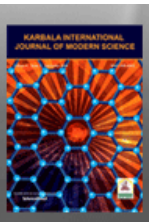
Karbala International Journal of Modern Science

Manuscript 3365

Antioxidant, photoprotective, and cytotoxic activities of Tristaniopsis merguensis leaf fractions with molecular docking study of potential fraction

Boima Situmeang

Respati Tri Swasono

Tri Joko Raharjo

Antioxidant, photoprotective, and cytotoxic activities of Tristaniopsis merguensis leaf fractions with molecular docking study of potential fraction

Abstract

Through experimental along with molecular docking techniques of potential fraction, this study intended to evaluate the essential phytochemical elements of Tristaniopsis merguensis leaflets as well as potential antioxidant, photoprotective, along with cytotoxic activities. The DPPH and ABTS tests were used to evaluate the antioxidant efficacy of the n-hexane (HPF), ethyl acetate (EPF), and methanol (MPF) fractions. Using an in-vitro solar protection factor (SPF) assessment, the photoprotective ability of T. merguensis components against UV damage was examined. Subsequently, invitro cytotoxic research were carried out against MCF-7 cell line (breast cancer line) assay. This was followed by molecular docking stimulation testing against HER-2 protein through in-silico of potential cytotoxic fraction and phytochemical analysis of potential fraction using LC-HRMS instrument. Based on the results, MPF was categorized as having a very strong antioxidant activity with an IC50 value of 3.31 ± 0.06 µg/mL, whereas EPF showed a significant cytotoxic impact targeting MCF-7 malignant cells at an IC50 value of 25.85 µg/mL.Photoprotective abilities of EPF and MPF showed significant value in the development of new formulation sources. The existence of several secondary metabolites was also demonstrated by the LC-HRMS analytical findings. Asiatic acid (7) showed stable protein-ligand complex interaction with HER-2 protein at binding energy -9.1 kcal/mol. The binding energies of asiatic acid were -5.87 kcal/mol as well as -9.1 kcal/mol, respectively, lower than those of the natural ligand (3rcd).

Keywords

Tristaniopsis merguensis; antioxidant; photoprotective; cytotoxic

RESEARCH PAPER

Antioxidant, Photoprotective, and Cytotoxic Activities of *Tristaniopsis merguensis* Leaf Fractions With Molecular Docking Study of Potential Fraction

Boima Situmeang [a,b], Respati T. Swasono [a], Tri J. Raharjo [a,*]

[a] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia
[b] Department of Chemistry, Sekolah Tinggi Analis Kimia Cilegon, Banten, Indonesia

Abstract

This paper evaluated the essential phytochemical components of *Tristaniopsis merguensis* leaves and their antioxidant, photoprotective, and cytotoxic activities through experimental and molecular docking techniques. The antioxidant efficacy of n-hexane (HPF), ethyl acetate (EPF), and methanol (MPF) fractions was assessed using DPPH and ABTS tests. The photoprotective ability of *T. merguensis* components against UV damage was examined using an *in vitro* solar protection factor (SPF) assessment. *In vitro* cytotoxic studies were conducted on the MCF-7 breast cancer cell line, followed by molecular docking simulations against the HER-2 protein with TAK-285 as the native ligand. Meanwhile, phytochemical analysis was performed using LC-HRMS. The results showed that MPF exhibited very strong antioxidant activity with an IC_{50} value of 3.31 ± 0.06 μg/mL in DPPH and 13.745 ± 0.14 μg/mL in ABTS, while EPF demonstrated significant cytotoxic effects on MCF-7 cells with an IC_{50} value of 25.85 μg/mL. Both EPF and MPF displayed significant photoprotective properties, highlighting their potential in new formulation development. LC-HRMS analysis revealed the presence of several secondary metabolites. Asiatic acid (7) formed a stable protein-ligand complex with HER-2 protein, showing a binding energy of −9.1 kcal/mol, compared to the binding energy of the native ligand (TAK-285) at −5.87 kcal/mol.

Keywords: *Triptaniopsis merguensis*, Antioxidant, Photoprotective, Cytotoxic

1. Introduction

Tristaniopsis merguensis, belonging to *Myrtaceae* family, is naturally grown in the rainforest of Namang city, Bangka Island, Indonesia, where it is locally known as *pelawan*. The *Myrtaceae* family consists of 155 genera and 4000 species [1,2]. This plant species contains numerous bioactive compounds such as phenolics, flavonoids, triterpenoids, steroids, glycosides, saponins, and tannins [3]. *T. merguensis* leaves have economic importance mainly between local producers. Also, this leaf has been recognized for its high nutritional value and rich sources of bioactive compounds. These bioactive compounds contribute to *T. merguensis* biological activity, including antimicrobial, antidiabetic, antioxidant, anti-inflammatory, wound healing, and antidiarrhea activity [4]. As a result, *T. merguensis* has been employed as an antipyretic, diuretic, and cough suppressant as conventional medicine [5].

Although the leaves of *T. merguensis* is widely used in Bangka Belitung Island both as traditional medicine and tea, little scientific study has been published on its biological activity, including tests for antioxidant, photoprotective, along with citotoxity actions against MCF-7 cell lines. Roanisca et al. (2019) reported that the acetone extract of *T. merguensis* leaf contained a total phenolic content of 215.22 mg GAE/g and exhibited an antioxidant capacity with an IC_{50} value of 22.15 μg/mL [2].

Received 9 May 2024; revised 25 July 2024; accepted 29 July 2024.
Available online 28 August 2024

* Corresponding author.
E-mail address: trijr_mipa@ugm.ac.id (T.J. Raharjo).
Peer review under responsibility of University of Kerbala.

https://doi.org/10.33640/2405-609X.3365

Although previous investigations reported that the secondary compounds possess antibacterial and antioxidant activity, the medicinal properties have not been explored. Therefore, this research was conducted using *in vitro* antioxidant, photoprotective, and cytotoxic as well as *in silico* methods to assess the cytotoxity activity of *T. merguensis* leaves fractions against HER-2 breast cancer protein.

2. Materials and methods

2.1. Materials

In Namang City, Bangka Belitung, Indonesia, twelve kilograms of *T. merguensis* leaves were gathered. The chemicals utilized were DPPH (Sigma Aldrich), n-hexane, ethyl acetate, and methanol, as well as 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). Additionally, the materials used included, Trolox, 1.5 mL microtube (Merck Gene follower MCTB015), 15 mL tube (Merck Biologix 109151), 75 ml T-flask (Merck NEST 708001), 96 well plate (Merck NEST 701001), and Dimethyl sulfoxide (DMSO) (Merck D1435). The Chemicals utilized were phosphate buffered saline (PBS), antibiotic (Sigma Aldrich P4333), and cisplatin (EDQM C2210000). Trypan Blue (Sigma Aldrich T-8154), FBS (Gibco 10270-106), Trypsin-EDTA (Gibco 25200-056), and RPMI (Gibco 11875-093) were additional materials needed.

The devices used included an Ultra High-Performance Li connected to an untargeted superior performance Mass Spectroscopic (Thermo Scientific Dionex Ultimate 3000) RSLC Nanotechnology UHPLC. This device was then connected to the a Thermo Fisher Scientific Q Extraction (Thermo Fisher Scientific, Massachusetts, USA), microscope (EVOS XL Core, Thermo Scientific), centrifuge (microCL17), CO_2 incubator (series 8000DH), and multiple modes of reader (Tecan Infinite M200 PRO).

2.2. Determination and fractionation of plant

T. merguensis leaves were identified and deposited in the Laboratory of Plant Systematics at Gadjah Mada University, Special Region of Yogyakarta, Indonesia with a voucher specimen (No. 0262/S. Tb/II/2023). The leaves were collected and dried at 25 °C. 4.3 dry powdered samples were then fractionated using n-hexane, ethyl acetate, and methanol. After that, these fractions were filtered using 15–20 μm pore size filter paper, followed by evaporation under reduced pressure.

2.3. Qualitative test for T. merguensis fractions

The colorimetric approach was employed in a qualitative test to identify metabolites that were secondary in the MPF, EPF, and HPF samples. Subsequently, several experiments were conducted using phytochemical testing methods to identify alkaloids, phenols, tannins, flavonoids, steroids, and terpenoids [6].

2.4. Total phenolic content (TPC)

Five milliliters of 7.5% Folin-Ciocalteu were added to one milliliter of each extract and fraction solution, totaling 250 μg/mL. The solution was allowed to stand for a period of 8 min before four milliliters of 1% NaOH was added. The mixture was then incubated for a further hour. Basic solutions containing 60, 50, 40, 30, and 20 μg/mL of gallic acid were prepared. The coefficient of absorption at the wavelength of 743 nm was measured using spectrophotometry [7,8].

2.5. Total flavonoid content (TFC)

2.8 mL of water, 0.1 mL of 1% $AlCl_3$, 0.1 mL of 1 M Na acetate, 1.5 mL of ethanol, and a total of 250 μg/mL of extract and fraction were added to each 0.5 mL of extract and fraction. Standard solutions were prepared using 80, 70, 60, 50, and 40 μg/mL of quercetin. After shaking and digesting the solutions for a period of 30 min at the ambient temperature, the absorbance of the solution was checked at 426 nm [9].

2.6. DPPH radical scavenging assay

DPPH radical's scavenging potential of *T. merguensis* leaf fractions was evaluated using Quang et al.'s methodology with slight modifications [10]. The reaction mixture consisted of 600 μL of methanol DPPH solution (1000 μg/mL) and 2400 μL of *T. merguensis* fractions at varying concentrations. The absorbance ability of each treatment was measured at 515 nm following a 30-min incubation period at room temperature. Ascorbic acid (vitamin C) and Trolox were used as the positive control, while the graph's IC_{50} was visually obtained by charting the fraction quantity against the inhibitory percentage. The IC_{50} value was determined by plotting the proportion of DPPH free radicals that were scavenged against the concentration of test sample concentration. The half-maximum inhibitory concentration, or IC_{50}, has been employed to quantify a

sample's capacity to block a procedure; low IC_{50} denotes significant antioxidant activity [11].

2.7. ABTS radical scavenging activity

The ABTS radical cation ($ABTS^{\bullet+}$) bleaching test was used to measure the fractions' capacity to scavenge free radicals [12]. A spectrophotometer was utilized to measure the concentration of $ABTS^{\bullet+}$ after the freshly made solution had been mixed with water that was deionized. Subsequently, the samples were prepared in methanol at various concentrations ranging from 20 μg/mL to 100 μg/mL). Trolox and ascorbic acid in 2, 4, 6, 8, and 10 μg/mL dosage were used as the standards [(A control − A sample)/A control] × 100 was the formula used to determine the ABTS inhibition percentage. The A control represented the absorbance of the control reaction (which contained all components except the test sample), while the A sample represented the absorbance of test sample. The amount of the specimen dosage needed for ABTS•+ to neutralize 50% of the free radical is shown by the IC_{50} values [13].

2.8. Photoprotective activity

Using in-vitro SPF testing, the photoprotective potential of *T. merguensis* ingredients upon UV damage was examined. Sample absorbance values were measured using a UV spectrophotometer (Thermo Scientific) with a 290–320 nm wavelength spectrum [14]. The sample had been diluted to 2 mg per milliliter in 100% methanol. The SPF was calculated using the following equation.

$$SPF = CF \times \sum_{320}^{290} EE\,(\lambda) \times I \times Abs(\lambda)$$

The sun intensity spectrum is denoted by I(λ), while the erythemal effect spectrum is represented by EE(λ). Abs (λ) stands for absorption, and CF (CF = 10) is the correction factor. Furthermore, the values of EE (λ) × I (λ) remain constant [15].

2.9. Cytotoxic activity determination

The PrestoBlue® assay was utilized to assess the cytotoxicity of HPF, EPF, along with MPF using a cell viability test [16]. Initially, the cells were cultured in a medium obtained from RPMI and enhanced with 10% (v/v) FBS and 1 μL/1 mL antibiotics (1% Penicillin-Streptomycin). On 96 multi-well culture plates, MCF-7 cells were placed at a dosage of 1.7 × 104 cells/well. It was subsequently maintained at 37 °C in a moist condition with five percent carbon dioxide. Following a 24-h period, fresh samples with varying doses of 7.81, 15.63, 31.25, 62.50, 125.00, 250.00, 500.00, and 1000.00 μg/mL were added to the medium, and then control was added. The sample was then added to the PrestoBlue® solution (resazurin dye) and processed for 48 h. At 570 nm, the results of the PrestoBlue® test were extracted employing a multimode reader, and the IC_{50} concentrations were ascertained using the GraphPad Prism program and the linear regression approach. The absorbance in control represents 100% viability, and the IC_{50} value relates to the quantity of fractions that reduces cell survival by 50% [17].

2.10. Targeted liquid chromatography-high resolution mass spectrometry (LC-HRMS)

Targeted high-performance MS and Thermo Scientific Dionex Ultimate 3000 RSLCnano UHPLC System, in conjunction with the Scientific Q Extractive (Thermo Fisher Scientific, Massachusetts, USA) ultra-high-performance LC were utilized to examine the EPF and MPF samples. HRMS was conducted using Acclaim™ Vanquish C18 Dim (mm) 150 × 2.1, with 40 μL/min flow velocity, 5 μL injection size, and a solvent slope over a 25-min observation period. Mobile stage A and B consisted of 0.1 percent formic acid in water and formic acid in methanol, respectively. The gradient was coded as follows: 60% of mobile stage B was achieved in 15 min, 95% in 22 min, 95% in 25 min, 5% in 25.1 min, and 5% in 30 min. Then, test were conducted utilizing heated electrospray and positive ionization with parallel reaction monitoring at a resolution of 35,000 FWHM, and the data analysis was performed using Thermo Scientific software [18,19].

2.11. Molecular docking method

The molecular docking protocol in this study was conducted according to the procedure previously reported, with some minor modifications. The HER-2 receptor from *Homo sapiens* was prepared using Chimera. Initially, non-standard residues such as water molecules and native ligands were removed from the PDB (3RCD) file. Hydrogen atoms were then added to the protein to make it suitable for docking [20]. The 3D structures of secondary metabolites (1–10) were drawn using GaussView 5.0 and were optimized using the AM1 method in Gaussian-09 Revision D.01. The optimized data were saved in PDB format. Redocking was performed using AutoDock4 with a grid box of 50 × 50 × 50 Å and 100 runs of the Lamarckian Genetic Algorithm (LGA). The method was deemed

acceptable for further docking analysis if the RMSD value was less than 2 Å. The 2D structures of the secondary metabolites (1–10) are shown in Fig. 5. The same parameters as the redocking analysis (grid map size and LGA) were used. All compounds were docked into the receptor binding sites [20,21].

2.12. Statistical analysis

The one-way ANOVA test was used in statistical evaluation, and data representation was performed using the GraphPad Prism 10.1.2 software.

3. Results

3.1. Phytochemical screening

The phytochemical evaluation of HPF, EPF, and MPF of *T. merguensis* leaf is displayed in Table 1. Based on the results, HPF was found to contain alkaloids, flavonoids, triterpenoids, and steroids. EPF contained alkaloids, flavonoids, triterpenoids, steroids, and phenolic/tannins, while MPF had alkaloids, flavonoids, triterpenoids, and phenolic/tannins. These findings, which demonstrated the presence of secondary metabolites in leaves, were in conformity with other studies.

3.2. Total phenolic content, total flavonoid content, and antioxidant activity assay

MPF of *T. merguensis* leaves revealed the highest flavonoid content compared to HPF and EPF, suggesting the need for further investigation. Table 2 displays the results of TPC and TFC study for HPF, EPF, and MPF. Employing DPPH techniques, the effectiveness of antioxidants was measured *in vitro* then contrasted with ascorbic acid, the appropriate reference. The results showed that the MPF value of *T. merguensis* leaf had the greatest concentration of detoxifying free radicals when compared to HPF, EPF, and ascorbic acid. Additionally, Figs. 1 and 2 display the percentages of DPPH and ABTS free radical scavenging, respectively.

Table 1. Phytochemical screening of HPF, EPF, and MPF of T. merguensis.

Bioactive compounds	Reagent	HPF	EPF	MPF
Alkaloids	Dragendrof, wagner, meyer	+	+	+
Flavonoids	$AlCl_3$ 5 %	+	+	+
Triterpenoids	Liebermann-Burchard	+	+	+
Steroids	Liebermann-Burchard	+	+	−
Phenolic/tannins	$FeCl_3$ 5 %	−	+	+

+ = present, − = absent.

3.3. Photoprotective activity

UV spectrophotometry was utilized in this study to evaluate the SPF value of *T. merguensis* fractions in relation to two commercial sunscreens and established standards. Table 3 displays the computed SPF at a dosage of 2 mg/mL. Both MPF and EPF had values of 74.19 and 55.78, respectively, indicating their potential as natural sources of UV protection.

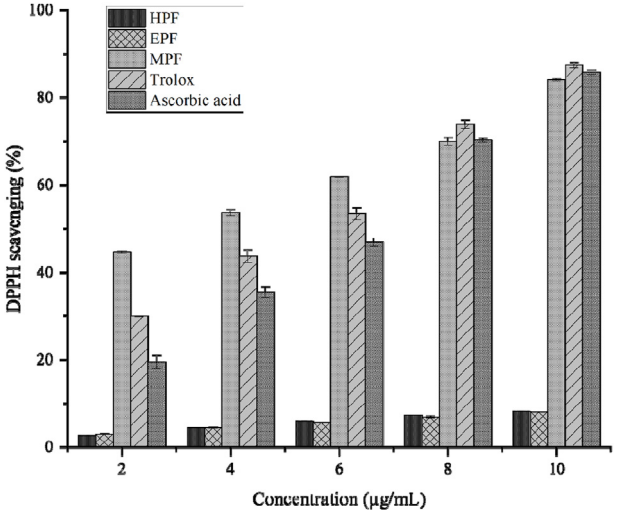

*Fig. 1. The radical scavenging activity of DPPH in ascorbic acid, HPF, EPF, MPF, and Trolox. The data is presented as the mean (n = 3) plus the standard deviation. The threshold for statistical significance was *p < 0.05.*

Table 2. TPC, TFC, and antioxidant activity assay of HPF, EPF, and MPF.

Sample	TPC (mgGAE/g ext)	TFC (mgQAE/g ext)	DPPH (IC_{50} µg/mL)	ABTS (IC_{50} µg/mL)
HPF	13.67 ± 1.7	38.22 ± 1.5	91.092 ± 0.44[a]	79.737 ± 0.03
EPF	128.67 ± 4.7	247.11 ± 4.2	43.386 ± 2.09[b]	54.741 ± 0.08
MPF	420.33 ± 2.4	278.22 ± 1.6	3.313 ± 0.06[b]	13.745 ± 0.14
Ascorbic acid	–	–	5.817 ± 0.05[b]	7.71 ± 0.04[b]
Trolox	–	–	9.590 ± 0.05[b]	4.950 ± 0.05

Note: Concentration sample (MPF and Ascorbic acid) 2, 4, 6, 8,10 µg/mL; (EPF) 25, 50, 75, 100, 125 µg/mL; (HPF) 50, 100, 150, 200, 250 µg/mL for evaluation of DPPH radical scavenging activity [22].

[a] Moderate antioxidant activity.

[b] Active antioxidant activity.

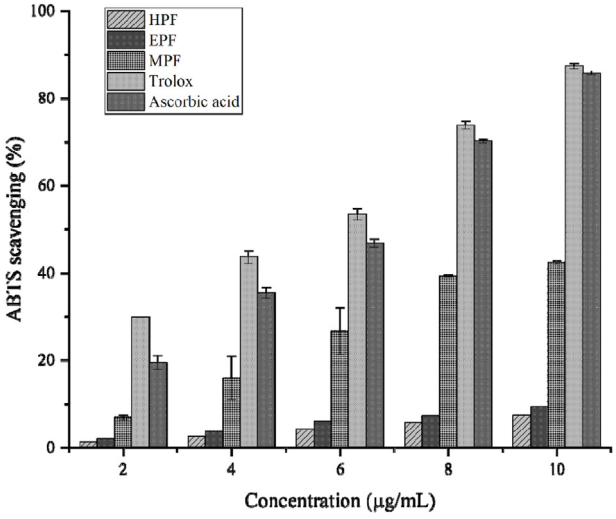

*Fig. 2. Ascorbic acid, HPF, EPF, MPF, and Trolox all exhibit 2,2′-azinobis (ABTS) radical scavenging action. The data are displayed as the mean ± standard deviation (n = 3), with *p < 0.05 indicating statistical significance.*

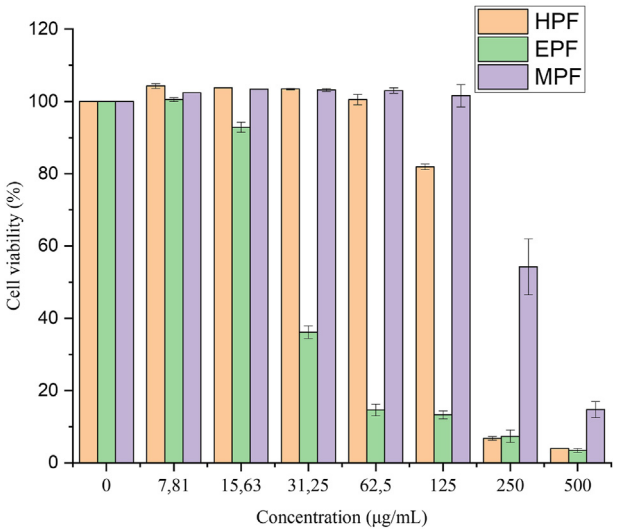

*Fig. 3. The influence of MPF, HPF, and EPF on the longevity of MCF-7 cells. The viability was evaluated using the MTT assay after a one-day treatment period. The data were shown as mean ± SD and the significance threshold was chosen at *p < 0.05.*

Table 3. SPF values of T. merguensis fractions.

λ (nm)	HPF	EPF	MPF
290	3.88 ± 0.04	7.72 ± 0.04	9.28 ± 0.03
295	4.60 ± 0.05	8.25 ± 0.05	11.53 ± 0.17
300	8.07 ± 0.05	9.77 ± 0.05	21.01 ± 0.04
305	6.80 ± 0.04	11.54 ± 0.05	10.43 ± 0.03
310	4.57 ± 0.05	7.63 ± 0.05	9.02 ± 0.04
315	2.86 ± 0.04	6.18 ± 0.05	8.17 ± 0.03
320	1.62 ± 0.05	4.69 ± 0.09	4.75 ± 0.04
Total	32.40	55.78	74.19
	Standards SPF for commercial products		
	50.10 ± 0.53		
	44.22 ± 0.34		

SPF: sun protection factor; HPF: n-hexane fraction; EPF: ethyl acetate fraction; MPF: methanol fraction.

3.4. Cytotoxicity assay MCF-7 cell lines

The MCF-7, a breast cancer cell type, was used to assess the cytotoxic effects of HPF, EPF, and MPF, with cisplatin acting as the control agent. Based on the results on percentage cell viability shown in Fig. 3, EPF exhibits the highest amount of cytotoxic effect towards cells, with an IC_{50} value of 25.85 µg/mL.

The morphology cell lines of MCF-7 after incubation are presented in Fig. 4a–c.

3.5. Compounds of EFP and MFP of Triptaniopsis merguensis leaves based on LC-HRMS/MS data

As can be seen in Table 4, LC-HRMS findings for EPF revealed 10 major chemicals: (1) (2R)-5-hydroxy-7-methoxy-2-phenyl-3,4-dihydro-2H-1-(benzopyran-4-one, (2) 5,6-dimethoxy-2-(2-methoxyphenyl)-4H-chromen4-one, (3) 5-hydroxy-6,7-dimethoxy-2-phenyl-4H-chromen-4-one, (4) Myricetin, (5) 7-Methoxy-5,3′,4’ trihydroxyflavanone, (6) oleanolic acid, (7) asiatic acid, (8) betulin, (9) ursolic acid, and (10) siaresinol.

3.6. Molecular docking analysis

A total of ten active compounds were extracted from EPF. The compounds were created by employing the DFT approach with the B3LYP hybrid function as well as 6-31G (d,p) basis set in order to maximize their structure and acquire the most constant energy with Gaussian 09W. The improved structures were then verified using AutoDockTools. Table 5 displays the degree of connection between active substances and putative target proteins for breast cancer. Target proteins used in cancer treatments were utilized for *in silico* assessment of the active chemicals. Furthermore, as Fig. 5 illustrates, the protein code employed in this study was TAK-285 (Her-2) (see Fig. 6).

4. Discussion

HPF included alkaloids, flavonoids, triterpenoid, as well as steroid compounds, according to phytochemical screening. The results also showed that EPF contained alkaloids, flavonoids, triterpenoids, steroids, and phenolic/tannin. Meanwhile, MPF comprised alkaloids, flavonoids, triterpenoids, and phenolic/tannins. As presented in Table 2, TPC and TFC results demonstrated the biological activity of

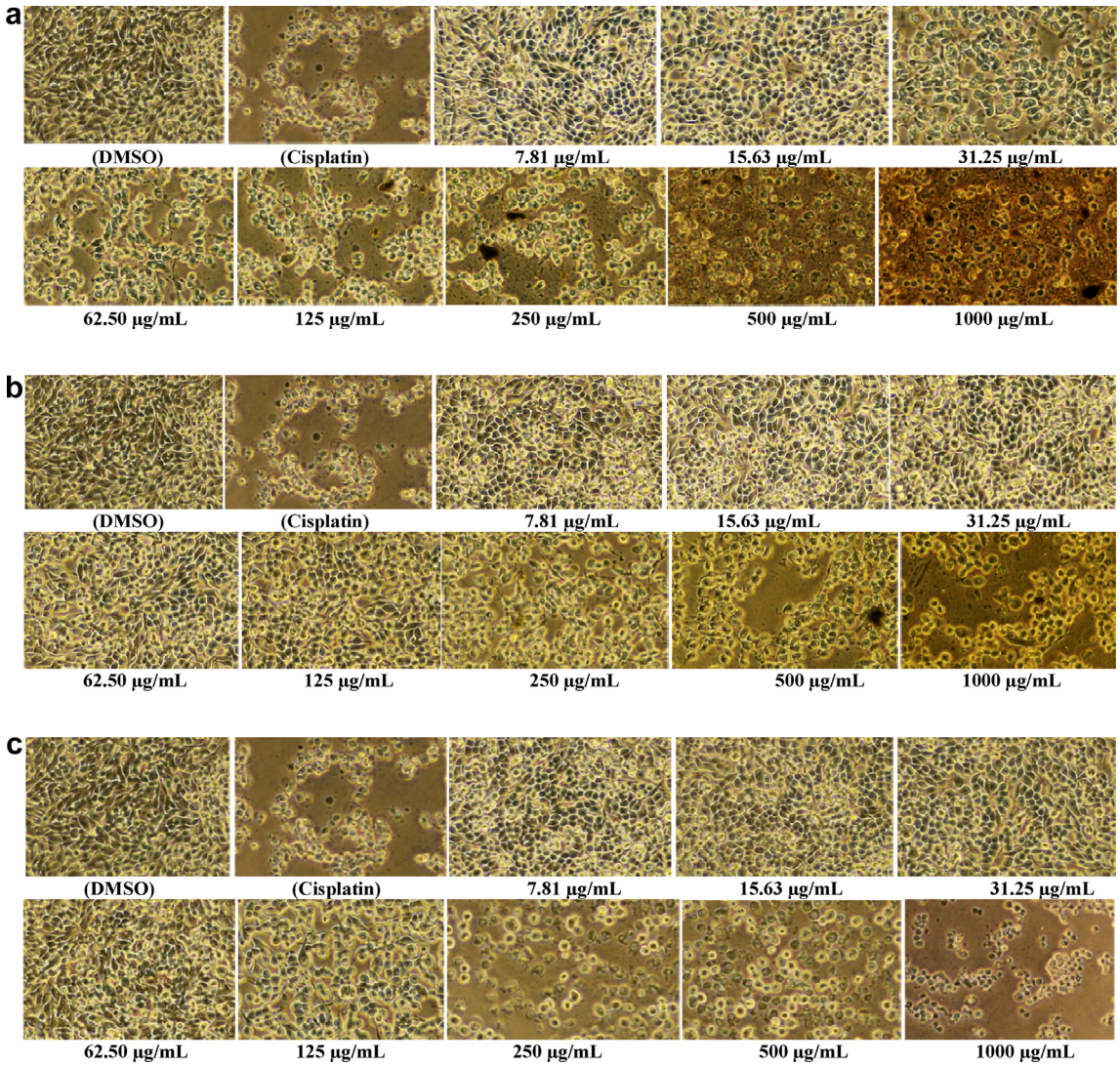

*Fig. 4. **a.** The morphology of MCF-7 cell line of ethyl acetate fraction (EPF) with a scale bar indicating 20 times magnification. **b.** The morphology of MCF-7 cell line of methanol fraction (MPF) with a scale bar indicating 20 times magnification. **c.** The morphology of MCF-7 cell line of n-hexane fraction (HPF) with a scale bar indicating 20 times magnification.*

Table 4. Main constituents detected from EPF of T. merguensis using targeted LC-HRMS.

Compounds	Name	Formula	Calculated Mass	Observed Mass (*m/z*)	RT (min)	Absolute Error (ppm)
1	(2R)-5-hydroxy-7-methoxy-2-phenyl-3,4-dihydro-2H-1-benzopyran-4-one	$C_{16}H_{14}O_4$	270.08873	271.09583	11.226	2.7028
2	5,6-dimethoxy-2-(2- methoxyphenyl)-4H-chromen4-one	$C_{18}H_{16}O_5$	312.09985	313.10617	12.782	4.8382
3	5-hydroxy-6,7-dimethoxy-2- phenyl-4H-chromen-4-one	$C_{17}H_{14}O_5$	298.08419	299.09070	11.143	4.4283
4	Myricetin	$C_{15}H_{10}O_8$	318.03757	319.04419	5.677	3.8046
5	7-Methoxy-5,3′,4′ trihydroxyflavanone	$C_{16}H_{14}O_6$	302.07904	303.08612	8.221	2.4828
6	Oleanolic acid	$C_{30}H_{48}O_3$	456.36059	457.36691	14.597	3.3088
7	Asiatic acid	$C_{30}H_{48}O_5$	488.35019	487.34244	11.498	−1.6393
8	Betulin	$C_{30}H_{50}O_2$	442.38107	443.38779	15.097	2.5092
9	Ursolic acid	$C_{30}H_{48}O_3$	456.36035	457.36710	11.739	2.3666
10	Siaresinol	$C_{30}H_{48}O_4$	472.35526	473.36258	13.665	1.1644

Table 5. Connection affinity between EPF's active ingredients and putative target proteins for breast cancer.

Compounds	Binding Energy (kcal/mol)	RMSD	H-Bonding	other interaction
TAK-285	−5.87	1.19	Met801, Asp863	Leu796, Leu852, Phe864, Thr862
(2R)-5-hydroxy-7-methoxy-2-phenyl-3,4-dihydro-2H-1-benzopyran-4-one	−6.71	0.98	Met801	Thr798, Gln799, Leau800, Gly804, Cys805, Asp863, Thr862
5,6-dimethoxy-2-(2- methoxyphenyl)-4H-chromen4-one	−7.26	0.45	Lys753, Thr 798, Ser783	Ala751, Ile752, Thr862, Val797, Arg784, Met774, Phe864, Asp863, Leu852
5-hydroxy-6,7-dimethoxy-2- phenyl-4H-chromen-4-one	−8.56	0.18	Ser783, Thr862	Leu785, Gly727, Asp863
Myricetin	−6.64	0.22	Asp863, Lys753	Gly804, Phe1004, Met801, Thr862, Leu796, Thr798, Leu785, Val797, Ile752
7-Methoxy-5,3′,4′ trihydroxyflavanone	−7.86	0.4	Ser783, Thr862	Gly727, Leu726, Thr798, Asp863
Oleanolic acid	−7.74	0.37	Glu770, Gly865, Lys753, Asp863, Thr862, Thr798, Ser783	Ile767, Phe731, Arg784, Met774, Phe864
Asiatic acid	−9.1	0.43	Ala751, Leu796, Leu785	Ser783, Thr798, Ile752, Val797, Thr862, Asp863, Asn850, Val851, Arg849
Betulin	−8.73	0.21	Thr862, Ser783	Leu785, Thr798, Asp863
Ursolic acid	−8.07	0.54	Asp863, Lys753	Asp808, Thr862, Met801, Gly727
Siaresinol	−7.3	0.64	Met801, Asp863, Lys753	Thr862

total phenolic as free radical scavengers and antioxidants. The study found that the redox properties of *T. merguensis* leaves, which acted as singlet oxygen quenchers, reducing compounds, and donations of hydrogen, were the main source of the leaves' antioxidant activity. The greatest phenolic concentration was found in *T. merguensis* leaves, with MPF measuring 420.33 ± 2.4 mg/GAE.g^{-1}, EPF came in second with 128.67 ± 4.7 mg GAE.g^{-1}, and HPF came in third with 13.67 ± 1.7 mg GAE.g^{-1}.

Flavonoid is among the largest natural phenolic compounds, commonly found in the extract and fractions of all plants. Table 2 exhibits that MPF had the highest flavonoid content of 278.22 ± 1.6 mgQE.g^{-1}, followed by EPF at 247.11 ± 4.2 mgQE.g^{-1}, and HPF at 38.22 ± 1.5 mgQE.g^{-1}.

The DPPH free radicals has been employed to test antioxidant activity [23]. It measures the change in violet color caused by electron transfer. At room temperature, it is a stable free radical that creates a diamagnetic molecule by accepting electrons or other free radicals, with the highest absorbance of 517 nm due to the existence of an odd electron. As antioxidant molecules quench DPPH free radicals and lower absorbance, the freshly generated DPPH solution transform from deep blue to colorless. Table 2 demonstrates the potent antioxidant activity of MPF and EPF, with IC_{50} levels of 3.313 ± 0.059 ppm and 43.386 ± 2.090 ppm, respectively. Tajammal et al. [20] classified an antioxidant as extremely potent if its IC_{50} value was less than 50 ppm. The IC_{50} value of ascorbic acid used as a positive control in this study was observed at 5.817 ± 0.045 ppm, indicating very significant antioxidant activity. Furthermore, with a score of 91.092 ± 0.435 ppm, HPF was classified as having significant antioxidant actions.

MPF had the strongest antioxidant activity, according to the ABTS assay, with an IC_{50} of 5.498 μg/mL. EPF and HPF came in second and third, respectively, at 54.737 and 79.371 μg/mL. The Trolox as positive control had antioxidant activity of 4.950 μg/mL, indicating a very strong category, while HPF and EPF exhibited strong radical scavenging capacity. In comparison, Trolox had higher antioxidant activity than MPF and EPF, while HPF showed significantly different results in DPPH and ABTS. A favorable association was seen in the investigation of the correlation between the radical scavenging activity toward TPC and TFC, and the elevated concentrations of total phenolic in MPF were shown to play a major role as antioxidants [24,25]. Furthermore, other phenolic compounds such as tannin, alkaloid, and terpenoid contributed as antioxidant [26].

The SPF was evaluated in this study utilizing the methodology proposed by Mansur. Only protection against erythemally efficacious solar UV, which was mostly constrained to UVB (290–320 nm) wavelengths, was indicated by SPF [27]. A UV spectrophotometer was employed for the analysis, and Table 3 exhibits the SPF values. To effectively prevent skin damage, sunscreen should absorb a broad spectrum, ranging from 290 to 320 nm. Phenolic and flavonoid compounds have the ability to generally suppress UV-induced lipid peroxidation and the oxygen-free radicals production associated with

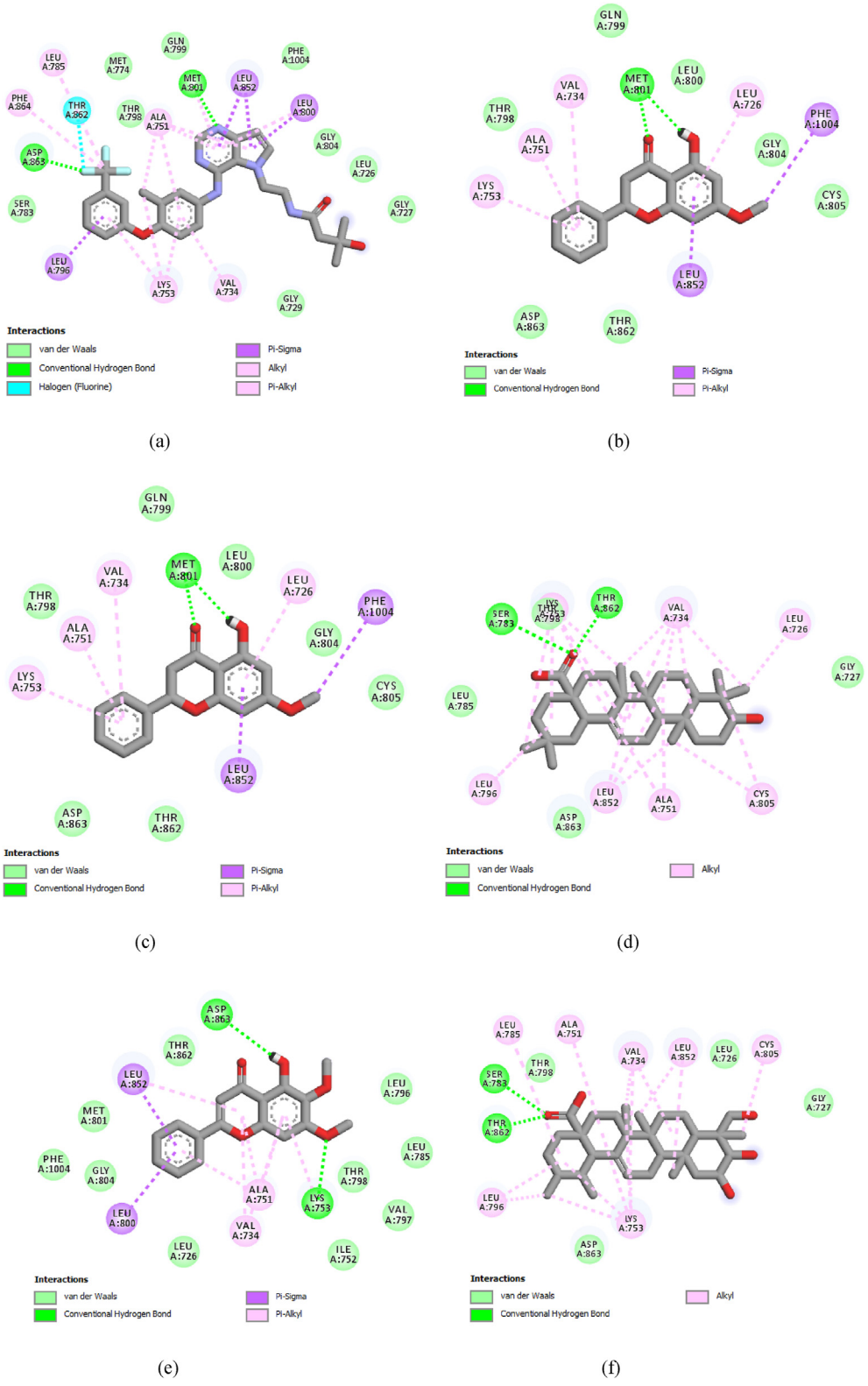

Fig. 5. 2-D molecular docking interactions between active drugs and target proteins in breast cancer: (a) Native ligand TAK-285, (b) (2R)-5-hydroxy-7-methoxy-2-phenyl-3,4-dihydro-2H-1-benzopyran-4-one, (c) 5,6-dimethoxy-2-(2- methoxyphenyl)-4H-chromen4-one, (d) 5-hydroxy-6,7-dimethoxy-2-phenyl-4H-chromen-4-one, (e) myricetin, (f) 7-Methoxy-5,3′,4′ trihydroxyflavanone, (g) oleanolic acid, (h) asiatic acid, (i) betulin, (j) ursolic acid, and (k) siaresinol.

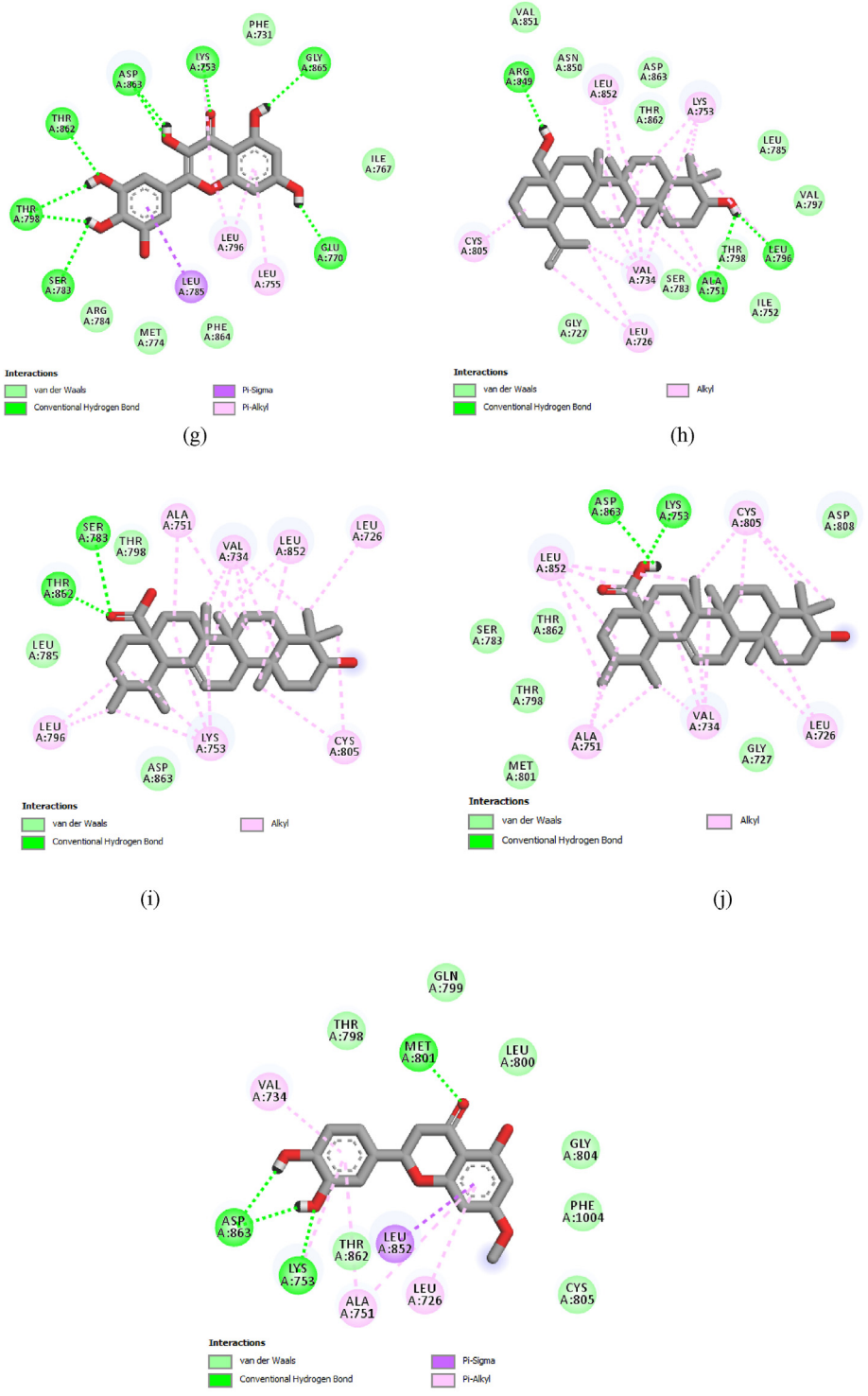

Fig. 5 (Continued).

pathological illnesses, hence preserving DNA from damage [28]. Based on the results, a positive correlation was observed between SPF as well as phenolic and flavonoid content of *T. merguensis* fractions. With SPF values of 55.78 ± 0.05 or 74.19 ± 0.04, respectively, EPF and MPF demonstrated a noteworthy capacity for photo-screening; these values are deemed high when compared to

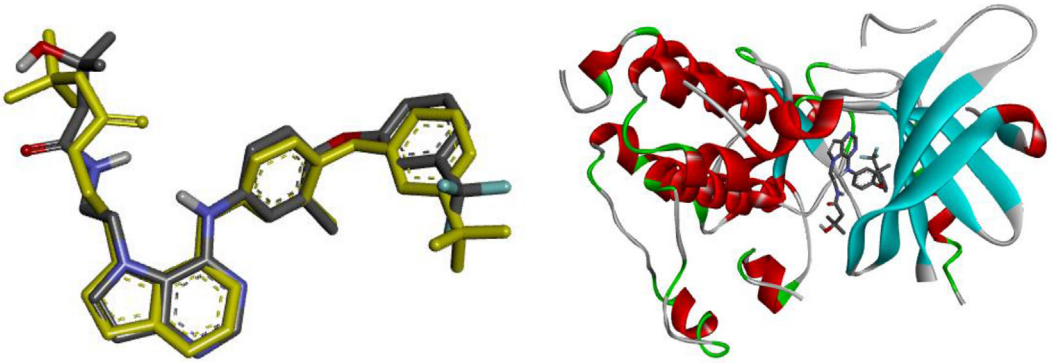

Fig. 6. The superimposed structure of TAK-285 as the native ligand before (gold) and after (black) re-docking process; (b) The visualization of chemical interactions between TAK-285 with the active site of HER-2 enzyme.

the SPF values of commercial sunscreens (refer to Table 3). These fractions also revealed significant antioxidant activity with TPC, TFC, and SPF value, indicating superior effectiveness compared to other commercial sunscreen products [29].

The process of photolysis involves the creation of singlet oxygen, which can cause permanent changes in skin proteins, resulting in tissue photodegradation alongside other negative consequences [30]. This finding makes sense given that EPF contains a large number of phenolic and flavonoid compounds which are known to act as photoprotective agents against UV radiation [31]. As a result, EPF and MPF of *T. merguensis* can be used in sunscreen compositions. These findings are in line with those of previous studies on crude extracts with antioxidant capacity produced from the *Myrtaceae* family. According to another study, plants' chemical composition, particularly their flavonoid and phenolic content, affects their ability to absorb UV light [32]. Thus, *T. merguensis's* antioxidant and photoprotective properties have significance for the creation of novel photoprotective formulations.

Using cisplatin as a favorable control, the cytotoxic activity of HPF, EPF, along with MPF towards the MCF-7breast cancer cell line was assessed. With an IC_{50} value of 25.85 μg.mL-1, the analysis results presented in Fig. 1 demonstrates that EPF had the best cytotoxic activity and was placed in the strong category [33]. The EPF value indicates that the addition of triterpenoid and flavonoid components enhanced cytotoxic action based on the LC-HRMS data. Additionally, as demonstrated in Fig. 2, MPF and HPF with IC_{50} values of 251.19 and 154.90 μg. mL^{-1}, respectively, had mediocre effectiveness against MCF-7 cell lines. The correlation analysis of radical scavenging activity against cytotoxic activity showed a weak positive correlation.

LC-HRMS findings for EPF (Table 4) revealed ten chemicals. Based on the polarity of ethyl acetate, the semi-polar compounds such as flavonoid, phenolic, and triterpenes can be solved [34]. According to *in silico* research, asiatic acid and ursolic acid compounds showed significant potential to improve cytotoxic activity.

The molecular docking analysis of EPF compounds was shown in Table 5, while the interaction of hydrogen bond was presented in Fig. 5. Utilizing the 6-31G (d, p) foundation set and the DFT method, all molecules were synthesized having B3LYP composite functionality, adjusting their structure to reach the best stable energy using Gaussian 09W. These structures were then verified with AutoDockTools. The three compounds from triterpenoid classes, namely asiatic acid (7), betulin (8), and ursolic acid (9) showed good binding energy with −9.1, −8,73, and −8,07 kcal/mol respectively for breast cancer protein target HER-2. According to Pantia et al. [35], asiatic acid has the potential to impede NPC cell viability and motility, and may cause cell death by elevating the production of cleaved caspase-3. In NPC cells, aspartic acid decreased the production of claudin-1 and prevented the phosphorylation of STAT3. Meanwhile, there was a small reduction in cell viability due to STAT3 or claudin-1 knockdown, suggesting that the anti-proliferative impact did not significantly improve. The results also indicate that asiatic acid might be a viable candidate for NPC-targeting medications since it inhibits the proliferation, migration, and death of human colon cancer cells by regulating Pdcd4 via the PI3K/Akt/mTOR/p70S6K communication pathway. Compared to native ligands (3rcd), asiatic acid has lower binding energy, with −5.87 and −9.1 kcal/mol, respectively.

5. Conclusion

In conclusion, this research demonstrates that the MPF and EPF fractions from *T. merguensis* leaves

exhibit strong antioxidant and cytotoxic activities against MCF-7 cell lines. MPF showed optimal antioxidant activity, particularly in DPPH scavenging and reducing power, surpassing HPF and EPF. The IC_{50} values of MPF and EPF were 3.313 ± 0.059 μg/mL and 43.386 ± 2.090 μg/mL, respectively, in DPPH assays, indicating very strong antioxidant potential. In ABTS assays, MPF and EPF showed IC_{50} values of 13.745 ± 0.14 μg/mL and 54.741 ± 0.08 μg/mL, respectively, further highlighting their robust antioxidant properties. MPF exhibited higher activity than the standard ascorbic acid. Furthermore, EPF and MPF demonstrated significant photoprotective capabilities, suggesting their potential for new formulation development. EPF exhibited superior cytotoxic activity compared to HPF and MPF. Additionally, *in silico* studies revealed that asiatic acid had the lowest binding energy of −9.1 kcal/mol against the HER-2 protein, underscoring its potential therapeutic relevance.

Acknowledgements

The author gratefully acknowledges the scholarship awarded to Boima Situmeang in 2022 by Indonesia Endowment Funds for Education (LPDP) and Center for Higher Education Funding (BPPT), as stated in decree number 02012/J5.2.3./BPI.06/9/2022.

References

[1] G.F. Kusuma, R.G. Mahardika, F.I.P. Sari, Ekstrak batang pelawan (tristaniopsis merguensis Griff.) sebagai antibakteri pada Staphylococcus aureus dan Escherichia coli, Stannum J. Sains Dan Terap. Kim. 4 (2022) 40–46, https://doi.org/10.33019/jstk.v4i2.3063.

[2] O. Roanisca, R.G. Mahardika, F.I.P. Sari, Total phenolic and antioxidant capacity of acetone extract of tristaniopsis merguensis leaves, Stannum J. Sains Dan Terap. Kim. 1 (2019) 10–13, https://doi.org/10.33019/jstk.v1i1.1274.

[3] A.P.G. da Silva, W.G. Sganzerla, A.P. Jacomino, E.P. da Silva, J. Xiao, J. Simal-Gandara, Chemical composition, bioactive compounds, and perspectives for the industrial formulation of health products from uvaia (Eugenia pyriformis Cambess – myrtaceae): a comprehensive review, J. Food Compos Anal. 109 (2022) 104500, https://doi.org/10.1016/j.jfca.2022.104500.

[4] E.E. Aung, A.N. Kristanti, N.S. Aminah, Y. Takaya, R. Ramadhan, H.T. Aung, Anticancer activity of isolated compounds from syzygium aqueum stem bark, Rasayan J. Chem. 14 (2021) 312–318, https://doi.org/10.31788/RJC.2021.1416106.

[5] T. Triadiati, A.R. Hidayanti, N. Sukarno, Growth and development of tristaniopsis merguensis seedling inoculated by natural ectomycorrhiza, J. Trop. Life Sci. 10 (2020) 89–95, https://doi.org/10.11594/jtls.10.02.01.

[6] C. Bitwell, S.I. Sen, C. Luke, M.K. Kakoma, UHPLC-MS/MS phytochemical screening, polyphenolic content and antioxidant potential of Diplorhynchus condylocarpon (Müll.Arg.) Pichon (Apocynaceae), a medicinal plant, Sci. African 20 (2023), https://doi.org/10.1016/j.sciaf.2023.e01712.

[7] A.N. Tavanappanavar, S.I. Mulla, C. Shekhar Seth, Z.K. Bagewadi, M. Rahamathulla, M. Muqtader Ahmed, S. Ayesha Farhana, Phytochemical analysis, GC–MS profile and determination of antibacterial, antifungal, anti-inflammatory, antioxidant activities of peel and seeds extracts (chloroform and ethyl acetate) of Tamarindus indica L, Saudi J. Biol. Sci. 31 (2024) 103878, https://doi.org/10.1016/j.sjbs.2023.103878.

[8] F. Ali, Z. Saddiqe, M. Shahzad, A. Rafi, M. Javed, F. ul Haq, S. Saleem, S. Kusar, Crotalaria medicaginea Lamk.: an unexplored source of anticancer, antimicrobial and antioxidant agents, Eur. J. Integr. Med. 58 (2023) 102226, https://doi.org/10.1016/j.eujim.2023.102226.

[9] H. Rao, I. Rao, L. Saeed, H.Y. Aati, S. Aati, M. Zeeshan, K. ur Rehman Khan, Phytochemical analysis and bioactivity assessment of five medicinal plants from Pakistan: exploring polyphenol contents, antioxidant potential, and antibacterial activities, Saudi J. Biol. Sci. 30 (2023) 103783, https://doi.org/10.1016/j.sjbs.2023.103783.

[10] T.H. Quang, N.X. Cuong, C. Van Minh, P. Van Kiem, New flavonoids from Baeckea frutescens and their antioxidant activity, Nat. Prod. Commun. 3 (2008) 755–758, https://doi.org/10.1177/1934578x0800300515.

[11] B. Ludwaba, M.O. Jimoh, C.P. Laubscher, F. Nchu, Insecticidal and antioxidant potential of volatile compounds and nanoparticles from Tulbaghia violacea Harv. inoculated with endophytic fungus Beauveria bassiana (Bals.-Criv.) Vuill, South African J. Bot. 165 (2024) 246–256, https://doi.org/10.1016/j.sajb.2023.12.028.

[12] S. Bakari, H. Hajlaoui, A. Daoud, H. Mighri, J.M. Ross-Garcia, N. Gharsallah, A. Kadri, Phytochemicals, antioxidant and antimicrobial potentials and LC-MS analysis of hydro-alcoholic extracts of leaves and flowers of Erodium glaucophyllum collected from Tunisian Sahara, Food Sci. Technol. 38 (2018) 310–317, https://doi.org/10.1590/fst.04517.

[13] D.O. Idowu, O.O. Aiyelaagbe, P.A. Idowu, Chemical composition and biological activities of volatile oil of the stem of Dombeya buettneri K. Schum. (Sterculiaceae), Sci. African 20 (2023) 1–10, https://doi.org/10.1016/j.sciaf.2023.e01624.

[14] W.A.d.S. Almeida, A. dos S. Antunes, R.G. Penido, H.S.d.G. Correa, A.M.d. Nascimento, Â.L. Andrade, V.R. Santos, T. Cazati, T.R. Amparo, G.H.B. de Souza, K.M. Freitas, O.D.H. dos Santos, L.R.D. Sousa, V.M.R. do Santos, Photoprotective activity and increase of SPF in sunscreen formulation using lyophilized red propolis extracts from Alagoas, Rev. Bras. Farmacogn. 29 (2019) 373–380, https://doi.org/10.1016/j.bjp.2019.02.003.

[15] T. Roquete Amparo, A. Cherem Peixoto da Silva, J. Brandão Seibert, D. dos Santos da Silva, V. Martins Rebello dos Santos, P. Melo de Abreu Vieira, G. Célio Brandão, G. Henrique Bianco de Souza, B. Aloise Maneira Corrêa Santos, In vitro and in silico investigation of the photoprotective and antioxidant potential of Protium spruceanum leaves and its main flavonoids, J. Photochem. Photobiol. Chem. 431 (2022), https://doi.org/10.1016/j.jphotochem.2022.114037.

[16] N.D. Amalina, M. Suzery, B. Cahyono, Cytotoxic activity of hyptis pectinate extracts on MCF-7 human breast cancer cells, Indones. J. Cancer Chemoprevention 11 (2020) 1, https://doi.org/10.14499/indonesianjcanchemoprev11iss1pp1-6.

[17] Y. Herdiana, N. Wathoni, S. Shamsuddin, M. Muchtaridi, Cytotoxicity enhancement in MCF-7 breast cancer cells with depolymerized chitosan delivery of α-mangostin, Polymers (Basel) 14 (2022), https://doi.org/10.3390/polym14153139.

[18] G.S. Lima, N.M. Lima, J.V. Roque, D.V.A. de Aguiar, J.V.A. Oliveira, G.F. dos Santos, A.R. Chaves, B.G. Vaz, LC-HRMS/MS-Based metabolomics approaches applied to the detection of antifungal compounds and a metabolic dynamic assessment of orchidaceae, Molecules 27 (2022), https://doi.org/10.3390/molecules27227937.

[19] L. Giannetti, V. Gallo, F. Necci, F. Marini, A. Giorgi, E. Sonego, F. D'Onofrio, B. Neri, LC-HRMS analysis of 13 classes of pharmaceutical substances in food supplements, Food Addit. Contam. Part B Surveill. 16 (2023) 253–265, https://doi.org/10.1080/19393210.2023.2214883.

[20] A. Tajammal, A. Siddiqa, A. Irfan, M. Azam, H. Hafeez, M.A. Munawar, M.A.R. Basra, Antioxidant, molecular docking and computational investigation of new flavonoids, J. Mol. Struct. 1254 (2022) 132189, https://doi.org/10.1016/j.molstruc.2021.132189.
[21] A. Gorbatenko, R. Søkilde, E.E. Sorensen, I. Newie, H. Persson, B. Morancho, J. Arribas, T. Litman, C. Rovira, S.F. Pedersen, HER2 and p95HER2 differentially regulate miRNA expression in MCF-7 breast cancer cells and downregulate MYB proteins through miR-221/222 and miR-503, Sci. Rep. 9 (2019) 1–16, https://doi.org/10.1038/s41598-019-39733-x.
[22] W.J.A. Musa, N. Bialangi, A.K. Kilo, B. Situmeang, N.T. Susparini, I.D. Rusydi, Antioxidant, cholesterol lowering activity, and analysis of Caesalpinia bonducella seeds extract, Pharmacia 70 (2023) 97–103, https://doi.org/10.3897/pharmacia.70.e96817.
[23] S.Y. Kim, The antioxidant and anti-complementary activities of crude polysaccharides from trifoliate orange (poncirus trifoliate) seeds, Prev. Nutr. Food Sci. 28 (2023) 321–327, https://doi.org/10.3746/pnf.2023.28.3.321.
[24] Y.J. Lee, H.J. Kang, S.H. Yi, Y.H. Jung, Antioxidant properties of kombucha made with tartary buckwheat tea and burdock tea, Prev. Nutr. Food Sci. 28 (2023) 347–352, https://doi.org/10.3746/pnf.2023.28.3.347.
[25] Mikkili Indira, Karlapudi Abraham Peele, Srirama Krupanidhi, Kodali Vidya Prabhakar, K.B.S. Vimala, P. Satya kavya, Isani Sravya, T.C. Venkateswarulu, In vitro assessment of the bioactive compounds and anticancer potential of citrus medica leaf extract, Trop Life Sci. Res. 34 (2023), https://doi.org/10.21315/tlsr2023.34.3.11.
[26] N. Jiangseubchatveera, C. Saechan, A. Petchsomrit, T. Treeyaprasert, N. Leelakanok, C. Prompanya, Phytochemicals and antioxidant activities of red oak, red coral and butterhead, Trop Life Sci. Res. 34 (2023) 1–17, https://doi.org/10.21315/tlsr2023.34.1.1.
[27] K.B. Wróblewska, A.R. Baby, M.T. Grombone Guaratini, P.R.H. Moreno, In vitro antioxidant and photoprotective activity of five native Brazilian bamboo species, Ind. Crops Prod. 130 (2019) 208–215, https://doi.org/10.1016/j.indcrop.2018.12.081.
[28] J.M.T. de Alencar Filho, P.A. Sampaio, I.S. de Carvalho, A.L. Guimarães, I.A.e. Amariz, E.C.V. Pereira, P.J. Rolim-Neto, L.A. Rolim, E.C. da C. Araújo, Flavonoid enriched extract of Alternanthera brasiliana with photoprotective effect: formulation development and evaluation of quality, Ind. Crops Prod. 149 (2020) 112371, https://doi.org/10.1016/j.indcrop.2020.112371.
[29] N.R. Mohamad, S.S.A. Gani, R.A. Wahab, U.H. Zaidan, In-vitro photoprotective activities of different solvent extraction of hibiscus sabdariffa, Malaysian J. Anal. Sci. 22 (2018) 1078–1083, https://doi.org/10.17576/mjas-2018-2206-18.
[30] J.G. Ferreira Macedo, M. de Oliveira Santos, C. de F.A. Nonato, G.J. Torres Salazar, F.F. Galvão Rodrigues, J.W. Almeida-Bezerra, Â.M. de Miranda Freitas, C.E. Barnes Proenca, J.G. Martins da Costa, M.M. de Almeida Souza, Chemical composition, antioxidant, antibacterial and modulating activity of the essential oil of psidium L. species (Myrtaceae Juss.), Biocatal. Agric. Biotechnol. 42 (2022), https://doi.org/10.1016/j.bcab.2022.102363.
[31] M.M. Cascaes, G.M.S.P. Guilhon, M. das G. Zoghbi, E.H.A. Andrade, L.S. Santos, J. Kelly R. da Silva, A.P. Trovatti Uetanabaro, I.S. Araújo, Flavonoids, antioxidant potential and antimicrobial activity of Myrcia rufipila mcvaugh leaves (myrtaceae), Nat. Prod. Res. 35 (2021) 1717–1721, https://doi.org/10.1080/14786419.2019.1629912.
[32] T.P. Paixão, M.T. Silva, M.A. de Andrade, N.R.B. Raposo, J.L.F. Vieira, Plants from Brazil with potential photoprotective activity: a review, Pharmacogn. Rev. 17 (2023) 204–213, https://doi.org/10.5530/097627870191.
[33] H.M. Mashar, I. Annah, Cytotoxicity of kelakai (stenochlaena palustris) extract to MCF-7 breast cancer cell, J. Fitofarmaka Indones. 7 (2020) 5–9, https://doi.org/10.33096/jffi.v7i3.590.
[34] S. Amari, A. Karbab, N. Charef, L. Arrar, M.S. Mubarak, Anti-urolithiatic, antibacterial, anti-inflammatory and analgesic effects of Erica arborea flowers and leaves hydromethanolic extracts: an ethnopharmacological study, Saudi J. Biol. Sci. 30 (2023) 103785, https://doi.org/10.1016/j.sjbs.2023.103785.
[35] S. Pantia, T. Kangsamaksin, T. Janvilisri, W. Komyod, Asiatic acid inhibits nasopharyngeal carcinoma cell viability and migration via suppressing STAT3 and claudin-1, Pharmaceuticals 16 (2023), https://doi.org/10.3390/ph16060902.