

Ameliorative Effect of *Tapinanthus globiferus* on Sodium Arsenite Induced Carcinogenesis in Canton-S strain of *Drosophila melanogaster*. In-vivo and In-silico Studies

Jane-Rose Oche

ocheja@unijos.edu.ng

University of Jos Faculty of Medical Sciences

Jonathan D. Dabak

University of Jos

Titilayo O. Johnson

University of Jos

Research Article

Keywords: Tapinanthus globiferus, anticancer, carcinogenesis, sodium arsenite, Drosophila melanogaster

Posted Date: December 21st, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-5673577/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

Tapinanthus globiferus is a hemi-parasitic plant that grows on vascular trees. It is often regarded as an economic menace due to the damages it causes on trees with economic values. However, despite the perception of the ever-green plant as an economic problem, traditional medicine makes use of the plant in the treatment of different diseases including cancer. This study aimed to investigate the anticancer property of the leaf of *Tapinanthus globiferus* using *Drosophila melanogaster* and to identify the potential anticancer bioactive compounds. To determine a safe dose of the crude extract and fractions of *T. globiferus* to be used for the studies, the survival rates of the flies were evaluated at different concentrations (0, 0.5, 1.0 and 2.0 mg/10 g diet) for 14 days. Carcinogenesis was induced in *Drosophila melanogaster* using sodium arsenite (SA) and the SA-exposed flies were treated with 1.0 mg/10 g diet of the three fractions for 10 days. Biochemical parameters of oxidative stress (hydrogen peroxide, non-protein thiol (NPSH), total thiol, nitric oxide, protein carbonyl, malondialdehyde and glutathione-S-transferase (GST) activity), cell viability, negative geotaxis and gene expressions (*p53* and *Ras*) were used to evaluate the ameliorative activity of the fractions on the carcinogenic effects of SA in the flies. HPLC-DAD analysis was carried out to determine the bioactive compounds present in the active fraction. Molecular Docking analysis of the compounds against selected cancer drug targets (VEGF-A and Ras) was done followed by ADMET studies. The results of biochemical analyses showed that the three fractions ameliorated the SA-induced carcinogenic effects in the flies. The butanol fraction showed higher ameliorative activity. HPLC-DAD results showed the presence of hydroxybenzoate derivatives, hydroxycinnamate derivatives and flavonoids in this fraction. Result of the molecular docking shows that Rutin had higher binding with *HsVEFGA* with score of -9.793 kcal/mol and with *DmVEFGA* with score of -8.890 kcal/mol, compared to the standard inhibitor. Also, isoquercetin had higher docking score compared with the standard inhibitor drug of Ras protein of both humans (-8.587 kcal/mol) and *Drosophila melanogaster* (-11.883 kcal/mol). Both compounds showed low bioavailability as drug candidates. While rutin showed class 5 toxicity, isoquercetin showed class 4 toxicity. This suggests that *Tapinanthus globiferus* may possess potential anticancer attributes that can be associated to the presence of the polyphenolic compounds, which could be explored further for the purpose of anticancer drug design.

INTRODUCTION

Cancer is a metabolic disease characterized by uncontrolled proliferation of cells in the body. It is one of the burden that is widespread globally and also one of the major causes of death. Cancer mortality is one of the biggest challenges in Sub-Saharan Africa, although it is not as prevalent as it is in high income countries (Adeloye et al., 2018). The primary cause remains unknown, however, has been attributed to lifestyle (Nenclares & Harrington, 2020). There are different types of cancer including gastrointestinal cancer. Gastrointestinal cancers are diverse gastrointestinal diseases caused by a combination of non-modifiable and modifiable risk factors that lead to the conversion of normal cells into precursor, premalignant, and malignant cells, with varying etiologies and clinical management (Morgado-Diaz, 2022). Cancers of the gastrointestinal tract (GI), which include those of the stomach, liver, oesophagus, pancreas, and colon, account for more than 25% of all cancer cases and are becoming more common with an high overall burden, which could be worse than those of non-GI tumours (Jardim et al., 2023). Gastrointestinal cancers accounts for 1 in 4 cases, (about 4.8 million cancer cases) with 1 in 3 deaths (about 3.4 million) globally, coupled with variation in the rate of incidence and mortality (IARC, 2024). In Nigeria for instance, it accounts for approximately 7.8–18.4% of cancer cancers reported by various epidemiological studies (Ray-Offor & Obiorah, 2021). Due to certain factors, a large population of people in Africa, including Nigeria, regularly use medicinal plants for cancer treatment (Fakudze et al., 2023).

The use of medicinal plant for primary healthcare and cancer management can be attributed to different factors. Most common are financial insecurities, lack of access to affordable and effective treatment strategies partly due to the inadequacies of public health services in most areas., (Arjona-García et al., 2021). Plants play a very crucial role in

treating various ailments due to their potentially high therapeutic values and level of acceptance by patients, irrespective of the peculiarities of their diseases (Barkat et al., 2021). The use of plants in traditional medicine has been an age long activity that has existed for centuries. This practice has been passed down from generations. Till date medicinal plants are still used for treatment of ailments including cancer, and more are still being explored for their biological activities and the therapeutic potentials. The biological activities of plants are attributed to their bioactive components. Various plants, including *Tapinanthus globiferus*, are utilized in the treatment of diseases. Although there is a lack of extensive scientific literature regarding their anticancer properties, anecdotal reports show that these plant are widely employed in traditional medicine for cancer treatment.

Tapinanthus globiferus, also referred to as African mistletoe is a hemi-parasitic plant that grows on vascular trees. While it is capable of photosynthesizing its own organic matter, they are dependent on their host's xylem for nutrients and water. Unlike epiphytic or mycoheterotrophic plants, they are affixed to their host by a modified root known as the haustorium. (Muche et al., 2022). Mistletoes are used in South West Nigeria in the treatment of cancer alongside other ailments (Ogunmefun et al., 2013). The application of *Tapinanthus globiferus* in traditional medicine does not depend on species difference but on the host trees obtained from, which in most cases is related to the traditional use of the host tree for treatment of diseases. For instance, undocumented reports from different traditional healthcare, use mistletoes from guava (*Psidium guajava*) for treatment of cancer. A study by Oriola et al. (2018) showed that mistletoes from *Leucena leucocephala*, *Citrus paradisi* and *Ficus sur* all had anticancer activity. Mistletoes from cocoa tree (*Theobroma cacao*) have been reported to have anti-malarial properties (Olanlokun et al., 2021). Another study showed that mistletoe obtained from River tamarind (*Leucena leucocephala* (Lam)) can be used for management of rheumatism (Oriola et al., 2021). Anti-diabetic activities of mistletoes from *Psidium guajava* (guava) have been reported (Afuye & Omotayo, 2024). Mistletoe obtained from host plants contains and has the efficacy of drugs associated to them, making it suitable for treating diseases (Isikhuemen et al., 2020).

Different models are adopted for cancer research. Cancer models are systems that mimic human cancers, either naturally occurring or created by artificial means. Cell lines and species like fruit flies (*Drosophila melanogaster*) and genetically engineered mice are used as experimental systems to research human cancer pathophysiology and biochemical or genetic mechanisms. *Drosophila melanogaster* as a model has been attributed to its unique genetic features. *Drosophila melanogaster* as an amenable animal model can be used to investigate gene expression and gene regulatory activities (Iftikhar et al., 2017). An estimated 75% of human disease-related genes have functional homologs in *Drosophila*, including important genes related to apoptosis, cell adhesion, differentiation, migration, polarity, and the cell cycle. Compared to mammals, *Drosophila* have less genetic redundancy, which results in a lower frequency of these genes (Munnik et al., 2022). Most signalling mechanisms that regulate cell growth and invasion in mammals are conserved in flies, enabling the availability of models that can mimic tumour biology in the fly (Mirzoyan et al., 2019). Therapeutic and biological activities of plants can be evaluated using *Drosophila melanogaster*. It is easy to maintain in the laboratory and has a lifespan of between 45 to 90 days compared to rodents with more than 12 months. In addition, the fly also poses fewer ethical restrictions compared to rodents (Rocha, 2013). Molecules and biomarkers can be validated on a functional large-scale analysis, rapidly, compared to rodent-based studies (Moraes & Montagne, 2021). The aim of this study was to evaluate the ameliorative effect of *Tapinanthus globiferus* in SA-induced carcinogenesis using *Drosophila melanogaster* and identify bioactive compounds with potential anticancer properties

MATERIALS AND METHODS

Chemical Reagents

Sodium arsenite was acquired from Oxford Lab Fine Chem LLP Vasai East, Palghar-410210, Maharashtra, India. 1-chloro-2,4-dinitrobenzene, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB or Ellman's reagent) etc, were purchased from

Sigma Aldrich (St. Louis; MO), and Trizol reagent, DNase I, complementary deoxyribonucleic acid (cDNA), polymerase chain reaction (PCR) buffer, primers, magnesium chloride (MgCl₂), deoxynucleotide trisphosphate (dNTPs), SYBR Green I, Taq DNA polymerase were obtained from Inqaba Biotec West Africa Ltd. The chemicals used for the study were commercially obtained and were of analytical grade.

Plant Samples

Fresh leaf samples of *Tapinanthus globiferus* Lam found on *Psidium guajava* (Guava tree) located at Alheri, (Latitude: 9.92000 E° and Longitude: 8.89000 N°) Jos North L.G.A of Plateau State Nigeria was harvested during the dry season. The whole plant was authenticated at Federal College of Forestry, Jos, Plateau State, Nigeria and was deposited at the herbarium with voucher number FHJ284. The leaves were air dried in a well-ventilated room after washing under clean running water. Dried samples were pulverized and subjected to extraction using maceration method.

Extraction and Fractionation of *Tapinanthus globiferus* Leaf

The maceration extraction process was carried out using 70% methanol. Using the ratio 1:10, for 72 Hours the pulverized leaves was soaked in the 70% methanol with intermittent stirring. Afterwards, the mixture was filtered, and the filtrate was dried at 60 °C in an oven to obtain the dried crude extract. Afterwards Vacuum Liquid Chromatography (VLC) was used to fractionate the crude extract into different fractions butanol, ethyl acetate and methanol.

Experimental Flies

Mixed sex of Canton-S strain of *Drosophila melanogaster*, not more than 3 days old were used for the study. The flies were cultured on a cornmeal medium containing 1% w/v brewer's yeast, 1% w/v agar and 0.08% v/w nipagin at constant temperature and humidity (22–25°C; 60–70% relative humidity) under 12 h dark/light cycle conditions, at the *Drosophila* Laboratory, Department of Biochemistry, College of Medicine, University of Ibadan, Oyo State, Nigeria. The flies were obtained directly from the Federal University of Santa Maria in Brazil, originally obtained from the National Stock Centre in Bowling Green, Oklahoma, in the United States of America.

In vivo Studies

Exposure and preparation of *Drosophila melanogaster* for biochemical assays

Toxicity of *Tapinanthus globiferus* in *Drosophila melanogaster* was carried out using varying concentrations. The flies were exposed to 0, 0.5, 1.0, and 2.0 mg of the crude extract and different fractions of *Tapinanthus globiferus* in a 10 g diet. The crude extract and fractions were dissolved in 200 mL of distilled water (200 mL) and fed to the flies for 14 days to evaluate survival rates. Thereafter, the flies were exposed to the concentration of crude extract and fractions with the highest survival rate for 10 days to investigate biochemical parameters. The flies were divided into 4 experimental groups of 5 replicates with 50 flies per vial (replicate). Afterwards, the flies were anaesthetized in ice, and homogenized in 0.1 M potassium phosphate buffer of pH 7.4 (1:10 (flies/volume (μL))), after weighing them. Subsequently the homogenates were centrifuged at 4,000 g for 10 min at 4°C. The supernatants were used to determine the biochemical parameters.

Treatment

Butanol, ethyl acetate and methanol fractions (1.0 mg/10 g diet) of *Tapinanthus globiferus* (TG) were used to treat flies exposed to SA (concentration) to investigate the ameliorative effect of *Tapinanthus globiferus* on carcinogenesis induced in the gut of *Drosophila melanogaster* after 10 days. This was grouped as described in Table 1. Locomotive ability of the flies were observed in live flies. The samples were prepared for analysis using the process described earlier. The supernatants obtained were store at -20°C, which were used to evaluate hydrogen peroxide, nitric oxide, non-protein thiol, total thiol, protein carbonyl, malondialdehyde (MDA), cell viability (MTT), Glutathione-S-Transferase activity

Table 1
Treatment Groups

Group	Treatment
Group 1	Positive Control (Distilled water only)
Group 2	Negative Control (Exposed to NaASO ₂ and untreated
Group 3	NaASO ₂ + 1.0 mg/10 g diet of TG butanol fraction
Group 4	NaASO ₂ + 1.0 mg/10 g diet of TG methanol fraction
Group 5	NaASO ₂ + 1.0 mg/10 g diet of TG ethyl acetate fraction

n = 5: 5 replicates per group; 50 flies/replicate

Protein Determination

The method described by Lowry was used determine protein concentration. The protein concentration of the various sample was determined using modified Lowry method as described by Everette et al. (2010). **Survival Rate of the *D.melanogaster***

The flies were sorted into 9 experimental groups, each consisting of 5 vials containing 50 flies each. The flies were subjected to several concentrations of the crude extract and fractions of *T.globiferus* (0, 0.5, 1.0 and 2.0 mg/10g diet) dissolved in 200 mL distilled water, for a duration of 14 days. Distilled water was given to control flies. Every day, the number of live and dead flies in each vial was counted and recorded. After analysis of the data, the Kaplan Meier survival test was used to create a survival curve. A preliminary study was carried out to determine the concentration SA that was used to induce carcinogenesis. The duration of treatment was determined by the survival assay data of the SA exposure (Johnson, Abolaji, et al., 2021).

Hydrogen Peroxide Determination

Hydrogen peroxide (H₂O₂) concentration was determined based on the method of (Wolff, 1994). A mixture was made using 10 µL of the sample and 590 µL of Ferrous Oxidation-Xylenol orange (FOX-1) reagent. FOX-1 reagent was constituted by dissolving xylenol orange (0.76 mg/ mL), sorbitol (0.19 g/mL), and acidified ferrous sulfate (0.196 mg/mL), in distilled water. The mixture was incubated for 30-min incubation at room temperature and the absorbance was read at 560 nm. H₂O₂ generated was expressed as µmol/mL which was determined using the extinction coefficient of H₂O₂.

Total Thiol Concentration

The total thiol level was analysed based on the method of Ellman (1959). To 35 μL of 1 mM DTNB 510 μL of 0.1 M phosphate buffer (pH 7.4), was added, followed by 35 μL of distilled water, and 20 μL of sample. The mixture was mixed thoroughly and incubated for 30 minutes at room temperature. Absorbance was read at 412 nm.

Reduced Glutathione Concentration

Determination of reduced glutathione (GSH) was carried out using the method described by Beutler et al. (1963), was used concentration. Serial dilution of GSH stock solution containing 20–200 μg of reduced glutathione were prepared in different test tubes and made up to 100 μL with 0.1M phosphate buffer, pH 7.4. About 900 μL of Ellman's reagent was then added to each sample tube. Readings were taken immediately as there could a loss of 1–2% of colour 5–10 minutes after the addition of Ellman's reagent. The GSH concentration in each test tube was determined and absorbance was read at 412nm.

Glutathione-S-Transferase Activity

The method described by Habig and Jakoby (1981) was used to measure GST activity. To prepare Solution A used for the assay, 20 μL of 0.25 M potassium phosphate buffer was mixed with 500 μL of 0.1 M GSH, 10.5 μL of distilled water and 2.5 mM EDTA, at pH 7.0 and temperature of 25°C. Then 20 μL of the sample (1:5 dilution) was added to 270 μL of the solution A, followed by 10 μL of 25 mM CDNB. At an interval of 10 seconds, absorbance was read at 340 nm for 5 minutes.

Nitric Oxide Concentration

Greiss method as described by Johnson, Abolaji, et al. (2021) was used to analyse the concentration of nitric oxide. 250 μL of the sample was added to 250 μL of Griess reagent and incubated for 20 minutes at room temperature, afterwards it was read at 550 nm. The absorbance of the sample was compared with that of a standard solution with a known nitrite concentration.

Protein Carbonyl Determination

The protein carbonyl concentration was determined based on the method described by Wehr and Levine (2013). 1000 mL 2 M HCl was used to dissolve 2.0 g DNPH (dry weight) and stirred in the dark. Followed by filtration and then concentrated by precipitation in 10% trichloroacetic acid using a slow speed centrifugation to form a loose, easily dispersed pellet for 2minutes at 2,000 $\times$ g. Then 0.5 mL of the solution was added to the sample and vortexed to suspend the sample. The mixture was allowed to stand for 10 minutes at room temperature, vortexing occasionally. 10% TCA was added to precipitate the pellets, using slow speed centrifugation it was recovered. 1 mL ethanol-ethyl was used to rinse the free DNPH thrice, after the supernatant was drained off. This was followed by centrifugation at 5000 $\times$ g for 2 minutes. The supernatant was drained and the pellets were dried. The dried pellets were re-dissolved in 200 μL 6.0 M guanidine 500 mM KCl mixture at pH 2.5 and centrifuged. The absorbance was taken at 370 nm and 276 nm.

Protein carbonyl was determined using a molar absorption coefficient of 22,000 $\text{M}^{-1} \text{cm}^{-1}$

Lipid Peroxidation (Malondialdehyde Concentration)

The levels of malondialdehyde (MDA) was evaluated using thiobarbituric acid reactive substances (TBARS) formation for MDA based on the method described by based on Ohkawa et al. (1979). To 105 μL of distilled water, 5 μL of 10 mM butyl-hydroxytoluene (BHT), 200 μL of 0.67% Thiobarbituric acid and 600 μL of 1% O-phosphoric acid were added and mixed properly. Then 90 μL of supernatant was incubated with the mixture for 45 min at 90°C. Absorbance was read at 535 nm and results were expressed as μM of MDA/mg protein.

Cell Viability (MTT Assay)

The fly homogenate was incubated in MTT dye for 4 hours at 37°C. Afterwards the insoluble formazan crystals were solubilized using dimethyl Sulfoxide (DMSO) by shaking the mixture. Then the absorbance was read using a microplate reader at wavelength 570nm. The result was expressed, relative to the control, as percentage.

Gene Expressions

The ameliorative effect of *Tapinanthus globiferus* on the effect of sodium arsenite induced carcinogenesis on *Ras* and *p53* gene were carried using mRNA expression kits. Extraction of mRNA was carried out using the Quick-RNA™ MiniPrep Plus Kit and cDNA was synthesized using the ProtoScript II First Strand cDNA Synthesis Kit according to the protocols of the manufacturer. Quantitative real-time PCR was carried out to evaluate the expression of the identified gene of the flies. RNA isolation and extraction was carried out rapidly under ice to prevent degradation by RNase. The primers used are presented in table 2:

Table 2
Reverse and Forward Primers used for gene expression studies

Gene	Sequence
p53 R	5' CGACCTCCGTGGAGTCATCC 3'
F	5' ATCCAGCCTACGGAGGCAAC 3'
RAS R	5' TCGGCTTGTTTCATTTGCGG 3'
F	5' ACGGCAAATCGAAAACGGAC 3'

Statistical Analysis

Data was statistically analysed using GraphPad Prism Software, San Diego, CA, USA, version 9.5.1. The results were presented as mean + standard deviation (SD). Tukey's post hoc test was used to assess significant differences, whereas the statistical significance of survival rate was determined using the Log rank (Mantel Cox) test.

Determination of Polyphenolic Compounds using HPLC

Methanol was used to resuspend the dried butanol fraction, which was then diluted in the initial mobile phase and filtered using a 0.22 µm PTFE syringe filter (Millipore®, São Paulo, Brazil). The quantification of phenolic compounds was carried out in accordance with the method described by Quatrin et al. (2019). The CBM-20A Prominence HPLC (Shimadzu, Kyoto, Japan) was utilised for analysis. It was coupled with a DGU20A5 prominence degasser and a CTO-20A prominence column oven, which was connected to a DAD detector (SPDM-20A prominence, Shimadzu, Japan). In a C-18 Hypersil Gold column (5-µm particle size, 150 mm, 4.6 mm; Thermo Fisher Scientific, Massachusetts, USA), samples were injected (20 µL) at 38°C. The mobile phase consisted of 0.1% (v/v) of formic acid in acetonitrile as eluent B and 5% (v/v) of methanol in acidified water as eluent A at 1 millilitre per minute. Reverse phase mode was used for the chromatographic separation: 4% B was kept for 0 min, 16% B for 21.1 min, 50% B for 55.1 min, 100% B for 70.1 min, 100% B for 80 min, 0% B for 80.1 min, 83 min, and then kept for 92.1 min at a flow rate of 1 mL min⁻¹. The absorption spectra were recorded between 200 and 800 nm, and the phenolic compounds present in the sample were determined by comparing the spectral data acquired from UV-vis absorption spectra with the retention time of genuine standards. As suggested by Quatrin et al. (2019), the quantification proposal was made for the derivatives of hydroxybenzoate (280

nm), hydroxycinnamate (320 nm), flavonoids (360 nm), and total phenolic components. The results were reported as mg per 100 g of dry fraction weight.

In silico Studies

Protein and Ligands

The structures of the target proteins implicated in human gastrointestinal cancer, VEGF-A (504E), and Kras (7083) were retrieved from RCSB Protein Data Bank (<https://www.rcsb.org/>). The Structure Data Format (SDF) of the structures of the ligands identified in the butanol fraction of *Tapinanthus globiferus* were obtained from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Homology modelling panel of maestro 13.4 (Schrödinger suite 2022-4) was used to model the VEGF-1 and Ras protein of *Drosophila melanogaster*. The GW786034 and ChEMBL5183988 co-crystallized with the VEGF-A and Ras proteins were used respectively as the standard.

Protein Preparation

The protein preparation wizard panel was used to prepare the protein structures using Glide (Schrodinger "Suite 2022-4"). In the course of preparation, side chains and loops that were absent were substituted with primary, disulphide bonds were formed, hydrogen atoms were added, and bond ordering were assigned. After eliminating water molecules that were more than 3.0 Å from the heteroatoms, OPLS4e was used to minimise the protein structure and PROPKA was used to optimise it (Johnson, Adegboyega, et al., 2021).

Ligand preparation

The bioactive components of *Tapinanthus globiferus* were prepared as ligands based on the outcomes of HPLC-DAD analysis. The compounds and standard ligand for each protein were synthesised to generate low-energy 3D structures with appropriate chiralities using the Ligprep panel of Maestro 13.4, Schrodinger Suite 2022-4. At a physiological pH of 7.2 ± 0.2 , the ionisation state of every ligand structure was produced. For each ligand, the stereoisomers were calculated with specific chiralities held constant and others altered.

Grid Generation

Receptor grids were generated to show the location and dimensions of the active site of the proteins for ligand docking, following the procedure outlined by Johnson et al. (2021). The Schrodinger Maestro 13.4 receptor grid generating tool was used for this. The scoring grid was based on the location of the co-crystallized ligand within the active sites of the proteins.

Protein-Ligand docking

The Glide Ligand Docking panel of Maestro 13.4 on Schrodinger Suite 2022-4 was used for the molecular docking investigations. After importing the receptor grid file and the prepared ligands into Maestro workspace, the ligands were docked into the binding pockets of the target proteins using extra precision (XP) docking. The vdW radius scaling factor of each protein was set to 0.8, the ligand sampling was set to flexible, and ligand atom partial charge cut-off was set to 0.15. The interaction fingerprints in the Discovery informatics and QSAR of maestro was used to analyse the different interactions between the amino acids of the individual proteins and the compounds including the standard inhibitors.

ADME and toxicity profiling

Using in silico integrated model predictions at SWISS-Adme and Protox II online servers, ADME and toxicity (ADME-Tox - Absorption, Digestion, Metabolism, Excretion and Toxicity) profiling of the substances identified by HPLC-DAD was done. The following ADME properties of the bioactive compounds were determined using the SwissADME server: drug-likeness based on the bioavailability score and Lipinski and Veber rules, water solubility (ESOL Log S), and lipophilicity (Log P). The pharmacokinetics prediction was based on the following: gastrointestinal (GI) absorption, blood brain barrier (BBB) permeant, permeability glycoprotein (Pgp) binding, and Cytochrome P450 (CYP) inhibition (Daina et al., 2017). The bioactive compounds' acute toxicity class, LD50, hepatotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and immunotoxicity were all predicted using the Protox II web server (Banerjee et al., 2018).

RESULTS

Exposure to the Fractions of *Tapinanthus globiferus* Improved the Survival Rate of *D.melanogaster*

Following 14 days exposure to the crude extract and fractions of *T.globiferus*, there was improved survival rate in the *D. melanogaster* compared with the control flies. However, higher survival rate was observed in the group treated with butanol fraction (0.5 and 1.0 mg/ 10 g diet, 79% and 77% respectively) there was 25% and 23% increase in the survival rate for both concentrations, with a corresponding increased probability of survival (Fig. 1b). Flies exposed to methanol showed improved survival at 1.0 mg/10 g diet, with 10% increased survival and 63.3% probability of surviving as of day 14, in comparison to the control group (Fig. 1c). Improved survival rate was observed with higher concentration of ethyl acetate concentration (2.0 mg/10 g diet), there was an increased survival by 7% and 61% probability of survival (Fig. 1d). The flies exposed to the crude extract did not show improved survival rate compared to the control flies (Fig. 1a).

Biochemical Analysis

Following the outcome of the survival rate, the crude extract of *Tapinanthus globiferus* was not used to carry out amelioration studies. All three fractions showed improved survival even at high concentrations. However 1.0 mg/10 g diet of all three fractions was used for further studies.

Fractions of *Tapinanthus globiferus* ameliorated the levels of oxidative stress markers in SA-induced carcinogenesis in *Drosophila melanogaster*

Following 10 days treatment, from the graph in Fig. 2a, it was observed that all three fraction of *Tapinanthus globiferus* significantly decreased H_2O_2 generation ($p < 0.05$) in the SA-induced carcinogenesis in the flies. Also the decreased T-SH which was observed in the SA exposed-untreated flies showed increase in the groups co-treated with the fractions, with a significant increase ($p < 0.05$) observed in the methanol fraction of *Tapinanthus globiferus* (Fig. 2b). Reduced glutathione (GSH) levels were significantly restored ($p < 0.05$) in the groups treated with the three fractions when compared with the SA exposed-untreated group (Fig. 2c). GST activities in Fig. 2d, shows there was decreased activity following SA-exposure, which was significantly increased ($p < 0.05$) upon administration of the butanol and methanol fractions. Nitric oxide (NO) concentrations were significantly decreased in the SA-induced untreated group, but the groups treated with the three fractions showed significant appreciable increase when compared with the negative control. (Fig. 2e). The product of protein oxidation (protein carbonyl), in Fig. 2f, was increased in the SA-induced untreated group, which was significantly decreased ($p < 0.05$) in the SA-induced groups treated with the three fractions. The production of malondialdehyde, an indicator of lipid peroxidation was significantly decreased ($p < 0.05$) following treatment with the three fractions especially in the group treated with the methanol fraction (Fig. 2g).

Fractions of *Tapinanthus globiferus* modulated the excess metabolic activity induced by SA

Sodium arsenite exposure showed high MTT values which indicates excess metabolic activity in the cells, an attribute of cancer cells. However treatment of the flies with the three fractions showed that the MTT levels were decreased significantly (Fig. 3).

Fractions of *Tapinanthus globiferus* improved the climbing ability of the flies and modulated SA-altered mRNA expression of p53 and Ras

There was a significant decline ($p < 0.05$) in the climbing rate of the flies in the SA-induced-untreated group, which was increased significantly when treated with the three fractions (Fig. 4a). SA down-regulated the expression of *p53*, which was reversed by the three fractions, also the overexpressed *Ras* was modulated by the three fractions.

Bioactive compounds present in the active fractions

Although both butanol and methanol fractions had overall best ameliorative activities, only the butanol fraction showed presence of quantifiable polyphenolic compounds when subjected to the HPLC-DAD analysis. The major polyphenols identified in the butanol fraction of *Tapinanthus globiferus* are: the hydroxybenzoate derivates which includes Gallic acid, protocatechuic acid and syringic acid; hydroxycinnamate derivatives including caffeic acid, chlorogenic acid, p-coumaric acid and t-ferulic acid; flavonoid derivatives comprising rutin, quercetin-3-glucoside, catechin, epigallocatechin, epicatechin, kaempferol derivatives (kaempferol 3-o-β-D-glucopyranoside) and quercetin derivatives (Table 3)

Table 3
Polyphenols identified in *Tapinanthus globiferus* using HPLC-DAD

		Quantification mg/100 g of lyophilized extract*
	λ (nm)	Butanol
Hydroxybenzoate derivatives	280 nm	1723.54 ± 19.55
Hydroxycinnamate derivatives	320 nm	196.71 ± 4.94
Flavonoid derivatives	360 nm	22179.34 ± 114.85
Total phenolic compounds		24099.6 ± 100.25

Results express as mean ± standard deviation

*Hydroxybenzoate derivatives (HBD) were quantified as equivalent to gallic acid; hydroxycinnamate derivatives (HCD) were quantified as equivalent to p-coumaric acid; flavonoid derivatives were quantified as equivalent to kaempferol 3-o-β-D-glucopyranoside.

	λ (nm)	Butanol
Hydroxybenzoate derivates (HBD)		
Gallic acid	280 nm	< LoQ
Protocatechuic acid	280 nm	23.85 $\pm$ 1.23
Syringic acid	280 nm	24.95 $\pm$ 4.26
Hydroxycinnamate derivatives (HCD)		
Caffeic acid	320 nm	< LoD
Chlorogenic acid	320 nm	< LoQ
<i>p</i> -Coumaric acid	320 nm	< LoQ
<i>t</i> -Ferulic acid	320 nm	< LoQ
Flavonoids		
Rutin	360 nm	1633.77 $\pm$ 7.71
Quercetin-3-glycoside	360 nm	1318.37 $\pm$ 1.70
Catechin	280 nm	192.04 $\pm$ 23.96
Epigallocatechin	280 nm	< LoD
Epicatechin	280 nm	32.49 $\pm$ 0.16
Kaempferol derivatives*	360 nm	8848.81 $\pm$ 12.70
Quercetin derivatives**	360 nm	7517.89 $\pm$ 74.25
Total phenolic compounds***		24099.60 $\pm$ 100.25

Results express as mean $\pm$ standard error. *Kaempferol derivatives were quantified as equivalent to kaempferol 3-o- β -D-glucopyranoside. ** Quercetin derivatives were quantified as equivalent to quercetin-3-glycoside ***Total phenolic compounds were determined through the sum of the hydroxybenzoate derivatives, hydroxycinnamate derivatives, and flavonoids; LoQ: limit of quantification.

In silico Studies

To determine the bioactive compounds with anticancer potential in *Tapinanthus globiferus*, butanol fraction, the compounds were used to dock two target proteins which are implicated in gastrointestinal cancers; VEGF-A (table 4) and Ras protein (Table 5).

Molecular Docking analyses of the Bioactive Compounds in Butanol fraction of *Tapinanthus globiferus* against Vascular Endothelial Growth Factor

The results in table 4 shows the binding affinities of the different compounds with VEGFs in both humans (*Hs*VEGF-A) and *Drosophila melanogaster* (*Dm*VEGF-1). Rutin showed the highest binding affinity with the lowest energy score in both *Hs*VEGF-A and *Dm*VEGF-1. In *Hs*VEGF-A, it had a score of -8.890 kcal/mol and - 9.793 kcal/mol in *Dm*VEGF-1. The binding affinity of isoquercetin in both organisms was higher than that of the standard inhibitor, which had - 3.560 kcal/mol in *Hs*VEGF-A and - 1.284 kcal/mol in *Dm*VEGF-1. As illustrated in Fig. 6, in the binding pocket of *Hs*VEGF-A, rutin interacted with the backbone of PRO 247, SER 375, ASP 376, ALA378 and VAL 379, while within the binding pocket

of *Dm*VEGF-1, interacted with the backbone of ALA 126, ALA 129, CYS 162, GLY 164, THR 169, LEU 170, ILE 171, SER 172, CYS 173 and GLN 174. Interactions were also made it the sidechains of ALA 126, THR 127, ALA 129, CYS 162, SER 172, CYS 173, GLN 174 and PRO 175 of the *Dm*VEGF-1 and with the sidechains of LYS 246, PRO 247, LYS 248, ARG 255, SER 375, ASP 376, ILE 377, ALA 378, VAL 379, GLU 380, THR 393 and LEU 406 in the binding pocket of *Hs*VEGF-A. Polar interactions were formed with LYS 246, LYS 248, ARG 255, SER375, ASP 376, GLU 380 and THR 393 in the pocket of *Hs*VEGF-A and with THR 127, SER 172 and GLN 174 of *Dm*VEGF-1. Hydrophobic interaction was established within the binding pocket of *Hs*VEGF-A with PRO 247, ILE 377, ALA 378, VAL 379 and LEU 406, and in the *Dm*VEGF-1 with amino acids ALA 126, ALA 129, CYS 162, CYS 173 and PRO 175. Hydrogen bond formation between ASP 376, ILE 377, GLU 380, SER 375 and VAL 379 was observed in *Hs*VEGF-1 and between GLY 164, THR 169, LEU 170, CYS 173 and ALA 129 in the pocket of *Dm*VEGF-1. Electrostatic interactions were formed with the amino acids LYS 246, LYS 248, ARG 255, ASP 376 and GLU 380 of the *Hs*VEGF-A but none was formed within the *Dm*VEGF-1. Molecular interactions between the standard inhibitor and the *Hs*VEGF-A. On the other hand interactions with *Dm*VEGF-1 include interactions with the backbones of SER 117, ALA 126, SER 130, CYS 131, CYS 162, GLY 164, CYS 165, THR 169 and CYS 173; interactions with the side chains of SER 117, THR 127, ALA 129, SER 132, CYS 165, THR 169, SER 172 and GLN 174; polar interactions with SER 117, ALA 126, THR 127, SER 132, THR 169, SER 172 and GLN 174; Hydrophobic interactions with ALA 129, CYS 165, SER 130 and CYS 131 (Fig. 6A-D).

Table 4
Docking score of the Bioactive compounds of *Tapinanthus globiferus* against Vascular Endothelial Growth Factor (VEGF) in Humans and *Drosophila melanogaster*

Compounds	CID	<i>Hs</i> VEGF-A Kcal/mol	<i>Dm</i> VEGF-1 Kcal/mol
Rutin	5280805	-8.890	-9.793
Trifolin	5282149	-7.041	-6.261
Epigallocatechin	72277	-6.554	-5.375
Epicatechin	72276	-6.375	-5.933
Gallic acid	370	-6.337	-5.001
Isoquercetin	5280804	-6.271	-8.378
Protocatechuic acid	72	-5.772	-4.597
Chlorogenic acid	1794427	-5.397	-6.483
Syringic acid	10742	-5.269	-3.797
Catechin	9064	-5.211	-5.760
Caffeic acid	689043	-4.411	-5.227
GW786034	10113978	-3.560	-1.284
T-Ferulic acid	445858	-3.120	-4.368
P-coumaric acid	637542	-2.783	-3.680

Molecular Docking analyses of the Bioactive Compounds in Butanol fraction of *Tapinanthus globiferus* against Ras Protein

The docking of the compounds with the Ras protein in table 5, showed higher binding affinity with isoquercetin in both human and *Drosophila* homolog, with a docking score of -8.587 kcal/mol in the Ras of humans (*HsRas*) and - 11.883 kcal/mol in the growth factor of *Drosophila melanogaster* (*DmRas*), when compared with the co-crystallized ligand, which had - 2.177 kcal/mol with *HsRas* and - 5.558 kcal/mol with *DmRas*. The interactions of the compounds with the amino acids of the proteins showed that isoquercetin had high level of interactions with the amino acids within the binding pocket of the growth factor. Isoquercetin interacted with the backbones of the amino acids in the *HsRas* namely THR 58, ALA 59, GLU 62, GLN 99, TYR 64, SER 65 and ASP 69. In *DmRas*, isoquercetin had interactions with the backbone of GLY10, THR 58, ALA 59, GLN 61, GLU 62 and GLU 63. It also interacted with the sidechains of THR 58, GLU 62, TYR 64, ARG 68, ASP 69, TYR 96, GLN 99, ARG 102 and VAL 103 in the *HsRas*, whereas with the *DmRas*, isoquercetin interacted with LYS 16, THR 58, GLU 62, ARG 68, MET 72, TYR 96, GLN 99, ILE 100, ARG 102 and VAL 103. Polar interactions was established between isoquercetin and *HsRas* via THR 58, GLU 62, ARG 68, ASP 69, GLN 99, ARG 102 and ILE 100, and with *DmRas* via LYS 16, THR 58, GLU 62, ARG 68, ASP 69, GLN 99 and ARG 102. Hydrophobic contact with the *HsRas* was with TYR 64, TYR 96, VAL 103, ASP 69 and ALA 59, and in *DmRas* with MET 72, TYR 96, ILE 100 and VAL 103. Hydrogen bonds formed with *HsRas* were with ALA 59, GLU 62, SER 65, ASP 69 and GLN 99 whereas with *DmRas* was with SER 65, ASP 69, THR 58, GLY 10 and TYR 96. Pi-pi ($\pi - \pi$) stacking were formed with TYR 64 and TYR 96 in the *HsRas* binding pocket and with TYR 96 in the binding pocket of *DmRas*. Electrostatic interactions were established with the GLU 62, ARG 68, ASP 69, MET 72, ARG 102 and VAL 103 in the binding pocket of *HsRas* and were formed with SER 65, ASP 69, THR 58, and TYR 96 of the *DmRas*. The co-crystallized ligand interacted the backbone of the amino acids in the *HsRas* namely, GLY 13, VAL 14, LYS 16, SER 17 and ALA 18 and with the amino acids in *DmRas* VAL 9, GLY 10, THR 58, ALA 59, GLU 62, GLU 63 and TYR 64. Interactions were also established with the side chains of amino acids LYS 16, SER 17, ALA 18, PHE 28, TYR 32, PRO 34 and LYS 117 in *HsRas* and side chains of amino acids VAL 9, LYS 16, THR 58, TYR 64, ARG 68, MET 72, TYR 96 and GLN 99 of the *DmRas*. Polar interactions with the *HsRas* were with LYS 16, SER 17 and LYS 117, whereas it was with LYS 16, THR 58, ARG 68 and GLN 99 in the *DmRas*. Hydrophobic interactions were formed with ALA 18, PHE 28, TYR 32 and PRO 34 in the binding pocket of *HsRas*, and with amino acids VAL9, TYR 64, MET 72 and TYR 96 of *DmVEGF-1*. Hydrogen bonds were formed with LYS 16, SER 65 and ASP 69 within the binding pocket of *HsRas* and amino acid GLY 10, only, in the binding pocket of *DmRas*. Also the standard inhibitor formed aromatic interactions with PHE 28 and TYR 32 in the pocket of *HsVEGF-A*, whereas in the pocket of *DmRas*, it was formed with TYR 96 and TYR 64. Electrostatic interactions were formed with LYS 16 and LYS 117 amino acids of *HsRas* and with amino acids LYS 16 and ARG 68 of the *DmRas*. There was also a metal coordination between the CYS 12 of *HsRas* and the standard inhibitor (Fig. 7A-D).

Table 5
Docking scores of the Bioactive compounds of *Tapinanthus globiferus* against Ras protein in Human and *Drosophila melanogaster*

Compounds	CID	Docking scores <i>HsKRAS</i>	Docking scores <i>DmRAS</i>
Isoquercetin	5280804	-8.914	-11.883
Chlorogenic acid	1794427	-8.587	-7.370
Rutin	5280805	-8.390	-7.928
Epigallocatechin	72277	-6.939	-9.904
Gallic acid	370	-6.736	-6.489
Catechin	9064	-6.267	-9.122
Epicatechin	72276	-5.863	-9.547
Trifolin	5282149	-5.284	-10.11
Syringic acid	10742	-5.199	-5.541
Protocatechuic acid	72	-5.043	-5.685
Caffeic acid	689043	-4.816	-5.839
T-Ferulic acid	445858	-4.35	-5.883
P-coumaric acid	637542	-4.279	-4.602
Sotosarib	156124857	-4.446	-4.288
CHEMBL5183988 (Co-rystallized ligand)	146250992	-2.177	-5.558

ADMET Profile

The predicted ADME properties of the compounds shows that the compounds are water soluble based on the solubility (Log S) values. The values of their Lipophilicity (Log P) ranged from – 3.89 to 0.7. The prediction of drug-likeness based on Lipinski's rule of five (Ro5) showed that the rule was violated by only chlorogenic acid (1), isoquercetin (2), rutin (3), and epigallocatechin (1). The Veber's rules on the other hand was violated by chlorogenic acid (1), isoquercetin (1) and rutin (1), whereas other compounds did not violate the rule. The bioavailability score of chlorogenic acid was 0.11, isoquercetin, rutin and trifolin had 0.17, which predicts low oral availability; however, kaempferol, quercetin, epigallocatechin, epicatechin and catechin had 0.55, protocatechuic acid, gallic acid, syringic acid and caffeic acid had 0.56, whereas P-coumaric acid and t-ferulic acid had 0.85, which suggests high probability of being orally available. PAINS alert shows that some of the compounds contained promiscuous groups, which may influence their binding affinities, as indicated by 1 and 0 which indicated the absence of any (table 6).

Table 6
Lipophilicity, Solubility, Druglikeness, and Oral bioavailability of Bioactive compounds of *Tapinanthus globiferus*

Compound	Molecular Weight	Mean LOGP	Silicos-IT LogSw	Silicos-IT class	Lipinski violations	Veber violations	Bioavailability Score	PAINS alerts
Protocatechuic acid	154.12	0.40	-0.60	Soluble	0	0	0.56	1
Gallic acid	170.12	-0.16	-0.04	Soluble	0	0	0.56	1
Syringic acid	198.17	0.49	-1.46	Soluble	0	0	0.56	0
Chlorogenic acid	354.31	-1.05	0.40	Soluble	1	1	0.11	1
Isoquercetin	464.38	-2.59	-1.51	Soluble	2	1	0.17	1
Rutin	610.52	-3.89	-0.29	Soluble	3	1	0.17	1
T-ferulic acid	194.18	1.00	-1.42	Soluble	0	0	0.85	0
Kaempferol	286.24	-0.03	-3.82	Soluble	0	0	0.55	0
Quercetin	302.24	-0.56	-3.24	Soluble	0	0	0.55	1
P-coumaric acid	164.16	1.28	-1.28	Soluble	0	0	0.85	0
Caffeic acid	180.16	0.70	-0.71	Soluble	0	0	0.56	1
Epigallocatechin	306.27	-0.29	-1.56	Soluble	1	0	0.55	1
Epicatechin	290.27	0.24	-2.14	Soluble	0	0	0.55	1
Catechin	290.27	0.24	-2.14	Soluble	0	0	0.55	1

Pharmacokinetic predictions of the bioactive compounds in the *Tapinanthus globiferus* Butanol fraction

Based on the pharmacokinetic prediction shown in table 7, it indicates that only chlorogenic acid, isoquercetin and rutin had low ability to cross the gastrointestinal tract (GI). T-ferulic acid and P-coumaric acid showed they have ability to cross the Blood-brain Barrier (BBB) whereas other compounds had low permeability. Only rutin, epicatechin and catechin were predicted to be substrates of the Permeability glycoprotein (P-gp), which could be effluxed out of the cell. Kaempferol and quercetin are predicted to have inhibitory activities on CYP1A2 and CYP2D6. None of the compounds were predicted to be potential inhibitors of CYP2C19 and CYP2C9. The prediction shows that protocatechuic acid, gallic acid, kampferol and quercetin have the ability to inhibit CYP3A4.

ProTox-II-predicted toxicity profile of the compounds in the *Tapinanthus globiferus* Butanol fraction

As shown in Table 8, quercetin was predicted to have LD₅₀ of 159 mg/kg whereas other compounds showed high LD₅₀ especially Epigallocatechin, epicatechin and catechin showing highest LD₅₀ of 10000 mg/kg. Predicted toxicity shows that quercetin has class 3 toxicity, protocatechuic acid, gallic acid, syringic acid and T-ferulic acid have class 4 toxicity, chlorogenic acid, isoquercetin, rutin and p-coumaric acid trifolin have class 5 toxicity, while epigallocatechin, epicatechin and catechin belong to the class 6 toxicity.

Based on the organ toxicity profile of the compounds, none of the compounds were predicted to be toxic except nephrotoxicity. However, Protocatechuic acid, gallic acid, quercetin and caffeic acid prediction showed potential carcinogenicity. Immunotoxicity was predicted in chlorogenic acid, isoquercetin, rutin, and t-ferulic acid. Only quercetin showed predicted mutagenicity and none of the compounds were cytotoxic.

Table 7
Pharmacokinetic predictions of the bioactive compounds in the *Tapinanthus globiferus* Butanol fraction

Pharmacokinetics Prediction of the Bioactive Compounds of *Tapinanthus globiferus*

Compounds	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
Protocatechuic acid	High	No	No	No	No	No	No	Yes
Gallic acid	High	No	No	No	No	No	No	Yes
Syringic acid	High	No	No	No	No	No	No	No
Chlorogenic acid	Low	No	No	No	No	No	No	No
Isoquercetin	Low	No	No	No	No	No	No	No
Rutin	Low	No	Yes	No	No	No	No	No
T-ferulic acid	High	Yes	No	No	No	No	No	No
Kaempferol	High	No	No	Yes	No	No	Yes	Yes
P-coumaric acid	High	Yes	No	No	No	No	No	No
Caffeic acid	High	No	No	No	No	No	No	No
Epigallocatechin	High	No	No	No	No	No	No	No
Epicatechin	High	No	Yes	No	No	No	No	No
Catechin	High	No	Yes	No	No	No	No	No

Table 8

Protox II Predicted Toxicity Profile of Bioactive Compounds in *Tapinanthus globiferus*

Compound	LD50	Toxicity Class	Hepa	Neuro	Nephro	Cardio	Carcino	Immuno	Muta	Cyto
Protocatechuic acid	2000 mg/kg	4	-	-	+	-	+	-	-	-
Gallic acid	2000 mg/kg	4	-	-	+	-	+	-	-	-
Syringic acid	1700 mg/kg	4	-	-	+	-	-	-	-	-
Chlorogenic acid	5000 mg/kg	5	-	-	+	-	-	+	-	-
Isoquercetin	5000 mg/kg	5	-	-	+	-	-	+	-	-
Rutin	5000 mg/kg	5	-	-	+	-	-	+	-	-
T-Ferulic acid	1772 mg/kg	4	-	-	+	-	-	+	-	-
Kaempferol	3919 mg/kg	5	-	-	+	-	-	-	-	-
P-Coumaric acid	2850 mg/kg	5	-	-	+	-	+	-	-	-
Caffeic acid	2980 mg/kg	5	-	-	+	-	+	-	-	-
Epigallocatechin	10000 mg/kg	6	-	-	+	-	-	-	-	-
Epicatechin	10000 mg/kg	6	-	-	+	-	-	-	-	-
Catechin	10000 mg/kg	6	-	-	+	-	-	-	-	-

Hepa = hepatotoxicity; Neuro = Neurotoxicity; Neph = Nephrotoxicity; Cardio = Cardiotoxicity; Carcino = Carcinogenicity; Immuno = Immunotoxicity; Muta = Mutagenicity; Cyto = Cytotoxicity

DISCUSSION

With so many people worldwide exposed to cancer-predisposing variables, particularly in developing and under-developed nations, cancer is a global health concern. Traditional medicine has been embraced in response to the difficulty of accessing conventional medications for the treatment of cancer, with the usage of medicinal herbs rising in underdeveloped nations. Secondary metabolites, often known as bioactive molecules, are responsible for the therapeutic capabilities of herbal plants. Plant secondary metabolites are essential to human health and serve as the building blocks of many pharmacological medications; in fact, many of the medications now on the market are derived from plants (Yeshi et al., 2022). The anticancer properties of this *Tapinanthus globiferus*, despite its widespread use in traditional medicine for cancer therapy, has remained unexplored. Therefore the major aim of this study was to investigate this potential anticancer properties and identify the bioactive compounds with the anticancer properties, which prompted the investigation as the potential anticancer activity of *Tapinanthus globiferus*. Based on traditional practices, they are used for treatment of cancers and other ailments, which could be attributed to their phytochemical components. These metabolites often possess distinct chemical characteristics that are released from the plant cells

by various extraction techniques, as observed in the unrefined techniques employed in traditional medicine. Cells from mistletoes often have greater amounts of most of these chemical compounds than those of their hosts. (Griebel et al., 2017).

The results from this study, which are consistent with earlier research, indicate that *T. globiferus* may be a viable source of therapeutic molecules for anticancer treatment (Gade et al., 2022). This is especially true given the bioactive compounds that were identified and their demonstrated anticancer efficacy, as protein target inhibitors, which could be attributed to its antioxidative properties (Oche et al., 2019). The butanol fraction was used to further investigate the anticancer potential of *Tapinanthus globiferus* using in silico studies, because the overall outcome of the in vivo anticancer/ameliorative studies showed that it had better results. Although the methanol fraction also showed good activities, the result of the HPLC-DAD did not produce quantifiable amount of the bioactive compounds, which was one of the major limitation of this study.

Hydrogen peroxide modulates and acts a signal molecule in the redox metabolism of cells by acting as a messenger alongside hydrogen sulfide (H_2S) and the nitric oxide radical ($\cdot NO$), activating specific oxidations that determine the metabolic response. The reaction that is triggered determines cell survival or apoptosis, depending on which downstream metabolic pathways are activated (Andrés et al., 2022). Arsenic is known to compromise cellular respiration in the mitochondria thereby disrupting mitochondria activities in the cell, which leads to cell damage and programmed apoptosis (Zhong et al., 2022). This eventually leads to increased hydrogen peroxide generated in the mitochondria, which accumulates and induces oxidative stress. In certain cancer types, high levels of H_2O_2 can trigger proliferation of the cells (Andrés et al., 2022). In this study, the level of hydrogen peroxide generated was decreased following treatment with the three fractions of *Tapinanthus globiferus*, which agrees with the report of Elekofehinti et al. (2019) that *Tapinanthus globiferus* can modulate H_2O_2 production. This could suggest that *Tapinanthus globiferus* has the potential of restoring mitochondria function (Elekofehinti et al., 2019) by reversing the effect of the NAD-dependent pathways which invariably reduces excess hydrogen peroxide generated. Also, following treatment with the fractions, an increase in total thiol level was observed in all three fractions especially the methanol fraction. Since arsenite have high affinity for thiol groups, probably, by binding of the antioxidants present in the plant fractions to these arsenite molecules, it may spare the thiol pool thus increasing the thiol concentration of the antioxidant system. In addition, the observed increase in thiol groups observed in the treated groups may be attributed to the thiols present in the plant itself. Thiols are also components of the plant defense systems, which are usually synthesized upon metal-induced stress in the plants, by activating the pathways involved in their synthesis. These thiol groups are usually incorporated in amino acids such as cysteine and methionine, which serve as sources of these thiol groups present in the plant (Pál et al., 2018). Increased GSH level was observed after treatment with the fractions of *T. globiferus*. GSH is a vital endogenous antioxidant involved in mitigating oxidative stress. However in the presence of arsenite, GSH levels deplete due to the high affinity for thiols by arsenite. The increase in GSH could be due to the presence of bioactive compounds such as flavonoids, which scavenge free radicals thus sparing the system of depleted GSH levels, which invariably increases the rate of detoxification of the metals (Madhu et al., 2023). In addition, plants can be major sources of sulphur due to the presence of cysteine, which acts as the precursor for synthesis of glutathione thereby replenishing depleted levels (Galant et al., 2011). GST is an enzyme involved in the conjugation of xenobiotics, including metals like arsenic, using GSH. Arsenite on the other hand has the ability to inhibit the activities of this enzyme due to the presence of thiols groups, in addition arsenite can cause down regulation of GST genes, which decreases GST synthesis (Porter et al., 1999). There was increased GST activity after treatment with the fractions, especially the butanol fraction. The observed increased may be due to the presence of metal chelators (Oche et al., 2019), which could bind to the arsenite thus preventing it from binding to the GST (Guneidy et al., 2020). Nitric oxide is another signaling molecule, which is vital in immune response. However, in oxidative stress, the level is usually altered, which could affect its activity. Arsenite has the ability to increase its concentration thus potentiating its activity as a radical involved in oxidative stress or it could

lead to a decreased concentration, depending on the affected cells or tissues. Depletion of NO by arsenite is usually via inhibition of endothelial Nitric Oxide synthase (eNOS) (Seo et al., 2014). From the result of this study, arsenite caused depletion in NO levels which were restored following treatment with the three fractions. However, a higher improvement was observed in the group treated with the butanol fraction. An overwhelming increase in the group treated with the ethyl acetate fraction could be a pointer to the fact that the ethyl acetate fraction may possess bioactive components capable triggering increased NO, which could be detrimental. Arsenite is known to cause an increased oxidation of macromolecules such as DNA, lipids and proteins, which leads to accumulation of oxidized products, as an indication of oxidative stress. These accumulated compounds have been associated with carcinogenesis. Protein oxidation gives rise to protein carbonyls, which can cause genomic instability, which is a hallmark of cancer (Rodríguez-García et al., 2020), and products of lipid peroxidation such as malondialdehyde can form adducts with DNA, which accumulates and disrupts cellular activities which promotes cancer (Morabito et al., 2004). Aldehydes derived from lipid peroxidation can also lead to oxidation of these proteins, which increases the protein carbonyl levels as well (Akagawa, 2021). These processes are known to induce inflammation, which leads to cellular damages that promotes carcinogenesis (Rodríguez-García et al., 2020). There was a decrease in the elevated levels of protein carbonyl and malondialdehyde after treatment with the three fractions. Lipid peroxidation was lower in the group treated with the butanol fraction. Based on this general outcome, the plant has shown to have ameliorative activity against damages induced by carcinogenesis, as such this means that the plant possess the ability to reduce most of the side effects of cancer, especially oxidative stress, thereby possessing the potential of improving the quality of life of cancer patients and also contributing to their survival rates as well. This feature of improving the survival rate could be deduced from the outcome of the survival assay of the different fractions of the plants, which the flies were exposed to. Although a higher survival rate was prominent in the group treated with butanol fraction, the level of survival with the methanol and ethyl acetate fractions also indicates that *Tapinanthus globiferus* can improve survival rate, which may also be applied in aging apart from disease conditions, as most diseases, including cancer, can reduced survival rates of individuals and drastically reduce the quality of life as well.

Increased metabolic activity is one of the characteristics of cancerous cells, which can be detected using cell viability assays. MTT measures the cell viability of cells and can also be used to measure the level of metabolic activities in the cells, with increased value associated with carcinogenesis and decrease linked to cytotoxicity. From this study, there was a higher MTT value compared with the cell viability of the positive control group. This suggests the presence of cancerous cells, with increased metabolic activity, suggesting cell proliferation. When treated with the fractions, there was a decrease in MTT value with suggests that the fractions had cytotoxic ability or the ability to prevent or arrest continuous proliferation of cells. Higher decline was observed in the groups treated with butanol and methanol fractions. With an increase in lipid peroxidation and protein oxidation, it is expected that cell viability would be lower in the group exposed to SA but this was not observed in the study. An increased value which was observed despite cellular damage, as evidenced by lipid peroxidation and protein oxidation, could be attributed to the Warburg effect, which enables cells to carry out glycolysis due to depleted ATP, caused by cellular damage. Damaged cells are characterized by decreased ATP levels due to disruption of the mitochondria function. In cancer cells, there is a switch to glycolysis, which leads to fermentation, due to absence of oxygen. This drives carcinogenesis, which could be responsible for the increased MTT value despite the presence of high lipid peroxidation and protein oxidation, which confirms the carcinogenicity of arsenic.

The alterations in the expression of *p53* and *Ras* observed with exposure to SA were reversed by the three fractions. The could be due to the presence of bioactive compounds that can bind to transcription factors which can modulate the expression of genes (Cho et al., 2022). Generally, these bioactive compounds are known to modulate gene expressions (Jantan et al., 2015). Alteration in genes is one of the major hallmarks in cancer. Tumour suppressor genes and oncogenes are most implicated in cancer. A loss of function of tumour suppressor genes and a gain of function of

oncogenes have direct link with cancer progression. *p53* is a tumour suppressor gene that acts as a regulator of other genes especially genes involved in cell proliferation. Loss of its function have been implicated in several cancers, as this could lead to gain of function of the oncogenes such as the *Ras*, which invariably leads to overexpression of the gene (Buganim et al., 2010). Arsenite have been reported to downregulate *p53* via oxidative stress which damage DNA and trigger other biological processes that cause *p53* to be downregulated (Yu et al., 2013). *Ras* on the other hand can also be overexpressed by arsenite via DNA methylation and histone modification, which promotes cell proliferation and tumorigenesis (Wāng et al., 2024).

The result of HPLC-DAD showed that *Tapinanthus globiferus* has high concentration of polyphenols especially flavonoids. This corroborates with our initial study, which showed that *Tapinanthus globiferus* has high concentration of flavonoids (Oche et al., 2019). Flavonoids are well known polyphenols that have therapeutic benefits and their presence in the different part of a plant makes them possess biological activity and therapeutic potentials (Ullah et al., 2020). Different studies have shown that they possess anticancer activities (Rathee et al., 2024) which they are able to carry out via different mechanism which includes antioxidative activities (Li et al., 2020), decreasing the section of pro-inflammatory agents involved in inflammation (Kim et al., 2004) cell cycle modulation (Kopustinskiene et al., 2020), induction of apoptosis (Raina et al., 2020) and inhibition of angiogenesis (Okamura et al., 2020).

Based on the compounds that were identified, in silico studies were carried out to predict compounds with potential anticancer activities based on their ability to inhibit proteins implicated in gastrointestinal cancer in humans alongside their homolog in *Drosophila melanogaster*. This was carried out to not only identify the compounds but to also elucidate their possible mechanism of action as inhibitors of target proteins implicated in gastrointestinal cancers, notably VEGF-A and the Ras protein. From the results of this study, isoquercetin and rutin were identified as the top scorers with high binding affinities for VEGF-A and Ras protein respectively, which suggests their anticancer activities as inhibitors of the proteins. VEGF-A is a signalling molecule that is involved in angiogenesis, which is the development of new blood vessels. Tissue repair and develop also depends on the activities of VEGF-A and dysregulation of the growth factor can lead to disease conditions including cancer. An overexpression of VEGF-A has been associated with different types of cancers including colon cancer. By supplying blood vessels to cancer cells, VEGF-A plays crucial role in supplying rapidly dividing cells with the requirement for their growth process. Endothelial cell migration, proliferation, and tube formation are primarily mediated by VEGF-A (Ye et al., 2021). This makes them suitable target proteins for target therapy in cancer (Patel et al., 2023). By inhibiting the activity of VEGF-A, their angiogenic activity is impaired, which could prevent further growth and proliferation of the cancer cells. In the binding pocket of VEGF-A the ASP, GLU, ARG, HIS and LYS amino acid residues play important roles of binding of the protein to the receptor site (Keyt et al., 1996). Invariably, binding of the compounds to these amino acids present in the binding site of the VEGF-A could affect the binding of VEGFR1 and VEGFR2 to the receptor binding site of the protein. This suggests inhibitory activity by rutin, as such could be a potential anti-VEGF-A molecule for treatment of solid tumors. Ras protein is usually activated by GTP. Upon activation, it plays major roles in downstream activation of various pathways. The activated RAS, bound to GTP, interacts with downstream effectors including RAF, PI3K, and Ral guanine exchange factors (RalGEFs), transducing the signal to regulate biological activities (Chen et al., 2021). However an overexpression of Ras proteins trigger downstream signalling pathways, which promote the migration, survival, and multiplication of malignant cells. When these pathways are overexpressed, malignant transformation occurs, which gives cancer cells the ability to proliferate uncontrollably. Moreover, overexpression disrupts the regular regulation of the cell cycle, leading to increased mitotic activity and genomic instability—two important factors in the development of cancer (Chen et al., 2021). In this study isoquercetin had higher affinity to Ras protein in both humans and *Drosophila melanogaster*. Isoquercetin established favourable interactions with the Ras protein, which may be capable of preventing its activation by GTP.

These polyphenols are bioflavonoids whose anticancer activities have been reported by several studies. Isoquercetin prevents metastasis of cancer cells and enhances the efficacy of cancer chemotherapy (Zahra et al., 2024). It is also

known to be involved in the modulation of signaling pathways involved in cancer progression (Pyo et al., 2024). Rutin is another flavonoid well known for its antioxidative properties and its use for treatment of cancer. It is known to prevent proliferation of tumour cells and modulation of signalling pathways (Pandey et al., 2021).

ADMET studies were carried out to determine the druglikeness of the bioactive compounds present in *Tapinanthus globiferus*. This is necessary to ensure drug efficacy and safety prior to their use for therapeutic purposes. The occurrence of drug failures have been attributed to poor pharmacokinetics and toxicity of compounds (Beck & Geppert, 2014). Most of the compounds identified with potential anticancer potentials, based on their high affinity for target proteins, showed favourable ADMET properties. Notwithstanding, some of them showed unfavourable properties such as Chlorogenic acid, isoquercetin and rutin, which were the only compounds with low GI absorption, as such affects their level of oral bioavailability is relatively low and cannot be administered orally. This could affect the pharmacokinetics, which could downplay their efficacy and of their use as anticancer drugs. Also, the toxicity profile of the compounds, rutin and isoquercetin showed that both compounds belong to toxicity class 5 and have a high LD₅₀ of 5000 mg/kg each. This shows that the compounds are relatively safe. However, based on the prediction, both have nephrotoxicity and immunotoxicity. This does not rule out the possibility of their use as anticancer drugs as both lead compounds can be optimized to express better druglike features with desirable qualities for therapeutic purposes.

CONCLUSION

Cancer is a global burden with the need for therapeutic interventions, which will not only cure the disease but be safe as well. *Tapinanthus globiferus*, based on this study have shown to possess anticancer properties, which could be due to the presence of phytochemicals which have been shown to have this potentials, notably rutin and isoquercetin. Their limitations as drug candidates for anticancer therapy can be circumvented via optimization and further validation of their properties for drug development.

References

1. Adeloje D, Bowman K, Chan KY, Patel S, Campbell H, Rudan I (2018) Global and regional child deaths due to injuries: an assessment of the evidence. *J global health*, 8(2)
2. Afuye O, Omotayo S (2024) Evaluation of In vitro Anti-Diabetic Properties of Biosynthesized Magnesium Oxide Nanoparticles from *Vernonia amygdalina* (Bitter Leaf) Aqueous Leaf Extract. *Eur J Med Plants* 35(1):45–55
3. Akagawa M (2021) Protein carbonylation: molecular mechanisms, biological implications, and analytical approaches. *Free Radic Res* 55(4):307–320. <https://doi.org/10.1080/10715762.2020.1851027>
4. Andrés CMC, de la Pérez JM, Juan CA, Plou FJ, Pérez-Lebeña E (2022) Chemistry of Hydrogen Peroxide Formation and Elimination in Mammalian Cells, and Its Role in Various Pathologies. *Stresses*, 2(3), 256–274. <https://www.mdpi.com/2673-7140/2/3/19>
5. Arjona-García C, Blancas J, Beltrán-Rodríguez L, López Binnquist C, Colín Bahena H, Moreno-Calles AI, Sierra-Huelsz JA, López-Medellín X (2021) How does urbanization affect perceptions and traditional knowledge of medicinal plants? *J Ethnobiol Ethnomed* 17(1):1–26
6. Banerjee P, Dehnbostel FO, Preissner R (2018) Prediction is a balancing act: Importance of sampling methods to balance sensitivity and specificity of predictive models based on imbalanced chemical data sets. *Front Chem*, 362
7. Barkat MA, Goyal A, Barkat HA, Salauddin M, Pottoo FH, Anwer ET (2021) Herbal medicine: clinical perspective and regulatory status. *Comb Chem High Throughput Screen* 24(10):1573–1582
8. Beck B, Geppert T (2014) Industrial applications of in silico ADMET. *J Mol Model* 20(7):2322. <https://doi.org/10.1007/s00894-014-2322-5>

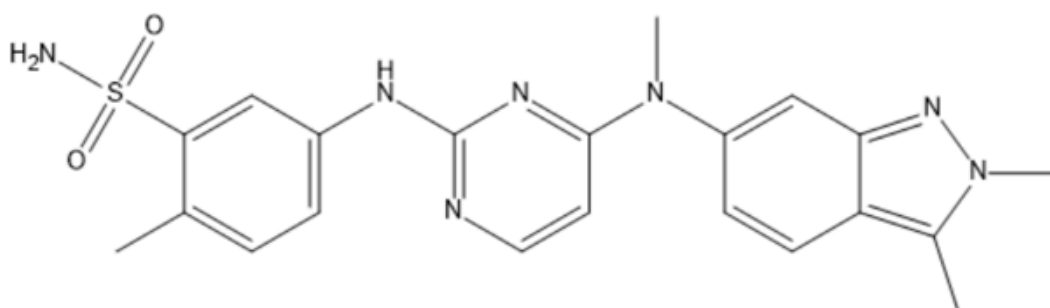
9. Beutler E, Duron O, Kelly BM (1963) Improved method for determination of blood glutathione
10. Buganim Y, Solomon H, Rais Y, Kistner D, Nachmany I, Brait M, Madar S, Goldstein I, Kalo E, Adam N (2010) p53 Regulates the Ras circuit to inhibit the expression of a cancer-related gene signature by various molecular pathways. *Cancer Res* 70(6):2274–2284
11. Chen K, Zhang Y, Qian L, Wang P (2021) Emerging strategies to target RAS signaling in human cancer therapy. *J Hematol Oncol* 14(1):116. <https://doi.org/10.1186/s13045-021-01127-w>
12. Cho JH, Hong YD, Kim D, Park SJ, Kim JS, Kim H-M, Yoon EJ, Cho J-S (2022) Confirmation of plant-derived exosomes as bioactive substances for skin application through comparative analysis of keratinocyte transcriptome. *Appl Biol Chem* 65(1):8. <https://doi.org/10.1186/s13765-022-00676-z>
13. Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 7(1):42717
14. Elekofehinti OO, Kamdem JP, Saliu TP, Famusiwa CD, Boligon A, Teixeira Rocha JB (2019) Improvement of mitochondrial function by *Tapinanthus globifer* (A.Rich.) Tiegh. Against hepatotoxic agent in isolated rat's liver mitochondria. *J Ethnopharmacol* 242:112026. <https://doi.org/https://doi.org/10.1016/j.jep.2019.112026>
15. Ellman GL (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys* 82(1):70–77
16. Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW, Walker RB (2010) Thorough study of reactivity of various compound classes toward the Folin – Ciocalteu reagent. *J Agric Food Chem* 58(14):8139–8144
17. Fakudze NT, Sarbadhikary P, George BP, Abrahamse H (2023) Ethnomedicinal Uses, Phytochemistry, and Anticancer Potentials of African Medicinal Fruits: A Comprehensive Review. *Pharmaceuticals*, 16(8), 1117. <https://www.mdpi.com/1424-8247/16/8/1117>
18. Gade IS, Chadeneau C, Simo T, Atchade R, Talla ADT, Seite E, Vannier P, Guillard B, Laurent J, S., Henoumont C (2022) A new flavonoid glycoside from *Tapinanthus* sp.(Loranthaceae) and evaluation of anticancer activity of extract and some isolated compounds. *Nat Prod Res* 36(16):4085–4093
19. Galant A, Preuss ML, Cameron JC, Jez JM (2011) Plant glutathione biosynthesis: diversity in biochemical regulation and reaction products. *Frontiers in plant science*, 2, 45
20. Griebel A, Watson D, Pendall E (2017) Mistletoe, friend and foe: synthesizing ecosystem implications of mistletoe infection. *Environ Res Lett* 12(11):115012
21. Guneidy RA, Gad AM, Zaki ER, Ibrahim FM, Shokeer A (2020) Antioxidant or pro-oxidant and glutathione transferase P1-1 inhibiting activities for *Tamarindus indica* seeds and their cytotoxic effect on MCF-7 cancer cell line. *J Genetic Eng Biotechnol* 18(1):74. <https://doi.org/10.1186/s43141-020-00077-z>
22. Habig WH, Jakoby WB (1981) [27] Glutathione S-transferases (rat and human). In *Methods in Enzymology* (Vol. 77, pp. 218–231). Academic Press. [https://doi.org/https://doi.org/10.1016/S0076-6879\(81\)77029-0](https://doi.org/https://doi.org/10.1016/S0076-6879(81)77029-0)
23. Iftikhar H, Schultzhaus JN, Bennett CJ, Carney GE (2017) The in vivo genetic toolkit for studying expression and functions of *Drosophila melanogaster* microRNAs. *RNA Biol* 14(2):179–187
24. International Agency for Research on Cancer (IARC) (2024) Global burden of gastrointestinal cancers. Available from Global burden of gastrointestinal cancers (who.int) Retrieved on 17/08/2024
25. Isikhuemen E, Olisaemeka U, Oyibotie G (2020) Host specificity and phytochemical constituents of mistletoe and twigs of parasitized plants: Implications for blanket application of mistletoe as cure-all medicine. *J Med Herbs Ethnomed* 6:30–37
26. Jantan I, Ahmad W, Bukhari SNA (2015) Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. *Front Plant Sci* 6:655
27. Jardim SR, de Souza LMP, de Souza HSP (2023) The Rise of Gastrointestinal Cancers as a Global Phenomenon: Unhealthy Behavior or Progress? *International Journal of Environmental Research and Public Health*, 20(4), 3640.

28. Johnson TO, Abolaji AO, Omale S, Longdet IY, Kutshik RJ, Oyetayo BO, Adegboyega AE, Sagay A (2021) Benzo[a]pyrene and Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide induced locomotor and reproductive senescence and altered biochemical parameters of oxidative damage in Canton-S *Drosophila melanogaster*. *Toxicol Rep* 8:571–580. <https://doi.org/https://doi.org/10.1016/j.toxrep.2021.03.001>
29. Johnson TO, Adegboyega AE, Adeyemi OE, Okonkwo FO, Ejembi SA, Oche J-RI, Oluwarotimi EA, Bashar F, Johnson GI, Adeyemi OS (2021) Computer-Aided Identification of Bioactive compounds of *Azadirachta indica* (Neem) with Potential activity against SARS-CoV-2 main protease. *BMC Plant Biology*
30. Keyt BA, Nguyen HV, Berleau LT, Duarte CM, Park J, Chen H, Ferrara N (1996) Identification of Vascular Endothelial Growth Factor Determinants for Binding KDR and FLT-1 Receptors: GENERATION OF RECEPTOR-SELECTIVE VEGF VARIANTS BY SITE-DIRECTED MUTAGENESIS (*). *J Biol Chem* 271(10):5638–5646
31. Kim HP, Son KH, Chang HW, Kang SS (2004) Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. *J Pharmacol Sci* 96(3):229–245. <https://doi.org/10.1254/jphs.CRJ04003X>
32. Kopustinskiene D, Jakstas V, Savickas A, Bernatoniene J (2020) Flavonoids as Anticancer Agents. *Nutrients* 12:457. <https://doi.org/10.3390/nu12020457>
33. Li G, Ding K, Qiao Y, Zhang L, Zheng L, Pan T, Zhang L (2020) Flavonoids Regulate Inflammation and Oxidative Stress in Cancer. *Molecules*, 25(23), 5628. <https://www.mdpi.com/1420-3049/25/23/5628>
34. Madhu, Sharma A, Kaur A, Tyagi S, Upadhyay SK (2023) Glutathione Peroxidases in Plants: Innumerable Role in Abiotic Stress Tolerance and Plant Development. *J Plant Growth Regul* 42(2):598–613. <https://doi.org/10.1007/s00344-022-10601-9>
35. Mirzoyan Z, Sollazzo M, Allocca M, Valenza AM, Grifoni D, Bellosta P (2019) *Drosophila melanogaster*: A model organism to study cancer. *Front Genet* 10:51
36. Morabito F, Cristani M, Saija A, Stelitano C, Callea V, Tomaino A, Minciullo PL, Gangemi S (2004) Lipid peroxidation and protein oxidation in patients affected by Hodgkin's lymphoma. *Mediat Inflamm* 13(5–6):381–383
37. Moraes KC, Montagne J (2021) *Drosophila melanogaster*: A powerful tiny animal model for the study of metabolic hepatic diseases. *Front Physiol* 12:728407
38. Morgado-Diaz JA (2022) Gastrointestinal Cancers [Internet]
39. Muche M, Muasya AM, Tsegay BA (2022) Biology and resource acquisition of mistletoes, and the defense responses of host plants. *Ecol Processes* 11(1):24. <https://doi.org/10.1186/s13717-021-00355-9>
40. Munnik C, Xaba MP, Malindisa ST, Russell BL, Sooklal SA (2022) *Drosophila melanogaster*: A platform for anticancer drug discovery and personalized therapies. *Front Genet* 13:949241
41. Nenclares P, Harrington K (2020) The biology of cancer. *Medicine* 48(2):67–72
42. Oche J-RI, Johnson TO, Akinsanmi AO, Jaryum KH, Francis T (2019) In vitro antioxidant activity and inhibition of Fe²⁺ + and SNP lipid peroxidation of African Mistletoes (*Tapinanthus globiferus*) from three selected host plants in Jos Plateau State Nigeria. *J Appl Life Sci Int* 20(4):1–10
43. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95(2):351–358. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
44. Okamura N, Ohta T, Kunimasa K, Uto Y, Kumazawa S (2020) Antiangiogenic Activity of Flavonols in Chorioallantoic Membrane (CAM) Assay. *Food Sci Technol Res* 26(6):891–896. <https://doi.org/10.3136/fstr.26.891>
45. Olanlokun JO, Ekundayo MT, Ebenezer O, Koorbanally NA, Olorunsogo OO (2021) Antimalarial and erythrocyte membrane stability properties of *globimetula braunii* (engle van tiegh) growing on cocoa in plasmodium berghei-infected mice. *Infect Drug Resist*, 3795–3808

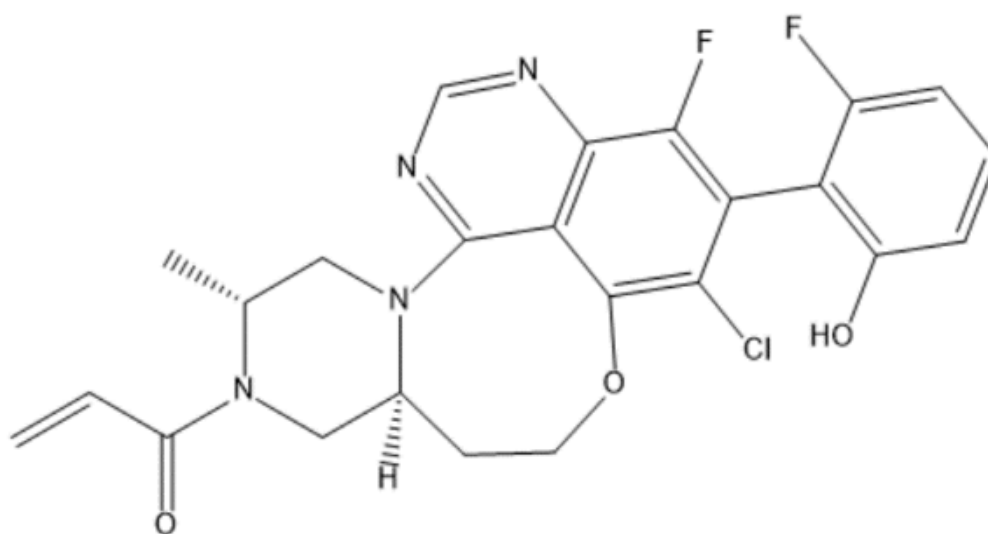
46. Oriola A, Aladesanmi A, Arthur G (2018) Anticancer activity of three African mistletoes. *Nigerian J Nat Prod Med* 22:129–134
47. Oriola AO, Aladesanmi AJ, Idowu TO, Akinwumi FO, Obuotor EM, Idowu T, Oyedeji AO (2021) Ursane-type triterpenes, phenolics and phenolic derivatives from *Globimetula braunii* leaf. *Molecules* 26(21):6528
48. Pál M, Janda T, Szalai G (2018) Interactions between plant hormones and thiol-related heavy metal chelators. *Plant Growth Regul* 85(2):173–185. <https://doi.org/10.1007/s10725-018-0391-7>
49. Pandey P, Khan F, Qari HA, Oves M (2021) Rutin (Bioflavonoid) as Cell Signaling Pathway Modulator: Prospects in Treatment and Chemoprevention. *Pharmaceuticals*, 14(11), 1069. <https://www.mdpi.com/1424-8247/14/11/1069>
50. Patel SA, Nilsson MB, Le X, Cascone T, Jain RK, Heymach JV (2023) Molecular mechanisms and future implications of VEGF/VEGFR in cancer therapy. *Clin Cancer Res* 29(1):30–39
51. Porter AC, Fanger GR, Vaillancourt RR (1999) Signal transduction pathways regulated by arsenate and arsenite. *Oncogene* 18(54):7794–7802
52. Pyo Y, Kwon KH, Jung YJ (2024) Anticancer Potential of Flavonoids: Their Role in Cancer Prevention and Health Benefits. *Foods*, 13(14), 2253. <https://www.mdpi.com/2304-8158/13/14/2253>
53. Quatrin A, Pauletto R, Maurer L, Minuzzi N, Nichelle S, Carvalho J, Junior MM, Rodrigues E, Bochi V, Emanuelli T (2019) Characterization and quantification of tannins, flavonols, anthocyanins and matrix-bound polyphenols from jaboticaba fruit peel: A comparison between *Myrciaria trunciflora* and *M. jaboticaba*. *J Food Compos Anal* 78:59–74
54. Raina R, Hussain A, Sharma R (2020) Molecular insight into apoptosis mediated by flavones in cancer. *World Acad Sci J* 2(3):1–1
55. Rathee S, Patil UK, Jain SK (2024) Exploring the Potential of Dietary Phytochemicals in Cancer Prevention: A Comprehensive Review. *J Exploratory Res Pharmacol* 9(1):34–47. <https://doi.org/10.14218/jerp.2023.00050>
56. Ray-Offor E, Obiorah CC (2021) Topography and Morphology of Gastric Cancer in Nigeria: A Dual Institution Review of 622 Upper Gastrointestinal Endoscopies. *Cureus* 13(4):e14693. <https://doi.org/10.7759/cureus.14693>
57. Rocha J (2013) *Drosophila melanogaster* as a promising model organism in toxicological studies. *Archives Basic Appl Med* 1(1):33–38
58. Rodríguez-García A, García-Vicente R, Morales ML, Ortiz-Ruiz A, Martínez-López J, Linares M (2020) Protein Carbonylation and Lipid Peroxidation in Hematological Malignancies. *Antioxidants*, 9(12), 1212. <https://www.mdpi.com/2076-3921/9/12/1212>
59. Seo J, Lee JY, Sung M-S, Byun CJ, Cho D-H, Lee H-J, Park J-H, Cho H-S, Cho S-J, Jo I (2014) Arsenite acutely decreases nitric oxide production via the ROS–protein phosphatase 1–endothelial nitric oxide synthase-thr497 signaling cascade. *Biomolecules Ther* 22(6):510
60. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M (2020) Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules* 25(22). <https://doi.org/10.3390/molecules25225243>
61. Wāng Y, Ma L, Wang C, Gao T, Han Y, Xu D-X (2024) Cardiovascular adverse effects and mechanistic insights of arsenic exposure: a review. *Environ Chem Lett* 22(3):1437–1472. <https://doi.org/10.1007/s10311-023-01677-0>
62. Wehr NB, Levine RL (2013) Quantification of protein carbonylation. *Cell Senescence: Methods Protocols*, 265–281
63. Wolff SP (1994) [18] Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods in enzymology*, vol 233. Elsevier, pp 182–189
64. Ye X, Gaucher JF, Vidal M, Broussy S (2021) A Structural Overview of Vascular Endothelial Growth Factors Pharmacological Ligands: From Macromolecules to Designed Peptidomimetics. *Molecules* 26(22). <https://doi.org/10.3390/molecules26226759>

65. Yeshi K, Crayn D, Ritmejerytė E, Wangchuk P (2022) Plant secondary metabolites produced in response to abiotic stresses has potential application in pharmaceutical product development. *Molecules* 27(1):313
66. Yu Y, Huang H, Li J, Zhang J, Gao J, Lu B, Huang C (2013) GADD45 β mediates p53 protein degradation via Src/PP2A/MDM2 pathway upon arsenite treatment. *Cell Death Dis* 4(5):e637–e637
67. Zahra M, Abrahamse H, George BP (2024) Flavonoids: Antioxidant Powerhouses and Their Role in Nanomedicine. *Antioxidants* 13(8):922. <https://www.mdpi.com/2076-3921/13/8/922>
68. Zhong G, Hu T, Tang L, Li T, Wu S, Duan X, Pan J, Zhang H, Tang Z, Feng X, Hu L (2022) Arsenic causes mitochondrial biogenesis obstacles by inhibiting the AMPK/PGC-1 α signaling pathway and also induces apoptosis and dysregulated mitophagy in the duck liver. *Ecotoxicol Environ Saf* 230:113117. <https://doi.org/https://doi.org/10.1016/j.ecoenv.2021.113117>

Figures



GW786034



CHEMBL5183988

Figure 1

Standard inhibitors

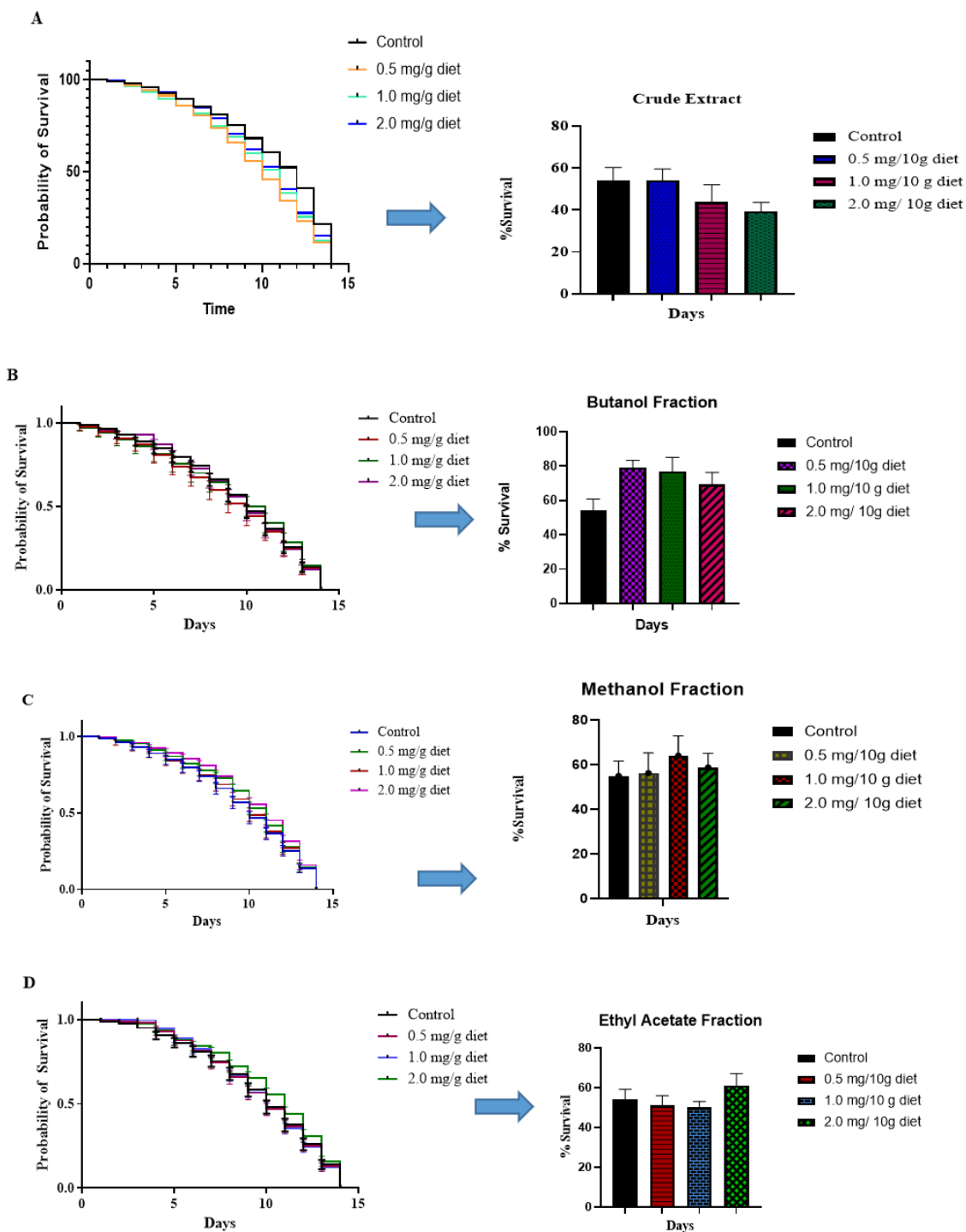


Figure 2

Figure 1 Survival Rate of *Drosophila melanogaster* exposed to different concentrations of crude extract and fractions of *Tapinanthus globiferus*

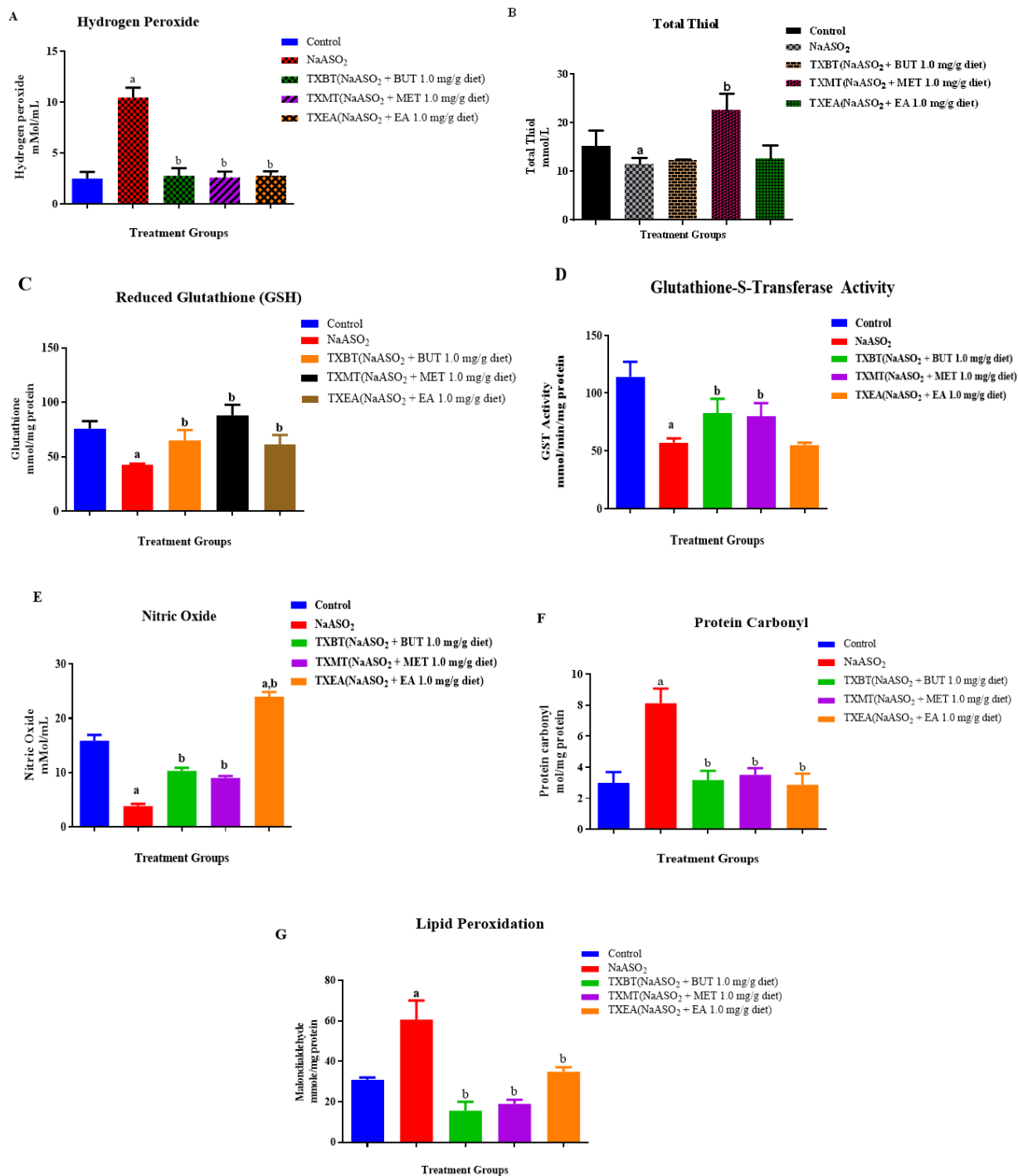


Figure 3

Figure 2 Effects of *Tapinanthus globiferus* Fractions on Oxidative Stress Markers in *Drosophila melanogaster* with SA-Induced Carcinogenesis (A) Hydrogen peroxide generation (B) Total thiol (C) Reduced glutathione (D) Glutathione-S-Transferase activity (E) Nitric Oxide Level (F) Protein Carbonyl (G) Lipid peroxidation. a: significant difference compared with the control group ($p < 0.05$); b: significant difference compared with the SA-induced untreated group.

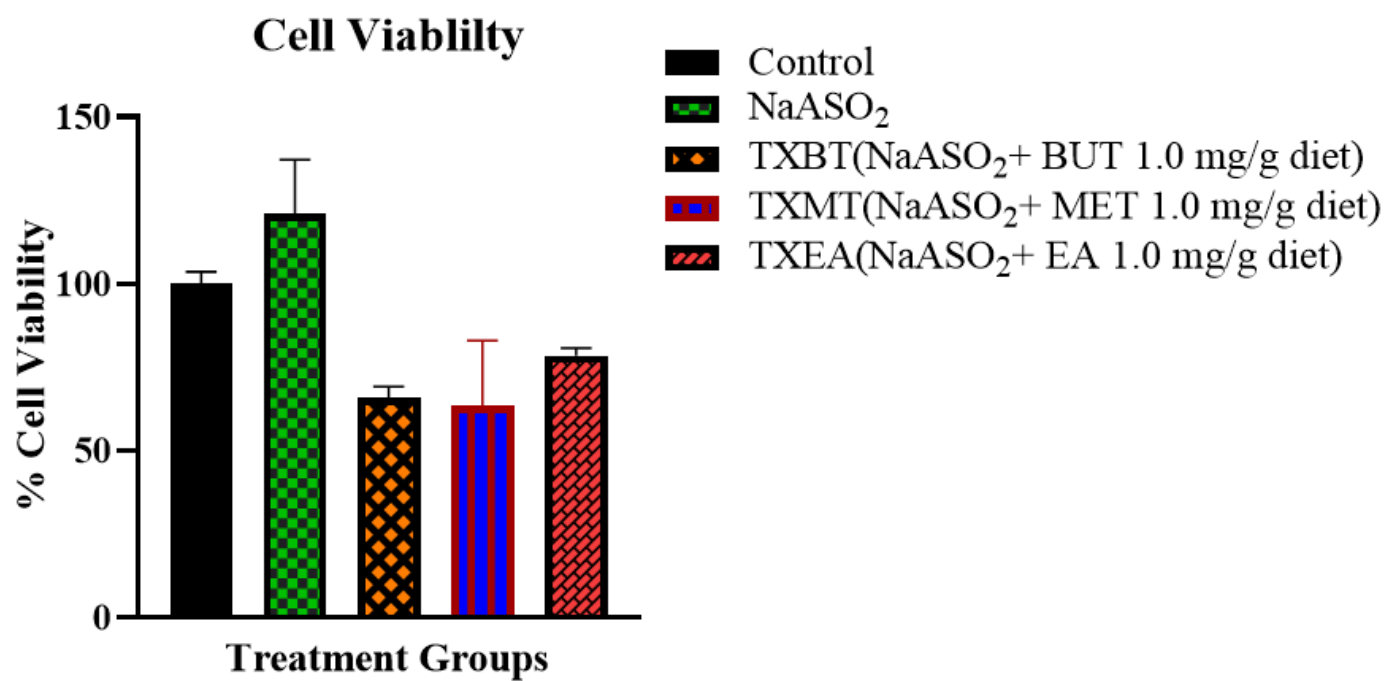


Figure 4

Figure 3 Effect of *Tapinanthus globiferus* Fractions on Cell Viability

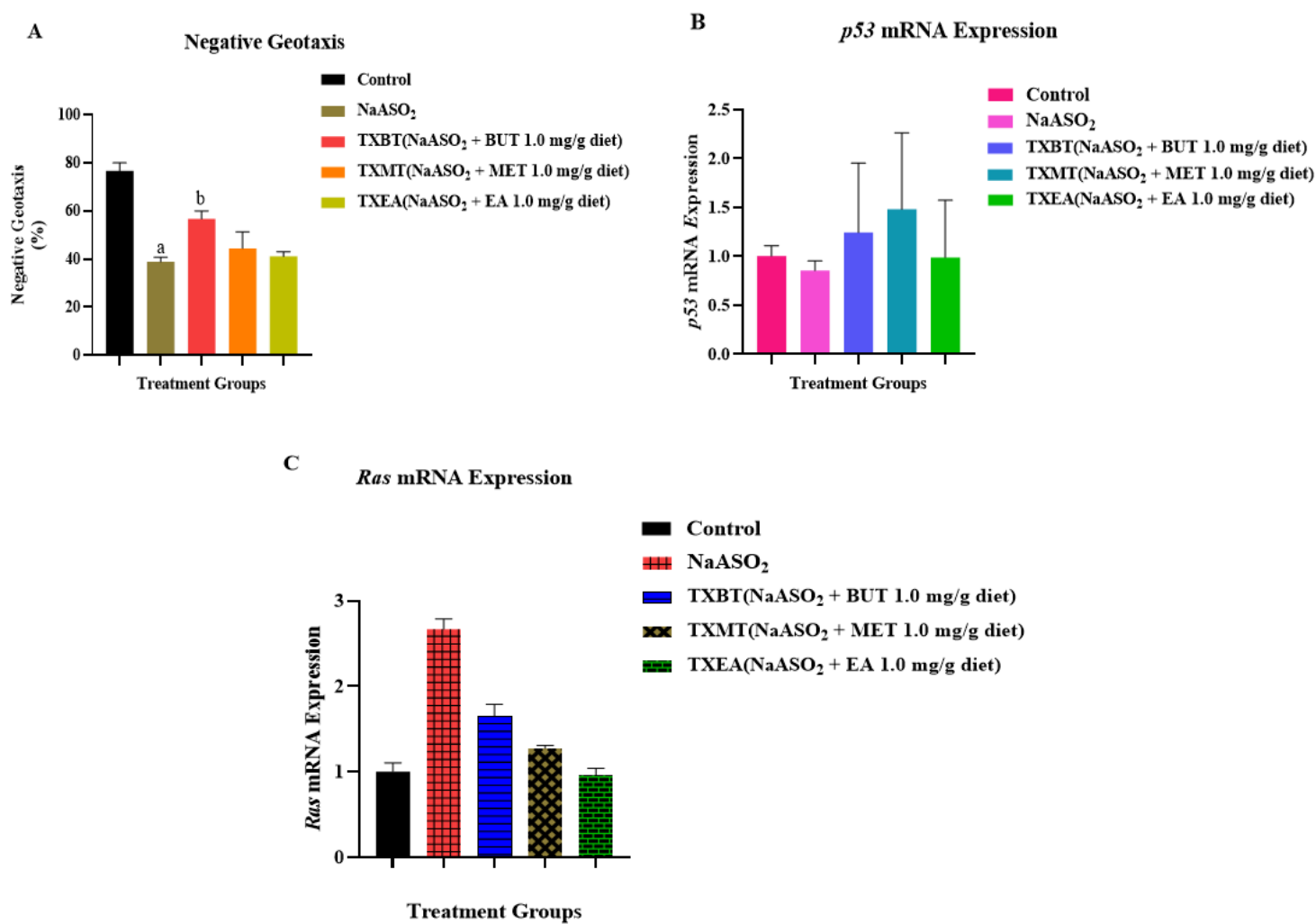
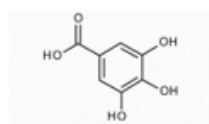


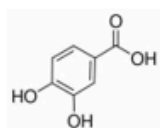
Figure 5

Figure 4 Effect of *Tapinanthus globiferus* Fractions on (A) Negative geotaxis (B) mRNA expression of *p53* (C) mRNA expression of *Ras* in *Drosophila melanogaster* with SA-induced carcinogenesis

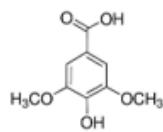
Hydroxybenzoate Derivatives



Gallic acid (CID: 370)

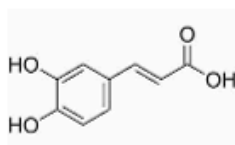


Protocatechuic acid (CID: 72)

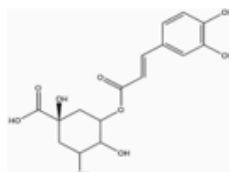


Syringic acid (CID: 10742)

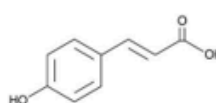
Hydroxycinnamate Derivatives



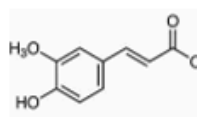
Caffeic acid (CID: 689043)



Chlorogenic acid (CID: 1794427)

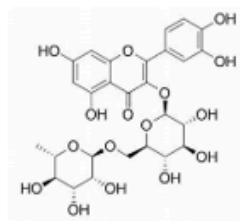


P-coumaric acid (CID: 637542)

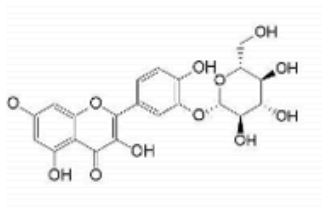


T-Ferulic acid (CID: 445858)

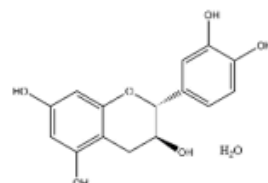
Flavonoids



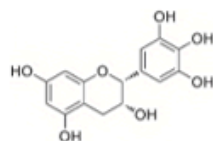
Rutin (CID: 5280805)



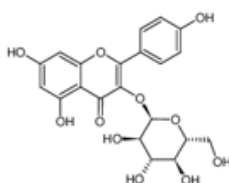
Quercetin-3- glycoside (Isoquercetin)
(CID: 5280804)



Catechin (CID: 9064)



Epigallocatechin (CID: 72277)



Kaempferol Derivative
kaempferol 3-o-beta-D-glucopyranoside
(CID: 5282102)

Figure 6

Figure 5 Bioactive polyphenols identified in *Tapinanthus globiferus* using HPLC-DAD

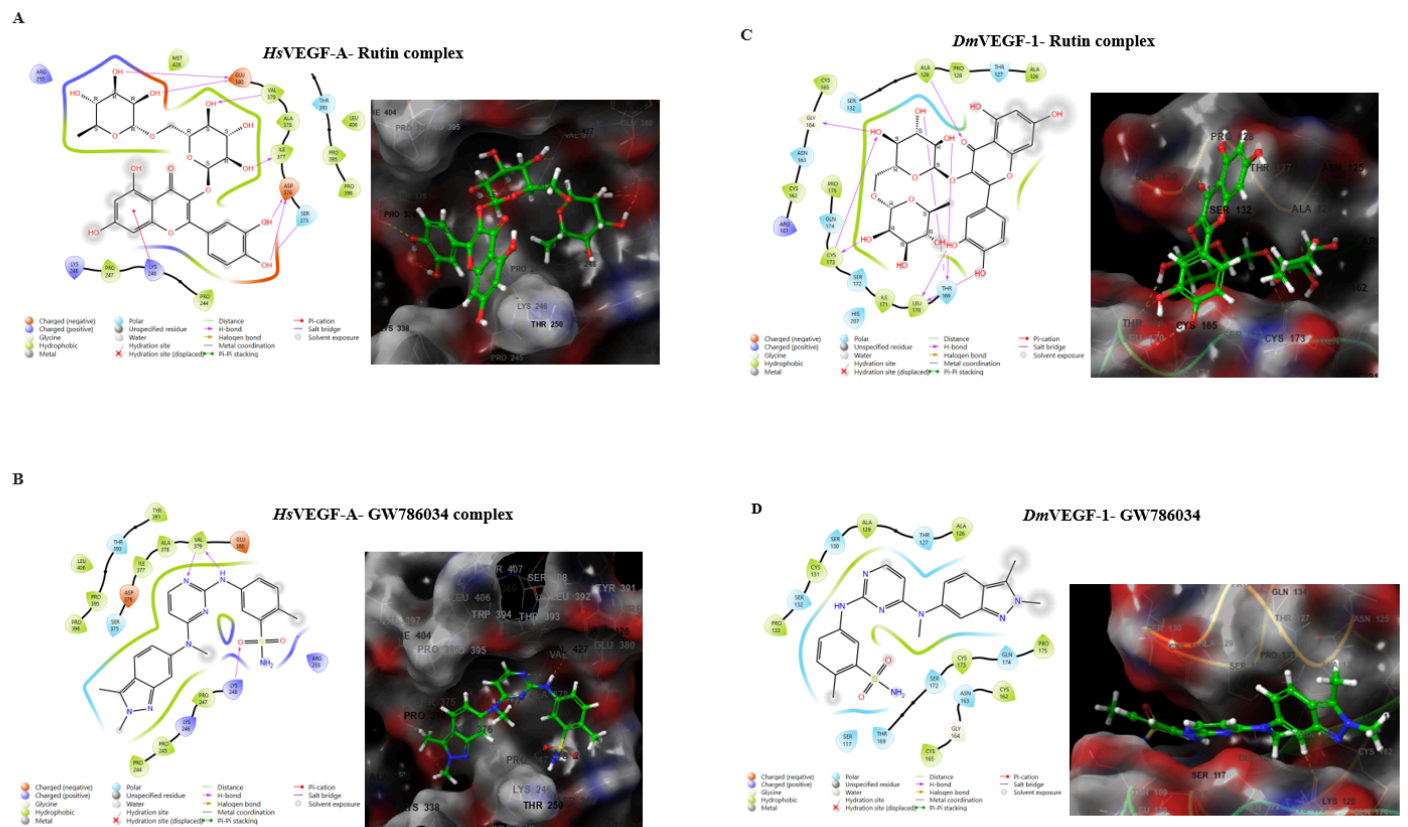


Figure 7

Figure 6 Molecular Interaction with the amino acids of VEGF-A protein (A) Human (*Hs* - *Homo sapiens*) with Rutin (B) *Hs*VEGF-A and GW786034 (C) *Drosophila melanogaster* (*Dm*) Ras with Rutin (D) *Dm*VEGF-1 and GW786034.

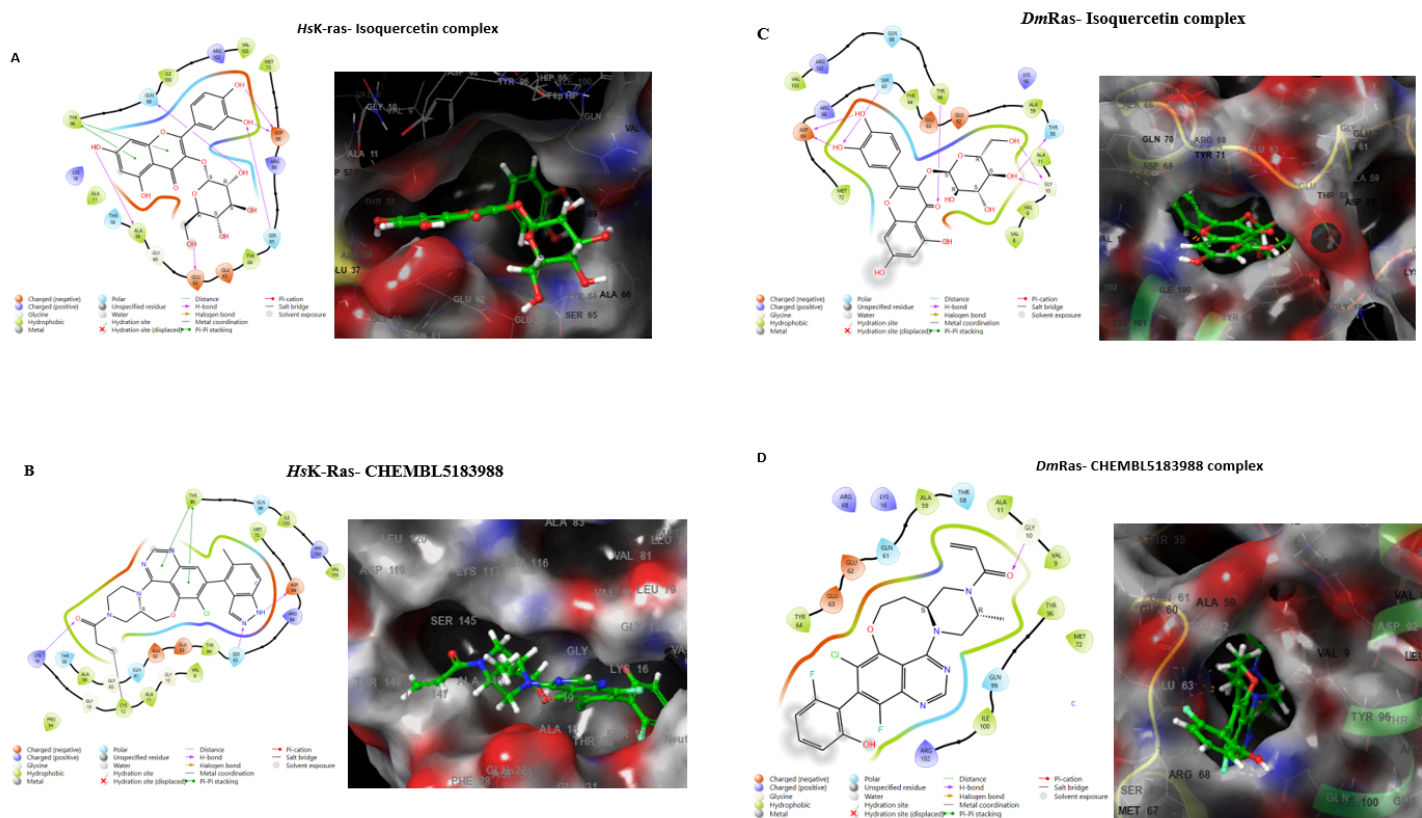


Figure 8

Figure 7 Molecular Interaction with the amino acids of Ras protein (A) Human (*Hs* - *Homo sapiens*) with isoquercetin (B) *Hs*Ras and CHEMBL5183988 (C) *Drosophila melanogaster*(*Dm*) Ras with isoquercetin (D) *Dm*Ras and CHEMBL5183988.