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Antioxidant, α-glucosidase inhibitory, and cytotoxic activities of *Mangifera rufocostata* extract and identification of its compounds by LC-MS/MS analysis

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ABSTRACT

Mangifera rufocostata, a member of the Mangifera genus, is a source of secondary metabolites and has been used traditionally as antidiabetic agent. Therefore, this study aims to determine the total flavonoid contens (TFC), total phenolic contents (TPC), antioxidant, α-glucosidase inhibitory, and cytotoxic activities of *M. rufocostata* stem bark extract. Antioxidant activity were evaluated by 2-2″-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing-antioxidant power (FRAP) tests. α-glucosidase inhibitory to prediction antidiabetic activity, and cytotoxic activity were studied against HT-29, HeLa, and MCF-7 cancer cell lines. The results showed that the methanol extract have the highest TFC, TPC, antioxidant, and α-glucosidase inhibitory activity. Furthermore, the methanol extract has strong cytotoxicity againts HT-29 cancer cells. Based on LC-MS/MS, the methanol extract contain several major compounds such as mangiferin, quinic acid, gallic acid, and 3-methoxy-4-hydroxy-phenyl glycol.

1. Introduction

The Mangifera genus consist of 69 species spread across Sumatra, Borneo, and the Malay Peninsula (Kostermans & Bomphard, 1993). They are rich in various secondary metabolites and display various bioactivities. About 23 species are found in Kalimantan (Uji, 2004), with some being active as antioxidants, antidiabetics, and anticancer.

One species that is active as an antidiabetic is *M. indica* as indicated by the leaf (Gazwi & Mahmoud, 2019; Mohammed & Rizvi, 2016; Ngo et al., 2019), fruit peel (Chowdhury et al., 2017), and stem bark extract (Bhowmik et al., 2009), which demonstrated significant hypoglycemic activity in diabetic-induced mice (Ojewole, 2005). Methanol extract of fruit peel with concentrations of 200 and 400 mg/kg can reduce plasma glucose by 13.95 to 26.18 % after 90–150 min, compared to standard glibenclamide (14.90—20.67 %) (Chowdhury et al., 2017). The leaves extract of *M. indica* is active as antioxidant with IC_{50} values of 3.18 to 13.37 μg/mL (Fitria et al., 2016; Itoh et al., 2020; Mohan et al., 2013; Park et al., 2015; Prommajak et al., 2014), while the peel has an antioxidant capacity of 23 ± 2.85 to 53.9 ± 4.2 (mM Trolox/100 g DW) (Marcillo-Parra et al., 2021), and 2986 ± 380 μmol QE/100 g FW (Abbasi et al., 2017). *M. indica* fruit extract can inhibit free radicals up to 45 % (Septembre-Malaterre et al., 2016), while the bark is active as an antioxidant with an IC_{50} 12 μg/mL (Itoh et al., 2020). The kernel, peel, pulp, and seeds of the fruits are cytotoxic by inhibiting the growth of breast cancer cells MCF-7 and MDAMB-231 (Abdullah et al., 2014, 2015; Arbizu-Berrocal et al., 2019; Banerjee et al., 2015; Wilkinson et al., 2011), prostate LNCaP (S. Prasad et al., 2007), leukemia HL-60 (Percival et al., 2006), HepG2 (Abbasi et al., 2017), cervix HeLa (Timsina & Nadumane, 2015), lung A-549, leukemia Molt-4, and colon SW480 (Noratto et al., 2010) with IC_{50} 15 to 30 μg/mL. *M. indica* pulp at a dose of 0.3 % can inhibit the growth of colon cancer in mice by up to 60 % ($p = 0.05$), after 10 weeks of treatment (Corrales-Bernal et al., 2014). The

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bark also showed toxic effects on pancreatic carcinogen PANC-1 (Nguyen, Do, et al., 2016), ovarian cell line SKOV-3, as well as breast MCF-7, and MDAMB-231 (Ediriweera et al., 2016). In contrast, *M. indica* leaf was less active in inhibiting gastric carcinoma Kato-III, Hepatoblastoma HepG2, ductal carcinoma BT474, colon adenocarcinoma SW 620, and bronchogenic carcinoma Chago K1 (Ganogpichayagrai et al., 2017). Other mangifera species also has been proven to be active as antioxidants, including *M. casturi* (Lestari et al., 2021; Pardede & Koketsu, 2017), and *M. longipes* (Guha et al., 2021). α-Glucosidase inhibitory from *M. foetida*, and *M. mekongensis*, with IC_{50} 13.49 mg/mL and 1.71 μg/mL, respectively (Nguyen et al., 2021; Nguyen, Le, et al., 2016; Yusro et al., 2016).

The mangifera species usually contain similar compounds, such as mangiferin, gallic acid, and quercetin. Mangiferin is an active compound that functions as an anticancer agent (Núñez Selles et al., 2016). It actively inhibited the growth of breast cell cancer MCF-7 and MDAMB-231(Fernández-Ponce et al., 2017), lung cancer in white mice *in vivo* (Rajendran et al., 2014; Singh et al., 2018), leukemia carcinogenic cells (Zhang et al., 2014), and neuroblastoma IMR-32 (Das et al., 2011). The compound has been shown to strongly inhibits the activity of α-amylase and α-glucosidase (Kulkarni & Rathod, 2018; Sekar et al., 2019). α-Glucosidase is an enzyme that catalyzes the cleavage of polysaccharides into glucose. Therefore, its inhibitors have been used to treat type 2 DM (diabetes mellitus).

M. rufocostata is a Mangifera species found in Kalimantan and is also known as Asem Tanduy in South Kalimantan (Kostermans & Bomphard, 1993). Traditionally, the community use the boiled water of the bark as a medicine for diabetes and mild stroke. Although *M. rufocostata* shows potential benefits in traditional medicine, research on the biological activity of *M. rufocostata* stem bark is limited to antioxidant activity. The ethanol extract of *M. rufocostata* stem bark was reported to be active as an antioxidant with an IC_{50} of 8.254 ppm (Sutomo et al., 2023), with a total phenolic content (TPC) of 471.3126 mg GAE/g, and total flavonoid content (TFC) of 872.075 mg QE/g (Susiani et al., 2023). Phytochemical screening studies show that the extract contains tannins, phenols, flavonoids and saponins (Sutomo et al., 2023). Therefore, this study aims to evaluate TFC, TPC, the activity of antioxidants ((2-2″-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1.1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing-antioxidant power (FRAP)), α-glucosidase inhibitory, and cytotoxic activity against cancer cells HT-29, HeLa, MCF-7 of *M. rufocostata* stem bark extract comprehensively. LC-MS/MS was also used to identify the compounds present in the active extract.

2. Material and methods

2.1. Plant material

The stem bark of *M. rufocostata* was obtained in September 2021 at Barabai, Hulu Sungai Tengah, South Kalimantan. The samples were identified by Dr. Gunawan, M.Si the Plant botanist of the Faculty of Mathematics and Science, Lambung Mangkurat University, Banjarbaru, Indonesia.

2.2. Extraction

M. rufocostata bark is dried at 40 °C, then ground into powder with a size of 60–80 mesh. The dry powder 25.0 g each was extracted with 300 mL *n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and methanol (MeOH) (Merck) for 24 h at room temperature. Each extract was concentrated using a rotary evaporator to produce dry extracts.

2.3. Total flavonoid contents (TFC)

The TFC value of *M. rufocostata* stem bark extract was measured using the method of Idris et al. (2022). Extracts including *n*-hexane, CH_2Cl_2, EtOAc, and MeOH with a certain concentration were taken up to 1 mL and mixed with 1 mL of $AlCl_3$ solution (2 %) in methanol (Merck), then the mixture was incubated for 1 h. The absorbance of the mixture was measured using a UV–vis spectrophotometer (415 nm). The standard curve of quercetin (Sigma-Aldrich) ranged from a concentration of 0–60 mg/L and the TFC value is equivalent to quercetin (mg QE)/g dry extract.

2.4. Total phenolic content (TPC)

The total phenolic content of *M. rufocostata* stem bark extract was measured using the Follin-Ciocalteu method (Idris et al., 2022). Each extract including *n*-hexane, CH_2Cl_2, EtOAc, and MeOH with a certain concentration was taken up to 0.5 mL each, then added with sodium carbonate solution (7.5 %; 2.0 mL), and 2.5 mL of Follin–Ciocalteu solution (10 %) (Sigma-Aldrich). After the mixture was incubated (1 h), it was measured using a UV–vis spectrophotometer (765 nm) to determine the absorbance. The standard curve of gallic acid (Sigma-Aldrich) has a 0–130 mg/L concentration. The calculated TPC value was equivalent to gallic acid (mg GAE)/g dry extract.

2.5. ABTS radical scavenging assay

Free radical scavenging activity of *M. rufocostata* stem extract was performed using the ABTS method, as reported by Idris et al. (2022) with slight modifications. About 5 mL of 7 mM ABTS (Sigma-Aldrich) was mixed with 8.8 mL of 1.40 mM potassium persulfate (Merck) and stored in a dark place at room temperature for 12 to 16 h. The mixture was diluted with ethanol (Merck) to obtain an absorbance of 0.700 ± 0.015 at a wavelength of 734 nm and was referred to as the ABTS working solution. Furthermore, 3 mL of ABTS working solution and 30 μL of the extract with various concentrations were vortexed for 10 s and incubated at 30 °C for 4 min. The absorbance of each solution was measured with a UV–vis spectrophotometer at a wavelength of 734 nm. Gallic acid (Sigma-Aldrich) was used as a positive control.

2.6. DPPH radical scavenging assay

The antioxidant activity of *M. rufocostata* stem bark extract was analyzed using the modified DPPH method (Kuntorini et al., 2022). The *n*-hexane, CH_2Cl_2, EtOAc, and MeOH extracts were diluted to a certain concentration in methanol. Afterward, 2 mL of DPPH (0.15 mM) (Sigma-Aldrich) was mixed with 2 mL of sample, then the mixture was incubated for 30 min in the dark at room temperature. The absorbance of the mixture was measured using a UV/Vis spectrophotometer (517 nm). The antioxidant activity was measured in moles of Trolox equivalent (Sigma-Aldrich)/g dry extract (μg TE/g). Gallic acid (Sigma-Aldrich) was used as a positive control.

2.7. Ferric-reducing antioxidant power (FRAP) assay

The antioxidant activity of *M. rufocostata* stem bark was also measured using the FRAP method modified by Kuntorini et al. (2022). The TPTZ in HCl (2.5 mL; 10 mM) (Sigma Aldrich), 25 mL of acetate buffer (300 mM) with a pH of 3.6, and 2.5 mL of $FeCl_3{\cdot}6H_2O$ (20 mM) solution (Merck) is referred to as FRAP solution. Extracts including *n*-hexane, CH_2Cl_2, EtOAc, and MeOH with a certain concentration of 1 mL were reacted with the FRAP solution of 3 mL. The mixture was incubated for 15 min at room temperature in a dark place and the absorbance of the mixture was measured using a UV/Vis spectrophotometer at a wavelength of 597 nm. $FeSO_4{\cdot}7H_2O$ (Merck) and Trolox (Sigma-Aldrich) were used as standard curves, while the antioxidant capacity was expressed as the mole equivalent of Trolox (TE)/g dry extract (μg TE/g). Gallic acid (Sigma-Aldrich) was used as a positive control.

2.8. α-glucosidase inhibitory activity

Stem bark of *M. rufocostata* was carried out using *in vitro* based assay againts the inhibition of α-glucosidase enzymes (rat intestinal acetone powder, Sigma) (Fatmawati et al., 2011; Idris et al., 2022). About 10 μL of *M. rufocostata* stem bark extract (10 mg/mL in DMSO) was reacted with phosphate buffer (50 μL; 0.1 M) at pH 6.9 and maltose substrate (20 μL; 10 mM) in 0.1 M phosphate buffer (Merck). Then 80 μL of glucose kit (Human) and α-glukosidase enzyme solution (20 μL) were added. Then the mixture was incubated (37 °C; 10 min) and the absorbance was measured using a microplate reader at 520 nm (Spectrostar nano, BMG Labtech).

2.9. Cytotoxic activity

The *in vitro* cytotoxicity assay was carried using MTT assay againts HT29 (human colon cancer from ATCC, HTB-38), HeLa (human cervical adenocarcinoma from ATCC, CCL-2), and MCF-7 (human breast adenocarcinoma from ATCC, HTB-22) cells (Ali et al., 2021). HT29 was grown in McCoy's 5A Medium (Gibco), while HeLa and MCF-7 were grown in DMEM (Gibco). They were supplemented with 10 % fetal bovine serum (FBS, Gibco), sodium pyruvate 1 % (Gibco), and penicillin/streptomycin 1 % (Gibco). They were preserved in complete medium containing 10 % (v/v) PBS in 96 well plate cultures at a density of 185,000 cells per mL of medium, then incubated for 24 h at 37 °C (5 % CO_2). Furthermore, the MeOH extract with a concentration of 7.8 to 1000 μg mL^{-1} was added to the media and incubated for 48 h. At 42 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) solution (20 μL, 5 mg mL^{-1}) was added to each well and incubation continued for six hours at 37 °C. The absorbance of the mixture was measured at 570 nm using an ELISA reader (Zenix). The negative control was in the form of media, while cisplatin (Sigma-Aldrich) was used as the positive control. Cell morphology was observed using an inverted microscope.

2.10. LC-MS/MS analysis

About 10 mg/mL of the MeOH extract was dissolved in methanol and filtered through a 0.2 μm PTFE membrane, then measured with UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS ThermoScientific at column temperature of 30 °C; flow rate 0.2 mL/min, mobile phase H_2O + 0.1 % formic acid (A) and acetonitrile + 0.1 % formic acid (B), and elution gradient, 0–1 min (5 % B), 1–25 min (5–95 % B), 25–28 min (95 % B), and 28–30 min (5 % B). The mass spectrometry conditions electrospray ionization (ESI) source, scanning range of 100–1500 *m/z* with the ChemSpider and Mzcloud database (Resida et al., 2021).

2.11. Statistical analysis

The data obtained were summarized using the standard deviation formula including antioxidant activity, TFC and TPC values, α-glucosidase inhibitory which were calculated with the linear regression equation. Meanwhile, cytotoxic activity against several cancer cells was calculated using Probit analysis with $p < 0.05$. Differences in the antioxidant, TPC, TFC, and α-glucosidase inhibitory of each extract were analyzed by Student's *t*-test with $p < 0.01$. The relationship between antioxidant, TPC, TFC, and, α-glucosidase inhibitory was analyzed using the Pearson Correlation test with $p < 0.01$.

3. Results and discussion

3.1. Extraction yield

The solvents chosen for extraction of *M. rufocostata* stem bark were *n*-hexane, dichloromethane, ethyl acetate and methanol. The solvents were chosen based on differences in polarity, because the polarity of the solvent greatly influences the yield of the extract. The order of polarity of the solvent used is *n*-hexane < CH_2Cl_2 < EtOAc < MeOH. The extraction results of the four solvents are presented in Table 1. It can be seen that the greatest extract yield was obtained from extraction with methanol in the order MeOH > EtOAc > CH_2Cl_2 > n-hexane. This is caused by the polar protic nature and dielectric constant of methanol which is greater than other solvents. This shows that the secondary metabolite compounds in the stem bark of *M.rufocostata* are dominated by polar compounds. This trend is in line with the results of extraction of *N. leucophylla* plants (Sharma & Cannoo, 2017).

However, the opposite results were found in the extraction results of the *D. pentandra* (L.) plant, where the extract from the non-polar solvent (*n*-hexane) was greater than the polar solvent (ethanol) (Kristiningrum et al., 2018). Therefore, the choice of solvent for extraction depends on the type and polarity of the secondary metabolite compounds of a plant material (El Mannoubi, 2023).

Table 1
Extraction yield of *M. rufocostata* stem bark.

Extract	Yield	
	weight (g)	w/w (%)
n-Hexane	0.50	2.00
CH_2Cl_2	0.75	3.00
EtOAc	1.50	6.00
MeOH	2.47	9.88

Table 2
TFC and TPC value of *M. rufocostata* stem bark[a].

Extract	TFC (mg QE g^{-1} dry extract)	TPC (mg GAE g^{-1} dry extract)
n-Hexane	1.74 ± 0.05^{b}	19.29 ± 0.08^{d}
CH_2Cl_2	1.93 ± 0.03^{c}	22.72 ± 0.26^{d}
EtOAc	$9.18 \pm 0.07^{b,c}$	27.05 ± 0.14^{d}
MeOH	$195.44 \pm 0.71^{b,c}$	446.07 ± 3.01^{d}

[a] Data represent mean ± SD (triplicate experiments), ^{b,c,d} $p < 0.01$ vs each extract.

3.2. Total flavonoids and phenolics contents

The total flavonoids and phenolic content of *M. rufocostata* stem bark extract is shown in Table 2.

The TFC value of the *M. rufocostata* extract is equivalent to that of quercetin, hence, it was determined by the linear standard curve of quercetin. The results of the TFC showed significant differences among all extracts, except between *n*-hexane and CH_2Cl_2 extracts (t-Test; $p < 0.01$). Moreover, the MeOH extract showed the highest flavonoid content compared to other extract, in the order MeOH > EtOAc > CH_2Cl_2 > n-hexane. These results indicate that *M. rufocostata* flavonoids accumulated more in polar extracts and less in the semipolar and non-polar. This trend is in line with several other research results, including on the flavonoid content in plants *Gracilaria changii* (Chan et al., 2015) and *N. leucophylla* (Sharma & Cannoo, 2016). The results of research by Susiani et al. (2023) regarding the TFC test on *M. rufocostata* stem bark using EtOH solvent produced TFC levels of 872.075 mg QE/g. The results of this study were greater when compared with the MeOH extract in this study. This condition is possible if the flavonoid compounds in the bark of *M. rufocostata* are more easily extracted into EtOH. However, the flavonoid content in the stem bark of *M. rufocostata* MeOH extract in this study was greater when compared to other mangifera species (*M. foetida* and *M. kemanga*) even though EtOH solvent was used. The TFC values of *M. foetida* and *M. kemanga* EtOH extracts were 94.34 ± 0.24 and 87.46 ± 0.35 mg QE/g dry extract, respectively (Fitmawati et al., 2020).

The TPC was determined from a linear gallic acid standard curve and the results of each extract showed a significant difference (*t*-test; $p < 0.01$). TPC value trends are in line with TFC. The trend in TPC values is

Table 3
The antioxidant activity of *M. rufocostata* stem bark [a.]

Extracts	ABTS	DPPH	FRAP
	IC_{50} (μg mL^{-1})	Antioxidant Capacity (μg TE/g dry extract)	Antioxidant Capacity (μg TE/g dry extract)
n-Hexane	10,780 ± 255.85[b]	2,789.17 ± 57.50[c]	7,356.95 ± 34.55[d]
CH_2Cl_2	2,035.00 ± 25.47[b]	3,317.73 ± 99.59[c]	7,680.18 ± 22.62[d]
EtOAc	1,137.44 ± 4.07[b]	6,407.78 ± 152.13[c]	19,805.85 ± 56.93[d]
MeOH	30.84 ± 0.05[b]	377,961.70 ± 18,426.9[c]	173,031.40 ± 345.55[d]
Gallic Acid	2.02 ± 0.01[b]	560,925.10 ± 6,003.2[c]	203,998.10 ± 513.32[d]

[a] Data represent mean ± SD (triplicate experiments), [b,c,d] $p < 0.01$ vs each extract.

in line with TFC, where the phenolic compounds also accumulates more in MeOH as a polar solvent, and becomes smaller with decreasing polarity (MeOH > EtOAc > CH_2Cl_2 > n-hexane). This result is also in line with the results of TPC analysis on plants *G. changii* (Chan et al., 2015) and *N. leucophylla* (Sharma & Cannoo, 2016). The phenolic content in the MeOH extract of *M. rufocostata* stem bark in this study was also lower when compared to the EtOH extract (471.3126 mg GAE/g) reported by Susiani et al. (2023). This result is in line with the TPC value of the EtOH extract of *M. caesia* fruit which is greater than the MeOH extract (Yunus et al., 2021). The TPC value of the MeOH extract of *M. rufocostata* stem bark in this study was also greater than the EtOH extract of stem bark *M. caesia* (48.54 ± 1.75 mg GAE/crude extract) (Yunus et al., 2021), *M. foetida* (100.65 ± 1.94 mg GAE/g) and *M. kemanga* (90.65 ± 0.59 mg GAE/g) (Fitmawati et al., 2020).

3.3. Antioxidant activity

The antioxidant activity of the extract was analyzed by ABTS, DPPH, and FRAP methods. The test results are shown in Table 3.

As shown in Table 3, the test results between extracts indicated significant differences in antioxidant activity (*t*-test; $p < 0.01$).

The ABTS method is used to evaluate the antioxidant activity through free radical scavenging with a proton donor (Aguirre-Becerra et al., 2020; Chohra et al., 2020). The test results showed that the MeOH extract have the highest antioxidant activity, with IC_{50} value of 30.84 ± 0.05 μg mL^{-1}. This value indicates that the antioxidant activity of the MeOH extract is very strong, although it is still weaker compared to the positive control of gallic acid. Gallic acid is a polyphenolic compound that is very active as an antioxidant which can act as a neuroprotective agent in oxidative stress, neurodegeneration and neurotoxicity (Daglia et al., 2014). Antioxidant activity decreased in descending order MeOH > EtOAc > CH_2Cl_2 > *n*-hexane.

The DPPH method was used to test the antioxidant activity using free radicals derived from DPPH compounds. The radical derived electrons or hydroxyl radicals from the extract's antioxidant compounds and became stable diamagnetic compounds. The presence of antioxidant activity was indicated by the change in color of the solution, from purple to yellow. Based on the results, the MeOH extract had the greatest antioxidant activity as shown in Table 3. The antioxidant capacity of MeOH extract is almost 63 times the antioxidant capacity of EtOAc, although it is smaller compared to the antioxidant capacity of gallic acid (1.5 x).

The FRAP method was used to evaluate the antioxidant activity based on the capacity of the sample to participate in a redox reaction with the FRAP reagent. The MeOH extract also showed the highest activity as shown in Table 3. In contrast, the smallest antioxidant activity was obtained in the other extract (EtOAc > CH_2Cl_2 > *n*-hexane), although it was smaller than the antioxidant capacity of gallic acid (1.2 x).

The results of antioxidant tests using three different methods (ABTS, DPPH, FRAP) in this study showed the same trend. These results are in line with the results of the antioxidant test of the *Garciinia forbesi* King plant (Wairata et al., 2022). MeOH extract has the highest inhibitory activity against free radicals, because MeOH extract contains the most flavonoid and phenolic compounds compared to other extracts, as evidenced by the high TFC and TPC (Table 2).

Table 4
The α-glucosidase inhibitory activity of *M. rufocostata* stem bark[a.]

Extracts	α-glukosidase	
	% inhibition ± SD (10 mg mL^{-1})	IC_{50} (μg/mL)
n-Hexane	16.64 ± 3.09[b,c]	nt
CH_2Cl_2	40.28 ± 6.04[b,c]	nt
EtOAc	67.24 ± 6.45[b,c]	nt
MeOH	99.07 ± 0.50[b]	49.57 ± 15.56
Acarbose	99.47 ± 0.19[c]	10.42 ± 2.39

[a] Data represent mean ± SD (triplicate experiments), [b,c] $p < 0.01$ vs each extract and positive control, nt; not tested.

3.4. α-glucosidase inhibitory

The results of the α-glucosidase inhibitory activity assay of stem bark *M. rufocostata* extract are shown in Table 4.

Table 4 shows the α-glucosidase inhibitory activity screening results of *n*-hexane, CH_2Cl_2, EtOAc, MeOH extracts, and acarbose as positive controls. The MeOH extract showed the highest activity with inhibition of 99.07 ± 0.50 % in concentration 10 mg mL^{-1} but not significantly different compared to acarbose as a positive control (*t*-test, $p < 0.01$). The EtOAc, CH_2Cl_2 and *n*-hexane extracts showed less inhibitory activity compared to acarbose in the same concentration but have a significant difference (*t*-test; $p < 0.01$). Several concentrations series of MeOH extract in the α-glucosidase enzyme inhibition produced an IC_{50} value of 49.57 ± 15.56 μg mL^{-1}, although the value is weaker compared to acarbose with $IC_{50} = 10.42 \pm 2.39$ μg mL^{-1}. The results of α-Glucosidase inhibition from *M. rufocostata* stem bark extract are smaller when compared to other mangifera species, such as *M. mekongensis* and *M. foetida*. The *n*-hexane extract of *M. mekongensis* stem bark was able to inhibit the α-glucosidase enzyme with an IC_{50} of 1.71 μg/mL, while the MeOH extract of *M. foetida* had an IC_{50} of 13.49 mg/mL. Several compounds that were isolated from *M. reba*, and *M. gedebe* were also active in inhibiting α-glucosidase with IC_{50} 28.5 to 162.8 mM, and 45.3 to 142.6 μM, respectively (Duong et al., 2017; Nguyen et al., 2021; Nguyen, Le, et al., 2016; Yusro et al., 2016). This shows that plants from the genus Mangifera have great potential as sources of phytopharmaceuticals.

This study is the first to report on the inhibitory activity of α-glucosidase *in vitro* by the stem bark extract of *M. rufocostata*. The results provide support for further investigations on the utilization of *M. rufocostata* stem bark extract as an antidiabetic therapy, through *in vivo* study and isolation of active compounds. Based on the screening results, MeOH extract showed the most potential with the highest inhibition. One therapy for treating type 2 DM is through the inhibitory activity of the α-glucosidase enzyme. The enzymes located on the border of the small intestine function to break down complex carbohydrates into glucose. When the α-glucosidase enzyme is inhibited, then the metabolism of complex carbohydrates will be delayed, thereby reducing glucose levels in the blood.

Table 5
The in-vitro anticancer activity of *M. rufocostata* stem bark[a].

Extract	IC_{50} (μg/mL)		
	HT-29	HeLa	MCF-7
MeOH	0.25 ± 2,74	801.93 ± 0.02	3,579.33 ± 0.05
Cisplatin	3.00 ± 0.04	50.00 ± 0.03	100.00 ± 0.08

[a] Data represent by Probit analysis ($p < 0.05$).

3.5. Cytotoxic activity

Cytotoxic activity of *M. rufocostata* stem bark was evaluated using the cell viability of the MTT method against three cancer cells, namely HT-29 (colon), HeLa (cervix), and MCF-7 (breast). The reason for selecting these three cancer cells in this research is based on literature studies which state that extracts from the other plants from genus mangifera, consist of *M. indica* and *M. Pajang* are toxic to colon, breast and cervical cancer cells line (Ahmad et al., 2015; Navarro et al., 2019). The MeOH extract of stem bark *M. rufocostata* was evaluated for its toxicity effect on these three cells and the test results are shown in Table 5.

Table 5 shows that the MeOH extract has less citotoxic against HeLa cancer cells with an IC_{50} value of 801.93 ± 0.02 μg/mL, less potential when compared to cisplatin as a positive control. This result is also less potent when compared with the cytotoxicity of MeOH extract of *M. pajang* stem bark against HeLa cells (>30 μg/mL) (Ahmad et al., 2015). However, it was more potent against HT-29 with an IC_{50} value of 0.25 ± 2.74 μg/mL. It was also more active than cisplatin as a positive control. This result is also more toxic when compared with the toxicity of MeOH extract of *M.pajang* stem bark against HT-29 (>30 μg/mL). These results are also very potential when compared with Cetuximab, the drug for colorectal cancer accepted by the FDA (Wu et al., 2022). Cetuximab

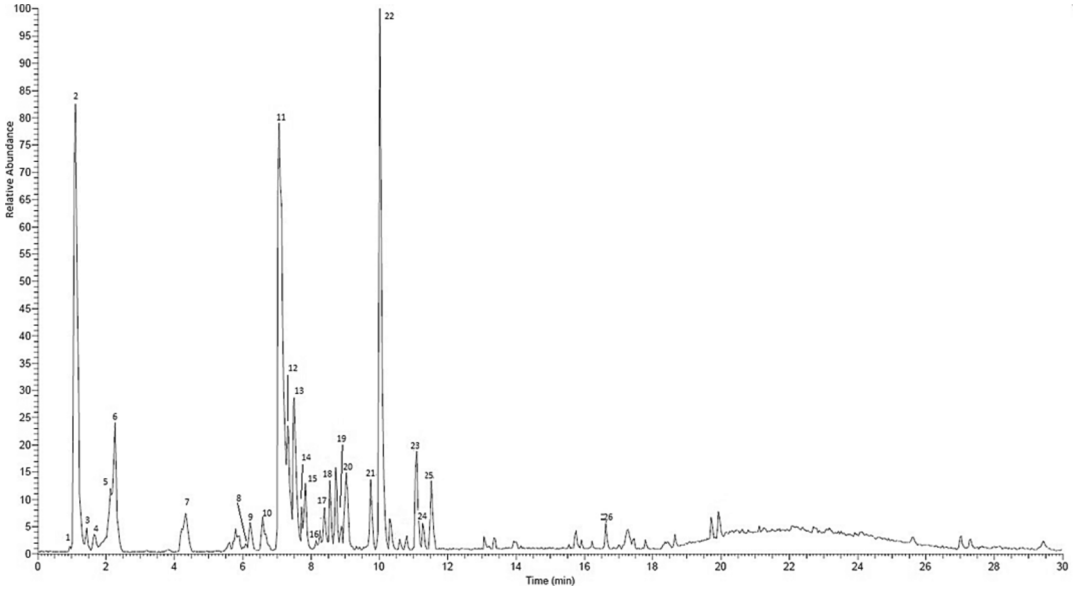

Fig. 1. LC-MS/MS Chromatogram of methanol extract stem bark *M. rufocostata* in a negative ion mode.

Table 6
List of compounds detected from methanol extract of *M. rufocostata* stem bark observed by LC-MS/MS negative ions.

No	T_R (min)	Molecular formula	Major ion $[M-H]^-$ (*m/z*)	Calculate Molecular Weight	Tentative Assignment
1	1.513	$C_{14}H_{24}O_{12}$	383.1268	384.12678	1-O-acetyl-alpha-maltose
2	1.522	$C_7H_{12}O_6$	191.0626	192.06264	Quinic acid
3	1.529	$C_6H_8O_7$	191.0267	192.02669	Citric acid
4	1.543	$C_4H_6O_5$	133.0206	134.02057	Malic acid
5	2.088	$C_4H_6O_4$	117.0257	118.02571	Methylmalonic acid
6	2.670	$C_7H_6O_5$	169.0208	170.02075	Gallic acid
7	4.750	$C_7H_6O_4$	153.0259	154.02589	Gentisic acid
9	6.305	$C_{22}H_{28}N_4O_6$	443.2000	444.19996	Mitoxantrone
10	6.650	$C_8H_8O_5$	183.0367	184.03667	3.4-Dihydroxyphenyl glycolic acid
11	7.507	$C_{19}H_{18}O_{11}$	421.0849	422.08494	Mangiferin
13	7.658	$C_{20}H_{22}O_{10}$	421.1193	422.11925	Catechin 7-O-ß-D-xyloside
14	7.730	$C_{11}H_{14}O_8S$	305.0411	306.04109	4-Hydroxy-5-(dihydroxyphenyl)-valeric acid-O-sulphate
15	7.929	$C_{20}H_{20}O_{11}$	435.1006	436.1006	Irisxanthone
16	8.150	$C_{13}H_{16}O_7$	283.0898	284.0898	4-Methylphenyl-ß-D-glucopyranosiduronic acid
17	8.679	$C_8H_8O_4$	167.0416	168.04162	5-Methoxysalicylic acid
18	8.970	$C_{21}H_{20}O_{10}$	431.1061	432.10609	Apigetrin
19	9.149	$C_{22}H_{22}O_{10}$	445.1215	446.12153	NP-018731
20	9.413	$C_{19}H_{16}O_{10}$	403.0745	404.07451	Urolithin A-8-O-glucuronide
21	9.466	$C_{22}H_{22}O_{10}$	445.1213	446.12153	Glycitin
22	10.450	$C_9H_{12}O_4$	183.0728	184.07281	3-Methoxy-4-hydroxy-phenylglycol
23	11.495	$C_9H_{14}O_4$	185.0886	186.08859	1-(Carboxymethyl) cyclohexane carboxylic acid
24	11.557	$C_{15}H_{10}O_8S$	349.0098	350.00981	Apigenin 7-sulfate
25	11.940	$C_{15}H_{10}O_7$	301.0428	302.0428	Quercetin
26	17.047	$C_{18}H_{22}0_6$	333.1418	334.14183	Adlerol

Table 7
Pearson correlation coefficient of TFC, TPC, antioxidant and antidiabetic activity.

	TFC	TPC	DPPH	FRAP	ABTS	α-Glukosidase
TFC	1					
TPC	1.000**	1				
DPPH	0.998**	0.998**	1			
FRAP	0.999**	0.998**	0.997**	1		
ABTS	−0.489*	−0.482*	−0.475*	−0.507*	1	
α-Glucosidase	0.822**	0.812**	0.807**	0.838**	−0.830**	1

*Correlation is moderate at $p < 0.01$, ** correlation is strongly at $p < 0.01$.

toxicity test results against colon cancer cells (E705) with IC_{50} 0.165 ± 0.047 μg/mL (Bovio et al., 2020). But, the MeOH extract did not potentially inhibit MCF-7 cells, as indicated by the high IC_{50} value, while the MeOH extracts of *M. pajang* and *M. indica* are toxic with $IC_{50} > 30$ and 15 μg/mL, respectively (Ahmad et al., 2015; Navarro et al., 2019).

Several investigations have been carried out on the development of effective source of anticancer drugs using plants with diverse chemical structures. This study shows that *M. rufocostata* has potential as a source of new phytopharmaceuticals. The toxycity of *M. rufocostata* methanol extract originates from the combination of the phenolic and flavonoid content. These compounds including mangiferin, gallic acid, and quercetin are commonly found in the Mangifera genus.

3.6. LC-MS/MS analysis

The compounds in the methanol extract of *M. rufocostata* stem bark were analyzed using LC-MS/MS. The total ion current chromatogram in negative ESI mode is displayed in Fig. 1, and the compounds tentatively detected are summarized in Table 6.

Based on the LC-MS/MS results, the dominant compounds present in the MeOH extract are mangiferin, quinic acid, gallic acid, 3-methoxy-4-hydroxy-phenyl glycol, as well as a quercetin compound. These compounds play a role in antioxidant, antidiabetic, and anticancer activities. According to previous studies, mangiferin is active as an anticancer (du Plessis-Stoman et al., 2011; Jung et al., 2012; Núñez Selles et al., 2016; Peng et al., 2004; Shoji et al., 2011), antidiabetic (Ganogpichayagrai et al., 2017; Kulkarni & Rathod, 2018; Muruganandan et al., 2005), while quinic acid also has bioactivity as an antioxidant, antidiabetic, and anticancer (Benali et al., 2022). Moreover, gallic acid is active as an antioxidant (Badhani et al., 2015), anticancer (Subramanian et al., 2015), and antidiabetic (Variya et al., 2020). Another polyphenol compound, quercetin, is also an antioxidant and anticancer agent (Baghel et al., 2012). Mangiferin, gallic acid, and quercetin compounds were also identified in other Mangifera species, such as *M. indica* (Gu et al., 2019; Ronchi et al., 2015) and *M. pajang* (Bakar et al., 2010 (a); 2010 (b); Prasad et al., 2011). Based on taxonomy, some species have close kinship relationships because they belong to the same genus.

The combination of several secondary metabolite compounds in the flavonoid and polyphenol groups such as mangiferin, gallic acid quinic acid and quercetin in the MeOH extract of *M. rufocostata* allows these compounds to jointly inhibit cancer cells. The ability of flavonoids and polyphenols to capture free radicals can help regulate cell metabolism and prevent diseases caused by oxidative stress. There is a lot of evidence showing that these two groups of compounds have anticancer activity, but the molecular mechanism is not completely clear. Cancer is a disease caused by uncontrolled cell proliferation processes, resulting in abnormal cell growth. Oxidative stress, and reduced apoptotic function are the main causes of internal cancer. Therefore, the ability of flavonoid and phenolic compounds to capture free radicals such as ROS can prevent cell damage (Kopustinskiene et al., 2020).

3.7. Correlation of TFC, TPC, antioxidant and antidiabetic activity

Pearson's test was used to determine the correlation between TFC, TPC, as well as the antioxidant and antidiabetic activities of *M. rufocostata* stem bark extract. As shown in Table 7, the total flavonoids positively correlated with total phenolics ($r = 1.000$, $p < 0.01$), meaning that the higher the TFC, the higher the TPC content. Both TFC and TPC play an important role in antioxidant activity as indicated by a strong positive correlation between TFC vs DPPH and TPC vs DPPH $r = 0.998$ ($p < 0.01$). There was also a strong positive correlation between TFC vs FRAP ($r = 0.999$; $p < 0.01$) as well as TPC vs FRAP with $r = 0.998$ ($p < 0.01$). Meanwhile, the correlation between TFC and TPC with ABTS was negative. The greater the flavonoid and phenolic content, the smaller the IC_{50} (ABTS) value and the more active the extract is as an antioxidant. The level of correlation obtained was moderate in TFC vs ABTS ($r = -0.489$; $p < 0.01$) and TPC vs ABTS with $r = -0.482$ ($p < 0.01$). Furthermore, DPPH, FRAP, and ABTS were strongly correlated with the inhibitory activity of α-glucosidase enzymes, with Pearson's coefficient (r) of 0.807, 0.838, and −0.830 ($p < 0.01$), respectively. This data shows that there is a correlation between antioxidant and antidiabetic activity as also reported by Wairata et al.,(2022).

A correlation between antioxidants and antidiabetes is possible, because as an antioxidant a plant can neutralize cell damage caused by reactive nitrogen and reactive oxygen species (ROS) such as singlet oxygen, free radicals and hydroperoxides. Cells damaged by free radicals appear to be the main cause of diabetes mellitus. Apart from that, it also has an impact on degenerative diseases such as cancer. Secondary metabolite compounds may help enzymes in the body to reduce free radicals.

4. Conclusions

This study showed that the methanol extract of *M. rufocostata* stems bark had the highest TFC and TPC content compared to other extracts. These results affected the highest antioxidant activity. The methanol extract inhibited the α-glucosidase enzyme, and strong cytotoxicity against HT-29 cells. Based on the LC/MS-MS results, the extract contains main compounds such as mangiferin, quinic acid, gallic acid, and 3-methoxy-4-hydroxy-phenyl glycol. This suggests that the methanol extract of *M. rufocostata* stem bark can be utilized as a source of biopharmaceutical in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2023.105391.

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