

## Reduction of anoxia-induced bioenergetic disturbance in astrocytes by methanol fruit extract of *Tetrapleura tetraptera* and *in silico* evaluation of the effect of its antioxidative constituents on excitotoxicity

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### ARTICLE INFO

Edited by DR. A.M Tsatsaka

**Keywords:**

*Tetrapleura tetraptera*  
Astrocyte  
Hypoxia  
Oxidative stress  
Binding affinity  
Glutamine synthetase

### ABSTRACT

Oxidative stress and excitotoxicity are some of the pathophysiological abnormalities in hypoxia-induced brain injury. This study evaluated the intrinsic antioxidant property of methanol fruit extract of *Tetrapleura tetraptera* (TT), traditionally used for managing brain diseases such as cerebral infarction in West Africa, and its ability to protect primary astrocytes from anoxia-induced cell death. The effect of the phytochemicals present in TT on excitotoxicity was assessed *in silico*, through docking with human glutamate synthetase (hGS). Chromatographic and spectrophotometric analyses of TT were performed. Primary astrocytes derived from neural stem cells were treated with TT and its effect on astrocyte viability was assessed. TT-treated astrocytes were then subjected to anoxic insult and, cell viability and mitochondrial membrane potential were evaluated. Molecular docking of hGS with detected phytochemicals in TT (aridanin, naringenin, ferulic acid, and scopoletin) was performed and the number of interactions with the lead compounds, aridanin, analyzed. HPLC-DAD analysis of TT revealed the presence of various bioactive phytochemicals. TT demonstrated notable antioxidant and radical scavenging activities. TT also protected astrocytes from anoxic insult by restoring cell viability and preventing alteration to mitochondrial membrane integrity. Aridanin, naringenin, ferulic acid, and scopoletin demonstrated good binding affinities with hGS indicating that *Tetrapleura tetraptera* is a potential source of new plant-based bioactives relevant in the therapy of neurodegenerative diseases.

### 1. Introduction

Reactive oxygen species (ROS) are produced within the body due to normal metabolic activities and its harmful effects are counteracted by endogenous antioxidants. The imbalance between free radical production and antioxidant defense results in oxidative stress which causes deregulation of cellular functions and cause the development of various disease conditions. Oxidative stress has been implicated in various neurodegenerative diseases such as ischemic stroke and cerebral hypoxia [1–7].

Mitochondria, an important source of ROS in most cells [8] and their integrity is therefore of utmost importance. During oxidative phosphorylation, ATP synthase uses mitochondrial membrane potential (MMP) to produce ATP [9]. MMP provides the driving force for the

production of cellular energy in the mitochondria, making ATP a compound buffering MMP. In hypoxic conditions, oxidative phosphorylation is halted, ATP generation is arrested and this leads to mitochondrial dysfunction [10].

Cerebral hypoxia is the reduction of cerebral oxygen and it is a part of physiological events involved in cerebral ischemic stroke [11,12]. Oxidative stress as a result of hypoxia causes mitochondrial dysfunction characterized by reduction in mitochondrial mass, change in membrane potential and change in oxidative proteins activities [5,8]. Cerebral hypoxia leads to various physiological dysfunctions such as oxidative stress and excitotoxicity, which culminate in the death of neuronal and non-neuronal cells [13]. In cerebral ischemia, excitotoxicity which is characterized by an increase in glutamate level accompanied by a decrease in glutamine level is observed. The decrement in the amount of

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glutamine has been explained to be due to the inhibition of glutamine synthetase [15]. Excitotoxicity induced marked expression of several pro-oxidant enzymes or mediators and therefore, alter brain synaptic plasticity and causes neurological dysfunction [16]. Glutamine synthetase catalyzes the condensation of glutamate and ammonia to form glutamine in an ATP-dependent mechanism [15] in other to mop up excess glutamate. Glutamine synthetase is highly expressed in astrocytes compared to other cells in the central nervous system [17].

Astrocytes are vital housekeeping non-neuronal cells of the central nervous system [18]. They perform a plethora of functions to maintain brain homeostasis. They scavenge the release of neurotransmitters during synaptic action, control the homeostasis of ions and water, release neurotrophic factors, transport metabolite and waste products, and participate in blood-brain barrier formation [18,19]. Breakdown of one or more of these astrocytes functions will either alone or in combination with other cell dysfunction, constitute a threat to neuronal survival [20]. Therefore, astrocytic cultures can be a useful model in determining whether the detrimental effects of anoxia can be overcome with therapeutic interventions.

*In vitro* models of neurological diseases have been reported to be useful for investigating the effects of preventive or therapeutic treatments on brain cells directly in both animal and human cultured cells [21,22].

Natural products such as medicinal plants contain pharmacologically active secondary metabolites [23]. These secondary metabolites are antioxidants and can safely interact with free radicals to stop the chain reaction that leads to damage of biomolecules through several mechanisms [24–27].

*Tetrapleura tetraptera* fruit is effective for treating cerebral ischemia [28]. It is found in the lowland forest of tropical Africa such as Nigeria, Ghana, and Uganda, and has been traditionally used for managing brain diseases [29]. Its stem, bark, root, leaves, and fruits have various medicinal and nutritional properties [30]. The medicinal effects of *Tetrapleura tetraptera* plants have been ascribed to the presence of polyphenol components such as phenols, flavonoids and saponins [31–33]. The fruit of *Tetrapleura tetraptera* has been shown to have antihypertensive, anticonvulsive, and cognitive enhancing properties [34–37].

We hypothesized that the methanol fruit extract of *Tetrapleura tetraptera* (TT) could be beneficial in scavenging free radicals and inhibiting lipid peroxidation that are implicated in cerebral ischemia and may probably be mechanisms used in protecting astrocytes from ischemia-induced mitochondrial biogenetic dysfunction.

Anoxic condition (chronic hypoxia) was employed in this study to mimic the oxygen deprivation in the situation of cerebral ischemia. In cerebral ischemia, the infarct zone has an ischemic core where the death of neurons is detected but the penumbra region (surrounding the ischemic core) does not immediately experience cell death and can be salvageable. In this study, we evaluate the antioxidative potential of TT to mitigate anoxia-induced bioenergetic disturbance in primary astrocytes, as well as examine the role of its phytoconstituents to optimize human glutamate synthetase (hGS) in abating excitotoxicity through

azoniacyclopenta-1,4-diene chloride (TPTZ), thiobarbituric acid (TBA), ammonium molybdate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), neocuproine, potassium ferricyanide, Trolox, sodium nitroprusside, 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) and 5,59,6,69-tetrachloro-1,19,3,39-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other materials employed in this study were analytical grade and were purchased from standard suppliers within Nigeria.

## 2.2. Plant material and preparation of TT

*Tetrapleura tetraptera* fruits were obtained in the afternoon of April 2016 at the Department of Botany and authenticated at the same Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, in a geographical coordinates 07°30'59.46" North and 04°31'42.82" East (Voucher number: IFE17720). The fruits were sundried (65 % humidity), powdered and 600 g was soaked for 72 h in 70 % methanol with constant stirring at every 12 h. It was then sieved with a cloth of 100 µm pore size and further filtered with Whatman filter paper no. 1. The filtrate was concentrated in a rotary evaporator and further concentrated to dryness to obtain the extract (TT). The percentage yield of the extract was 11.6 % of dried plant material. It was dissolved in distilled water and DMSO for biological activity and cell culture experiments, respectively.

## 2.3. HPLC-DAD quantification of compounds in methanol fruit extract of *Tetrapleura tetraptera* (TT)

Compounds were quantified in TT using High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) according to the previous method by Saliu et al. [28].

## 2.4. Evaluation of *in vitro* antioxidant activity

All experiments were run in triplicate and absorbance of the antioxidant assays were taken on a UV-vis spectrophotometer. Half maximal inhibitory concentration ( $IC_{50}$ ) of TT from the antioxidant assays was calculated using the inverse logarithmic method.

### 2.4.1. DPPH radical scavenging assay

The DPPH radical scavenging ability of TT was estimated using the method of Senguttuvan et al. [38]. This assay is based on the ability of polyphenol compounds to scavenge and neutralize DPPH radicals by donating one electron to the radical molecule [39]. DPPH radical solution (0.1 mM) was prepared in methanol and 0.5 mL was added to 0.5 mL of different concentrations of TT (10–100 µg/mL). The mixture was incubated in the dark at room temperature for 30 min. A control reference contained methanol instead of TT for baseline correction. After incubation, the absorbance (Abs) was taken at 517 nm. Ascorbic acid served as a standard antioxidant.

$$\% \text{ DPPH radical scavenging activity} = \frac{(Absorbance\text{ of control} - Absorbance\text{ of sample})}{(Absorbance\text{ of control})} \times 100$$

molecular docking.

## 2. Materials and methods

### 2.1. Chemicals

Bovine serum albumin (BSA), 2,3,5-triphenyl-1,3,4-triaza-2-

### 2.4.2. ABTS radical cation decolorization assay

The ability of TT to scavenge free radical was also determined by ABTS radical cation decolorization assay according to the method of Re et al. [40]. It is based on the ability of polyphenol compound to neutralize ABTS cation radicals by donating one electron to the radical molecule [39]. ABTS radical was produced by reacting 7 mM of ABTS and 2.45 mM potassium persulfate (1:1, v/v) and incubated in the dark at

room temperature for 14 h. ABTS<sup>+</sup> solution was then diluted with 80 % methanol to obtain an absorbance of 0.700 at 734 nm. Then, 100 µL of different concentrations of TT or Trolox (12.5 µg/mL – 100 µg/mL) was added to 2 mL of diluted ABTS<sup>+</sup> solution and the resulting mixture was incubated in the dark for 10 min and the absorbance (Abs) was taken at 734 nm. Control reference contained only ABTS<sup>+</sup> solution without TT for baseline correction. Percentage ABTS cation radical inhibition was calculated from the formula:

$$\begin{aligned} \text{\% ABTS cation radical scavenging activity} \\ = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100 \end{aligned}$$

#### 2.4.3. Hydroxyl radical scavenging activity

Hydroxyl (OH) radicals scavenging activity of TT was determined using the method described by Adjimani and Asare [41]. It is based on the ability of phytocompound to scavenge OH radicals by donating an electron to the radical molecule [39]. The Fenton reaction mixture was prepared by adding 0.2 mL FeSO<sub>4</sub>·7H<sub>2</sub>O (10 mM), 0.2 mL EDTA (10 mM), 0.2 mL 2-deoxyribose (10 mM) and 1.2 mL phosphate buffer (0.1 M, pH 7.4). Then, 0.2 mL of different concentrations of TT or mannitol (12.5 µg/mL – 100 µg/mL) were added, followed by the addition of 0.2 mL hydrogen peroxide (10 mM) and incubated for 4 h at 37°C. To the incubated mixture, TCA (1 mL, 2.8 % w/v) and TBA (1 mL, 1 % w/v) were added and further incubated in boiling water for 10 min. Thereafter, it was allowed to cool at room temperature and then, centrifuged

together. Then, 400 µL of TT (1 mg/mL) or freshly prepared Trolox solution of varying concentrations was added and diluted with 700 µL of deionized water. The mixture was incubated at room temperature for 30 min and the absorbance was taken at 450 nm. CUPRAC of TT was calculated with a molar extinction coefficient of 1.67 × 10<sup>-4</sup> L mol<sup>-1</sup> cm<sup>-1</sup> for Trolox using the formula:

$$\begin{aligned} \text{CUPRAC value} = & \frac{\text{Absorbance of Sample}}{\text{Extinction coefficient of Trolox}} \\ & \times \frac{\text{Total volume of reaction solution}}{\text{Volume of sample used}} \times (\text{dilution factor}) \\ & \times \frac{\text{volume of the stock sample}}{\text{Weight of stock sample used}} \end{aligned}$$

Where absorbance of sample is the absorbance of TT.

#### 2.4.6. Albumin anti-denaturation assay

Albumin anti-denaturation assay was carried out according to the method described by Sakat et al. [44]. The ability of polyphenols to hinder heat-induced protein denaturation was examined here. A reaction mixture containing 200 µL of 1 % bovine serum albumin (BSA) and 1 mL of varying concentrations of TT or ascorbic acid (as standard) was prepared, and the pH adjusted using 1 N HCl. The mixture was initially incubated at 37°C for 20 min, then also at 60°C for another 20 min, allowed to cool and the turbidity was measured at 660 nm. The control contained distilled water instead of TT or ascorbic acid. The percentage albumin denaturation inhibition was calculated as follows:

$$\begin{aligned} \text{\% Protein denaturation inhibition} = & \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100 \end{aligned}$$

at 4000 rpm for 4 min. A control reference that contained all solution except TT for baseline correction was used. The absorbance (Abs) was measured at 532 nm against a blank solution.

#### 2.4.7. Lipid peroxidation assay

The assay for lipid peroxidation was carried out according to Ohkawa et al. [45]. This is a spectrophotometry assay that is based on

$$\begin{aligned} \text{\% OH radical scavenging activity} = & \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100 \end{aligned}$$

#### 2.4.4. Ferric reducing antioxidant power (FRAP) assay

Benzie and Strain [42] method was followed for determining FRAP value of TT. This assay is based on the diminution of ferric iron-TPTZ complex (Fe<sup>3+</sup>-TPTZ) by antioxidants to its ferrous form [39]. This reduction is monitored spectrophotometrically by measuring the change in absorbance at 593 nm. FRAP reagent solution containing 300 mM acetate buffer, 10 mM TPTZ in 40 mM HCl, 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O (10:1:1, respectively) was prepared. FRAP reagent (3 mL) was mixed with 100 µL TT (1 mg/mL) and incubated for 30 min incubation at 37°C. The absorbance was then taken at 593 nm and FRAP value was extrapolated from a linear graph of FeSO<sub>4</sub> and expressed as mmol Fe<sup>2+</sup> equivalents per g dry weight.

#### 2.4.5. Cupric ion reducing antioxidant capacity (CUPRAC) assay

CUPRAC assay was performed according to the method described by Apak et al. [43]. The ability of antioxidants to reduced Cu<sup>2+</sup> to Cu<sup>+</sup> in the presence of neocuproine [43]. The reduction is spectrophotometrically monitored at 593 nm. Copper(II) chloride (1 mL, 0.01 M), neocuproine (1 mL, 0.0075 M), and 1 M NH<sub>4</sub>CH<sub>3</sub>COO solution were added

the ability of polyphenol to inhibit peroxidation of polyunsaturated fatty acids of biological membrane through inhibiting the formation of thiobarbituric acid reactive substances, TBARS [46]. The excised brain of Wistar rat was homogenized in ice-cold 0.1 M phosphate-buffered saline (PBS, 10 %) and centrifuged at 3000 rpm for 10 min to obtain the brain supernatant. To 0.5 mL of the brain supernatant, 0.5 mL of TT or ascorbic acid of different concentrations (12.5–200 µL of 1 mg/mL) and 0.05 mL of freshly prepared FeSO<sub>4</sub> (0.071 mM) were added, vortexed, and incubated at 37°C for 30 min. After incubation, 1.5 mL of 0.8 % TBA in 1.1 % sodium dodecyl sulfate was added followed by another incubation at 95°C for 1 h. It was allowed to cool and then centrifuged at 3000 rpm and the absorbance was taken at 532 nm. The control contained methanol instead of TT or ascorbic acid.

$$\% \text{ Lipid peroxidation inhibition} = \frac{(Absorbance\text{ of control} - Absorbance\text{ of sample})}{(Absorbance\text{ of control})} \times 100$$

## 2.5. Evaluation of the effect of *Tetrapleura tetraptera* methanol fruit extract on astrocytes derived from Neural Stem Cells subjected to anoxia

### 2.5.1. Differentiation of primary human astrocytes from Neural Stem Cells

Astrocytes derived from human induced pluripotent stem cell (hiPSC) were used for this experiment. Human fetal brain tissue was procured following the informed consent of the mother. The brain tissues were processed according to approval by the Institutional Human Ethics and Stem Cell and Research Committee of National Brain Research Centre, India, in compliance with the Indian Council of Medical Research (ICMR). The telencephalon of human aborted fetuses (10–15 weeks old) was used to isolate fetal Neural Stem Cells (fNSCs). Isolation and differentiation of fNSCs to astrocytes were done as described by Fatima et al. [47] and Bhagat et al. [48].

Briefly, isolated neural stem cells (NSCs) were cultured in poly-D-lysine (Sigma-Aldrich, St. Louis, MO, USA) coated culture dishes in neurobasal media (Invitrogen, San Diego, CA, USA). The media was supplemented with Neural Survival Factor-1, N2 supplement, bovine serum albumin, glutamine, 25 ng/mL basic fibroblast growth factor (bFGF), 20 ng/mL epidermal growth factor (EGF), penicillin and streptomycin solutions, and gentamycin. The expression of neural stem cell markers, SRY-Box Transcription factor 2 (SOX2) and Nestin, and lineage-specific markers, Glial fibrillary acidic protein (GFAP) and microtubule-associated protein 2 (MAP2) were assessed for NSCs characterization using western blotting and immunocytochemistry. Almost 99 % of fNSCs showed immunoreactivity towards SOX2 and Nestin, and were negative for both GFAP and MAP2. Differentiation of NSCs into astrocytes was achieved by withdrawing neurobasal media and growing NSCs in minimum essential medium (MEM) supplemented with 10 % fetal bovine serum (FBS). Cells were maintained in MEM with FBS for atleast 21 days before using them for the experiments. Following trypsinization at the confluence of 80–90 %, cells were seeded for experiments in 8 well chamber slides or 12 well plates.

### 2.5.2. Astrocytes counting and seeding

The prepared astrocytes were counted to know the number of cells per volume of the media. Briefly, 10 µL of the cell was added onto a hemocytometer and viewed under the microscope for cell counting. The population of cells was then calculated:

$$\text{No. of cells per ml} = \frac{\text{No. of cells counted on the four grids} \times \text{dilution factor}}{\text{No. of grids counted} \times 10^{-4}}$$

For the 12 well plates, 100,000 cells were seeded; and for the 8 well chamber slide, 20,000 cells were seeded.

### 2.5.3. Induction of anoxia in astrocyte culture

Severe hypoxic condition (anoxia) was set by flushing the hypoxia chambers with a gas mixture of ~0.2 % oxygen (replaced with 94.9 % nitrogen) and 5.1 % carbon dioxide [13], while normoxia chambers were maintained at 18.0 % oxygen and 5.1 % carbon dioxide and 76.9 % nitrogen, with a pressure of 1.75 bar for both conditions.

Viable but activated astrocytes were analyzed morphologically using the live/dead assay.

**2.5.3.1. Assessment of astrocyte viability by live/dead assay.** The live/dead assay is used for assessing cell viability/death and was carried out using a live/dead viability/cytotoxicity assay kit for mammalian cells (Invitrogen Detection Technologies, ThermoFisher, UK) according to the manufacturer's instructions. This method is used for assessing cell viability/death in live cell culture of cells. It uses two components,

namely; Calcein AM (CaAM), which is component A and Ethidium homodimer-1 (EthD-1), which is component B. Dead cells are characterized by intense fluorescence at over 600 nm and little fluorescence around 530 nm.

Astrocytes seeded in an 8-well chamber slide were allowed to adhere and grow to confluence for 14 h at 37 °C in a CO<sub>2</sub> incubator. Thereafter, cells were placed in a normoxic or severe hypoxic condition for 3 and 6 h. After 3 and 6 h, 100 µL of EthD-1 (1 µL/mL) and CaAM (0.2 µL/mL) were separately prepared in cell media (MEM), added to the cells, and then, incubated at 37 °C for 10 min. Fluorescent images of the cells were taken at excitation/emission(ex/em) wavelength of 495 nm/515 nm for the green filter (live cells) and ex/em wavelength of 495 nm/635 nm for the red filter (dead cells) using an Invitrogen Floid microscope (Fisher Scientific, Sweden). Percentage cell death was calculated to get the number of dead cells.

### 2.5.4. Determination of cytotoxicity of methanol fruit extract of *Tetrapleura tetraptera* on astrocyte cell culture

Astrocytes were seeded onto a 24 well-plate (Corning Flat Bottom Transparent Polystyrol well plate) at a density of 5 × 10<sup>4</sup> cells/well and were allowed to stay for 14 h at 37 °C in a CO<sub>2</sub> incubator. The cells (n = 3) were treated for 24 h with 0.5, 1 and 10 µg/mL of TT dissolved in 0.01 % DMSO in the culture medium. Treatment with DMSO (0.01 % v/v culture medium) alone serves as the control. Thereafter, MTT assay was carried out.

MTT is a colorimetry method used for assessing cell viability and cytotoxicity for drug screening [49]. The MTT assay is based on the reduction of MTT (yellow colored) due to the activity of NAD(P)H-dependent oxidoreductase [50].

After 24 h of astrocyte treatment with the extract, 10 µl of MTT working solution (5 mg/mL) was added and then incubated in a CO<sub>2</sub> incubator for 3 h at 37 °C. The medium was removed, and the formazan crystals formed were dissolved by adding 200 µl of DMSO and incubated in the dark inside a CO<sub>2</sub> incubator for 1 h at 37 °C. Finally, the purple color of the dissolved formazan crystals was quantified in a plate reader (Infinite 200Pro Tecan, Switzerland) at 650 nm.

### 2.5.5. Evaluation of the viability of astrocyte treated with methanol fruit extract of *Tetrapleura tetraptera* after anoxia induction

Cells (astrocytes) were seeded in a 24 well-plate at a density of 5 \* 10<sup>4</sup> cells/well (n = 3) and allowed to stay for 14 h at 37 °C in a CO<sub>2</sub> incubator. Treatment with TT was performed 4 h before exposure to either 3 h normoxic (normoxia) or anoxic (anoxia) conditions. Two concentrations of TT (1 µg/mL and 10 µg/mL) were used and the treatments were as follows: cells treated with 1 µg/mL TT prior to anoxia (anoxia + TT 1 µg/mL); cells treated with 10 µg/mL TT prior to anoxia (anoxia + TT 10 µg/mL); cells treated with 10 µg/mL TT prior to normoxia (normoxia + TT 10 µg/mL). DMSO was used as a vehicle for TT.

After treatment, MTT assay was carried out to determine the percentage cell viability as previously described. Each assay was repeated twice.

### 2.5.6. Measurement of mitochondrial membrane potential of astrocytes after anoxia induction

The mitochondrial membrane potential (MMP) was measured by staining astrocytes with JC-1 (5,59,6,69-tetrachloro-1,19,3,39-tetraethylbenzimidazolyl-carbocyanine iodide) according to the method of Korenic et al. [51].

JC-1 is a lipophilic and cationic dye that exhibits a fluorescence

emission shift upon aggregation from 530 nm (green monomer) to 590 nm (red “J-aggregates” monomer). In healthy cells with high MMP, JC-1 enters the mitochondrial matrix in a potential-dependent manner and forms aggregates.

Cells were seeded into groups as previously described. After treatment, cells were stained with 2.5 mg/mL JC-1 at 37°C for 15 min. Thereafter, cells were rinsed three times with PBS and the dye was allowed to equilibrate at room temperature for another 10 min before imaging using an Invitrogen Floid microscope (Fisher Scientific, Sweden). Stained polarized mitochondria were detected with red fluorescence while the loss of mitochondrial integrity was detected with green fluorescence. The fluorescence was read on a plate reader (Infinite 200Pro Tecan, Switzerland) with an excitation wavelength of 485 nm and the absorbance at 530 nm and 590 nm. The JC-1 fluorescence values were first normalized separately to their respective controls for red and green signals before the relative MMP was calculated.

## 2.6. Molecular docking

Docking study was carried out in order to examine the activity of some phytochemicals in TT against excitotoxicity. Pathophysiological mechanisms observed in cerebral ischemic stroke are multifactorial and involve both neuronal and non-neuronal cell dysfunction [18,22, 52–55]. An important target enzyme in the pathway is glutamine synthetase [15,56,57]. Glutamine synthetase (GS) catalyzes the biosynthesis of glutamine from glutamate and ammonia. In the human brain, GS regulates excitotoxicity by converting neurotoxic glutamate (cause of excitotoxicity) to harmless glutamine with simultaneous hydrolysis of ATP [15].

### 2.6.1. Selection of protein and ligands

The 3D structure file of the identified target protein, human glutamine synthetase, hGS (Fig. 6), was retrieved from RCSB Server in the PDB format (PDB ID: 2ojw; [www.rcsb.org](http://www.rcsb.org)).

Out of the compounds that were quantified from TT by HPLC, the most abundant phytochemical compounds from four different classes of phytoconstituents, namely, aridanin (saponin), ferulic acid (phenolic acid), naringenin (flavonoid), and scopoletin (coumarin) were selected as ligands (except aridanin which was selected based on the premise that it is a marker compound of *Tetrapleura tetraptera*). Their structures were adapted from PubChem NCBI database (<https://www.ncbi.nlm.nih.gov/pubmed/>), with ID number 73146, 1548883, 932, and 5280460, respectively (Fig. 7). The structures were delineated by ACD/ChemSketch 11.0 software and the MOL format of these ligands were generated. The structure of the ligands were brought to a ground state and PDB format generated by Avogadro 1.2 software.

### 2.6.2. Optimization and docking of protein and ligand

Human glutamine synthetase was optimized for protein-ligand

interaction studies by deleting all hetero atoms, ligands, and water molecules using Chimera software. The protein and ligands were loaded onto PyRx software in preparation for docking. The amino acid residue in the binding pocket, which includes the active site of hGS [58] was selected along with the ligands and toggled to set the grid parameters. The three-dimensional affinity grid parameters were set at 19.120, -13.908, -34.861 (x, y, z respectively) on the geometric center of the target protein and 40.177, 35.676, 36.044 (x, y, z respectively) as dimensions (Å). Autodock vina from PyRx was used in docking the ligands into the binding pocket of the protein to get the binding energy data. Protein-ligand complex was generated using PyMol software in PDB format, the complex was uploaded on “Protein-Ligand Interaction Profiler” (<https://projects.biotecltd.de/plip-web/plip>)” and analyzed for the number of interactions formed [59].

## 2.7. Statistical analysis

All data were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by post hoc Tukey's test was used for data analysis. Statistical significance was considered at values of  $p < 0.05$ . The statistical package used was GraphPad Prism 6 software (Graph Pad Software Inc., San Diego, CA, USA).

## 3. Results

### 3.1. Phytoconstituents

The analysis of compounds identified from the HPLC-DAD chromatogram of TT (Supplementary data) is presented in Table 1. It revealed the presence of isoliquiritigenin, naringenin, butein, scopoletin, aridanin, ferulic acid, echinocystic acid, umbelliferone, octodrine, piperazine and hentriacontane in TT. These compounds can be categorized into flavonoids, saponins, coumarins and phenolic acids. The most abundant phytochemical present was scopoletin (11.13 mg/g) and the least abundant were octodrine and isoliquiritigenin (0.17 and 0.12 mg/g, respectively).

### 3.2. Antioxidant activity of methanol fruit extract of *Tetrapleura tetraptera*

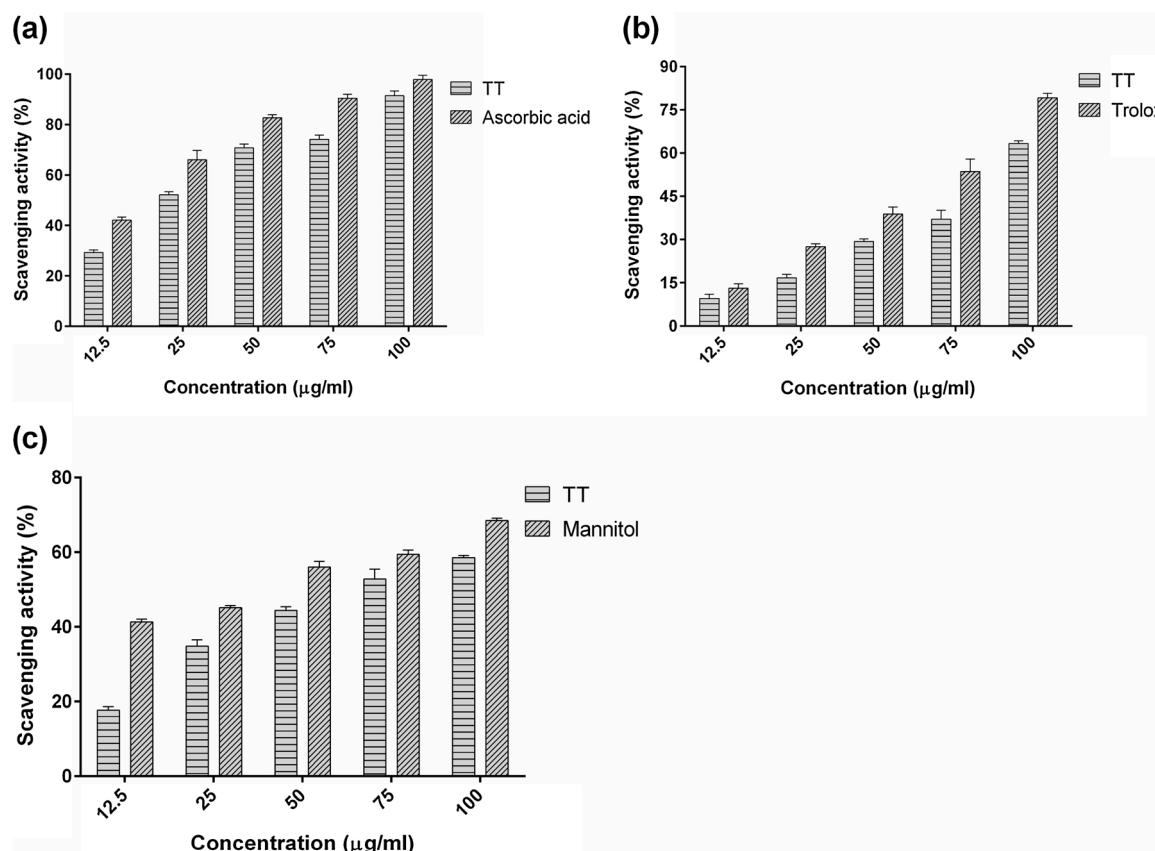
Antioxidant activity profile of TT shown in Figs. 1 and 2 indicate the robust ability of TT to scavenge DPPH radicals (Fig. 1a), ABTS radicals (Fig. 1b), and hydroxyl radicals (Fig. 1c), as well as inhibit lipid peroxidation (Fig. 2a) and denaturation of albumin by heat (Fig. 2b) *in vitro* in a concentration-dependent manner. Table 2 showed the antioxidant activity, inhibition of lipid peroxidation, and inhibition of heat-induced denaturation of protein albumin of TT in the tests in terms of its half-maximal inhibitory concentration ( $IC_{50}$ ) in comparison with reference standards. TT demonstrated appreciable antioxidant activity with  $IC_{50}$  values of  $20.96 \pm 0.65$ ,  $47.03 \pm 0.97$ ,  $20.63 \pm 0.68$  and  $47.36 \pm 0.67$  for DPPH radical scavenging activity, ABTS radical scavenging activity, hydroxyl radical, and lipid peroxidation inhibitory activity, respectively, compared with the  $IC_{50}$  values of reference standards ascorbic acid ( $15.36 \pm 0.29$ ), Trolox ( $39.88 \pm 0.52$ ), mannitol ( $11.06 \pm 0.87$ ) and quercetin ( $45.61 \pm 0.56$ ), respectively. The  $IC_{50}$  of TT in the CUPRAC and anti-denaturation assays were  $32.08 \pm 1.22$  and  $26.76 \pm 0.21$ , respectively, compared with the value of trolox ( $9.7553 \pm 0.89$ ) and ascorbic acid ( $12.15 \pm 0.21$ ), respectively, which were still appreciable. The FRAP value of the extract was  $117.81 \pm 4.066$  mM Fe/mg dry weight while the TEAC value was  $2.89 \pm 0.451$  mM Trolox/mg dry weight.

### 3.3. Cell death after anoxia

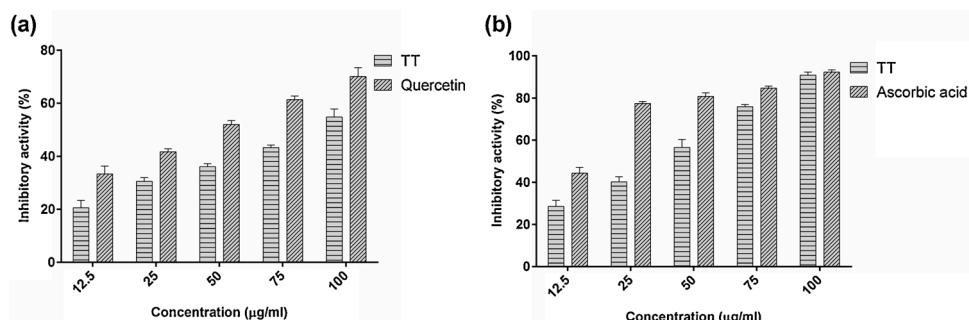
The survival rate of astrocytes following anoxic conditions (severe hypoxia) is shown in Fig. 3. When astrocytes were subjected to anoxia

**Table 1**  
HPLC-DAD quantified phytochemicals.

Standard compound	Standard retention time	Methanol fruit extract of <i>Tetrapleura tetraptera</i> (TT)	
		Area (mUA)	(mg/g)
Ferulic acid	1.27	1099.6020	1.38
Echinocystic acid	2.52	852.1330	1.07
Umbelliferone	2.75	1.384.0905	1.74
Piperazine	4.45	164.7690	0.21
Aridanin	4.70	285.5670	0.36
Octodrine	5.47	132.5145	0.17
Scopoletin	11.05	8866.8670	11.13
Hentriacontane	12.17	3123.0645	3.92
Naringenin	13.70	1581.5080	1.99
Butein	17.62	225.0670	0.28
Isoliquiritigenin	19.77	93.3595	0.12



**Fig. 1.** Scavenging activity of TT *in vitro*. (a) Percentage DPPH radical scavenging activity of TT; (b) Percentage ABTS<sup>+</sup> radical scavenging activity of TT; (c) Percentage hydroxyl radical scavenging ability of TT. Each bar represents the mean  $\pm$  SD of three replicates. TT: Methanol fruit extract of *Tetrapleura tetraptera*; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid).



**Fig. 2.** Inhibitory activity of TT against lipid peroxidation and protein denaturation. (a) Percentage lipid peroxidation inhibitory effect of TT in rat brain homogenate; (b) Percentage inhibitory effect of TT on denaturation of albumin. Each bar represents the mean  $\pm$  SD of three replicates. TT: Methanol fruit extract of *Tetrapleura tetraptera*.

for 3 h (Fig. 3a), the cell death was 15 % compared to normoxia ( $p < 0.01$ ), and when the severe hypoxic exposure was for 6 h, 55 % of astrocytic cell death occurred (Fig. 3b) compared to that in the normoxia condition ( $p < 0.01$ ). With 3 h anoxia exposure, a larger number of astrocytes with altered star shape were observed suggesting that they were morphologically impaired live cells. Therefore for further experiments, the 3 h anoxia where cells were morphologically impaired with less cell death was used because it was important to have morphologically impaired viable cells to test the neuroprotective property of TT.

All the cells that appeared red (bright or dull) are dead cells while most of the green cells are live cells. Some cells which appeared green but had round shape were counted as dead cell because the round shape indicated that cells were dying. Green cells with altered star shape were the astrocytes that have lost their morphology and were counted as live

cells.

#### 3.4. Cytotoxicity of methanol fruit extract of *Tetrapleura tetraptera* on astrocyte

Cytotoxicity of TT on astrocytes after 24 h of treatment is shown in Fig. 4a. Compared with the control (0.01 % DMSO), the three concentrations of TT (0.5  $\mu\text{g/mL}$ , 1.0  $\mu\text{g/mL}$  and 10.0  $\mu\text{g/mL}$ ) showed no cytotoxicity ( $p < 0.05$ ). This implied that the extract did not have deleterious effects on the viability of astrocytes up to 10  $\mu\text{g/mL}$ .

**Table 2**

IC<sub>50</sub> values of *Tetrapleura tetraptera* methanol fruit extract in *in vitro* antioxidant tests.

Antioxidant tests	TT IC <sub>50</sub> ( $\mu\text{g/mL}$ )	Reference standard	
		Compound	IC <sub>50</sub> ( $\mu\text{g/mL}$ )
DPPH	20.96 $\pm$ 0.65 <sup>a</sup>	Ascorbic acid	15.36 $\pm$ 0.29 <sup>b</sup>
ABTS <sup>+</sup>	47.03 $\pm$ 0.97 <sup>a</sup>	Trolox	39.88 $\pm$ 0.52 <sup>b</sup>
Hydroxyl radical	20.63 $\pm$ 0.68 <sup>a</sup>	Mannitol	11.06 $\pm$ 0.87 <sup>b</sup>
CUPRAC	32.08 $\pm$ 1.22 <sup>a</sup>	Trolox	9.76 $\pm$ 0.89 <sup>b</sup>
LPO	47.36 $\pm$ 0.67 <sup>a</sup>	Quercetin	45.61 $\pm$ 0.56 <sup>b</sup>
Protein anti-denaturation	26.76 $\pm$ 0.21 <sup>a</sup>	Ascorbic acid	12.15 $\pm$ 0.21 <sup>b</sup>

Values are expressed as mean  $\pm$  SD ( $n = 3$ ). IC<sub>50</sub> was calculated using the inverse logarithmic method. Values with the same letter across a row are not significantly different ( $p < 0.05$ ). TT: *Tetrapleura tetraptera* methanol fruit extract; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); CUPRAC: Cupric ion reducing antioxidant capacity; LPO: Lipid peroxidation.

### 3.5. Effect of methanol fruit extract of *Tetrapleura tetraptera* on cell viability following 3 h anoxia

There was a significant reduction ( $p < 0.05$ ) in viable astrocytes after 3 h of anoxia treatment compared to control (Fig. 4b). This showed that anoxia may result in a reduction of metabolic activity which could be a contributory mechanism in causing the death of cells. When cells were

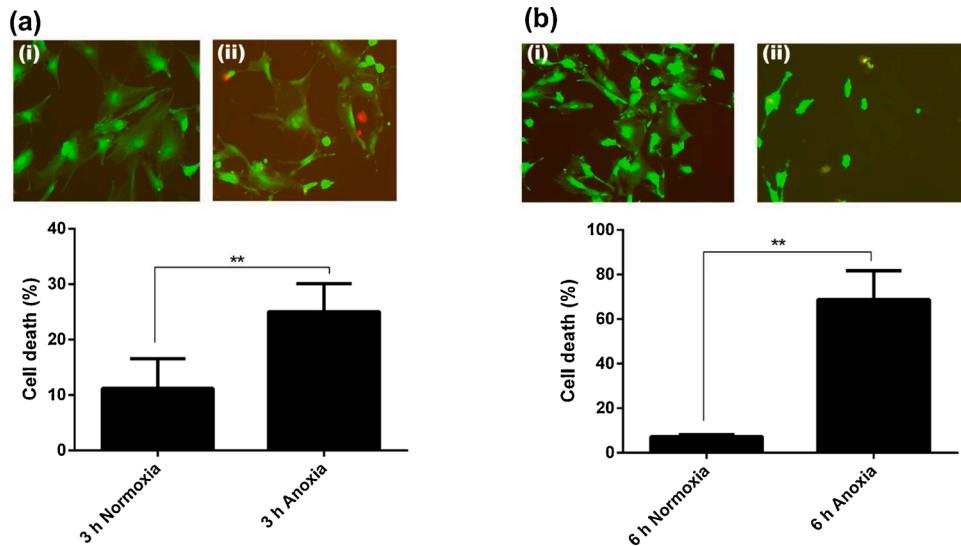
treated with TT before subjecting them to 3 h anoxic condition, the total number of viable cells was significantly higher ( $p < 0.05$ ) than untreated cells. This implied that TT protected cells against anoxic injury and death.

### 3.6. Effect of methanol fruit extract of *Tetrapleura tetraptera* on mitochondrial membrane potential following 3 h anoxia

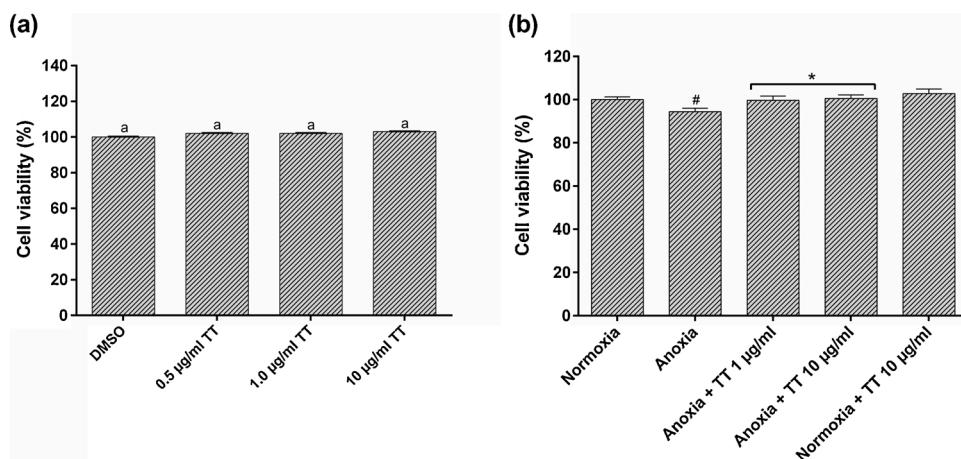
There was a significant decrease ( $p < 0.05$ ) in MMP of cells subjected to 3 h anoxia (severe hypoxia) compared to normoxia. This was characterized by marked staining of the "J-aggregate" form of JC- 1 (red fluorescence) with few monomer forms (green fluorescence) of normoxia-exposed cells compared to anoxia-exposed cells (Fig. 5). The difference in the loss of mitochondrial integrity of cells was visible with the loss of red stain (polarized J-aggregates form, ~590 nm excitation) on exposure to anoxic condition. In Fig. 5, astrocytes that were treated with 1 and 10  $\mu\text{g/mL}$  TT before anoxia exposure exhibited a marked restoration of MMP ( $p < 0.05$ ) in JC-1 staining compared to untreated anoxia-exposed astrocytes.

### 3.7. Molecular docking

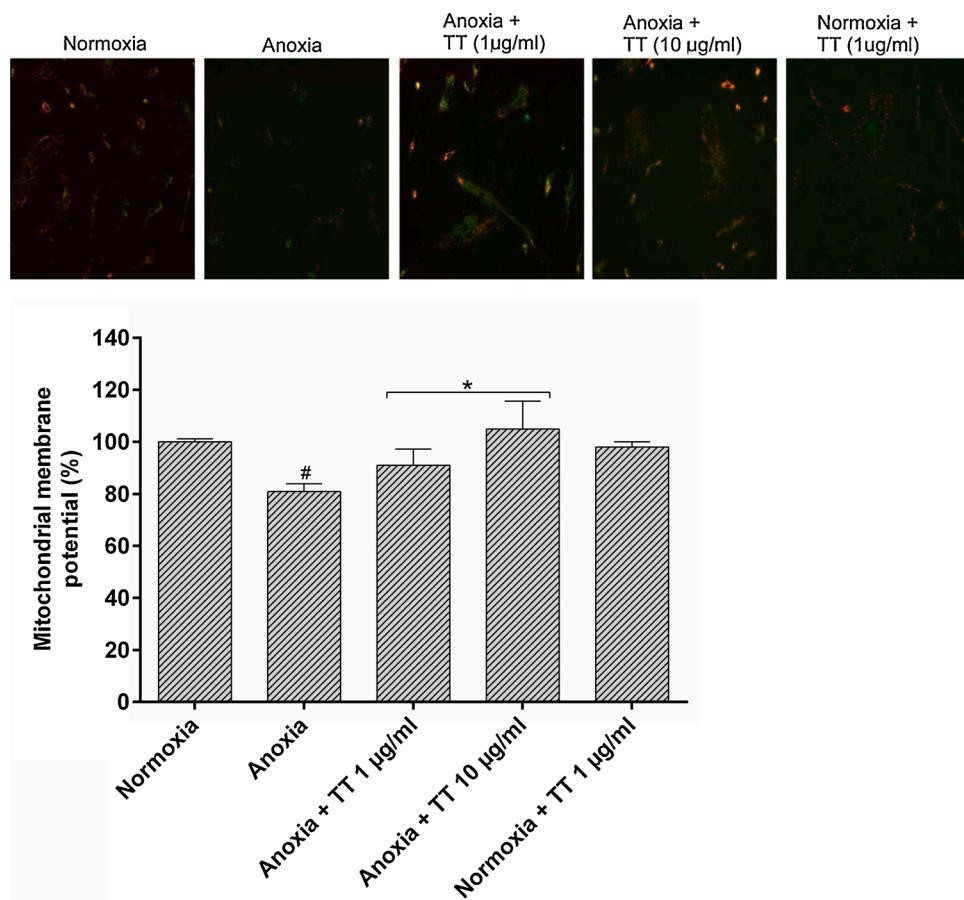
Table 3 shows that the 4 ligands (Fig. 7) possess good binding potential towards hGS. Aridanin showed the best binding potential with a minimum binding energy of -8.6 Kcal/mol, followed by naringenin (-8.3



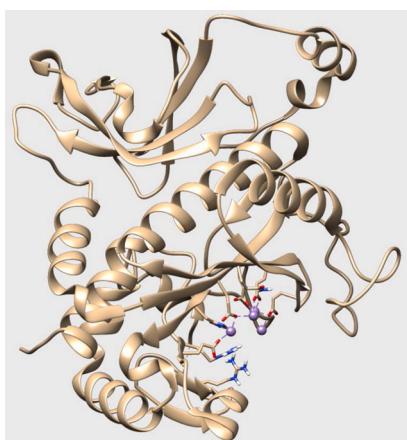
**Fig. 3.** Measure of astrocyte survival following hypoxic treatment. (a) Effect on survival of astrocytes after 3 h exposure to (i) normoxic or (ii) anoxic conditions. (b) Effect on survival of astrocyte after 6 h of (i) normoxic or (ii) anoxic conditions. Each bar is expressed as mean  $\pm$  SD ( $n = 3$ ). Significance with student *t*-test indicated \*\* $p < 0.01$ . All the cells that appeared red (bright or dull) are dead cells while most of the green cells are live cells. Some cells which appeared green but had round shape were counted as dead cell because the round shape indicated that cells were dying. Green cells with altered star-shape were the astrocytes that have lost their morphology and were counted as live cells.



**Fig. 4.** Effect of methanol fruit extract of *Tetrapleura tetraptera* (TT) on cell viability using the MTT method. (a) Effect of TT on cell viability of astrocytes. Astrocytes were treated for 24 h with varying concentrations of TT to assess its cytotoxicity using MTT method. There was no cell death observed at all doses. Bars with the same letters are not significantly different ( $p < 0.05$ ) compared to control. (b) Effect of TT pretreatment on the viability of astrocytes after 3 h normoxia and anoxia. A significant increase in viable cells indicated that astrocytes were protected during an anoxic condition when pretreated with TT. Each bar in (a) and (b) is expressed as mean  $\pm$  SD ( $n = 3$ ). #  $p < 0.05$  vs normoxia; \*  $p < 0.05$  vs anoxia. TT: Methanol fruit extract of *Tetrapleura tetraptera*.



**Fig. 5.** Effect of methanol fruit extract of *Tetrapleura tetraptera* on mitochondrial membrane potential of astrocytes. Each bar is expressed as mean  $\pm$  SD ( $n = 3$ ).  $^{\#}p < 0.05$  vs normoxia;  $^{*}p < 0.05$  vs anoxia. TT: Methanol fruit extract of *Tetrapleura tetraptera*. Red stain (polarized J-aggregates form) indicated high mitochondria membrane potential while green fluorescence (monomer form) indicates depolarized (low) mitochondrial membranes. Few/loss of red stain indicated low mitochondria membrane potential.



**Fig. 6.** Ribbon structure of Chain B from the homologous pentamer (A-E) of human glutamine synthetase (hGS) from RCSB (2ojw). The coloured part represents the funnel-shaped binding pocket.

kcal/mol), scopoletin (-7.1 kcal/mol), and ferulic acid (-6.4 kcal/mol). The binding affinity of the ligands having the lowest RMSD value (zero for upper bound and lower bound RMSD) with hGS showed a good binding pose and was considered and documented. Docking conformation was only analyzed for aridanin because unlike the other compounds, aridanin, a triterpenoid saponin, is a marker compound of *Tetrapleura tetraptera* and it has the best binding affinity with hGS. The result of docking analysis of aridanin from PLIP (Fig. 8) explicated its interactions with hGS through the formation of ten hydrophobic interactions with amino acid residues TRP 130, GLU 134, ALA 191, ASN

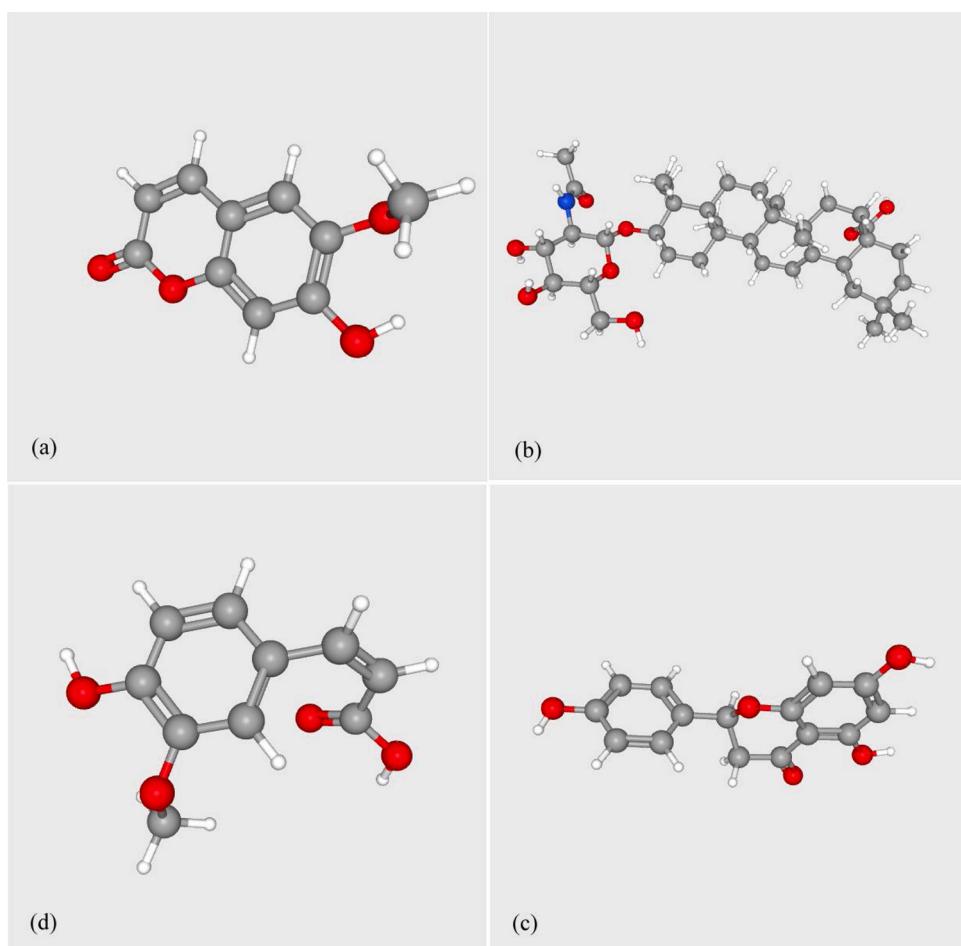
194, GLN 205, PRO 208, ASN 255 and TYR 336 (Table 4), two hydrogen bonds (H-bonds) with amino acids GLY 132 and SER 257 (Table 5) and one salt bridge formation with the amino acid residue ARG 319 (Table 6).

#### 4. Discussion

Medicinal plants are emerging as a promising option in the treatment of neurological diseases due to their pharmacologically active phyto-constituents [2,60]. *Tetrapleura tetraptera* has a long history of use for the traditional management of neurological diseases in West Africa. Studies have shown that its biological effects include antioxidative and anti-inflammatory properties [33,61,62] and also, its anticonvulsive [30,35] and anti-ischemic [28] properties. Polyphenols in *Tetrapleura tetraptera* may act synergistically against ischemia/reperfusion injury through abatement of oxidative stress, lipid peroxidation, and neurochemical dysfunctions [28].

HPLC-DAD quantification of TT revealed the presence of compounds that are pertinent in treating neurological disorders. Aridanin demonstrated anticonvulsive property by modulating nuerotransmitter and opioid receptors [35]. Cerebral infarct size in the brain of rats following cerebral ischemia was reduced by treatment with ferulic acid through antioxidative and anti-inflammatory effects [63] and naringenin regulated the expression of inflammatory molecules to give neuroprotection in rats after focal ischemia [64]. Isoliquiritigenin and scopoletin treatment protected the brain of rats following transient cerebral ischemic injury through antioxidative, anti-inflammatory, anti-convulsive and mitochondrial modulatory effect [65–67].

The *in vitro* antioxidant and various radical scavenging activity of phytoextracts offer an insight into their potential biological effects *in vivo*. The antioxidant property of medicinal plants has been associated



**Fig. 7.** 3D structure of phytochemical constituent. (a) Scopoletin (pubchem ID: 5280460) (b) Aridanin (pubchem ID: 73146) (c) Ferulic acid (pubchem ID: 1548883) (d) Naringenin (pubchem ID: 932). Red balls: Oxygen atoms; White balls: Hydrogen atoms; Grey balls: carbon atoms; Blue ball: Nitrogen atom.

**Table 3**

Binding affinity of some phytochemical compounds identified in *Tetrapleura tetraphylla* methanol fruit extract with human glutamine synthetase.

Ligand	Binding Affinity (Kcal/mol)	RMSD upper bound	RMSD lower bound	Molecular weight (g/mol)
Aridanin	-8.6	0	0	659.9
Naringenin	-8.3	0	0	272.25
Ferulic acid	-6.4	0	0	194.18
Scopoletin	-7.1	0	0	192.17

Aridanin, a lead compound that is peculiarly isolated from *Tetrapleura tetraphylla* fruit, has the minimum binding energy compared to other phytocompounds. The low value of RMSD (less than 1.5 Å, it is zero in this case) indicates the accuracy of docking. RMSD: Root mean square deviation.

with their therapeutic potentials [6,23,68,69]. Multiple *in vitro* antioxidant tests such as carried out in this study are often employed to account for several antioxidant mechanisms exhibited by phytochemicals [25,40,43,70].

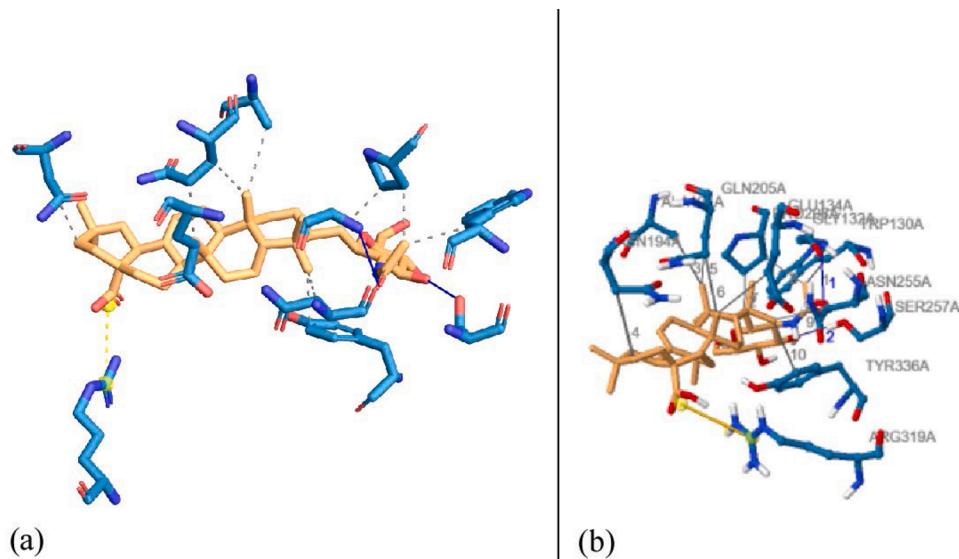
TT demonstrated appreciable radical scavenging and antioxidant activity notably against the highly damaging hydroxyl radical and against lipid peroxidation which is a major culprit in the etiology of many brain pathologies. The effectiveness of TT could be due to the presence of both hydrophilic and lipophilic antioxidants. The performance of TT in the various antioxidant tests with different underlying antioxidative mechanisms highlights its efficacy. Antioxidants play a vital role in reducing oxidative processes. The brain is susceptible to damage by oxidative injury and is also a target of lipid peroxidation

owing to its high metabolic rate and high levels of polyunsaturated lipids [1,71,72]. As observed in this study, the lipid peroxidation inhibitory and protein anti-denaturation activities of TT shows the ability of TT to prevent oxidative damage to biomolecules. The significant antioxidant activity of TT could be related to its reduction of anoxia-induced bioenergetic disturbance in astrocytes observed in this study.

In the present study, anoxia was employed to mimic the events that leads to changes in biochemical and morphological properties of cells in the penumbra region of ischemic brain and to determine what happens to cell metabolism and viability after chronic oxygen deprivation. The astrocytes were also cultured and placed in an anoxic chamber without removing dissolved glucose. Glucose and other nutrients of cell culture were retained in the cultured media and there was no flow of new media after anoxia. In the penumbra region of the ischemic zone, cross-talk between the neurons and astrocytes is vital for the survival of neurons, so maintaining astrocyte viability is critical. Viability of astrocytes support synaptic modification and neurite outgrowth to compensate for neuron loss during ischemic insult [19,20].

Astrocytes death recorded in this study may be as a result of severe hypoxia and acidosis [56,73]. No cytotoxicity was observed after astrocytes were treated with TT suggesting that TT is not toxic to the cells at the employed concentrations. Protection of the viability and metabolic activity of astrocytes after treatment with TT and subjecting them to the anoxic condition may be due to the protective action of its bioactive components. This suggests that TT treatment may restore the function of astrocytes to provide trophic and metabolic support for neurons during metabolic brain injury.

Alteration to mitochondrial membrane integrity could cause



**Fig. 8.** (a) 3D structure of interaction between protein (hGS) and ligand (aridanin); (b) 3D structure of interaction between hGS and aridanin showing the participating amino acid residue in the protein binding pocket. In (a) and (b): structure in gold colour represent the ligand (aridanin); blue (light and deep) represent amino acid residues from the binding pocket of the protein (hGS); blue line and blue number 1 and 2 represent hydrogen bond; dash and full yellow line represents salt bridge; dash grey lines in (a) and full grey lines which are numbered 1 to 10 in (b) represents hydrophobic interactions.

**Table 4**  
Hydrophobic interaction between hGS and ligand (aridanin).

S/ N	Amino acid residue with position	Distance between interacting C-atom (Å)	ID of ligand carbon atom	ID of protein carbon atom
1	TRP 130	3.34	3548	1298
2	GLU 134	3.85	3536	1341
3	ALA 191	3.69	3535	1862
4	ASN 194	3.97	3529	1882
5	GLN 205	3.52	3535	1989
6	GLN 205	3.36	3536	1990
7	PRO 208	3.15	3540	2016
8	PRO 208	3.18	3548	2015
9	ASN 255	3.33	3534	2448
10	TYR 336	3.23	3534	3223

The hydrophobic interaction between the amino acids of protein and ligand with the geometry of interaction (distance of interacting atoms). Hydrophobic interaction stabilizes the protein-ligand (hGS-aridanin) complex. C-atom: carbon atom.

**Table 5**  
Hydrogen bond between amino acid residue in the binding pocket of human glutamine synthetase (hGS) and aridanin.

S/ N	Amino acid residue with position	Distance H-A (Å)	Distance D-A (Å)	Donor Angle	Protein donor?	Side chain?
1	GLY 132	3.68	4.10	107.64	Yes	No
2	SER 257	2.03	2.98	170.68	Yes	Yes

Distance H-A: Distance between hydrogen and acceptor atoms; Distance D-A: Distance between donor and acceptor atom. Protein donor? Yes indicates protein is the donor group for the interaction. Sidechain? Yes indicates that the hydrogen bond is formed with the amino acid side chain while No indicates otherwise.

bioenergetic disturbances and cascade of events leading to cell death [9]. Also, MMP contributes to determining the driving force for calcium entry into the mitochondria and the extent of ROS production [8]. Under hypoxic condition, the mitochondria of astrocytes like other cells, are vulnerable to the production of free radicals that leads to the reduction in ATP generation which could result in cellular damage as observed in mitochondrial dysfunction [13]. In this study, pretreatment of astrocytes with TT increased the MMP of astrocytes subjected to anoxia probably by increasing the energy capacity of the inner mitochondrial

**Table 6**  
Salt bridge formation between the amino acid residue in the binding pocket of hGS and aridanin.

S/ N	Amino acid residue with position	Distance (Å)	Protein positive	Ligand group	Ligand atom
1	ARG 319	4.37	Yes	Carboxylate	3555, 3556

The protein provides a positive charge for the interaction with the carboxylate group of the ligand to form the salt bridge.

membrane and attenuating oxidative stress. Zorova et al. [9] posited that the higher the MMP, the higher the synthesis of ATP, since the energy capacity of the inner mitochondrial membrane becomes high. Therefore, a decrease in MMP will cause a reduction in mitochondrial ROS, however, this decrease must not be sustained inappropriately. Sustained inappropriate low levels of MMP could lead to reductive stress which is as detrimental as oxidative stress to mitochondrial homeostasis [74].

Needless accumulation of glutamate in the extracellular space (outside neuron, astrocytes and glia cells) is toxic to both neuronal and non-neuronal cell (excitotoxicity) and it may cause various neurological disorders if left unabated [75]. Park et al. [75] demonstrated that excitatory amino acid transporter 1, EAAC1, (not glutamate transporter 1) is the glutamate transporter responsible for transportation of glutamate in C6 cell and extracellular accumulation of  $\beta$ -amyloid may lead to decrease in EAAC1 expression which may result to excessive glutamate accumulation seen in Alzheimer's disease. GS regulates excitotoxicity by converting neurotoxic glutamate (cause of excitotoxicity) to harmless glutamine with concomitant hydrolysis of ATP [15]. Molecular docking was carried out to examine the role of some of the phytochemical constituents (aridanin, ferulic acid, naringenin, and scopoletin) of TT to improve the activity of human glutamine synthetase (hGS). The binding energy of the ligands to the protein is an indicator of the stability of the ligand-protein complex [76,77]. The results indicate the ability of the ligands to improve the activity of hGS. The effectiveness of the ligands is shown by the minimum binding energy in the following order; aridanin > naringenin > scopoletin > ferulic acid.

The improved activity of the protein by ligands could further be confirmed by the formation of hydrogen bonds and hydrophobic interaction [76]. The interaction of aridanin, the marker compound in TT [35,30], with hGS was confirmed through the formation of hydrogen

bonds with the formation of hydrophobic interaction and salt bridge in the neighborhood of the hydrogen bond. In this case, the side chain of the polar amino acid (SER257) participated in the hydrogen bond formation which corroborated the findings of Stojanovic and Zaric [78]. Using the following criteria; “H-A distance <2.7 Å, D-A distance <3.3 Å, D-H-A angle >90° and carbon atoms separated by <3.9 Å” SER257 is the only amino acid that is truly interacting with the ligand through hydrogen bonding, whereas, GLY132 is only interacting through hydrophobic contact [78,79]. Also, the shorter distance of the side chains to the ligand promote the formation of hydrogen bond and this may be due to the closeness of the rings of the ligand to the side chain residue (SER257) of the protein [77,78]. Therefore, the interaction of aridanin with hGS may improve its activity by positioning it to effectively condense ammonia and glutamate to form glutamine.

## 5. Conclusion

Methanol fruit extract of *Tetrapleura tetraptera* (TT) demonstrate impressive antioxidant activity through scavenging of free radical, and it is not cytotoxic. This study revealed that TT has the potential to assuage excitotoxicity, restore astrocytic viability, and reduce bioenergetic dysfunction/disturbance via its antioxidative properties in treating cerebral hypoxic conditions. This study also demonstrated that aridanin from TT could help in optimization of the activity of glutamine synthetase in order to attenuate the severity of excitotoxicity in neurological diseases. Therefore, *Tetrapleura tetraptera* could potentially be employed as a plant-based agent for the treatment of cerebral astrocytic hypoxia. The findings from the *in vitro* and *in silico* results in this study could be employed for further experiment in translational research.

## CRediT authorship contribution statement

**Ibrahim Olabayode Saliu:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Reshma Bhagat:** Validation, Formal analysis, Investigation, Writing - original draft. **Olubukola Benedicta Ojo:** Validation, Formal analysis, Investigation. **Afolabi C. Akinmoladun:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration. **M. Tolulope Olaleye:** Resources, Project administration. **Pankaj Seth:** Conceptualization, Methodology, Resources, Supervision. **Velayudhan Rema:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgements

The award of DBT-TWAS sandwich postgraduate fellowship provided by the Department of Biotechnology, Government of India, and The World Academy of Science to Ibrahim O. Saliu (FR number: 3240293151) is greatly acknowledged. Thanks to Tolulope Saliu for assistance on molecular docking. This research was partly supported by NBRC core fund.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.toxrep.2021.01.015>.

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