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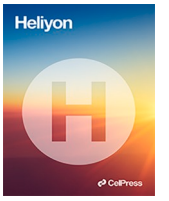

Determination of biological activities of malabar spinach (*Basellaalba*) fruit extracts and molecular docking against COX-II enzyme

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A B S T R A C T

To achieve the health benefit from the natural of *Basella. Alba*fruit. This study intended to figure out the bioactive compounds in the two varieties of *B. alba* (native and hybrid) fruit extract and measurement its biological activities like antioxidant, anti-inflammatory, cytotoxic activities and a molecular docking were performed to observed the pharmaceutical impact on the anti-inflammatory Cyclooxygenase-2 (COX-2) enzyme. The cold extractions along with GC-MS were used for the extraction of and analysis of phytoconstituents from *B. alba* fruit. The hemolytic inhibitory and BSA (Bovine serum albumin)-denaturation assay, DPPH(2,2-diphenyl-1-picrylhydrazyl) and H_2O_2-free radical scavenging analysis, and brine shrimp lethalness test were performed to measure the biological activities of the extracted The biological activities assay results showed that the ethanol extract of native malabar spinach exhibited dose-dependent antioxidant activity. The IC_{50} value 21.55 ± 1.51 μg/mL was for DPPH scavenging assay and 23.36 ± 0.36 μg/mL was for H_2O_2 scavenging analysis. In anti-inflammatory activity assessment study, the IC_{50} values of the ethanol extracts were 20.52 ± 0.91 μg/mL for BSA inhibition and 20.43 ± 1.30 μg/mL for RBC hemolytic inhibitory study. In this study, cytotoxicity test results reveal that aqueous extract exhibited no cytotoxicity as compared to ethanol and ethyl acetate extract (LD_{50} = 875.27 μg/mL). Conversely, the current study insist the *in silico* analysis, to find out the anti-inflammatory activity of the investigated two fruit varieties due to pharmacokinetics analysis, toxicity properties analysis, ADMETand molecular docking. The result of this study signified that both (native and hybrid) malabar spinach fruit varieties contain phytoconstituents with potent antioxidant, anti-inflammatory, and cytotoxic action.Moreover, the in vitro and *in silico* results suggest that the native and hybrid fruit varieties of the extracts could be a superior striver for future appraisal as a prospective therapeutically active ingredient.

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1. Introduction

Recently, the demand of natural color has intensified in foods, beverages and pharmaceutical industries as alternative for synthetic dye. Due to the conformation to the association of the synthetic dye for the development of cancer, evocation of allergic response and others chronic disease, the Food and Drug Administration (FDA) in USA, the European Food Safety Authority (EFSA) in Europe, and many other federal authorities throughout the world have impeded the utilizes of artificial dye in foods, beverages, and confectionery [1]. This is mostly owing to the raising consciousness of the consumer in connection with health and a safety issue by utilizing the artificial pigments in foods has expanded. The non-communicable diseases like cancer, obesity and diabetes have been reduced by devouring the natural color complexes. The devouring of the natural colored complexes has been involved to dissolution of non-communicable diseases such as diabetes, obesity, and cancer [2,3]. In views of these, the natural pigment utilization is encouraged. Thus, the tendency is running gradually favoring the utilization of natural color as supplements in the foods, beverages and pharmaceutical industries. As a result, it is necessary to search the cheapest and biologically valuable sources of natural color to meet their high demand in beverage, food and pharmaceutical industries. It is noted that plant extracts like roots, stems, leaves, fruits etc are good sources of natural color that have been utilized for diverse purposes such as food or textile industry [4]. *B. alba* is an important green leafy vegetable belongs to the family *Basellaceae* and is found usually in the hot region of the nature [5], known as malabar spinach and is very cheap in Bangladesh. In Bangladesh two varieties (native and hybrid) of *B. alba* are cultivated and consumed as a sources of food. But the cultivators are interested to cultivating the hybrid variety due to its low cultivation cost and high production rate compare to native one. Afterwards, the hybrid varieties are generally available in the market rather than native one. However, no investigation has been carried out to access the biological activities and pharmacokinetic properties of the *B. alba* fruits (native and hybrid) extracts. Even no more comparative study have been lunched on the two varieties of *B. alba* fruits (native and hybrid). Although the *B. alba* fruits have defensive properties against the disease are familiar. In this prospect, the biological activities and pharmacokinetics properties of the two varieties of *B. alba fruits* extracts have captured our interest.

Interestingly, the mature *B. alba* fruit with dark blue skin and deep red violet flesh is aexcellent source of natural color that could be used as food additives in food industries to upgrading the quality of the foods along with food preservatives [6].The bluish juice from fruits is utilized as a coloring agent in pastries and sweets [7]. Liberated colors are found to be a biological products, which have acquired rising to concern from health perceiving users and investigators. Natural color obtained from plants havea lot of interest owing to their potential uses in the food and cosmetics factories, as well as in nutraceutical and pharmacological research [8]. As a natural dye, extracts from *B. alba* fruits may be used in the food industry as a natural food preservatives and also used to intensify the texture of food. It is a more healthy and safe food color than synthetic azo-dye used in the food industry. Many studies have reported that the fruits of malabar spinach (*B. alba*) containing flavonoids and betalains possess antibacterial, anti-hyperglycemic, antioxidant, anti-inflammatory, cytotoxic, and anti-proliferative activity, according to *in-vitro*, preclinical, and clinical studies [9–11]. Moreover, antioxidant, anti-inflammatory and anti-cancer activities of malabar spinach fruits (*B.alba*) extracts in diverse solvents are yet to be investigated. Therefore, this study focused on investigating the extraction of coloring materials of malabar spinach fruits using different solvent (aqueous, ethanol and ethyl acetate) and to evaluate their antioxidant, anti-inflammatory and cytotoxic activity, to serve as food additives and ingredients in the food processing and pharmacological industry.

2. Materials and methods

2.1. Materials and chemicals

In this study, methanol, ethanol, ether, sodium chloride, anhydrous sodium sulfate were purchsed from BDH (UK), DMSO (Dimethyl Sulfoxide) from Merck (Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl), BSA (Bovine serum albumin), salicylic acid, ascorbic acid from Sigma-Aldrich (Germany), nutrient broth media and nutrient agar media from Merck (India), Whatman No. 1 filter paper (England) were all utilized for the investigation of *B. alba* fruit extracts.

2.2. Collecting and preparation

Two varieties of *Basella alba* (native - dark blue and hybird-deep red violet) were harvested at similar malabar spinach ground in Ambottala, Jashore, Bangladesh. The fruits were collected, cleaned, and dried up for 4 days in the shade before being smashed into a fine powder (HL7756/00, Phillips Electronics, Netherlands). The smashed materials were dried in an electric oven (PIMPF 50, Phoenix, Germany) at 55 °C for 3 h before being stocked at 4 °C until needed.

2.3. Preparation and collection of coloring materials from malabar spinach fruits(Basella albaL.) extract

The powdered malabar spinach fruit material (200 L) was first extracted via soaking into1.5 L water and then heated in the water bath (50 °C) for 3 h with shaking. The extract was then cooled and freeze-dried to obtain a brown powder of malabar spinach fruit crude aqueous extract by a minor modification of the method [12]. For ethanolic and ethyl acetate extraction, each 200 g of malabar spinach fruit powder was mixed with 95 % ethanol (1.5 L) and 1.5L ethyl acetate for 7 days at room temperature and was concentrated using a rotary vacuum evaporator (Witeg, 2600000, Germany) at 40 °C to yield a yellowish dark green powder of malabar spinach fruit crude ethanolic and ethyl acetate extract by a slight modification of the method [13]. These extracts were preserved at - 4 °C unless needed for the experiment.

2.4. Phytochemical screening of the extracted coloring materials

The different solvent extracts of malabar spinach fruits extract were examined for the existence of diverse phytochemical components through the standard procedure [14].

2.5. Total polyphenol content

The total phenolic content was assessed based on the method specified by Nur et al., 2022 [15]. The extract were mixed with Folin-Ciocalteu reagent (1:10 v/v), and sodium carbonate (7.5 %), then the reaction mixture were incubated for 30 min at 40 °C and determined its absorbance at 765 nm (Thermo Scientific Evolution 200, US). A gallic acid equivalent (GAE) quality was employed through determination of entire phenolic content.

$$\text{Total Phenolic Content } (A) = C * V/M,$$

Where A- Total phenolic content; C- Standard concentration (unit/mL); V- Fruits extract volume (mL); M − weight (g) of the utilized extract.

2.6. Total flavonoid content

The flavonoid content was determined by mixing extract with sodium nitrate (0.5 M) and distilled water, later on sodium hydroxide (1 M) and aluminum chloride (0.3 M) was added and the totalv olume was adjusted to 10 mL. After then incubated at 40 °C for 30 min and 510 nm filter was used to measured its absorbance [15]. Quercetin was functioned as standard for the measurement of total flavonoid content.

$$\text{Total flavonoid content } (A) = C * V/M,$$

Where A- Total flavonoid content; C- Standard concentration (unit/mL); V - Volume of the investigated fruit extract(mL); a M -Weight (g) of the investigated fruit extract.

2.7. Gas chromatography-mass spectroscopy (GCMS)

Gas chromatography-mass spectrometry assaying was conducted with Clarus 690 gas chromatograph (PerkinElmer, CA, USA) through a column (Elite-35, 30 m length, 0.25 mm diameter, 0.25 μm width of film) including with Clarus® SQ 8C mass spectrophotometer (PerkinElmer, CA, USA). 1 μL extract was injected (splitless mode) and pure Helium (99.999 %) was utilized as a supporting gas at a continuous flow rate (1 mL/min) of 40 min operating period High energy (70eV) electron ionization mode was need for sample analyzed. The input temperature was fixed at 280 °C, the column oven temperature was set at 60 °C (for 0 min), elevated at 5 °C–240 °C per minutes and kept for 4 min [16].

2.8. Analysis of biological properties

2.8.1. Determination of in vitro antioxidant activity

2.8.1.1. DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity. In DPPH assay, 2 mL newly produced DPPH (0.1 mM) mixed into 20–100 μg/mL serial diluted extract solution and then incumbent for 30 min at dark [17].

The DPPH scavenging capacity was measured as:

$$\text{Radical scavenging activity } (\%) = \left[\left(\text{Absorbance}\_(\text{control}) - \text{Absorbance}\_(\text{sample})\right)/\text{Absorbance}\_(\text{control})\right] \times 100.$$

The $IC\_(50)$ value was obtained using this data and compared to ascorbic acid.

2.8.1.2. Hydrogen peroxide (H_2O_2) free radical scavenging action. The antioxidant actions of malabar spinach fruit extraction were evaluated throughH_2O_2 (hydrogen peroxide) radical scavenging assay was accordingly to Khatun et al. 2021with minor alternation [18]. In this assay, 20–100 μg/mL serial diluted extracts, 0.6 mL hydrogen peroxide (43 mM, in 0.1 M phosphate buffer) and 3.4 mL phosphate buffer (0.1 M, pH 7.4) were mixing and incubated for 10 min. After then its absorbance were measured at 230 nm. Scavenging ability was evaluated in the following formula:

$$\text{Radical scavenging activity } (\%) = [1 - (\text{Absorbance of sample} / \text{Absorbance of control})] \times 100.$$

2.8.2. Assessment of anti-inflammatory action

2.8.2.1. Evaluation of bovine serum albumin (BSA) denaturation. Protein denaturation inhibition was evaluated accordingly to Amin et al. (2021) as mentioned with minor changes [17]. In this assay, 0.02 mL of extract (20–100 μg/mL) mixed with 4.78 mL of phosphate-buffered saline (PBS, pH 6.4), and 0.2 mL bovine albumin (1 %) for serial dilution and made final volume up to 5 mL. After then the mixed solution was incubated in a water bath (37 °C) for 15 min and then heated (70 °C) for 5 min and chilling. Later on, the

turbidity of the chilling reaction mixture was assessed through UV/VIS spectrometer (Thermo Scientific Evolution 200, US) at660 nm. The denaturation inhibition assessed in the following equation:

$$\text{Denaturation inhibition } (\%) = [1 - (\text{Absorbance of sample} / \text{Absorbance of control})] \times 100.$$

2.8.2.2. Hemolytic inhibitory assessment

2.8.2.2.1. Blood collection and processing of erythrocyte suspension. Freshly blood was received from a well-known healthful persons and it was kept in an EDTA tube. After then, it was centrifuged at 10000 rpm (5 min). Later on it was immersed through normal saline (0.9 % NaCl) and restored as a 10 % (v/v) suspension in isotonic buffer solution (10 mM sodium phosphate buffer, pH 7.4).

2.8.2.2.2. Heat-induced hemolysis. The heat-induced hemolysis inhibition been evaluated through a method as mentioned by Gunathilake et al., 2018 with light modification [19]. The erythrocyte suspension (0.1 mL) and tested extracts (20–100 μg/mL) mixture were incubated at 54 °C for 20 min and later centrifuged at 3000 rpm (5 min). After then, its absorbance was assessed at 540 nm for the assessment of the inhibition of hemolysis. Here, salicylic acid utilized as a positive control to compare the result.

The inhibition of hemolysis was measured through following equation

$$: \% \text{ (percentage) inhibition} = [1 - (\text{Absorbance of sample} / \text{Absorbance of control})] \times 100.$$

2.9. In vitro cytotoxicity test

2.9.1. Cytotoxicity test by brine shrimp lethalness test (Artemia salina)

In vitro cytotoxic test was executed as reported Amin et al., 2021 [17]. In this test, brine shrimp eggs containing sodium chloride brine solution (pH 8.5) were incubated for hatching under fluorescent light for 48 h. After hatching, the nauplii (ten couples) were then shifted into tested device with distinct concentrations (31.5, 62.5, 125, 250, 500, and 1000 μg/mL) of fruit extracts and incubation for 12 h. Later on the quantity of alive nauplii were counted and estimated the percent of fatality. Vincristine sulfate was employed as a positive reference of fatality, while, DMSO (1 %) was a negative reference, which was non-fatal. The percent of fatality were estimated though the following formula:

$$(\%) \text{ Percent of fatality} = (1 - \text{NL} / \text{NT}) \times 1008$$

Herein NT - quantity of picked nauplii (n = 10); NL – quantity of alive nauplii.

Microsoft Office Excel 2016 was used for brine shrimp lethality assay. The LC_{50} values were determined through operating a linear regression analysis of the percent fatality versus the concentrations of investigated substances (Microsoft Corp, Redmond, Washington).

2.10. Molecular docking analysis

2.10.1. Bioinformatics tools and software

The online Admet SAR 2.0, Protein Data Bank (PDB), Pub Chem database. Swiss ADME, PyRx, BIOVIA, tools and software were employed to perform molecular docking analysis. Pub Chem CIDs was utilized for the identification of the ligand complexes Protein Data Bank (PDB) database was used to retrieve the 3D structures of Cyclooxygenase-2. Swiss ADME (www.swissadme.ch) server has been used to analyze drug-likeness properties. Admet SAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2/) tools, the toxicity of the identified compounds. Auto DockVina wizard of PyRx virtual screening tool was utilized to figure out the binding affinity of the target protein (Cyclooxygenase-2) with identified phytochemicals (2-Butenoic acid, Phenylglyoxylic acid, 3-Methyl-2-(2-oxopropyl) furan etc.). The BIOVIA Discovery Studio Visualizer version 21.1.0.20298 software was utilized to visualize the complexes. High Performance Computing (HPC) Dell server with 64 GB RAM were utilized to perform the in sillico studies.

2.10.2. Identification of protein molecule and preparation

Molecular docking analysis in the computational biology is more convenient forms for the conversion of macromolecular structure of protein into its tertiary structural forms. To assess the anti-inflammatory action of the identified phytocompounds, Cyclooxygenase-2 (PDB ID: 5IKQ) selected for the target protein. The Protein Data Bank (https://www.rcsb.org/) was used to retrieve the 3D structure of the protein in PDB format. The target protein particles were formulated and boosted primarily by securing protein formulation wizard from UCSF Chimera version 1.16. The structures of the PDB designated proteins were consequently scavenge through consecutive stages: a) eviction of water, bound complex molecule and all the heteroatoms, b) insertion of polar hydrogen bond and non-polar H were incorporatedc) Gasteiger–Marsili charges were conferred. Such formulated structures were ultimately exploited to consecutive investigation.

2.10.3. Optimization of ligand complex

The 3D structure of identified ligand complex was downloaded in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Following are the PubChem CIDs for the ligands: 19,499 for 2-Butenoic acid, 14,496 for Cyclobutanone, 11,915 for Phenylglyoxylic acid, 102,684 for Photocitral A, 1032 for Propanoic acid, 545772 for 3-Methyl-2-(2-oxopropyl) furan. Subsequently, they were loaded in UCSF Chimera version 1.16, a comprehensive tool for energy minimization using Gasteiger mode. To prepare ligands for molecular docking, hydrogen atoms were added, and charges were made to zero, followed by the ligands exported as mol2 format [20].

2.10.4. Prediction of pharmacokinetic properties, ADMET (absorption, distribution, metabolism, excretion and toxicity), and drug-likeness properties of the phytochemicals

Pharmacokinetic properties are mostly the migration of drugs into, through and out of the body correlated to the intensity and time profile. In order to evaluate the pharmacokinetic properties of identified compounds, Swiss ADME (www.swissadme.ch) server has been used to analyze drug-likeness properties [21]. Therefore, to determine physicochemical properties and BBB(blood-brain barrier) permeation, selected compounds were conserved in smiles format and were uploaded to the Swiss ADME server. By evaluating the degree to which a chemical compound is hazardous to people or animals and can harm an organ, toxicity can be measured. Before a pharmaceutical trial, it is crucial to assess the adverse effects of chemical substances. Therefore, utilizing the web-based admet SAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2/) tools, the toxicity of the identified compounds has been analyzed [22].

2.10.5. Molecular docking analyzing and post-docking visualization

The molecular docking analysis was executed through AutoDock Vina wizard of PyRx virtual screening tool, to figure out the binding affinity of the target protein with identified phytochemicals [23]. The binding energy of the protein-ligand co-reaction and RMSD values were computed after molecular docking was done, these results were saved as CSV files for additional assessment as well as the docked models were in PDBQT format. The BIOVIA Discovery Studio Visualizer version 21.1.0.20298 software was then utilized to visualize and analyze the co reaction of the ligand-protein complex using the output docked model. 2D and 3D structures of the interacting protein-ligand complex can be visualized from the Receptor-ligand interaction section of the software.

2.11. Statistical analysis

Data from triplicate analysis for the same sample were subjected to one-way ANOVA. Means were separated at the significance level of $p < 0.05$. The statistical analysis was conducting through Graph-Pad Prism 8.1.0 (Graph Pad Software, Inc., USA).

3. Results

3.1. Extract preparation, qualitative phytochemical analysis and GC- MS analysis of B.alba fruit extracts

The yield obtained from different solvent (aqueous, ethanol and ethyl acetate) extracts of *B. alba* fruit was four per cent with reference to dry starting material. The phytochemical constituents of different solvent extracts of *B.alba* were represented in Table 1. From Tables 1 and it is observed that both the ethanol and ethyl acetate extracts of two varieties (native and hybrid) of malabar spinach (*B. alba*) fruits contains all the tested phytoconstituents but the terpenoid and alkaloid were totally absent in the aqueous extract. The total flavonoid and total phenolic contents of the ethanol extract of the native variety were 148.70 ± 9.63 and 181.99 ± 17.57 respectively, which was higher and significant ($p < 0.01$ and 0.05, respectively) than others two extracts (aqueous and ethyl acetate) of native and hybrid variety of malabar spinach (*B. alba*) fruits.

In this study, from Fig. 1, it is observed that the extract contain some important constituent such as 2-Butanoic acid (RT-14.074; MW-86), Propanoic acid (RT-15.514; MW-74), Cyclobutanone (RT-19.018; MW-70), Ecosaine (RT-20.742; MW- 282) and those are represented in Table 2. Table 4 represent the physicochemical properties (molecular weight (MW), HBA, HBD, RB, TPSA, cLogP, and LogS), lipophilicity, water solubility, toxicity, drug-likeness, and synthetic accessibility properties of phytoconstituents. The physicochemical properties of these phytoconstituents were induced them as a drugaspirants in pharmacokinetics (PK) based on their ADME properties. The evaluation and upgrade of such properties and those impacts on ADME features are required for the effective drug production.

Table 1
Preliminary phytochemical screening in the native and hybrid variety of malabar spinachfruit of three solvent extracts (aqueous, ethanol and ethyl acetate) and its fractions.

Bioactive Compounds	Test name	Native variety			Hybrid variety		
		Aqueous	Ethanol	Ethyl acetate	Aqueous	Ethanol	Ethyl acetate
Carbohydrate	Molish's test	+	+	+	+	+	+
Protein	Xanthoproteic test	+	+	+	+	+	+
Flavonoid	Alkaline reagent test	+	+	+	+	+	+
Glycoside	Killer-killani test	+	+	+	+	+	+
Phenol	Ferric chloride test	+	+	+	+	+	+
Tannin	Lead test	+	+	+	+	+	+
Terpenoid	Salkowski test	–	+	+	–	+	+
Alkaloid	Wagner test	–	+	+	–	+	–
Saponin	Foam test	+	+	+	+	+	+
Total phenolic content[a]	Folin-ciocalteu reagent test	133.07 ± 18.86	181.99 ± 17.57*	163.47 ± 9.57	125.32 ± 13.86	162.51 ± 18.15	134.92 ± 12.20
Total flavonoid content[b]	Aluminum chloride methods	111.14 ± 8.45	148.70 ± 9.63**	126.84 ± 7.96	110.25 ± 14.36	139.17 ± 8.15*	118.02 ± 6.27

Negative sign (−) indicate absence, positive sign (+) indicate presence. [a]mg gallic acid equivalent (GAE)/g sample, [b] mg quercetin equivalent (QE)/g sample. Results are represented as mean ± standard deviation (n = 3) of three independent experiments; Significant levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$ are considered significant (*), highly significant (**), and very highly significant (***), respectively.

3.2. Biological activity assay

3.2.1. Antioxidant activity assay

3.2.1.1. DPPH assay. Table 3 and Fig. 2a and b showed the free radical scavenging activities of the different fruit extracts of the two varieties (native and hybrid) of malabar spinach using DPPH. Table 3, displayed the IC50 values for the DPPH free radical scavenging activity of the aqueous, ethanol and ethyl acetate extracts of native variety of *B. alba* were34.37 ± 2.29, 21.55 ± 1.51, and 26.51 ± 0.87 μg/mL respectively, in contrast of hybrid variety, values were39.34 ± 2.11, 27.07 ± 2.11 and 33.11 ± 3.04 μg/mL respectively. The IC50 of positive control ascorbic acid was 17.54 ± 0.59 μg/mL(Table 3).

3.2.1.2. H_2O_2free radical scavenging assay. To confirm the antioxidant activities of the different extracts of the two varieties (native and hybrid) of *B. alba*, this study also performed H_2O_2free radical scavenging assay. Table 3 and Fig. 2c and d shown the anti-oxidant activities results of the two varieties (native and hybrid) of *B.alba* using H_2O_2free radical scavenging assay. In this assay, from Table 3 and it was observed that the IC50 values of three different extracts of native variety were46.57 ± 5.24, 23.36 ± 0.36 and 27.91 ± 1.12 μg/mL respectively, while for hybrid variety the IC50 values were 52.53 ± 9.27, 26.70 ± 2.62 and 33.18 ± 3.64 μg/mL respectively and for positive control (ascorbic acid), it was 18.20 ± 1.17 μg/mL (Table 3).

3.2.2. Anti-inflammatory assay

3.2.2.1. BSA denaturation assay. Table 3 and Fig. 3a and b demonstrated the anti-inflammatory activities of the different extract of the two varieties (native and hybrid) of malabar spinach (*B.alba*) fruits. IC_{50} values for anti-inflammatory action of the aqueous, ethanol and ethyl acetate extract of native variety of malabar spinach fruitfor BSA inhibitory assay were observed 25.26 ± 0.96, 20.52 ± 0.91 and 24.06 ± 1.70 μg/mL, conversely for hybrid variety, it was 27.28 ± 1.60, 22.73 ± 1.22 and 24.58 ± 0.40 μg/mL(Table 3).

3.2.2.2. Hemolytic inhibitory assay. Hemolytic inhibitory assay was also performed for the assessment of the anti-inflammatory activities of the native and hybrid varieties of the malabar spinach fruits extracts, which shown in Table 3 and Fig. 3c and d.From Table 3, the IC_{50} values of three different (aqueous, ethanol and ethyl acetate) extracts from native and hybrid varieties of malabar spinach fruits were 22.44 ± 0.99, 20.43 ± 1.30, 21.42 ± 1.07 μg/mL and 24.41 ± 1.15, 20.98 ± 0.33, 23.18 ± 0.51 μg/mL(Table 3). IC_{50} of positive control (salicylic acid) for both anti-inflammatory assay (BSA denature inhibition and hemolytic inhibition assay) were 15.38 ± 0.47 and 15.06 ± 0.50 μg/ml (Table 3and Fig. 3).

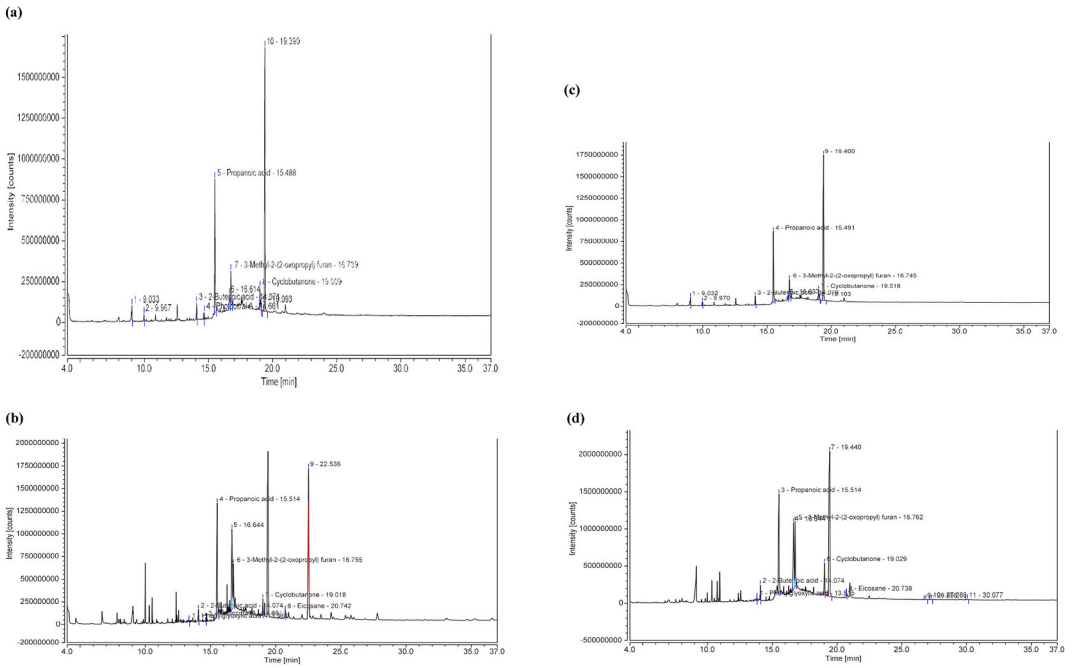

Fig. 1. Gas Chromatography Mass Spectrometry (GC-MS) chromatogram of the two varieties (native and hybrid) of malabar spinach fruit extracts.

Table 2
Phytochemical constituents identified in the extract of native and hybrid varieties of Malabar spinachfruit through Gas Chromatography Mass Spectrometry (GC-MS).

Peak No.	Retention time	Identified compounds	Molecular weight	Peak Area (%)	Formula
1	13.363	Phenylglyoxylic acid	150	0.33	$C_8H_6O_3$
2	14.074	2-Butenoic acid	86	1.47	$C_4H_6O_2$
3	14.661	Photocitral A	152	0.48	$C_{10}H_{16}O$
4	15.514	Propanoic acid	74	32.37	$C_3H_6O_2$
5	16.755	3-Methyl-2-(2-oxopropyl) furan	138	9.83	$C_8H_{10}O_2$
6	19.018	Cyclobutanone	70	3.22	C_4H_6O
7	20.742	Eicosane	282	0.72	$C_{20}H_{42}$

Table 3
IC_{50} values in the native and hybrid variety of malabar spinach fruitof three different solvent extracts (aqueous, ethanol and ethyl acetate) as measured via the two methods of in vitro antioxidant and anti-inflammatory activity assay.

Tested samples	Solvent extract	Antioxidant radical scavenging assay		Anti-inflammatory activity assay	
		IC_{50} of DPPH scavenging capacity (μg/mL)	IC_{50} of H_2O_2 scavenging capacity (μg/mL)	IC_{50} of BSA inhibitory assay (μg/mL)	IC_{50} Hemolytic inhibitory assay (μg/mL)
Native	Aqueous	34.37 ± 2.29	46.57 ± 5.24	25.26 ± 0.96	22.44 ± 0.99
	Ethanol	21.55 ± 1.51*	23.36 ± 0.36	20.52 ± 0.91**	20.43 ± 1.30***
	Ethyl acetate	26.51 ± 0.87***	27.91 ± 1.12*	24.06 ± 1.70***	21.42 ± 1.07***
Hybrid	Aqueous	39.34 ± 2.11	52.53 ± 9.27	27.28 ± 1.60	24.41 ± 1.15
	Ethanol	27.07 ± 2.11**	26.70 ± 2.62	22.73 ± 1.22***	20.98 ± 0.33***
	Ethyl acetate	33.11 ± 3.04***	33.18 ± 3.64*	24.58 ± 0.40***	23.18 ± 0.51***
L-Ascorbic acid (Standard)		17.54 ± 0.59	18.20 ± 1.17	–	–
Salicylic acid (Standard)		–	–	15.38 ± 0.47	15.06 ± 0.50

The results are shown as the mean ± standard deviation (n = 3) of carried out in triplicate; Level of Significant $p < 0.05$; $p < 0.01$ and $p < 0.001$ are considered as significant (*), highly significant (**), and very highly significant (***) respectively, according to Tukey's multiple comparisons test.

3.3. Cytotoxic assay

In brine shrimp lethalness test, it observed all three extracts (aqueous, ethanol and ethyl acetate) from 20 μg/mL to 50 μg/mL concentration did not exhibit remarkable effect on cells survival (no death of nauplii after 12 h) (shown in Table 4). From Tables 4 and it is observed that at concentration 250 μg/mL, 500 μg/mL and 1000 μg/mL of aqueous extract of native variety displayed death rate30 %, 40 % and 50 %. Whereas, at the same concentration ethanol extract and ethylacetate extract showed 30 %, 50 %, 60 % and 30 %, 50 % 70 %. In contrast to the hybrid variety, the aqueous extract showed mortality 30 %, 40 % and 50 %. Conversely, the mortality of the others two extracts (ethanol and ethyl acetate) were 40 %, 50 %, 70 % and 40 %, 60 % 70 %

(Table 4). Thus, the aqueous extract from native and hybrid showed 50 % mortality at 1000 μg/mL and 500 μg/mL concentrations and its LD_{50}-value was 875.27 μg/mL and 748.51 μg/mL, that was treated nontoxic. Whereas, others two extracts (ethanol and ethyl acetate) from native showed 50 % mortality at concentration 500 μg/mL and their LD_{50}-value was 701.66 and 622.99 μg/mL, respectively, in case of hybrid the ethanol and ethyl acetate extract showed 50 % and 60 % mortality at concentration 500 μg/mL but at 1000 μg/mL concentration both of two extracts (ethanol and ethyl acetate) exhibited 70 % mortality. The LD_{50}-values of ethanol and ethyl acetate extract from hybrid one were 603.59 μg/mL and 542.23 μg/mL respectively. These values were considered as toxic. Reference standard Vincrystine sulfate showed LC50-value (2.05 μg/mL). No death was found in negative control (1 % DMSO) group (Table 4).

3.4. In-silico studies

3.4.1. Molecular docking

To estimate pharmacokinetic properties, six compounds complied all of the criteria of the ADMET assessment and demonstrated drug-likeness properties. As a results those compounds were chosen for expedite molecular docking analysis. All of those six ligand molecules demonstrated a remarkable exudation tempo subsequently in the body metabolism. Additionally these were manifested optimum tolerance labels in ranges from 1.4864 to 2.0933 mg/kg/day (Tables 5–8).

3.4.2. ADME test

Table 7 also reveals the toxicity properties analysis of the chosen ligands compounds. From Tables 7 and it noted that Cyclobutanone, Phenylglyoxylic acid and Photocitral A were less toxic effect than other compounds and these compound could be a potential drug candidates. Molecular docking was executed to considering the assessment of the prominent drug aspirants through the

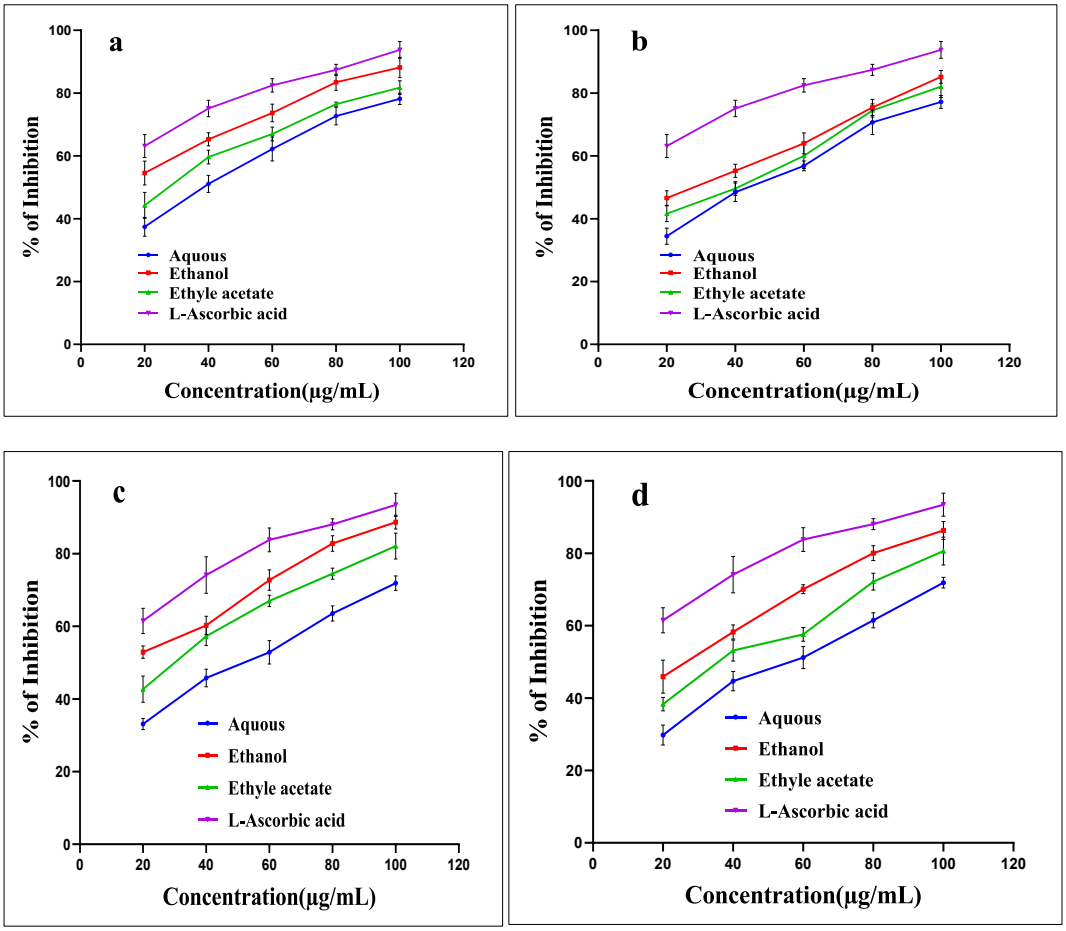

Fig. 2. The DPPH and hydrogen peroxide free radical scavenging potential of three solvent extracts (aqueous, ethanol and ethyl acetate) from the native and hybrid varieties of malabarspinachfruit.

specified ligands according to binding compatibility within protein and ligands. The superior binding energy of interacting protein and ligands revealed the perfect drug constituent. During this essay salicylic acid was utilized in term of controlled drug that exhibited the binding affinity of −5.8 kcal/moL and the dimension of the grid box was X, Y, and Z at 77.1165 Å, 62.5380 Å and 57.5579 Å respectively. Among the ligands Photocitral A displayed the perfect binding score of 6.3 kcal/moL, even though Cyclobutanone was at 3.8 kcal/mol. Some other ligands, Phenylglyoxylic acid, 3-Methyl-2-(2-oxopropyl) furan, 2-Butenoic acid and Propanoic acid presented the binding score of 6.1, 5.9, 4.5 and 4.1kcal/moL, respectively (Table 6). Furthermore, each and every adjoining compounds binding grade were presented in Table 6.Cyclobutanone displayed no more hydrogen bond interacting with COX-2 protein, although those showed few hydrophobic interactions (Table 6). Salicylic acid served as a control, made compound through the COX-2 protein via one hydrogen bond [Trp387(2.36 Å)] and two hydrophobic bond interactions [Gln 203 (A), Ala 202 (A)] (Fig. 4). Conversely, among all the ligands Propanoic acid exhibited highest three hydrogen bonds [Thr 129-A (1.91 Å), Asp125-A (2.23 Å), Ala151-A (3.66 Å)] interaction toward COX-2 protein. Moreover, two ligands like 2-Butenoic acid, 3-Methyl-2-(2-oxopropyl) furan separately formed three hydrogen bonds [Thr129-A (1.81 Å), ASP125-A(2.87 Å)]; [Ser530-A (2.37 Å), Met522-A(3.55 Å)] as well as few non-covalent bonds as shown [Ala378 (A), Ile124(A), Phe529(A)]; [Leu352(A), Val523(A), Phe518 (A), Ala527 (A)]. Concurrently, COX-2 interacted against rest of two ligandssuch as Phenylglyoxylic acid, Photocitral A, by only one hydrogen bond [Leu352-A (2.95 Å)]; [His388-A(3.59 Å)] correspondingly and small number of hydrophobic bonds [Gly526 (A), Val523 (A), Ala527 (A)]; [Leu390 (A), Leu391(A)] into neighboring amino acid residues through 5 Å (Fig. 3).

4. Discussion

4.1. Extraction, phytochemical and GC-MS analysis of B. alba fruit extracts

In the present investigation, various extracts (aqueous, ethanol and ethyl acetate) of *B. alba* fruits showed presence of flavonoids, glycosides, carbohydrates, protein, phenols, saponins, steroid and alkaloid on qualitative phytochemical analysis (Table 1). Similar

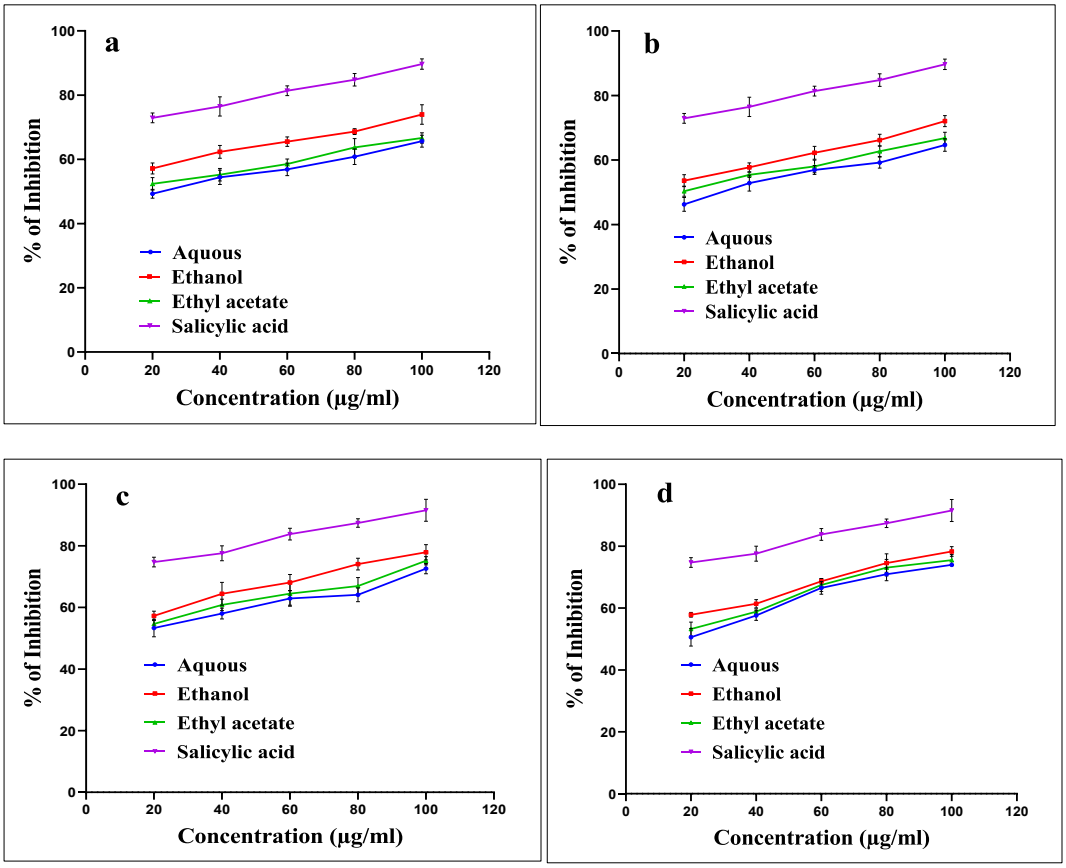

Fig. 3. Inhibition of BSA denaturation and erythrocyte hemolysis activity of three solvent extracts (aqueous, ethanol and ethyl acetate) from the native and hybrid varieties of malabar spinachfruit.

Table 4
Brine shrimp lethality bioassay in the native and hybrid variety of malabar spinachfruitof three different solvent extracts (aqueous, ethanol and ethyl acetate).

Sample Parameters	Tested solvent↓	Percent mortality under concentration studied (µg/mL)						LD_{50} (µg/mL)
	W/V→	31.25	62.5	125	250	500	1000	
Native	Aqueous	0	10	20	30	40	50	875.27
	Ethanol	0	10	20	30	50	60	701.66
	Ethyl acetate	0	10	20	30	50	70	622.99
Hybrid	Aqueous	0	10	20	30	40	60	748.51
	Ethanol	0	10	20	40	50	70	603.59
	Ethyl acetate	0	20	30	40	60	70	542.23
Vincristine sulfate (Positive control)	W/V→	0.25	0.50	1.00	2.00	5.00	10.00	2.05
		20	30	40	70	90	100	
DMSO (Negative control)	V/V→	0.5 %	1.0 %	1.5 %	2.0 %	2.5 %	3.0 %	
		0	0	0	0	0	0	

The test of each level of concentration was carried out in duplicate, n = 10 (no. of shrimps).

results have been observed by Anandara et al. 2011in the methanol extract of *B. alba* aerial part [24]. In other study, Reshmi et al., 2012 reported that the presence of found presence of phenols and flavonoids in *B. alba* fruit extract showed strong antioxidant activity [25].

B. alba fruit extract on GC-MS analysis revealed 07 (Seven) compounds out of which Phenylglyoxylic acid; 2-Butenoic acid; Photocitral A; Propanoic acid; 3-Methyl-2-(2-oxopropyl) furan; Cyclobutanone and Eicosane (Fig. 1 and Table 2). Majority of these compounds are belong to class of furan, phytol, flavonoids, phenols, glycerides, esters, fatty acids and sterols. in other study, Liu et al., 2015 identified 19 compounds including hexadecanoic acid, linoleic acid, palmitic acid and stearic acid by performing GC-MS analysis of *B. alba* seeds oil [26]. Similar GC-MS analysis results of *B. alba* leaves were also found by Baskaran et al., 2015 [27]. In other study, Vadivel et al., 2014 reported that 2-methoxy4-vinylphenol; a phenolic compound have antioxidant, anti-inflammatory, antimicrobial

Table 5
The pharmacokinetic and pharmacophore analysis of selected phytochemicals.

Properties		2-Butenoic acid	3-Methyl-2-(2-oxopropyl) furan	Cyclobutanone	Phenylglyoxylic acid	Photocitral A	Propanoic acid
Physicochemical Properties	MW (g/mol)	70.09	74.08	86.09	138.16	150.13	152.23
	Num. of HBA	2	2	1	3	1	2
	Num. of HBD	1	0	0	1	0	1
	Num. RB	1	2	0	2	2	1
	TPSA	37.3	30.21	17.07	54.37	17.07	37.3
Lipophilicity	cLog P*o/w*	0.56	1.54	0.76	0.99	2.42	0.3
Water Solubility	Log S (ESOL)	−0.76	−1.82	−0.3	−1.88	−2.46	−0.44
	Class	Very soluble	Very soluble	Very soluble	Very soluble	Soluble	Very soluble
Pharmacokinetics	GI absorption	High	High	High	High	High	High
Druglikeness	Lipinski	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)
Medicinal Chemistry	Synthetic accessibility	1.52	2.34	1.06	1.03	2.95	1

MW = molecular weight (g/mol); HBA = Hydrogen bond acceptor (acceptable range: ≤10); HBD = Hydrogen bond donor (acceptable range: ≤5); No. of Rotatable bonds (RB) optimal 0–11; Topological polar surface area optimal 0–140; Consensus LogP (cLogP) = High lipophilicity (expressed as LogP, acceptable range: 1 > and <5); LogS = Log of the aqueous solubility optimal: 4–0.5 log mol/L; GI = gastrointestinal.

Table 6
Binding affinity of ligands with targeted protein COX-II and comprehensive intermolecular interaction.

Ligands Name	Grid Box	Binding Affinity	Amino Acid Involved Interaction	Distance	Category
Salicylic acid (Control)	77.1165 × 62.5380 × 57.5579	−5.8	TRP387	2.36302	Hydrogen Bond
			GLN203	4.26084	Hydrophobic
			ALA202	4.43023	Hydrophobic
2-Butenoic acid		−4.5	THR129	1.81	Hydrogen Bond
			ASP125	2.86532	Hydrogen Bond
			ALA378	3.86809	Hydrophobic
			ILE124	4.14078	Hydrophobic
			PHE529	4.62881	Hydrophobic
3-Methyl-2-(2-oxopropyl) furan		−5.9	SER530	2.36777	Hydrogen Bond
			MET522	3.5523	Hydrogen Bond
			LEU352	3.96999	Hydrophobic
			VAL523	3.99049	Hydrophobic
			PHE518	5.41697	Hydrophobic
			ALA527	4.54603	Hydrophobic
Cyclobutanone		−3.8	THR206	2.81339	Other
Phenylglyoxylic acid		−6.1	LEU352	2.94908	Hydrogen Bond
			MET522	5.81764	Other
			GLY526	4.26044	Hydrophobic
			VAL523	5.41298	Hydrophobic
			ALA527	5.21793	Hydrophobic
Photocitral A		−6.3	HIS388	3.5994	Hydrogen Bond
			LEU390	5.40009	Hydrophobic
			LEU391	4.1813	Hydrophobic
Propanoic acid		−4.1	THR129	1.91046	Hydrogen Bond
			ASP125	2.2329	Hydrogen Bond
			ALA151	3.66397	Hydrogen Bond

and analgesic activity [28] and they also mentioned that 2, 3-dihydro-3, 5-dihydroxy6-methyl-4H-pyran-4-one (DDMP) compound have anti-inflammatory and antimicrobial activity. Thangam et al., 2013 stated that 2-pyrrolidinone has potent anticancer activity [29]. Phytol has strong anticancer activity through induction of apoptosis by ROS production [30]. Zhang et al., 2004 stated that palmitic acid has anticancer activity [31]. Bivalent β-carbolines has shown potent cytotoxic reaction against cancer cell lies [32]. In present study, three different extracts of *B. alba* (native and hybrid) fruits also contents 2-methoxy-4-vinylphenol, DDMP, 2- pyrrolidinone, phytol, palmitic acid, β-carbolines compounds that could be responsible for antioxidant and anticancer activity.

The secondary metabolites like phenols, sterols, flavonoids, alkaloids, tannins, saponin, terpenoids, glycosides, amines, hormones, and correspondent metabolites are synthesized by plants; those are widely utilized in the drug and pharmaceutical industries as an important ingredient [33]. In this study, it is observed that mentionable phytoconstituents were present in the different extracts of *B. alba* fruits extract (Table 1). Thus the present research focused on the detection of phytoconstituents and pharmacological properties of the *B. alba* fruit extract to see whether it has biological activities such as antioxidant, anti-inflammatory, toxic effects, along with a computational review of the investigated phytoconstituents, whether it has physicochemical, pharmacokinetics (ADME), and demonstrated drug-likeness characteristics outwardly intoxication.

Table 7
Toxicity properties analysis of selected ligands compounds.

Properties	2-Butenoic acid	3-Methyl-2-(2-oxopropyl) furan	Cyclobutanone	Phenylglyoxylic acid	Photocitral A	Propanoic acid
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	Weak inhibitor	Weak inhibitor	Weak inhibitor	Weak inhibitor	Weak inhibitor
AMES Toxicity	No	No	No	No	No	No
Carcinogens	Carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens	Carcinogens
Fish Toxicity	Yes	Yes	No	Yes	Yes	No
TetrahymenaPyriformis Toxicity	No	Yes	No	Yes	Yes	No
Honey Bee Toxicity	Yes	Yes	Yes	Yes	Yes	Yes
Biodegradation	Ready biodegradable	Ready biodegradable	Ready biodegradable	Ready biodegradable	Ready biodegradable	Ready biodegradable
Acute Oral Toxicity	III	II	II	III	III	III
Hepatotoxicity	No	No	No	No	No	No
Rat Acute Toxicity (LD50, mol/kg)	1.9038	2.0933	2.2323	1.7681	1.5407	1.4864

Table 8
Theabsorption, distribution, metabolism, and excretion (ADME) of phytoconstituents.

Properties		2-Butenoic acid	3-Methyl-2-(2-oxopropyl) furan	Cyclobutanone	Phenylglyoxylic acid	Photocitral A	Propanoic acid
Absorption	HIA	+	+	+	+	+	+
	HOB	+	+	+	+	+	+
	pglycoprotein inhibitor	NI	NI	NI	NI	NI	NI
	pglycoprotein substrate	NS	NS	NS	NS	NS	NS
	Caco-2 permeability	+	+	+	+	–	+
Distribution	BBB permeation	+	+	+	+	+	+
	PPB (%)	33.04 %	75.27 %	30.90 %	82.00 %	49.23 %	33.81 %
Metabolism	CYP4501A2 inhibitor	NI	I	NI	NI	NI	NI
	CYP4503A4 inhibitor	NI	NI	NI	NI	NI	NI
	CYP4502C9 inhibitor	NI	NI	NI	NI	NI	NI
	CYP4502C19 inhibitor	NI	NI	NI	NI	NI	NI
	CYP4502D6 inhibitor	NI	NI	NI	NI	NI	NI
	CYP4501A2 substrate	NS	NS	NS	NS	NS	NS
	CYP4503A4 substrate	NS	NS	NS	NS	NS	NS
	CYP4502C9 substrate	NS	NS	NS	NS	NS	NS
	CYP4502C19 substrate	NS	NS	NS	NS	NS	NS
	CYP4502D6 substrate	NS	NS	NS	NS	NS	NS
Excretion	Renal clearance (mL/min/kg	4.847	12.692	7.25	1.384	12.052	6.993

HIA=Human intestinal absorption; HOB=Human oral bioavailability; I=Inhibitor; NI=Non-inhibitor; NS= Non-substrate; PPB=Plasma protein binding <90 % is optimal; Clearance: High >15 mL/min/kg; Moderate: 5–15 mL/min/kg; Low <5 mL/min/kg.

4.2. Biological activity assay

4.2.1. Antioxidant activity assay

In DPPH scavening analysis the antioxidant actvities of the *B. alba* fruit extract were concentration dependent. Besides, the DPPH assay, hydrogen peroxide scavening assay also showed a similar concentation depenednt antioxidant activites (Table 3 and Fig. 2). In this study, GC–MS finding reveled the appearance of Propanoic acid and Cyclobutanone compounds in the ethanoic extracts of the both variety (native and hybrid) (Fig. 1) that could be concerned for free radical scavenging antioxidant action. In this study, *B. alba* fruit extract showed effective red blood cell membrane stability action over satisfactied percentage of preventive towards hydrogen peroxide and several compounds detected in the extract through GC-MS, exhibited antioxidantactions (Fig. 2). It has been reported that the compound Cyclobutanone (Z, Z) possess potential antioxidant [34]. The outcome of this study was in conformation with the previous statement claiming dragon fruit peel as a promising source of antioxidant phenolics [35]. Previously study reported aqueous B.alba extract with high content of phenolic compounds exhibited high antioxidant activity [36].Hence, the *B. alba* fruit extract may utilized as an important food and pharmaceutical ingradients with specific health benefits to consumers.

4.2.2. Anti-inflammatory assay

Inflammation is caused by the impairment of tissue due to the release of lysosomal enzymes and proteases from leukocytes. Free radical targeted the impaired cell membranes for secondary injury by inducing lipid peroxidation [37]. In anti-inflammatory response, the leakage of cytoplasmic components is inhibited, therefore tissue injury is reduced and the integrity of the cell membranes can

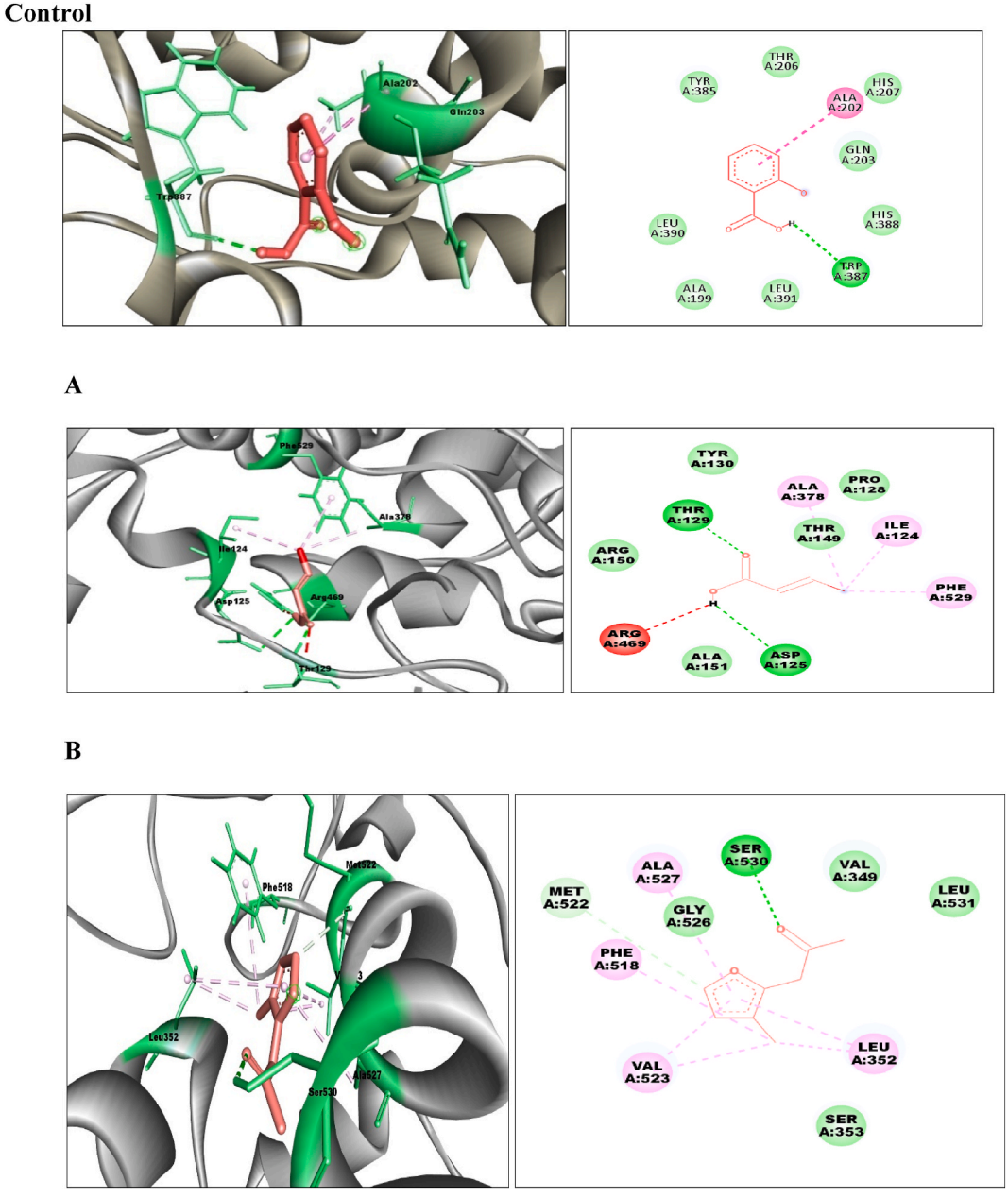

Fig. 4. Molecular docking analysis.Binding of compounds with COX-II.

retard or impede lysis. Protein denaturation is an important factor for inflammation [38]. The substances, which prevent protein denaturation that could be an effective factors for treating inflammatory disorder. Thus this study focused on to investigated the ability of *B.alba* fruit extract to impede red blood cell lysis and resist denaturation of BSA protein as well as anti-inflammatory action. In this study, *B.alba* fruit extract exhibited dose-dependent anti-inflammatory action through suppressed the lysis of red blood cell and denaturation of bovine serum albumin (BSA) protein (Fig. 3). The phyto constituents such as hydrocarbons, phenolics, ketone, esters, ether and terpenes in *B. alba* fruit extract could be causative agent for the anti-inflammatory action. Furthermore, the in vitro RBC lysis assay exhibited the impacts of Tetrapleura tetraptera, Olax sub-scorpioidea and Fagra zanthoxiloides on the stability of membrane. While,125–500 g/mL water extract of Albuca setosa prevent the lysis of RBC membrane through heat-induced hemolysis [39,40]. The inhibition of BSA protein denaturation through the effects of different parts of plants have been reported early, that incorporates methanolic and ethanolic extracts of *Wedelia trilobataon*, *Semecarpus anacardium* bark and *Justicia secunda* leaves [20]. In this study,

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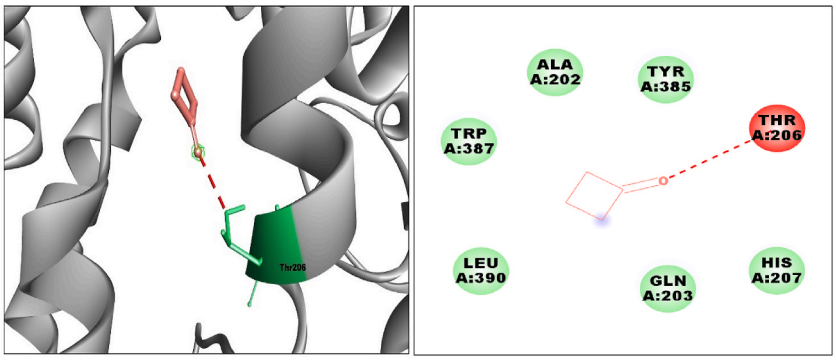

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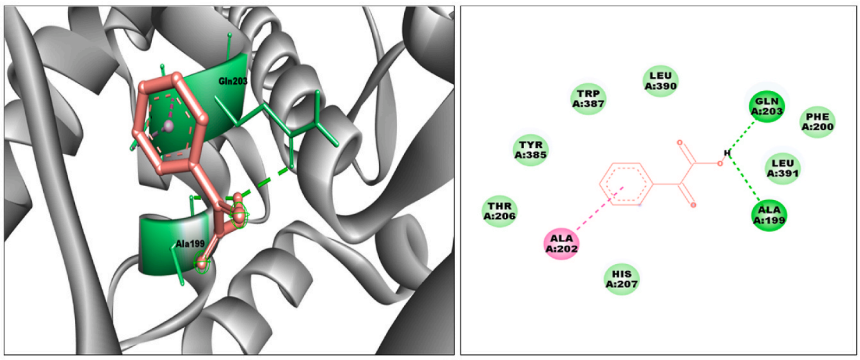

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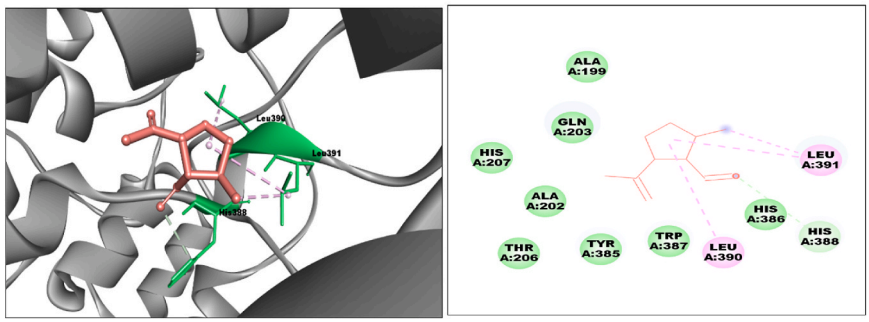

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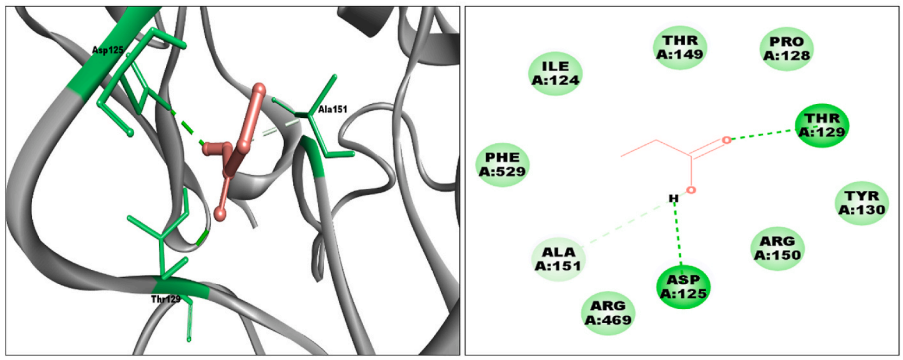

Fig. 4. (*continued*).

B. alba fruit extract showed effective red blood cell membrane stability action over satisfactied percentage of preventive towards hypotonic solution, hydrogen and heat-induced tests and several compounds detected in the extract through GC-MS, exhibited anti-inflammatory actions(Fig. 3). It has been reported that the compound Cyclobutanone(Z, Z) possess significant anti-inflammatory activities [41,42].

4.3. Cytotoxic assay

The present study, for toxicity analysis the Brine shrimp lethality assay was performed. The results obtained in this assay noted that the aqueous extract demonstrated 50 % death at 1000 μg/mL concentration and its LD_{50}-value was 875.27 μg/mL, that was treated as nontoxic compared to others two extracts (ethanol and ethyl acetate) of *B. alba* (Table 3). It is reported that cytotoxic activity assay of the methnolic extract of *Caesalpinia pulcherrima* wood on brime shrimp larvae has slight effects while aqueous extracts showed comparatively toxic impact [43]. But in the present study, the cytotoxicity experimental results indicated that aqueous extract of *B. alba*fruits are safe to serve as a food additives in food industries and a ingredients in pharmaceutical compare to the others two extracts (ethanol and ethyl acetate).

4.4. In-silico studies

Pharmacokinetic study assesses the drug-cognate properties of compound and treats with variant drugs depending on numerous parameters [44]. A significant result were reported by Pires et al., 2015 for good drugs candidate based some properties such as, numerous hydrogen bond donors and acceptors, numerous rotatable bonds, molecular weight and logP value being analyzed throughout SwissADME and pkCSM [44]. The results obtained in molecular docking analysis is cosistence to the finding of Olasupo et al., 2020 [45]. It is well recognized that a compound with low doses have higher level of intestinal absorption and showed maximum tolerance.

In clinical trials, the commonest causing drug failing is worse ADME. It is noted that pharmacokinetics are associated with the harmlessness and effectiveness of a drug. Thus, for a potential drug candidate the pharmacokinetic criterion have to be needed to maximize upon drug design and drug deliver for standard clinical trials. It is recognized that MW and polar surface topological area (TPSA) should affected the permeability drug candidates. Furthermore, the absorption of the drug in the body is affected by the lipophilicity (LogP) of a drug. The lowest LogS value is often selected of drug candidate molecules due to its effects on the solubility of drug candidate molecules [21].The drugs capacity to crossing the membrane bilayers in a particular range is influence by the number of hydrogen bond donors and acceptors. The phytoconstituents and their toxicity in the extracted samples were measured by using *in silico* methods. The data through *in silico* toxicity assay servers have used for the mutagenic characteristics analyze of the compounds and found to be non-mutagenic (Table 7). Furthermore, parent compounds structural alternations and synthesis of these biological active perfect phytochemicals derivatives could be useful to design new pharmacokinetic potential drugs.

It is also noted that all compounds displayed drug-like properties, even, those exhibited negative results for hepatotoxicity and AMES toxicity. Molecular docking is a leading sector in computer-aided drug design that manifested the excellent binding affinity toward receptor-ligands complex and python-based docking software PyRx V 0.8 becomed served tomolecular docking [46]. The best candidate can be selected based on the molecular docking score comprises through pharmacokinetic analysis. In the current study, Photocitral A exhibited higher binding energy (6.3 kcal/mol) compared with others compounds detected in the extract (Table 6 and Fig. 4). Even though owing to weak binding energy comparing to control, Propanoic acid interacted with three and 2-Butenoic acid and 3-Methyl-2-(2-oxopropyl) furan, interacted against COX-2 protein with two hydrogen bonds. Fukunishi and Nakamura, 2011 et al. reported that the compexity of a compound depend on the number hydrogen bond[47].In this study, Table 6 represents the outlines of overall hydrogen bond and hydrophobic bond interaction fromprotein COX-2 and Ligands. This binding affinity results supported the extract as a drug candidate according to the reported by Yuliana et al., 2013 [46]. In this study, consequential in vitro antioxidant, anti-inflammatoryimpacts on *B. alba* fruits extracts along with molecular docking analysis, confer a potent resarch based premise to utilization of *B. alba* fruits extract in the food industry asa dietary supplement for health benefit and as an ingradient in pharmaceutical industries for the medication of inflammatory associated aliments.

5. Conclusion

The current research reveals that different extract of *B.alba*fruitsextract showed significant anti-oxidant and anti-inflammatory activities. GCMS-based profiling of chemicals exposed the existence of six phytocompounds herein 2-Butenoic acid, 3-Methyl-2-(2-oxo-propyl) furan, Cyclobutanone, Phenylglyoxylic acid and Propanoic acid are the significant biologically-active compounds in the studied extract. Each of the two experimental form anti-oxidant, anti-inflammatory activities, and computer-based molecular docking analysis support/facilitate intense research confirmation to the utilization *B. alba* fruits extract as an excellent color accents in the food industry and also an important ingredient in the pharmaceutical industries.

CRediT authorship contribution statement

M. Ashaduzzaman Nur: Data curation, Formal analysis, Investigation, Writing – original draft. **Mubenul Islam:** Formal analysis, Methodology. **Sangram Biswas:** Formal analysis, Investigation, Visualization. **M. Nahid Hasan:** Data curation, Resources. **M. Mashiar Rahman:** Formal analysis, Writing – review & editing. **M. Jashim Uddin:** Conceptualization, Funding acquisition,

Supervision, Writing – review & editing. **Mohammed A. Satter:** Formal analysis, Software, Validation, Visualization. **M. Ziaul Amin:** Conceptualization, Funding acquisition, Investigation, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of Competing interest

Here by I am confirming that I would like to submit research article entiled "Determination of biological activities of Malabar spinach (Pui fall) (*Basella alba* L.) fruit extract colorants and molecular docking against COX-II enzyme" with my others co-author in your journal. This manuscript is original research and has not been published or considered for publication by any other journal or elsewhere, As a corresponding author I also declare that this study was performed according to the international, national and institutional rules considering animal experiments, clinical studies and biodiversity rights.

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References

[1] M. Carocho, M.F. Barreiro, P. Morales, I.C.F.R. Ferreira, Adding molecules to food, pros and cons: a review on synthetic and natural food additives, Compr. Rev. Food Sci. Food Saf. 13 (2014) 377–399, https://doi.org/10.1111/1541-4337.12065.
[2] A.M. Rodriguez, L.A.O. Martinez, S.M.G. Herrera, O.M.R. Quifiones, R.F.G. Laredo, B.O. Alonso, Natural pigments of plant origin: alassification, extraction and application in foods, Food Chem. 398 (2023), 133908, https://doi.org/10.1016/j.foodchem.2022.133908.
[3] J.L. Cooperstone, S.J. Schwartz, Handbook on Natural Pigments in Food and Beverages, Industrial Applications for Improving Food Color, 2016, pp. 473–497.
[4] T. Pujilestari, SumberdanPemanfaatanZatWarnaAlamUntukKeperluanIndustri. DinamikaKerajinandan Batik: MajalahIlmiah 32 (2) (2016) 93–106.
[5] S.A. Deshmukh, D.K. Gaikwad, A review of the taxonomy, ethnobotany, phytochemistry and pharmacology of *Basella alba* (*Basellaceae*), J. Appl. Pharmaceut. Sci. 4 (1) (2014) 153–165, https://doi.org/10.7324/JAPS.2014.40125.
[6] M.A. Nur, M. Khan, M.A. Satter, M.M. Rahman, M.J. Uddin, M.Z. Amin, Assessment of physicochemical properties and nutrient contents of native and hybrid varieties of Malabar spinach (*Basella alba*L.) fruits and the stability of their extracted colorant, Biocatal. Agric. Biotechnol. (2023), 102746, https://doi.org/10.1016/j.bcab.2023.102746.
[7] A.K. Azad, W.S.W. Azizi, Z.M. Babar, Z.K. Labu, S. Zabin, An overview on phytochemical, anti-inflammatory and anti-bacterial activity of *Basella alba* leaves extract, Middle East J. Sci. Res. 14 (5) (2013) 650–655.
[8] P. Brudzyńska, A. Sionkowska, M. Grisel, Plant-derived colorants for food, cosmetic and textile industries, Singap. Med. J. 14 (13) (2021) 3484, https://doi.org/10.3390/ma14133484.
[9] A. Ullah, S. Munir, S.L. Badshah, N. Khan, L. Ghani, B.G. Poulson, A.H. Emwas, M. Jaremko, Important flavonoids and their role as a therapeutic agent, Molecules 25 (22) (2020) 5243, https://doi.org/10.3390/molecules25225243.
[10] N.F. Shamsudin, Q.U. Ahmed, S. Mahmood, S.A.A. Shah, M.N. Sarian, M.M.A.K. Khattak, A. Khatib, A. Sukarni, M. Sabere, Y.M. Yusoff, J. Latip, Flavonoids as Antidiabeticand anti-inflammatory agents: a review on structural activity Relationship- based studies and Meta-Analysis.Int, J. Mol. Sci. 23 (20) (2022), 12605, https://doi.org/10.3390/ijms232012605.
[11] T.F. Rakotondrabe, M. Fan, M. Guo, Exploring potential antidiabetic and anti- inflammatory flavonoids from Euphorbia humifusa with an integrated strategy, Front. Pharmacol. 13 (2022), https://doi.org/10.3389/fphar.2022.980945.
[12] P. Peungvicha, S.S. Thirawarapan, R. Temsiririrkkul, H. Watanabe, J.K. Prasain, S. Kodata, Hypoglycemic effect of the water extract of Piper sarmentosum in rats, J. Ethnopharmacol. 60 (1998) 27–32.
[13] X.F. Zhang, B.K.H. Tan, Effects of an ethanolic extract of *Gynuraprocumbens* on serum glucose, cholesterol and triglyceride levels in normal and Steptozotocin-induced diabetic rats, Singapore Med. J. 41 (2000) 9–13.
[14] A. Harborne, Phytochemical Methods A Guide to Modern Techniques of Plant Analysis, Springer Netherlands, 1998, p. 60.
[15] M.A. Nur, M. Khan, S. Biswas, K.D. Hossain, M.Z. Amin, Nutritional and biological analysis of the peel and pulp of *Pouteriacampechiana* fruit cultivated in Bangladesh, Journal of Agriculture and Food Research (2022), 100296, https://doi.org/10.1016/j.jafr.2022.100296.
[16] M.S. Rahman, H. Riad, K.S. Forhad, M.R. Shaikh, K.S. Subbroto, H. Jongki, K. KiHyun, Insights into the in vitro germicidal activities of Acalyphaindica, Anal.Sci. Technol. 30 (2017) 26–31, https://doi.org/10.5806/AST.2017.30.1.26.
[17] M.Z. Amin, M. Afrin, M.A. Nur, M.M. Rahman, M.J. Uddin, Evaluation of medicinal effects of Gynuraprocumbens leave extracts on oxidative, glycemic, lipidomics, and enzymatic profiles in alloxan-induced diabetic mice, J. Food Sci. Technol. 12 (2021) 876.
[18] M. Khatun, M.A. Nur, S. Biswas, M. Khan, M.Z. Amin, Assessment of the antioxidant,anti-inflammatory and antibacterial activities of different types of turmeric (*Curcuma longa*) powder in Bangladesh, J. Agric Food Res. 6 (2021), 100201, https://doi.org/10.1016/j.jafr.2021.100201.
[19] M.Z. Amin, M. Afrin, N.S. Meghla, M.A. Nur, M.M. Rahman, M.J. Uddin, Assessment of antibacterial, anti-inflammatory, and cytotoxic effects of different extracts of Gynuraprocumbens leaf, Curr. Ther. Res. 95 (2021), 100636.
[20] K.D. Gunathilake, K.K. Ranaweera, H.P. Rupasinghe, In vitro anti-inflammatory properties of selected green leafy vegetables, Biomedicines 6 (4) (2018) 107, https://doi.org/10.3390/biomedicines6040107.
[21] Z. Yang, K. Lasker, D. Schneidman-Duhovny, B. Webb, C.C. Huang, E.F. Pettersen, T.D. Goddard, E.C. Meng, A. Sali, T.E. Ferrin, UCSF Chimera, MODELLER, and IMP: an integrated modeling system, J. Struct. Biol. 179 (2012) 269–278.
[22] A. Daina, O. Michielin, V. Zoete, Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. (2017) 7, https://doi.org/10.1038/srep42717.
[23] H. Yang, C. Lou, L. Sun, J. Li, Y. Cai, Z. Wang, W. Li, G. Liu, Y. Tang, Admet SAR 2.0: web-service for prediction and optimization of chemical ADMET properties, Bioinformatics 35 (6) (2019) 1067–1069, https://doi.org/10.1093/bioinformatics/bty707.
[24] S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx, Methods Mol. Biol. 1263 (2015) 243–250, https://doi.org/10.1007/978-1-4939-2269-719.
[25] K. Anandarajagopal, D. Sudhahar, T.V. Ajaykumar, G. Muthukumaran, Evaluation of CNS depressant activity of aerial parts of *Basella alba* Linn, IJPI'SJ Pharmacol.Toxicol. 1 (2011) 6.
[26] S.K. Reshmi, K.M. Aravindhan, P.S. Devi, The effect of light, temperature, pH on stability of betacyanin pigments in *Basella alba* fruit, Asian J. Pharmaceut. Clin. Res. 5 (4) (2012) 107–110.
[27] K. Liu, P.C. Liu, R. Liu, X. Wu, Dual AO/EB staining to detect apoptosis in osteosarcomacells compared with flow cytometry, Med.Sci.Monit.Basic Res. 21 (2015) 15–20, https://doi.org/10.12659/MSMBR.893327.

[28] G. Baskaran, S. Salvamani, S.A. Ahmad, N.A. Shaharuddin, P.D. Pattiram, M.Y. Shukor, HMG-CoA reductase inhibitory activity and phyto component investigation of Basella alba leaf extract as a treatment for hypercholesterolemia, Drug Des. Dev. Ther. 9 (2015) 509, https://doi.org/10.2147/DDDT.S75056.
[29] E. Vadivel, S. Gopalakrishnan, GC-MS analysis of some bioactive constituent of Mussaendafrondosa Linn, Int. J. Pharma Bio Sci. 4 (3) (2014) 226–234.
[30] R. Thangam, V. Suresh, M. Rajkuma, J.D. Vincent, P. Gunasekaran, C. Anbazhagan, Antioxidant and in vitro anticancer effect of 2-pyrrolidinone Rich fraction of Brassica oleracea var. capitata through induction of apoptosis in human cancer cells, Phytother Res. 27 (2013) 1664–1670, https://doi.org/10.1002/ptr.4908.
[31] P. Thakor, R.B. Subramanian, S.S. Thakkar, A. Ray, V.R. Thakkar, Phytol induces ROS mediated apoptosis by induction of caspase 9 and 3 through activation of TRAIL, FAS and TNF receptors and inhibits tumor progression factor Glucose 6 phosphate dehydrogenase in lung carcinoma cell line (A549), Biomed. Pharmacother. 92 (2017) 491–500, https://doi.org/10.1016/j.biopha.2017.05.066.
[32] L. Zhang, J. Ji, X.Y. Zhu, Y. Wu, H. Yu, B. Zhang, Palmitic acid induces apoptosis in human hepatoma cell line, HepG2 cells, Acta Acad. Med. Sin. 26 (2004) 671–676.
[33] B. Shi, R. Cao, W. Fan, L. Guo, Q. Ma, X.I. Chen, Design, synthesis and in vitro andin vivo antitumor activities of novel bivalent β-carbolines, Eur. J. Med. Chem. 60 (2013) 10–22.
[34] R.A. Hussein, A.A. El-Anssary, Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants, in: P.F. Builders (Ed.), Herbal Medicine, Intech Open, London, United Kingdom, 2019, pp. 11–30, https://doi.org/10.5772/intechopen.76139.
[35] B.R. Kim, H.M. Kim, C.H. Jin, S.Y. Kang, J.B. Kim, Y.G. Jeon, K.Y. Park, I.S. Lee, A.R. Han, Composition and antioxidant activities of volatile organic compounds in radiation-bred coreopsis cultivars, Plants 9 (2020) 717, https://doi.org/10.3390/plants9060717.
[36] L.C. Wu, H.W. Hsu, Y.C. Chen, C.C. Chiu, Y.A. Lin, Antioxidant and antiproliferative activities of red pitaya, Food Chem. 95 (2) (2006) 319–327.
[37] B.R. Kumar, A. Anupam, P. Manchikanti, A.P. Rameshbabu, S. Dasgupta, S. Dhara, Identification and characterization of bioactive phenolic constituents, anti-proliferative, and anti-angiogenic activity of stem extracts of *Basella alba* and *rubra*, J. Food Sci. Technol. 55 (5) (2018) 1675–1684, https://doi.org/10.1007/s13197-018-3079-0.
[38] E. Umapathy, E.J. Ndebia, A. Meeme, B. Adam, P. Menziwa, B.N. Nkeh-Chungag, J.E. Iputo, An experimental evaluation of Albucasetosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation, J. Med. Plants Res. 4 (9) (2010) 789–795, https://doi.org/10.5897/JMPR10.056.
[39] O.O. Oyedapo, A.J. Famurewa, Antiprotease and membrane stabilizing activities of extracts of fagarazanthoxyloides, olaxsubscorpioides and tetrapleuratetraptera, Int. J. Pharmacogn. 33 (1) (1995) 65–69, https://doi.org/10.3109/13880209509088150.
[40] K. Mazumder, A. Nabila, A. Aktar, A. Farahnaky, Bioactive variability and in vitro and in vivo antioxidant activity of unprocessed and processed flour of nine cultivars of Australian lupin species: a comprehensive substantiation, Antioxidants 9 (2020) 282, https://doi.org/10.3390/antiox9040282.
[41] K. Krishnamoorthy, P. Subramaniam, Phytochemical profiling of leaf, stem, and tuber parts of Solenaamplexicaulis (Lam.) gandhi using GC-MS, Int. Scholarly Res. Not. (2014), https://doi.org/10.1155/2014/567409.
[42] C.R. Pawar, R.E. Mutha, A.D. Landge, R.B. Jadhav, S.J. Surana, Antioxidant and cytotoxic activities of Caesalpiniapulcherrima wood, Indian J. Biochem. Biophys. 46 (2) (2009) 198–200.
[43] D.E. Pires, T.L. Blundell, D.B. Ascher, pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures, J. Med. Chem. 58 (2015) 4066–4072, https://doi.org/10.1021/acs.jmedchem.5b00104.
[44] S.B. Olasupo, A. Uzairu, G. Shallangwa, S. Uba, QSAR modeling, molecular docking and ADMET/pharmacokinetic studies: a chemometrics approach to search for novel inhibitors of norepinephrine transporter as potent antipsychotic drugs, J. Iran. Chem. Soc. 17 (2020) 1953–1966, https://doi.org/10.1007/s13738-020-01902-5.
[45] D. Yuliana, F.I.A. Bahtiar, Najib, Inscilico screening of chemical compounds fromroselle (*Hibiscus sabdariffa*) an angitotensin I-inhibitor converting enzyme inhibitor used PyRx program, ARPN J. Sci. Technol. 3 (2013) 1158–1160.
[46] Y. Fukunishi, H. Nakamura, Prediction of ligand-binding sites of proteins by molecular docking calculation for a random ligand library, Protein Sci. 20 (2011) 95–106, https://doi.org/10.1002/pro.540.