Vijayaraj and Kumaran 2017; Koch et al., 2019; Ashraf 2020; Pham et al., 2020; Lakshmanan et al., 2022). Earlier studies have confirmed that plant-derived compounds with antioxidant properties can help to reduce oxidative stress and protect against different illnesses and the harmful impacts of synthetic drugs (Koch et al., 2019; Stagos 2019; Suleman et al., 2019; Salehi et al. 2020; Mihailović et al., 2021; Lakshmanan et al., 2022).

The importance of biologically active compounds isolated from plants and their potential use against diseases has been emphasized in a study by Suleman et al. (2019). Among the most widely consumed plant-based beverages is *Camellia sinensis*, which is typically consumed as an aqueous infusion (Koch et al., 2019; Prasanth et al., 2019). This plant contains various metabolites that are effective as free radical scavengers, including antioxidant and anticancer compounds (Lobo et al., 2010; Coêlho et al., 2022; Shahbaz et al., 2022; Motyka et al., 2023; Janmeda and Chaudhary, 2021). Moreover, a variety of biological activities associated with *C. sinensis* extracts has been discovered (Fernando and Soysa, 2015; Ciampi et al., 2020). According to a literature review, the long-term health benefits of tea consumption have been an emerging field of study for less than three centuries (Chaudhary et al., 2023; Sharifi-Rad et al., 2022), highlighting the potential importance of further research into this area.

Currently, there is increasing attention towards the medicinal properties of both the flowers and leaves of *C. sinensis*. The objective of this research is to discover bioactive substances present in the plant's leaf buds and flowers that can function as free radical scavengers, which are involved in the development of several diseases. The study also examines the molecular interactions between these compounds and the NFE2L2 gene through in silico analysis of molecular docking. Additionally, the study analyzes the cytotoxic effects of these compounds using vero cell line and assesses their potential as therapeutic agents through analysis of their ADMET properties.

2. Material and methods

2.1. Processing of *Camellia sinensis* extraction

Fresh *Camellia sinensis* is commonly known as a tea plant. Its leaf buds and flowers were collected from dense Valparai tea plantation (Monica tea factory-wood briar group Tea Estates India limited), Coimbatore District, India. At 28 °C, the samples were shade dried and cut into small pieces. The shade dried leaf buds and flower samples were ground into a coarse powder using a mortar and pestle. The coarse plant materials were extracted with 70% methanol. Under low pressure, the solvent was removed to provide crude extract for future investigations.

2.2. Selection of solvent system for fractionation and purification of bioactive compounds

To begin the process, the crude extracts obtained from leaf buds and flowers were dissolved in chloroform (C). Fractionation was performed using Thin Layer Chromatography (TLC) and various solvent systems, including ethyl acetate (EA): hexane (H), chloroform (C): ethanol (E), chloroform (C): EA, H: EA, EA: E, and EA: M with different combinations and ratios as mobile phases. Finding the best eluent for extract column fractionation was the objective. The fractions were identified using iodine fumes, and the eluent that gave the best separation or resolution was chosen for future study. The concentrated crude extracts were then subjected to column chromatography (100-200 mesh, Merck, Germany) utilizing various ratios of EA:H mixtures (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9) to isolate the bioactive compounds (Brinkman et al. 1973; Monobe et al. 2008). To verify the purity of the isolated compounds, Thin Layer Chromatography (TLC) was conducted using aluminum sheets pre-coated with silica gel.

2.3. Antioxidant activity of the bioactive compound. *DPPH free radical scavenging activity*

To assess the antioxidant activity of the bioactive fractions, Hariprasath et al. (2015) used a range of stable DPPH free radical concentrations (50, 100, 150, 200, and 250 $g.mL^{-1}$). The bioactive compounds were diluted, combined with 2 mL of 0.16 mM DPPH in methanol, and vigorously shaken before being left at room temperature (30 °C) in the dark for 30 minutes. Using a spectrophotometer, the mixture's lowered absorbance was determined at 517 nm. The absorbance of the samples and the control solution (2 mL DPPH solution + 2 mL MeOH) was measured using spectrophotometric analysis. Ascorbic acid served as a positive control.

The following formula was used to determine the percentage of inhibition:

$$\text{Inhibition activity (\%)} = Abs_{(sample)} - Abs_{(control)}/Abs_{(sample)} \times 100$$

Where, Abs control stands for the absorbance of control responses, and Abs sample stands for the absorbance of test samples (contains all reagents except the test sample). Each experiment was performed three times.

2.4. Identification and Characterization of bioactive fraction

To identify the bioactive compounds, present in the bioactive fraction of *C. sinensis* leaf buds and flowers, GC (GC-7890A/MS-5975C) from Agilent Technologies was used. Retention time was utilized to recognize the bioactive components, and NIST libraries were used for their identification. The constituents were identified by comparing them with those in the computer library (NIST and Wiley) that was linked to the GC-MS instrument. The results were then tabulated.

2.5. Molecular docking studies of antioxidant markers

The study utilized molecular docking through the GOLD software to examine the binding mode of bioactive fractions. The bioactive chemicals were designed using ChemDraw and imported into Discovery Studio 2.5. The CHARMM force field was used to type compounds and the Momany-Rone option was used to compute partial charges. The structures were conjugate gradient reduced using the Smart Minimizer method. To better understand inhibitor binding, the structure of Antioxidant marker proteins (5FZN, 3WZN, 3ANS) was retrieved from the Protein Data Bank and used for docking studies. The active site in the antioxidant marker proteins complex was identified as an area with a radius of 10 nm, and water molecules were removed from the receptor. The

docking program's reproducibility was tested and compared to the original crystal structure with an RMS value of 0.95, confirming the repeatability of the GOLD program. The most promising lead compounds were chosen based on their active site binding orientation and GOLD score, and their pharmacokinetic assessment (ADMET) was performed using the pkCSM program. If any of a molecule's five anticipated conformations predicted over the rmsd value of 1.5, the early termination option was used.

2.6. Cytotoxicity Analysis

To assess the cytotoxic properties of the bioactive compounds, the study utilized the MTT assay method on Vero cell lines in vitro. After removing the culture medium, the Vero cells were subcultured separately in DMEM and treated with FCS (10%). The cells were then homogenized in DMEM (25 mL) by gently moving the pipette through the medium to suspend the cells. A 24 well culture plate was filled with one ml of the homogenized cell suspension and treated with various sample concentrations (50–250 g mL^{-1}). The plate was then placed in a humidified, CO2 (5%) incubator and kept at 37 °C. After a 48-hour incubation period, a cytotoxicity assay was conducted on the Vero cells, which were 80% confluent under an inverted microscope. 3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) was used in the experiment at different sample concentrations (50, 100, 150, 200, and 250 g mL^{-1}). Viable cells' mitochondrial enzymes broke down MTT, resulting in the production of a measurable purple formazan product. This formazan synthesis was negatively correlated with the level of cytotoxicity and positively correlated with the number of viable cells. MTT (5 g mL^{-1}) was applied to the wells after incubation, and the plate was then left at room temperature for 3 hours. The formazan crystals were then dissolved with the addition of 100 L of DMSO after the contents of the wells had been withdrawn using a pipette. In order to measure the absorbance, a Readwel Touch microplate reader was used at 570 nm (Mohammed et al., 2016).

3. Results and discussion

The *C. sinensis* plant which has various potential metabolites are used across multiple cultures as beverages, medicines, and cosmetics. Because of the unrivaled abundance of chemical constituents in variety, bioactive products from plants have provided the limitless potential for novel therapeutic leads in recent years (Vijayaraj et al., 2019). As a result,there is a demand raised across the world for screening drug components from natural sources (Kumar et al., 2019). The discovery of effective techniques for isolation, separation of bioactive compounds has remained a major problem for researchers in recent years (Modak et al., 2007).

Plant extracts commonly includes variety of bioactive chemicals with varying polarity, asa result, separating compounds remains a significant problem in the detection and characterization of bioactive fractions (Raks et al., 2018). Thin Layer Chromatography (TLC), Column Chromatography, and Gas Chromatography methods are often employed to acquire and identify pure bioactive substances and have been documented in isolation and identification approaches (Ashokkumar et al., 2020). In this research work, in *C. sinensis* through using the column chromatographymethod, four bioactive fractions (two from leaf buds and two in flowers) were purified. The purified bioactive fraction was subject to TLC for detection of purified compound spot, which was

exposed to UV light and iodine vapor. This validates the purity of the bioactive chemical extractedfrom *C. sinensis* leaf buds (CBLBF1) and flowers (CBFBF), as no other spot formed in the TLC sheet (Figure 1) and the Retention factor (Rf) values were recorded as 0.46, 52, 28 and 38 in CSLBF-1, CSLBF-2, CSFBF-1 and CSFBF-2, respectively in EA:H (7:3 ratio).

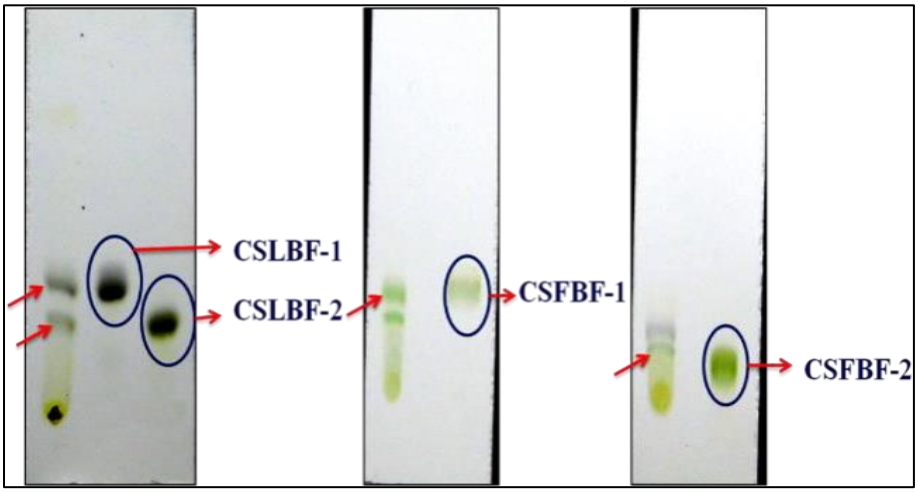


Figure 1. Purified bioactive compounds from crude leaf buds and flowers of C. *sinensis*

3.1. *In vitro* antioxidant activity

The purified 4 bioactive active fractions were examined in the present study using the DPPH free radical scavenging assay, a recognized method for evaluating the antioxidant activity of plant extracts. *C. sinensis* leaf buds and its flowers have been known to possess health promoting potential metabolites. Compounds belong to the class of pyrogallol, alkaloid, palmitic acid, polyphenols, diterpene, Tocopherol, and palmitic acid ethyl ester are present in both the extracts. These substances provide therapeutic benefits for a number of acute and chronic conditions brought on by free radicals, which are crucial in the pathophysiology of many diseases (Altemimi et al., 2017; Koch et al., 2019). Observation made by Tariq and Reyaz (2013) stated the methanol extract of *C. sinensis* showed highest antioxidant activity of 325.76±0.14 mg compared with the standard and Yang et al. (2009) concluded that the ethanol extract of tea flowers showed direct scavenging abilities against DPPH radicals (IC_{50} 47.6 µg mL^{-1}) and hydroxyl radical of IC_{50} 19.7 µg mL^{-1}. Consistent with the literature the isolated bioactive fractions from leaf and floral region were subjected to antioxidant assay and CSLBF-2 from *C. sinensis* leaf bud and CSFBF-2 from *C. sinensis* flowers showed the highest inhibiting activity against DPPH free radicals. The percentageof inhibition is 92.05% and 88.33% in CSLBF-2 and CSFBF-2, respectively at 250 µL of concentration (Figure 2). Inhibitory concentrations were calculated (IC_{50}; 132 and 147 µg mL^{-1}, ascorbic acid used as internal standard showed (IC_{50}; 57 µg mL^{-1}). The ability of bioactive components for scavenge free radicals (CSLBF-2 and CSFBF-2) tends to increase with increasingtheir concentration.

3.2. Identification of bioactive fraction

The identification and quantification of bioactive components from selected tea plant crudeextract and purified bioactive compounds has shown to be a powerful approach using gas GC-MS. In this connection, based on the *in vitro* study of free radical

scavenging activity the selected active fractions CSLBF-2 and CSFBF-2 were subjected for characterization and identification of bioactive compound using GC-MS techniques. The bioactive substances used in the current investigation was identified as 1,2,3-Benzenetriol from the crude extract of *C. sinensis* leaf bud (CSLBF-2) (Figure 2) and ADD from the crude extract of *C. sinensis* flower (CSFBF-2) (Figures 2-3). The confirmed that nature of the compounds Phenol and Glycoside, respectively and these compounds are responsible for antioxidant activity potentials (Sutanto et al., 2019).

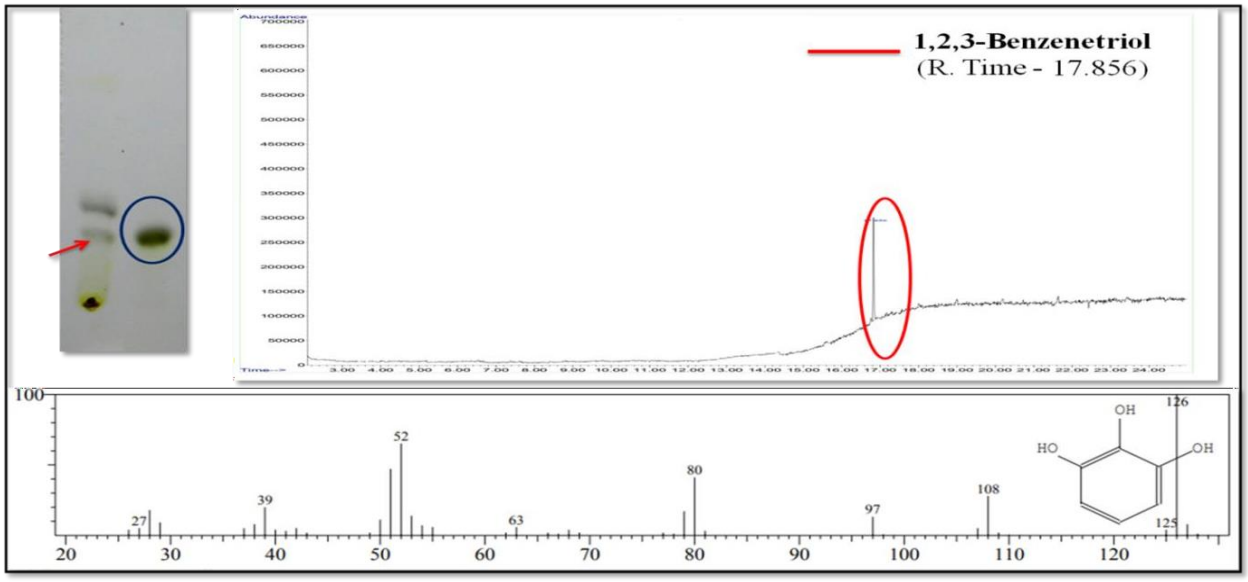


Figure 2. GC -MS analysis of CSLBF-2 (*C. sinensis* crude leaf buds extract)

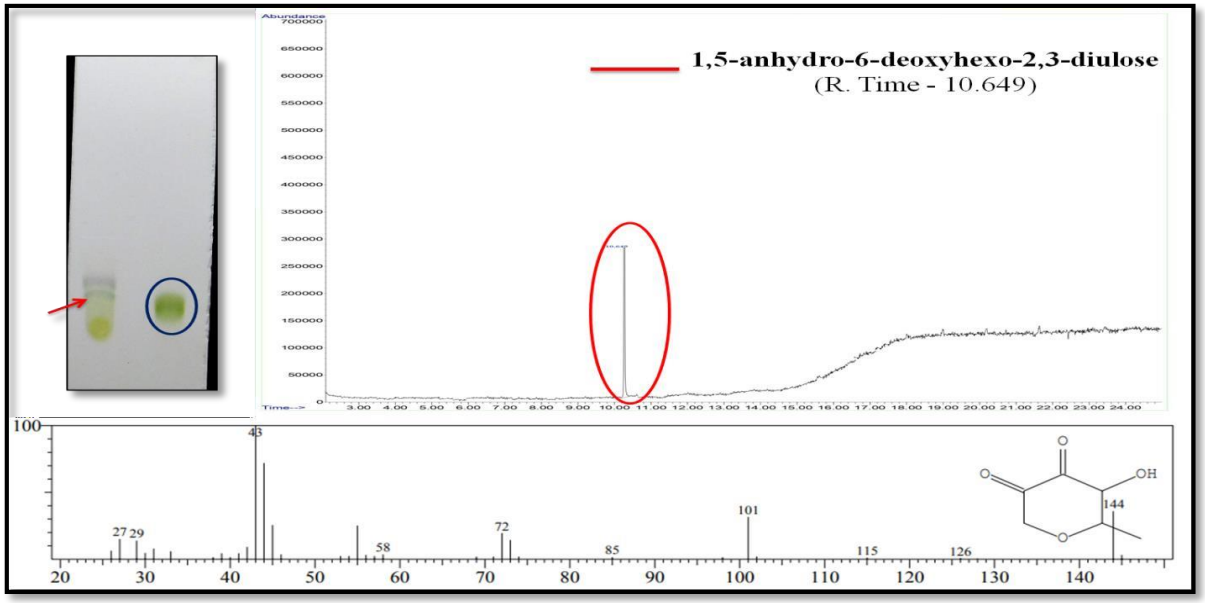


Figure 3. GC -MS analysis of CSFBF-2 (*C. sinensis* crude flowers extract)

3.3. Molecular Docking studies

In the field of drug development, molecular docking has become a widely used in silico approach in recent years. This technique involves predicting interactions between ligands and their targets at the molecular level, enabling the discovery of new medicinal drugs (Figures 4-9). In order to understand how small molecules, behave in target protein binding sites and in basic biochemical processes, molecular docking can also model the interactions between small molecules and proteins. Researchers have investigated the molecular docking properties of the NFE2L2 gene using purified bioactive compounds, namely 1,2,3-Benzenetriol and ADD. The expression of an antioxidant protein that guards against oxidative damage brought on by tissue damage is tightly controlled by the NFE2L2 gene. The gene also controls immunological responses, autophagy, proteostasis, inflammation, metabolism, and mitochondrial physiology. Research have demonstrated that oxidative stress-related disorders can be treated using bioactive substances that promote the NFE2L2 pathway. Thus, it is thought that the NFE2L2 gene is a prospective therapeutic target and a helpful biomarker for oxidative stress. The bioactive compounds 1,2,3-Benzenetriol and ADD have demonstrated good binding interactions with the NFE2L2 gene, with 1,2,3-Benzenetriol showing higher binding energy than ADD. Similarly, Mutha et al. (2021) reported that gallic acid and isovitexin exhibited high docking scores against NF-κB. (Qadir et al., 2020; Zhao et al., 2017; Rampogu et al., 2019; Iranshahy et al., 2018; He et al., 2020; Mutha et al., 2021).

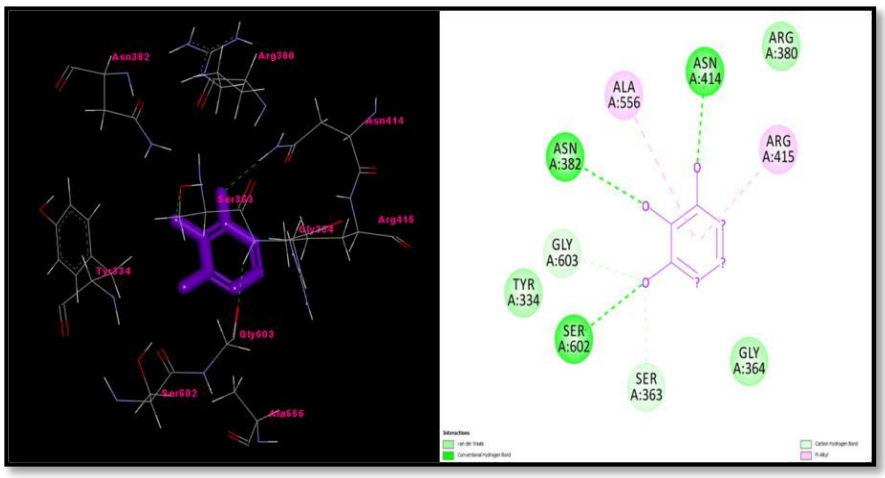


Figure 4. Docking of 1,2,3-Benzenetriol on binding pocket of 5FZN

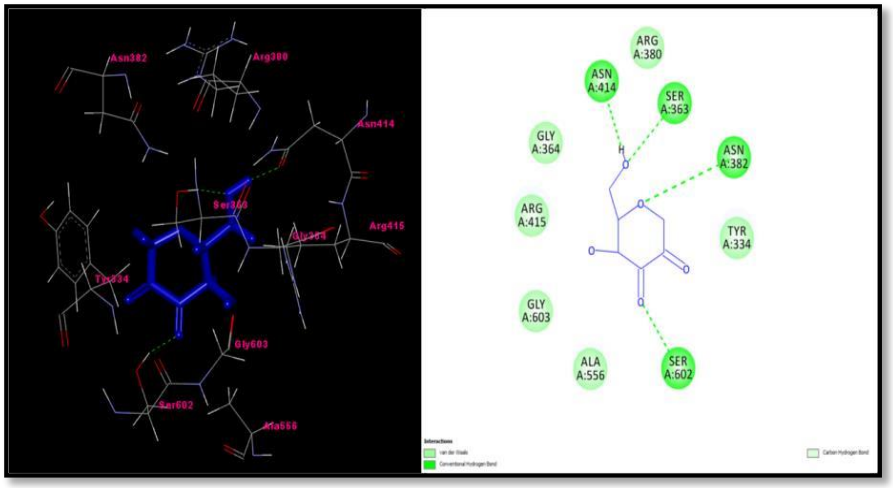


Figure 5. Docking of ADD on binding pocket of 5FZN

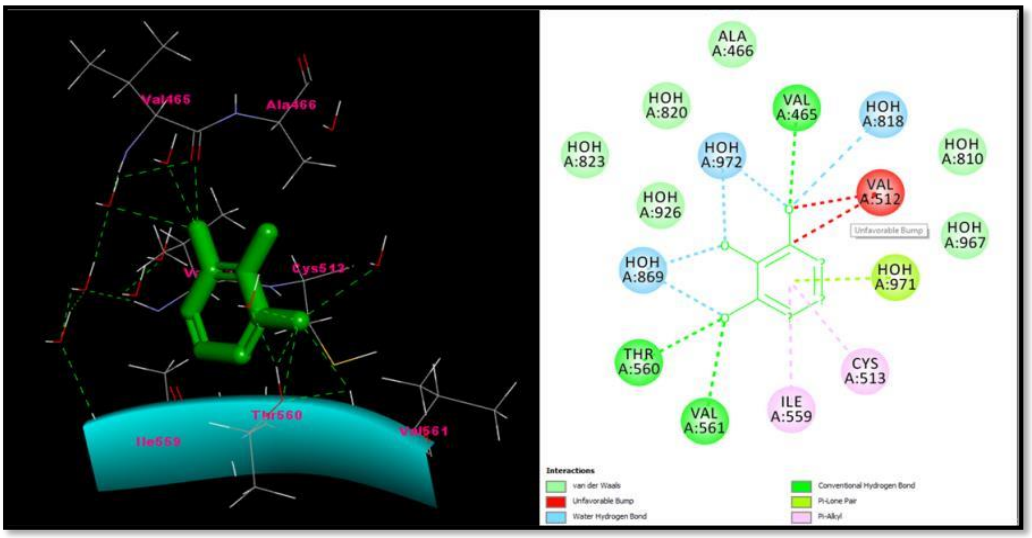


Figure 6. Docking of 1,2,3-Benzenetriol on binding pocket of 3WZN

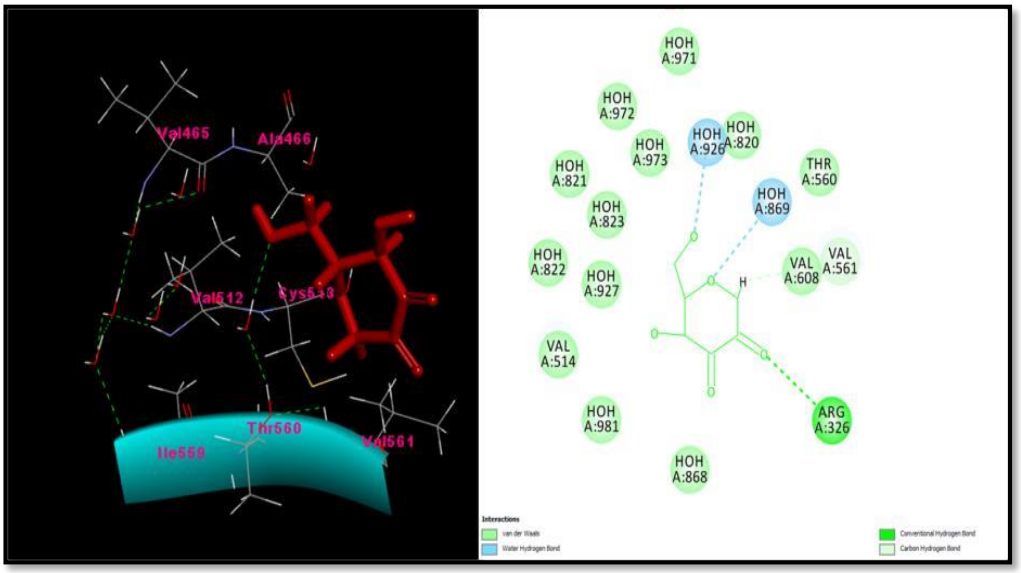


Figure 7. Docking of ADD on binding pocket of 3WZN

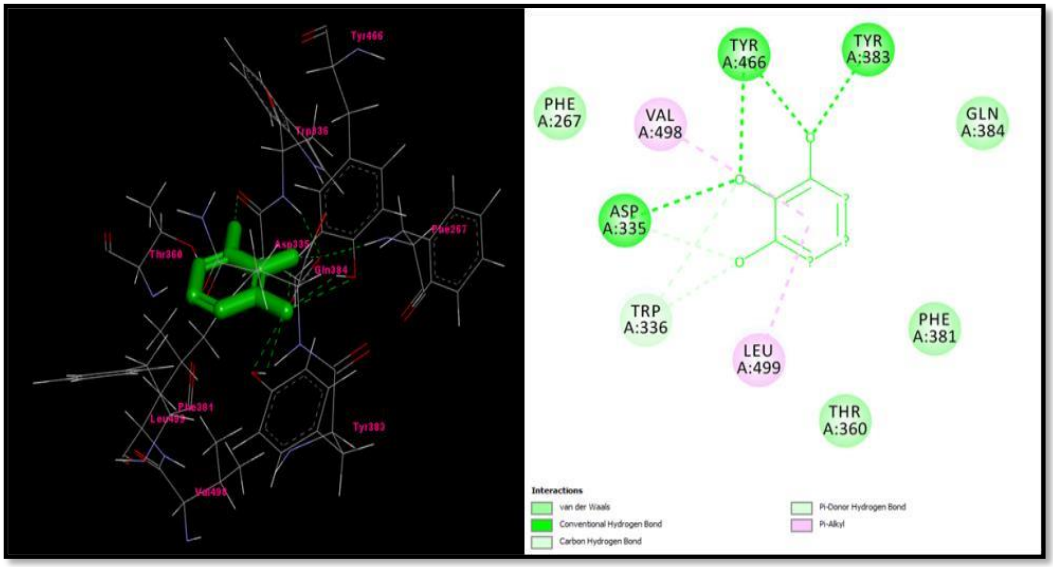


Figure 8. Docking of 1,2,3-Benzenetriol on binding pocket of 3ANS

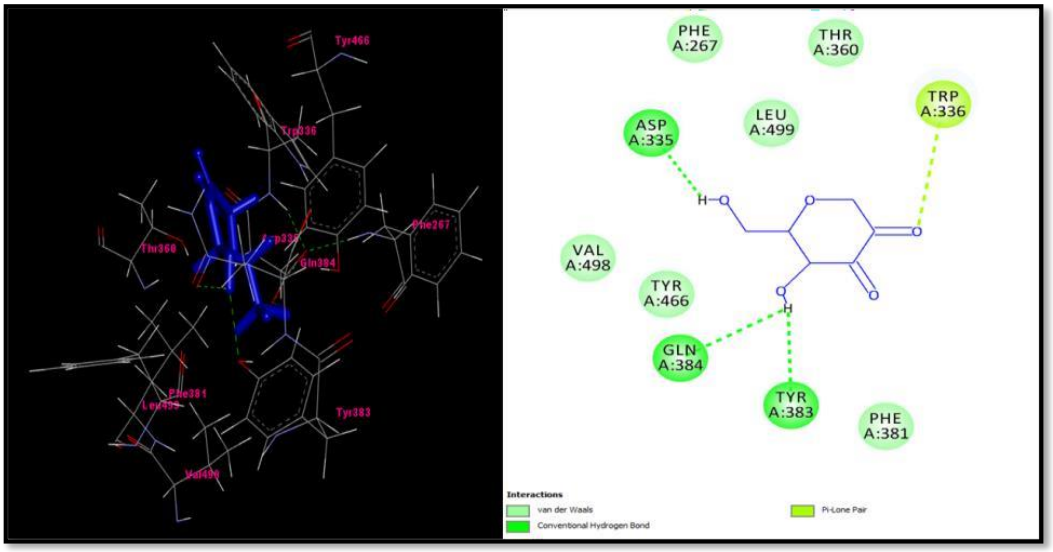


Figure 9. Docking of ADD on bindig pocket of 3ANS

Table 1: Molecular interaction of bioactive compounds

Compound	Binding Energy (kcal mol^{-1})		
	5FZN	3WZN	3ANS
1,2,3-Benzenetriol	-38.28	-27.68	-39.76
1,5-anhydro-6-deoxyhexo-2,3-diulose	-37.55	-27.03	-36.42

3.4. *In vitro* cytotoxicity activity against vero cell line

Cell vitality can be assessed using the sensitive and dependable MTT assay. This test depends on the cellular mitochondrial dehydrogenase enzyme's capacity to convert the yellow, water-soluble substrate 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) into the dark blue or purple, water-insoluble formazan product (Kpemissi et al., 2019). The number of cells in a variety of cell lines directly relates to the amount of formazan generated. Previous literature by (Kim et al., 2021b). evaluated the antioxidant effects of a turmeric leaf *in vero* cell lines (Kim et al., 2021a) and investigated the cytotoxic effect of nanoparticles synthesized from the plants against *vero* cell line. In the present study, the bioactive fractions 1,2,3-Benzenetriol and ADD were tested against Vero cell lines. The Figure 10 showed that different spectrum of purified compounds activity against Vero cells. By increasing sample concentration, the purified chemicals' anti-oxidant potential significantly increased their cytotoxicity, as seen in the data (Figures 11- 12). The findings revealed promising cytotoxic activity against Vero cell lines. In fact, at all tested sample concentrations, the Vero cell line displayed a good pattern of cytotoxicity activity, it also revealed that increased Cytotoxic effect of 1,2,3-Benzenetriol and ADD against Vero cell line concentration of drug shown high (90.21%; 78.05%) cytotoxicity of Vero cell lines. IC_{50} values were calculated (135 and 161 µg mL^{-1}).

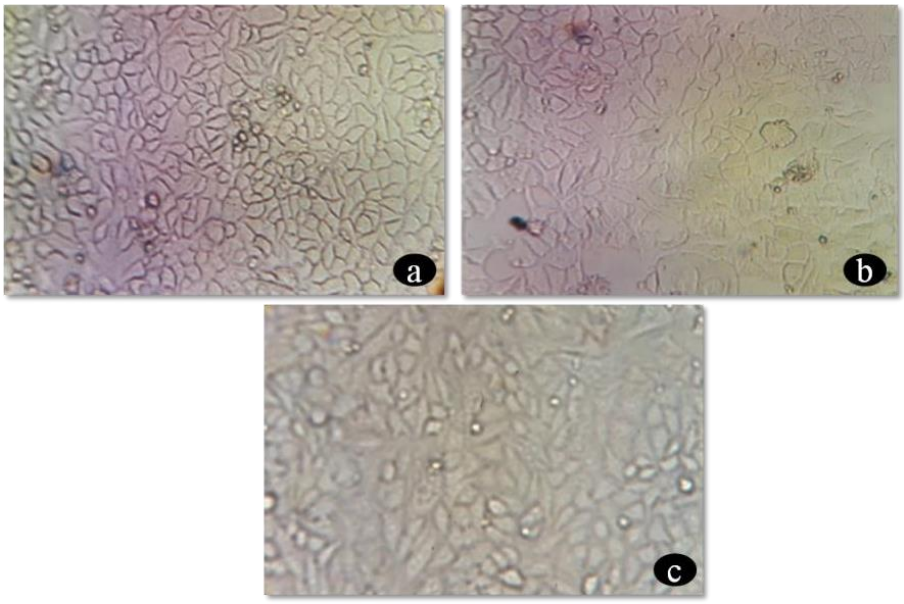


Figure10. Cytotoxic effect of 1,2,3-Benzenetriol against Vero cell line; a. control, b. treated with 1,2,3-Benzenetriol (150 µg mL^{-1}), c. treated with 1,2,3-Benzenetriol (250 µg mL^{-1})

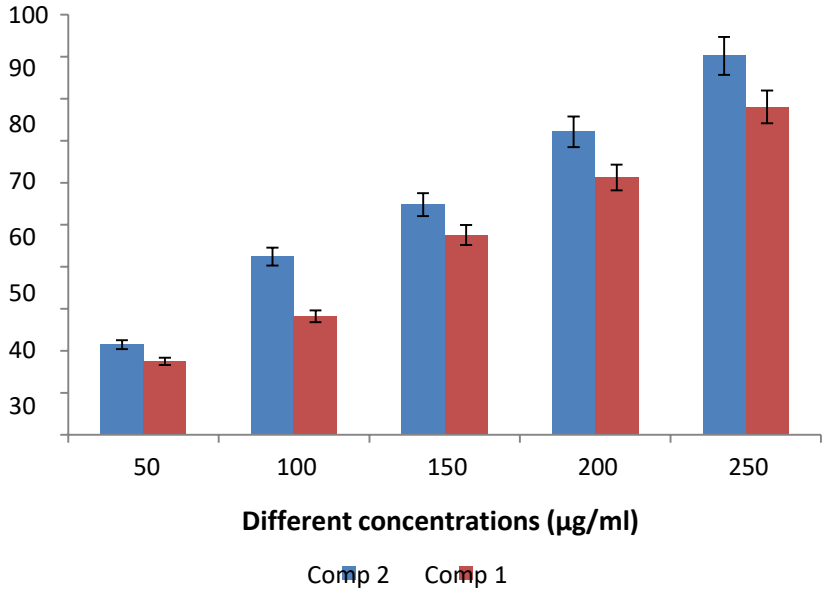


Figure 11. Cytotoxicity activity of purified compounds against Vero Cell lines

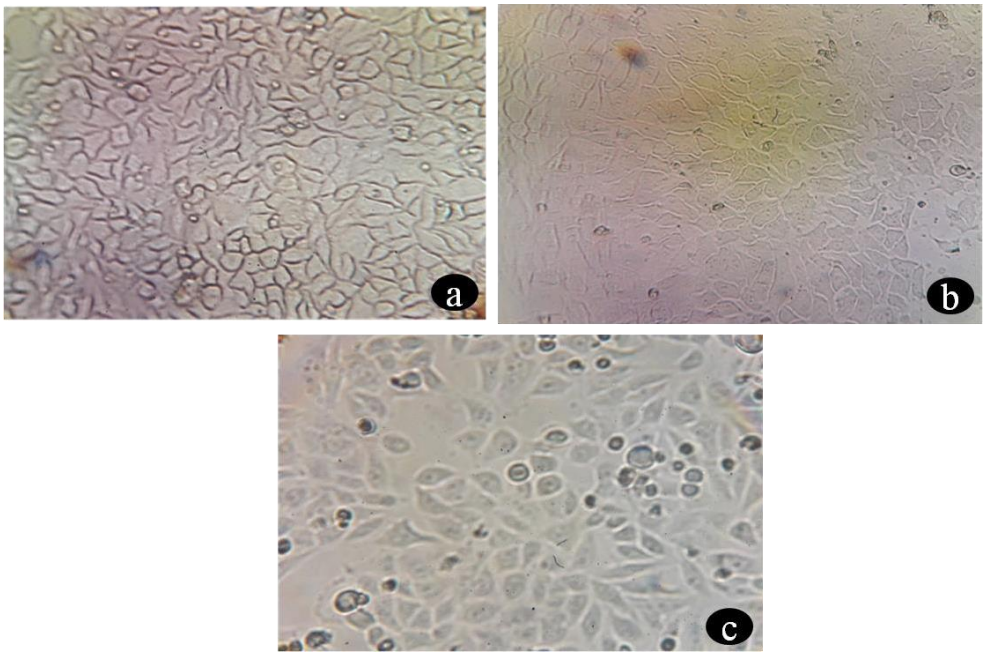


Figure12. Cytotoxic effect of ADD against Vero cell line, a. control, b. treated with ADD (150 µg mL^{-1}), c. treated with ADD (250 µg mL^{-1})

3.5. *In silico* pharmacokinetic properties (ADMET)

The ADMET (absorption, distribution, metabolism, excretion, and toxicity) characteristics of two bioactive substances, 1,2,3-Benzenetriol and 1,5-anhydro-6-deoxyhexo-2,3-diuloseADD, as determined by in silico methods, are shown in Tables 2-5 of this study. The absorption values for 1,2,3-Benzenetriol were determined to be 0.581 log mol L^{-1}, 1.102 log Papp, 87.43%, and -3.944 log K respectively, whereas those for 1,5-anhydro-6-deoxyhexo-2,3-diulose ADD were -1.408 log mol L^{-1}, 1.122 log Papp in 10-6 cm s^{-1}, 83.549%, and -3.137 log K, respectively. The distribution parameters, including VDss, fraction unbound, blood-brain barrier (BBB) permeability, and central nervous system (CNS) permeability, were also determined, and for 1,2,3-Benzenetriol, they were found to be 0.13 log L kg^{-1}, 0.712 Fu, -0.441 BB, and -3.252 PS, respectively, and for ADD they were -0.169 log L kg^{-1}, 0.809 Fu, -0.277 log BB, and -3.073 log PS, respectively. In terms of overall clearance, it was found that the excretion rates for 1,2,3-Benzenetriol and 1,5-anhydro-6-deoxyhexo-2,3-diulose ADD were 0.104 log mL min^{-1} kg^{-1} and 0.554 log mL min^{-1} kg^{-1}, respectively. There is no proof that 1,5-anhydro-6-deoxyhexo-2,3-diulose ADD and 1,2,3-benzenetriol are engaged in metabolic activities. The toxicity of these compounds was evaluated using various measures, including maximum tolerated dose, acute and chronic toxicity in rats when taken orally, toxicity in *Tetrahymena pyriformis*, and toxicity in minnows. All results were within normal ranges. The recorded values for the maximum tolerated dose, acute toxicity in rats when taken orally, chronic toxicity in rats when taken orally, toxicity in *Tetrahymena pyriformis*, and toxicity in minnows for 1,2,3-Benzenetriol and ADD are -0.269 log mg kg^{-1} day^{-1} 2.049 mol kg^{-1}, 2.374 log mg kg^{-1} bw day^{-1}, 0.127 log µg L^{-1} and 2.734 log nM,

respectively, and 1.121 log mg kg^{-1} day^{-1}, 1.967 mol kg^{-1}, 2.579 log mg kg^{-1} bw day^{-1}, -1.712 log µg L^{-1}, and 3.012 log mM, respectively. The in silico pharmacokinetic properties (ADMET) of the bioactive compounds, 1,2,3-Benzenetriol and ADD, indicate that their ADMET property parameters are within permissible limits. Furthermore, it was found that these compounds have good bioavailability for oral administration, and the in silico toxicity analysis indicated their non-toxic nature.

Table 2. ADME Properties of 1,2,3-Benzenetriol

Property	**Model Name**	**Predicted Value**
Absorption	WS	-1.408 log mol L^{-1}
	CP	1.122 log Papp in 10^{-6} cm s^{-1}
	IA	83.549 % Absorbed
	SP	-2.751 log Kp
	PGS	No
	PGI	No
	PGII	No
Distribution	VDH	0.13 log L kg^{-1}
	FU	0.712 Fu
	BP	-0.441 log BB
	CP	-3.252 log PS
Metabolism	CS1	No
	CS1	No
	CS3	No
	CS4	No
	CS5	No
	CS6	No
	CS7	No
Excretion	TC	0.104 log mL min^{-1} kg^{-1}
	ROS	No

Key: WS - Water solubility; CP - Caco2 permeability; IA - Intestinal absorption (human); SP - Skin Permeability; PGS - P-glycoprotein substrate; PGI - P-glycoprotein I inhibitor; PGII - P-glycoprotein II inhibitor; VDH - VDss (human); FU - Fraction unbound (human); BP - BBB permeability; CP - CNS permeability; CS1 -CYP2D6 substrate; CS1 - CYP3A4 substrate; CS3 - CYP1A2 inhibitor; CS4 - CYP2C19 inhibitor; CS5 - CYP2C9 inhibitor; CS6 - CYP2D6 inhibitor; CS7 - CYP3A4 inhibitor; TC - Total Clearance and ROS - Renal OCT2 substrate.

Table 3. *In silico* Toxicity of 1,2,3-Benzenetriol

Model Name	Predicted Value
AT	No
MTS	-0.269 log mg kg^{-1} day^{-1}
hIi	No
hIIi	No
ORAT	2.049 mol kg^{-1}
ORCT	2.374 log mg kg^{-1}_bw day^{-1}
HT	No

SS	No
TT	0.127 log μg L^{-1}
MT	2.734 log mM

Key: AT - AMES toxicity; MTS - Max. tolerated dose (human); hIi - hERG I inhibitor; hIIi - hERG II inhibitor; ORAT - Oral Rat Acute Toxicity (LD50); ORCT - Oral Rat Chronic Toxicity (LOAEL); HT - Hepatotoxicity; SS - Skin Sensitization; TT - T. pyriformis toxicity and MT - Minnow toxicity.

Table 4. *In silico* ADME Properties of 1,5-anhydro-6-deoxyhexo-2,3-diulose

Property	**Model Name**	**Predicted Value**
Absorption	WS	0.581 log mol L^{-1}
	CP	1.102 log Papp in 10^{-6} cm s^{-1}
	IA	87.43 % Absorbed
	SP	-3.944 log Kp
	PGS	No
	PGI	No
	PGII	No
Distribution	VDH	-0.169 log L kg^{-1}
	FU	0.809 Fu
	BP	-0.277 log BB
	CP	-3.073 log PS
Metabolism	CS1	No
	CS1	No
	CS3	No
	CS4	No
	CS5	No
	CS6	No
	CS7	No
Excretion	TC	0.554 log mL min^{-1} kg^{-1}
	ROS	No

Key: WS - Water solubility; CP - Caco2 permeability; IA - Intestinal absorption (human); SP - Skin Permeability; PGS - P-glycoprotein substrate; PGI - P-glycoprotein I inhibitor; PGII - P-glycoprotein II inhibitor; VDH - VDss (human); FU - Fraction unbound (human); BP - BBB permeability; CP - CNS permeability; CS1 -CYP2D6 substrate; CS1 - CYP3A4 substrate; CS3 - CYP1A2 inhibitor; CS4 - CYP2C19 inhibitor; CS5 - CYP2C9 inhibitor; CS6 - CYP2D6 inhibitor; CS7 - CYP3A4 inhibitor; TC - Total Clearance and ROS - Renal OCT2 substrate.

Table 5. *In silico* Toxicity of 1,5-anhydro-6-deoxyhexo-2,3-diulose

Model Name	Predicted Value
AT	No
MTS	1.121 log mg kg^{-1} day^{-1}
hIi	No
hIIi	No
ORAT	1.967 mol kg^{-1}
ORCT	2.579 log mg kg^{-1}_bw day^{-1}
HT	No
SS	Yes
TT	-1.712 log µg L^{-1}
MT	3.12 log mM

Key: AT - AMES toxicity; MTS - Max. tolerated dose (human); hIi - hERG I inhibitor; hIIi - hERG II inhibitor; ORAT - Oral Rat Acute Toxicity (LD50); ORCT - Oral Rat Chronic Toxicity (LOAEL); HT - Hepatotoxicity; SS - Skin Sensitization; TT - T. pyriformis toxicity and MT - Minnow toxicity.

4.Conclusions

This study demonstrates that 1,2,3-Benzenetriol and ADD, which were extracted from *C. sinensis* leaf buds and flowers, have a strong ability to inhibit DPPH free radicals and effectively bind with the NFE2L2 gene to protect against oxidative stress. The compounds were also tested for their cytotoxicity against vero cell lines in vitro. In addition, the ADMET properties of these bioactive compounds indicate that they have good drug bioavailability and could be used as an effective antioxidant agent for the development of a novel drug to treat various diseases.

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5. References

[1] Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot D.A., 2017. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants. 6, 42. doi: 10.3390/plants6040042

[2] Anderson, J. T., Huang, K.M., Lustberg, M.B., Sparreboom, A, Hu. S., 2020. Solute carrier transportome in chemotherapy-induced adverse drug reactions. Targets of Cancer Diagnosis and Treatment: Ion Transport in Tumor Biology. 177-215.

[3] Ashokkumar, K., Pandian, A., Murugan, M., Dhanya, M.K., Sathyan, T., Sivakumar, P., Raj, S., Warkentin, T.D., 2020. Profiling bioactive flavonoids and carotenoids in select south Indian spices and nuts. Nat. Prod. Res. 34, 1306-1310. doi: 10.1080/14786419.2018.1557179

[4] Ashraf, M. A. 2020. Phytochemicals as potential anticancer drugs: time to ponder nature's bounty. BioMed Research international 2020

[5] Brinkman, U. A.,. de Vries, T.G., Kuroda, R., 1973. Thin-layer chromatographic data for inorganic

substances. J. Chrom. A 85, 87-520. doi: https://doi.org/10.1016/S0021-9673(01)83160-5
[6] Chaudhary, P., Janmeda, P., 2022. Quantification of phytochemicals and in vitro antioxidant activities from various parts of *Euphorbia neriifolia* Linn. J. Appl. Biol. Biotech. 10, 133-145.
[7] Chaudhary, V., Chowdhury, R., Thukral, P., Pathania, D., Saklani, S., Rustagi, S., Kaushik, A., 2023. Biogenic green metal nano systems as efficient anti-cancer agents. Environ. Res. 115933.
[8] Ciampi, F., Sordillo, L., Gandy, J., Caroprese, M., Sevi, A., Albenzio, M., Santillo, A., 2020. Evaluation of natural plant extracts as antioxidants in a bovine in vitro model of oxidative stress. J. Dairy Sci. 103, 8938-8947.
[9] Coêlho, M. L., Islam, M. T., Laylson da Silva Oliveira, G., Oliveira Barros de Alencar, M. V., Victor de Oliveira Santos, J., Campinho dos Reis, A., Amélia de Carvalho Melo-Cavalcante, A., 2022. Cytotoxic and antioxidant properties of natural bioactive monoterpenes nerol, estragole, and 3, 7-dimethyl-1-octanol. Adv. Pharmacol. Pharmac. Sci. 2022.
[10] Fernando, C. D., Soysa, P., 2015. Extraction Kinetics of phytochemicals and antioxidant activity during black tea (*Camellia sinensis* L.) brewing. Nutrition. 14, 1-7.
[11] Hariprasath, L., Jegadeesh, R., Arjun, P., Raaman, N., 2015. In vitro propagation of *Senecio candicans* DC and comparative antioxidant properties of aqueous extracts of the in vivo plant and in vitro-derived callus. South Afr. J. Botany. 98, 134-141. doi: https://doi.org/10.1016/j.sajb.2015.02.011
[12] He, F., Ru, X., Wen, T., 2020. NRF2, a Transcription Factor for Stress Response and Beyond. Int. J. Mol. Sci. 21. doi: 10.3390/ijms21134777
[13] Iranshahy, M., Iranshahi, M., Abtahi, S.R., Karimi, G., 2018. The role of nuclear factor erythroid 2-related factor 2 in hepatoprotective activity of natural products: A review. Food Chem. Toxicol. 120, 261-276. doi: 10.1016/j.fct.2018.07.024
[14] Kim, S., Kim, M., Kang, M.-C., Lee, H. H. L., Cho, C.H., Choi, I., Park, Y., Lee, S.-H., 2021a. Antioxidant Effects of Turmeric Leaf Extract against Hydrogen Peroxide-Induced Oxidative Stress In Vitro in Vero Cells and In Vivo in Zebrafish. Antioxidants. 10, 112.
[15] Kim, S., Kim, M., Kang, M. C., Lee, H. H. L., Cho, C. H., Choi, I., Park, Y., Lee, S.H., 2021b. Antioxidant Effects of Turmeric Leaf Extract against Hydrogen Peroxide-Induced Oxidative Stress In Vitro in Vero Cells and In Vivo in Zebrafish. Antioxidants. 10, doi: 10.3390/antiox10010112
[16] Koch, W., Zagórska, J., Marzec, Z., Kukula-Koch, W., 2019. Applications of Tea (*Camellia sinensis*) and its Active Constituents in Cosmetics. Molecules. 24, 4277. doi: 10.3390/molecules24234277
[17] Kpemissi, M., Eklu-Gadegbeku, K., Veerapur, V.P., Negru, M., Taulescu, M., Chandramohan, V., Hiriyan, J., Banakar, S.M., Nv, T., Suhas, D.S., Puneeth, T.A., Vijayakumar, S., Metowogo, K., Aklikokou, K., 2019. Nephroprotective activity of Combretum micranthum G. Don in cisplatin induced nephrotoxicity in rats: In-vitro, in-vivo and in-silico experiments. Biomed. Pharmacoth. 116, 108961. doi: 10.1016/j.biopha.2019.108961
[18] Kumar, P. S., Premnath, D., Obadiah, A., Durairaj, A., Ramanathan, S., Vasanthkumar, S., 2019. Synthesis, Structure Characterization, and Biological Evaluation of 3-Amino-5-(5-Oxo-5H-Benzo [a] Phenothiazin-6-Ylamino) Benzoic Acid Derivatives via Molecular Docking, Cytotoxicity, and Antioxidant Studies. Curr. Pharmac. Rep. 5, 440-459.
[19] Lakshmanan, G., Selvam, L., Altemimi, A.B., Baldelli, A., Bharathi, A., Gopalakrishnan, B., Karthik, L., Saravanan, D., Kavitha, M., Mohammed, M.J., 2022. Phytochemical screening of ethanolic extracts of *Cuminum cyminum* L. seeds along with the evaluation of antidiabetic properties by molecular docking approach. Nat. Prod. Res., 37, 681-686.
[20] Li, C., Wang, J., Wang, Y., Gao, H., Wei, G., Huang, Y., Yu, H., Gan, Y., Wang, Y., Mei, L., 2019. Recent progress in drug delivery. Acta Pharmac. Sinica B. 9, 1145-1162.
[21] Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmac. Rev. 4, 118-126. doi: 10.4103/0973-7847.70902
[22] Mihailović, M., Dinić, S., Arambašić Jovanović, J., Uskoković, A., Grdović, N., Vidaković, M., 2021. The influence of plant extracts and phytoconstituents on antioxidant enzymes activity and gene expression in the prevention and treatment of impaired glucose homeostasis and diabetes complications. Antioxidants. 10, 480.
[23] Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., Devasagayam, T.P., 2007. Indian herbs and herbal drugs used for the treatment of diabetes. J. Clinic. Biochem. Nutr. 40, 163-173. doi: 10.3164/jcbn.40.163
[24] Mohammed, G. J., Kadhim, M.J., Hameed, I.H., 2016. Proteus species: Characterization and herbal antibacterial: A review. Int. J. Pharm. Phytochem. Res. 8, 1844-1854.
[25] Monobe, M., Ema, K., Kato, F., Maeda-Yamamoto, M., 2008. Immunostimulating Activity of a Crude Polysaccharide Derived from Green Tea (*Camellia sinensis*) Extract. J. Agric. Food Chem. 56,

1423-1427. doi: 10.1021/jf073127h
[26] Motyka, S., Jafernik, K., Ekiert, H., Sharifi-Rad, J., Calina, D., Al-Omari, B., Cho, W. C., 2023. Podophyllotoxin and its derivatives: Potential anticancer agents of natural origin in cancer chemotherapy. Biomed. Pharmac. 158, 114145.
[27] Mutha, R. E., Tatiya, A.U., Surana, S.J., 2021. Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. Futur. J. Pharm. Sci. 7, 25. doi: 10.1186/s43094-020-00161-8
[28] Pham, H. N. T., Vuong, Q.V., Bowyer, M.C., Scarlett, C.J., 2020. Phytochemicals derived from *Catharanthus roseus* and their health benefits. Technologies 8, 80.
[29] Prasanth, M. I., Sivamaruthi, B.S., Chaiyasut, C., Tencomnao, T., 2019. A review of the role of green tea (*Camellia sinensis*) in antiphotoaging, stress resistance, neuroprotection, and autophagy. Nutrients 11, 474. doi: 10.3390/nu11020474
[30] Qadir, A., Aqil, M., Ali, A., Ahmad, F.J., Ahmad, S., Arif, M., Khan, N., 2020. GC-MS analysis of the methanolic extracts of *Smilax china* and *Salix alba* and their antioxidant activity. Turkish J. Chem. 44, 352-363. doi: 10.3906/kim-1907-5
[31] Raks, V., Al-Suod, H., Buszewski, B., 2018. Isolation, Separation, and Preconcentration of Biologically Active Compounds from Plant Matrices by Extraction Techniques. Chromatographia 81, 189-202. doi: 10.1007/s10337-017-3405-0
[32] Rampogu, S., Baek, A., Park, C., Parate, S., Parameswaran, S., Park, Y., Shaik, B., Kim, J.H., Park, S.J., Lee, K.W., 2019. Discovery of Small Molecules that Target Vascular Endothelial Growth Factor Receptor-2 Signalling Pathway Employing Molecular Modelling Studies. Cells 8, 269. doi: 10.3390/cells8030269
[33] Salehi, B., Azzini, E., Zucca,P., Varoni E.M., Anil Kumar, N.V., Dini, L., Panzarini, E., Rajkovic, J., Valere Tsouh Fokou, P., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., El Beyrouthy, M., Setzer, W.N., Polito, L., Iriti, M., Sureda, A., Quetglas-Llabrés, M.M., Martorell, M., Martins, N., Sharifi-Rad, M., Estevinho, L.M., Sharifi-Rad, J., 2020. Plant-Derived Bioactives and Oxidative Stress-Related Disorders: A Key Trend towards Healthy Aging and Longevity Promotion. Appl. Sci. 10, 947.
[34] Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K., Latha, L.Y., 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr. J. Tradit. Complem. Altern. Medic. 8, 1-10.
[35] Stagos, D. 2019. Antioxidant activity of polyphenolic plant extracts. Antioxidants. 9, 19. https://doi.org/10.3390/antiox9010019
[36] Suleman, M., Khan, A., Baqi, A., Kakar, M.S., Ayub, M., 2019. Antioxidants, its role in preventing free radicals and infectious diseases in human body. Pure Appl. Biol. 8, 380-388.
[37] Shahbaz, A., Iqbal, J., Abbasi, B. A., Akhtar, W., Fatima, I., Zahra, S. A., Cho, W. C., 2022. Antioxidant, Anticancer, and PXR-Dependent CYP 3 A 4 Attributes of *Schweinfurthia papilionacea* (Burm. f.) Boiss., *Tricholepis glaberrima* DC. and *Viola stocksii* Boiss. Oxidat. Med. Cell. Long. 2022.
[38] Sharifi-Rad, J., Seidel, V., Izabela, M., Monserrat-Mequida, M., Sureda, A., Ormazabal, V., Zuniga, F.A., Mangalpady, S.S., Pezzani, R., Ydyrys, A., Tussupbekova, G., Martorell, M., Calina, D., Cho, W. C., 2023. Phenolic compounds as Nrf2 inhibitors: potential applications in cancer therapy. Cell Comm. Signal. 21, 1-18.
[39] Sutanto, H., Susanto, B.H., Nasikin, M., 2019. Solubility and Antioxidant Potential of a Pyrogallol Derivative for Biodiesel Additive. Molecules 24, 2439.
[40] Tariq, A. L., Reyaz, A. L., 2013. Antioxidant activity of *Camellia sinensis* leaves. Int. J. Curr. Microb. Appl. Sci. 2, 40-46.
[41] Vijayaraj, R., Sri Kumaran, N., Altaff, K., Ramadevi, S., Sherlin Rosita, A., 2019. In silico pharmacokinetics and molecular docking of novel bioactive compound (11-methoxy-2-methyltridecane-4-ol) for inhibiting carbohydrates hydrolyzing enzyme. J. Biol. Act. Prod. Nature 9, 445-456.
[42] Yang, Z., Tu, Y., Baldermann, S., Dong, F., Xu, Y., Watanabe, N., 2009. Isolation and identification of compounds from the ethanolic extract of flowers of the tea (Camellia sinensis) plant and their contribution to the antioxidant capacity. LWT-Food Sci. Technol. 42, 1439-1443.
[43] Zhao, H., Eguchi, S., Alam, A., Ma, D., 2017. The role of nuclear factor-erythroid 2 related factor 2 (Nrf-2) in the protection against lung injury. American journal of physiology. Lung Cell. Molec. Physiol. 312, L155-l162. doi: 10.1152/ajplung.00449.

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چکیده

خواص آنتی اکسیدانی مواد طبیعی نویدبخش مبارزه با اختلالات ناشی از استرس اکسیداتیو است. دو ماده از قبیل، Benzenetriol–۱،۲،۳ و 3-diulose (ADD).anhydro-6-deoxyhexo-2–1,5، مشتق شده از Camellia sinensis، برای پتانسیل آنتی اکسیدانی آنها مورد بررسی قرار گرفت. این مطالعه با هدف ارزیابی توانایی Benzenetriol–۱،۲،۳ و ADD در خنثی سازی رادیکال های آزاد و تنظیم بیومارکرهای استرس اکسیداتیو انجام شد. ترکیبات فعال با جداسازی آنها از برگ و گل گیاه کاملیا سیننسیس با استفاده از کروماتوگرافی ستونی با مخلوط اتیل استات و هگزان به دست آمد. خواص آنتی اکسیدانی آنها با استفاده از تکنیک های GC-MS ارزیابی شد. فراکسیون های زیست فعال، یعنی CSLBF-2 و CSFBF-2، به عنوان دارای بالاترین درصد مهار رادیکال های آزاد DPPH شناسایی شدند. غلظت مهاری برای CSLBF-2 و CSFBF-2 به ترتیب ۱۳۲ و ۱۴۷ میکروگرم در میلی لیتر در لیتر تعیین شد. مطالعات اتصال مولکولی با استفاده از نرم افزار GOLD برای ارزیابی تعاملات اتصال با NFE2L2، ژنی که در دفاع در برابر استرس اکسیداتیو نقش دارد، انجام شد. CSLBF-2 و CSFBF-2 درصد مهار قابل توجهی از رادیکال های آزاد DPPH را با مقادیر ۹۲/۰۵ و ۸۸/۳۳ درصد نشان دادند. مطالعات اتصال مولکولی برهمکنش‌های اتصال سودمند با NFE2L2 را نشان داد که مکانیسم‌های آنتی اکسیدانی بالقوه را نشان می‌دهد. قابل ذکر است که Benzenetriol–۱،۲،۳ قویترین انرژی اتصال را در بین ترکیبات مورد مطالعه نشان داد. علاوه بر این، ترکیبات خالص شده سمیت سلولی قابل توجهی را در برابر رده سلولی Vero نشان دادند و ویژگی‌های فراهمی زیستی دارویی مطلوبی از خود نشان دادند. این مطالعه نتیجه می گیرد که Benzenetriol–۱،۲،۳ و ADD خواص آنتی اکسیدانی امیدوارکننده و پتانسیل توسعه به عوامل دارویی جدید را نشان می دهند. اثربخشی آن‌ها در خنثی‌سازی رادیکال‌های آزاد و تنظیم بیومارکرهای استرس اکسیداتیو، راه امیدوارکننده‌ای را برای تحقیقات بیشتر و توسعه دارویی نشان می‌دهد.