Biocatalysis and Agricultural Biotechnology 39 (2022) 102235

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

In vitro antioxidant, antiglycation, and enzymatic inhibitory activity against α-glucosidase, α-amylase, lipase and HMG-CoA reductase of *Terminalia boivinii* Tul.

Bongani Sicelo Dlamini [a], Carlos Eduardo Hernandez [b], Chiy-Rong Chen [c], Wen-Ling Shih [d], Jue-Liang Hsu [d,e,f], Chi-I Chang [d,g,*]

[a] *Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung, 91201, Taiwan*
[b] *Laboratory of Food Quality Innovation, School of Agricultural Sciences, National University (UNA), Heredia, Costa Rica*
[c] *Department of Life Science, National Taitung University, Taitung, 95002, Taiwan*
[d] *Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung, 91201, Taiwan*
[e] *Research Centre for Austronesian Medicine and Agriculture, National Pingtung University of Science and Technology, Pingtung, 91201, Taiwan*
[f] *Research Centre for Tropic Agriculture, National Pingtung University of Science and Technology, Pingtung, 91201, Taiwan*
[g] *Research Centre for Active Natural Products Development, National Pingtung University of Science and Technology, Pingtung, 91201, Taiwan*

ARTICLE INFO

Keywords:
Antioxidant
Enzyme inhibition activity
Phytochemical analysis
Terminalia boivinii Tul

ABSTRACT

Phytoantioxidants from *Terminalia boivinii* Tul. are of medical interest given their potential inhibitory capacity against the formation of glycation end products and key enzymes associated with metabolic syndrome. The present study investigated and compared the polyphenolic content and biological properties of ethyl acetate, *n*-butanol and aqueous solvent fractions of *T. boivinii* fruits, leaves, stem and bark methanolic extracts. The total bioactive content of the tested materials was evaluated by spectrophotometric methods. The antioxidant activity was analyzed using DPPH, superoxide anion radical, metal chelation and FRAP assays. The enzyme inhibitory properties were tested on α-glucosidase, α-amylase, lipase and HMG-CoA reductase enzymes, while antiglycation activities were evaluated by the bovine serum albumin (BSA) - methylglyoxal (MG) method. Phytochemical analysis indicated that the ethyl acetate fractions had high levels of phenols (40.58 ± 2.94 mg GAE/g DW of leaves) and flavonoids (20.32 ± 1.62 mg QE/g DW of leaves) compared to the other extracts, which is directly related with their significant antiradical, reducing power, α-glucosidase, lipase and HMG-CoA reductase inhibitory ability. However, the water fraction with the lowest polyphenol content was more active in metal chelation. Moreover, the antiglycation activities followed a similar pattern to the radical scavenging activities. Our results showed that *T. boivinii* is a viable source of natural bioactive antioxidant and enzyme-inhibiting chemicals that maybe considered for commercial exploitation.

1. Introduction

Metabolic syndrome (MS) is a clinical multifactorial health problem characterized by interconnected abnormalities such as hypertension, hyperglycemia, and dyslipidemia that is most normally associated with obesity (Mongraw-Chaffin et al., 2018). MS is a

* Corresponding author Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung, 91201, Taiwan
E-mail address: changchii@mail.npust.edu.tw (C.-I. Chang).

https://doi.org/10.1016/j.bcab.2021.102235
Received 17 September 2021; Received in revised form 23 November 2021; Accepted 27 November 2021
Available online 29 November 2021
1878-8181/

complex health disease, and treatment options include both non-pharmacological and pharmaceutical treatments. Non-pharmacological treatment involves lifestyle modifications such as diet control, weight reduction and increased physical activity, which may be supplemented with psychiatric therapies if necessary (De la Iglesia et al., 2016). However, pharmacotherapies are frequently necessary due to the tremendous difficulties most patients have in maintaining significant weight loss requiring regular exercise program. Overall, a multifactorial pharmaceutical strategy is intended to modify the metabolic imbalances associated with diabetes while also lowering cardiovascular risk (Lim and Eckel, 2014). A multidrug program, or polypharmacy, is a serious challenge in the treatment of individuals with MS due to adverse effects and drug-drug interactions (Alwhaibi et al., 2018). Nonetheless, the efficacy of these multifactorial therapies can be limited by high patient noncompliance, which might be improved by combining two or more active ingredients in a single tablet (Artasensi et al., 2020).

Polypharmacy has attracted enormous interest in the pharmaceutical and academic fields as a prospective therapeutic solution to multifarious health problems like cancer, diabetes mellitus, cardiovascular complications and drug resistance phenomena (Talevi, 2015). Enzymes remain important targets for pharmacological intervention in human illnesses because of their crucial catalytic function in various physiological processes (Ramsay and Tipton, 2017). Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the primary and regulatory enzyme in the cholesterol biosynthetic pathway, results in a decrease in low-density lipoprotein (LDL) cholesterol, that is important for managing metabolic disorders (Liu et al., 2021). Another most effective therapeutic strategy for lipid lowering is the down regulation of pancreatic lipase. Pancreatic lipase is key enzyme in the hydrolysis dietary triglycerides and thus regulates the lipid absorption (Hou et al., 2020). Inhibition of α-glucosidase and α-amylase that are responsible for the breakdown of carbohydrates and glucose absorption leads to lower postprandial hyperglycemic, which is important in the treatment and management of diabetics (Gulati et al., 2012). Overall, the treatment of metabolic disorders involves the use of lipid-lowering, anti-obesity, anti-hypertensive, and anti-diabetic medicines that have both central nervous system and peripheral effects, raising concerns about polypharmacy (Lillich et al., 2020).

Polypharmacy related concerns can be curbed through the adoption of a comprehensive treatment approach for metabolic diseases. The development of a drug with antihypertensive, antihyperglycemic, and antidyslipidemic effects would be a great success in the treatment of metabolic disorders. Presently, there are no authorized medicines that can permanently relieve the various symptoms linked with MS (Handelsman and Jellinger, 2011). However, formulations made from a combination of phytomedicines and natural products from various plants found in most traditional medicines seem to adopt a versatile and all-inclusive approach. These pharmaceutical formulations comprise a number of natural ingredients that have synergistic effect on a range of targets via various methods to treat the illness more effectively (Zimmermann et al., 2007). As a result, it appears that the conventional, unidirectional treatment method in diabetes care is progressively being replaced by a more holistic, multidimensional approach (Oyenihi et al., 2014).

Terminalia is the second largest genus belonging to the family of Combretaceae with around 200 species (McGaw et al., 2001). *Terminalia boivinii* is indigenous to Madagascar, has spread to the tropical and subtropical zones of Africa and now it is also found in in southern Asia and Australia (McGaw et al., 2001). The genus has received significant pharmacological research because of its widespread usage in traditional Asian (Tibetan, Indian, and Chinese) medical systems (Fyhrquist et al., 2002; Okatch et al., 2012; Semenya et al., 2013; Senkoro et al., 2014; York et al., 2012). Modern pharmacological activities of the genus *Terminalia* were reported, including antibacterial (Datta et al., 2017), antioxidant (Kouassi et al., 2019), antiproliferative (Basu et al., 2017), antifungal (de Araújo et al., 2019), antiviral (Kesharwani et al., 2017), anti-inflammatory (Saleem et al., 2017), antinociceptive (Khan et al., 2018), and antidiabetic (Philip et al., 2017) therapeutic activities. However, *T. boivinii* have been underutilized, to the best of our knowledge few pharmacological studies were conducted on the plant. Most studies were directed to the Asian species for their phytochemical and biological properties (Cock, 2015). The extensive usage of *Terminalia* species in indigenous medicine for a variety of illnesses, as well as the considerable pharmacological activities displayed by some species, merits additional research into this plant for other putative biological activities. In this study, the antioxidant, antiglycation activities and potential inhibition of key metabolic enzymes of importance in the formulation of multifactorial therapeutic agents for the treatment and management of metabolic diseases by various extracts of *T. boivinii* were discussed.

2. Materials and methods

2.1. Plant collection and identification

Various parts (fruits, leaves, stem and stem bark) of *T. boivinii* were collected at National Pingtung University of Science and Technology, Pingtung County (Taiwan), and air dried at room temperature at the Department of Biological Science and Technology where a voucher specimen (No.BT524) was deposited. The plant material was authenticated by Professor Sheng-Zehn Yang, Herbarium Curator, Department of Forestry, National Pingtung University of Science and Technology.

2.2. General procedures

Alpha-glucosidase from *Saccharomyces cerevisiae*, α-amylase from porcine pancreas, pancreatic lipase, 4-p-nitrophenyl butyrate (pNPB), bovine serum albumin (BSA), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) and DL-3-hydroxy-3-methylglutaryl coenzyme A sodium salt were obtained from Sigma Aldrich (St. Louis, MO, USA). Acarbose, sodium azide, dithiothreitol, nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), methylglyoxal (MG), 4-p-nitrophenyl-α-D-glucopyranoside (pNPG) and β-nicotanamide adenine dinucleotide (NADH) were purchased from Acros Organics Company. Nicotinamide adenine dinucleotide phosphate (NADPH) and 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3) were obtained from Carbosynth Limited (Unit 8 & 9, Old Station Business Park, Compton, Berkshire, RG20 6NE, UK). All other chemicals and organic solvents were obtained from American Tedia Company and Acros Organics Company. The absorbance was recorded in a Thermo Fisher Scientific (Ratastie 2, FI-01620 Vantaa, Finland) spectrophotometer while fluorescence was measure in Thermo Scientific Varioskan LUX multimode microplate reader.

2.3. Extraction and partition

Fruits, leaves, stem and bark of *T. boivinii* were oven-dried at 60 °C for 24 h and crushed into powder, passing through a 100-mesh sieve. The plant materials (200 g) were extracted with 2 L of methanol at room temperature for 10 days and repeated three times. Each extract was concentrated to dryness under vacuum pressure using rotary evaporator to obtain crude extracts. The crude extracts (MeOH) were suspended in water then sequentially partitioned with ethyl acetate (EtOAc) and *n*-butanol (BuOH) to obtain EtOAc, *n*-BuOH and water-soluble fractions. Table 1 shows the dry weight and yield of the extracts.

2.4. Phytochemical analysis of extracts

2.4.1. Determination of total phenolic content

Total phenolic content was measured by the Folin-Ciocalteu colorimetric method with some minor modifications (Chang et al., 2001). The sample solutions (10 mg/mL) were prepared in methanol/water (50:50, v/v). In an eppendorf, 0.5 mL of sample solutions were mixed with 0.5 mL of 50% Folin-Ciocalteu reagent and allowed to stand at room temperature. After 5 min, 1.0 mL of 20% Na_2CO_3 was introduced into the reaction mixture and left to stand for 10 min. The mixture was then centrifuged at 1000 rpm for 8 min. The supernatant (200 μL) was transferred into a microplate and absorbance was measured at 730 nm. Gallic acid standard solutions were used to construct the calibration curve, and results are reported as gallic acid equivalents (GAE) in milligrams per gram of sample dry weight (mg GAE/g DW).

2.4.2. Determination of total flavonoid content

The total flavonoid content was evaluated by following the method of Quettier-Deleu et al. (2000) with minor changes. In a 96-well microplate, 20 μL of sample solution, 80 μL of deionized water and 5 μL of sodium nitrite solution (5% $NaNO_2$ dissolved in distilled water) were mixed and allowed to stand at room temperature for 5 min. Thereafter, 20 μL of aluminum trichloride solution (10% methanolic $AlCl_3{\bullet}6H_2O$) was added into the reaction mixture. After 1 min, 20 μL sodium hydroxide (1 M NaOH) was added and the volume of each well was adjusted to 200 μL with deionized water. The mixture was allowed to stand for 10 min, and absorbance was measured at 430 nm using microplate reader. The calibration curve was generated using quercetin, and the values were reported in milligrams of quercetin per 100 g dry weight (mg QE/g DW).

2.4.3. Liquid chromatography-mass spectrometry (LC-MS) analysis

LC-MS analysis was performed using TSQ Quantum^{TM} Access MAX Triple Quadrupole Mass Spectrometer (Thermo Scientific^{TM}) equipped with an Heated Electrospray Ionization (HESI) source in positive and negative modes (mass range of m/z 50–1000). The spectrometer was connected to a Shimadzu LC-20A system consisting of a LC-20AD pump, a DGU-20A_{5R} degassing unit, and a SIL-20A auto sampler (injection volume 5 μL) (Shimadzu Corporation, Japan). A Thermo Scientific SyncronisTM C18 column (150 mm × 2.1 mm, 5 μm particle size) with a mobile phase of water (solvent A) and acetonitrile (solvent B) was used for extract separation, with each solvent containing 0.1% formic acid. The solvent gradient was as follows: 15% (B) for 75 min, then 100% (B) from 75 to 85 min, and finally 15% (B) from 85 to 90 min at a flow rate of 0.35 mL/min. The temperature of the column was 25 °C. The experimental parameters were as follows: 3500 V spray voltage, collision gas pressure 1.17 Torr, capillary temperature 320 °C, and nitrogen was used as sheath gas. Xcalibur software version 2.1 was used for system control and data analysis. Compounds were identified based on the mass of compounds previously identified in the genus *Terminalia*.

Table 1
The percentage yield of the extracts from *T. boivinii*.

Extract	Amount (g)	Yield (%)
FME	52.0	26.1
FEF	3.8	1.9
FBF	6.2	3.1
FWF	41.6	20.8
LME	44.8	22.4
LEF	15.4	7.7
LBF	14.2	7.1
LWF	17.6	8.8
SME	12.2	6.1
SEF	1.8	0.9
SBF	1.2	0.6
SWF	8.0	4.0
BME	23.0	11.5
BEF	3.4	1.7
BBF	3.4	1.7
BWF	13.8	6.9

The plant materials used was 200 g per sample; FME, fruit methanol extract; FEF, fruit ethyl acetate fraction; FBF, fruit *n*-butanol fraction; FWF, fruit water fraction; LME, leaves methanol extract; LEF, leaves ethyl acetate fraction; LBF, leaves *n*-butanol fraction; LWF, leaves water fraction; SME, stem methanol extract; SEF, stem ethyl acetate fraction; SBF, stem *n*-butanol fraction; SWF, stem water fraction; BME, bark methanol extract; BEF, bark ethyl acetate fraction; BBF, bark *n*-butanol fraction; BWF, bark water fraction.

2.5. Antioxidant activities

2.5.1. DPPH scavenging assay

The antiradical effect of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) by the extracts was measured by a method previously used by Shimada et al. (1992), with few alterations. The samples and standards were dissolved in dimethyl sulfoxide (DMSO) while the DPPH radical was prepared in 5% DMSO in methanol. Briefly, 5 μL of samples and 45 μL of 5% DMSO in methanol were mixed with 150 μL of DPPH (400 μM) in a 96-well plate and incubated in the dark at room temperature. After 90 min, the absorbance of the extracts was recorded at 517 nm. Butylated hydroxytoluene (BHT) was used as a reference compound. The scavenging effect of the samples was determined using the following formula:

$$Scavenging\ \ effect(\%) = (\frac{A_c - A_s}{\mathrm{Ac}}) \times 100 \tag{1}$$

where A_c is the absorbance of the reaction in the absence of the samples or standards and A_s is the absorbance of the reaction in the presence of the samples.

2.5.2. Superoxide ion scavenging assay

The superoxide ion scavenging activity was monitored by the reduction of nitro blue tetrazolium (NBT) following the protocol of Siddiqui et al. (2016). The samples were dissolved in DMSO while the β-nicotanamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT) and phenazine methosulphate (PMS) were prepared in phosphate buffer (100 mM, pH 7.4). In a 96-well plate, each well contained 10 μL samples solution, 90 μL phosphate buffer, 40 μL NADH (200 μM), 40 μL NBT (80 μM). Twenty microliter of PMS (80 μM) was used to initiate the reaction. After 5 min incubation, the absorbance was measured at 560 nm using a microplate reader. Quercetin was used as a positive control. Equation (1) was employed to calculate the scavenging effect of the samples.

2.5.3. Ferrous ion chelating assay

The ferrous ion chelating capacity of the samples was evaluated in according to a method described by Dinis et al. (1994) with some modifications. Briefly, 0.25 μL sample solutions were mixed with 10 μL $FeCl_2$ (2 mM in distilled water) in a 96-well plate. Ten microliter of ferrozine (5 mM in distilled water) was introduced to the reaction mixture and left to stand for 10 min at room temperature. The absorbance of the solution was determined with a spectrophotometer at 562 nm. Equation (1) was used to determine the iron chelating effect.

2.5.4. Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was carried out with slight modifications according to Oyaizu (1986). In an eppendorf, 200 μL of samples solution was mixed with 400 μL of potassium phosphate buffer (0.2 M, pH 6.6) and 200 μL of potassium ferricyanide [$K_3Fe(CN)_6$], and incubated for 20 min at 50 °C. After incubation, 200 μL of 10% trichloroacetic acid (TCA) was added to the reaction and the solution was then centrifuged for 10 min at 3000 rpm. After centrifuge, the supernatant (100 μL) was mixed with distilled water (80 μL) and 0.1% $FeCl_3$ (20 μL), and left to react for 10 min at room temperature. After reaction, the absorbance was measured at 700 nm. The values were presented as ascorbic acid equivalents (μg AAE/mL).

2.6. Enzymatic activities

2.6.1. α-Amylase inhibition assay

The inhibition of α-amylase by the tested plant extracts was conducted following the procedures of Okutan et al. (2014) with minor adjustments. Briefly, 5 μL of the extract solution (0–1000 μg/mL, dissolved in DMSO), 160 μL of phosphate buffer (0.1 M, pH 6.0) with 0.02% sodium azide, and 10 μL of α-amylase (0.05 U/mL, final concentration) were mixed in a 96-well microplate and incubated at 37 °C for 5 min. After incubation, initiated the reaction with 10 μL of pNPG solution (25 mM, in phosphate buffer) and incubated at 37 °C for 60 min. After incubation, 5 μL NaOH (5M in distilled water) was introduced to the reaction mixture and the absorbance was recorded at 405 nm using a microplate reader. Acarbose (0–25 μg/mL) was used as reference drug. The percentage of inhibition was calculated using the following formula.

$$Inhibition\ \ effect(\%) = (\frac{A_c - A_s}{\mathrm{Ac}}) \times 100 \tag{2}$$

where A_c denotes enzyme reaction without the samples or standard and A_s represents the enzyme reaction with the samples. IC_{50} values were calculated using variable slope nonlinear regression (GraphPad Prism 5.0.1, GraphPad Software, San Diego, California USA).

2.6.2. α-Glucosidase inhibition assay

The α-glucosidase inhibitory properties of the plant extracts were evaluated according to the methods of Lin et al. (2015) and Morocho et al. (2018) with minor changes. Briefly, 10 μL of α-glucosidase (1 U/mL, dissolved in phosphate buffer) was pre-incubated with 5 μL of the extract solution (0–1 mg/mL, dissolved in DMSO) and 170 μL of phosphate buffer (0.2 M, pH 6.8) in a 96-well microplate. After 5 min of incubation at 37 °C, the reaction was initiated by adding 10 μL of 4-p-nitrophenyl-α-D-glucopyranoside (pNPG) solution (25 mM in phosphate buffer) as substrate and incubated for 60 min at 37 °C. After incubation, the reaction was terminated with the addition of 5 μL NaOH (5 M in distilled water) and the absorbance was measured at 405 nm using a spectrophotometer. Acarbose (0–1 mg/mL) was used as positive control. The amount of DMSO [2.5% (v/v)] used did not affect the experiment. Equation (2) was used to estimate the percentage of inhibition.

2.6.3. Lipase inhibitory assay

The inhibition of lipase enzyme was assessed by measuring the enzymatic hydrolysis of 4-p-nitrophenyl butyrate (pNPB) to p-nitrophenol, according to the protocol of Chen et al. (2019) with slight changes. A volume of 50 μL lipase solution (1.2 mg/mL in phosphate buffer 0.1M, pH 7) was mixed with 0.75 μL plant extract solution (0–1 mg/mL, dissolved in DMSO) and pre-incubated on ice for 5 min. Thereafter, 225 μL phosphate buffer and 20 μL pNPB (10 mM in phosphate buffer) were added into the mixture and incubated at 37 °C for 20 min. The reaction was terminated by the addition of 5 μL hydrochloric acid (2N HCl) and the absorbance was recorded at 405 nm. The inhibitory activity was calculated according to equation (2) and ursolic acid was used as a reference compound.

2.6.4. HMG-CoA reductase inhibition assay

HMG-CoA reductase inhibitory capacity of the extracts was evaluated using spectrophotometric method, according to Baskaran et al. (2015). The sample solutions (10 μg/mL) were prepared in DMSO while nicotinamide adenine dinucleotide phosphate (5 mM NADPH), HMG-CoA sodium salt solution (400 μM) and HMG-CoA reductase stock solution (0.75 mg/mL) were prepared in a phosphate buffer (100 mM, pH 7.4) containing potassium chloride (120 mM), ethylenediaminetetraacetic acid (1 mM), and dithiothreitol (5 mM). Each sample (0.2 μL) was mixed with NADPH (5 μL), HMG-CoA sodium salt (10 μL), potassium phosphate buffer (55 μL), followed by the addition of HMG-CoA reductase (2 μL). The reaction was incubated for 10 min at 37 °C and absorbance was measured at 340 nm. Lovastatin was used as a standard. Equation (2) was used to calculate the HMG-CoA reductase inhibition effect.

2.7. Non-enzymatic activities

2.7.1. BSA-MG assay

The test was performed according to the protocol described by Siddiqui et al. (2016), with slight modifications. This model is used to examine the intermediate protein glycation that results from the interaction of bovine serum albumin (BSA) with methylglyoxal (MG). Briefly, 0.5 μl of the sample solutions (various concentrations in DMSO) were mixed with 50 μL BSA solution (10 mg/mL), 100 μL sodium azide solution (6 mM), and 50 μL methylglyoxal solution (14 mM) in a 96-well microplate. All reagent solutions were prepared in phosphate buffer (100 mM, pH 7.4). The reaction mixture was incubated at 37 °C for 7 days with constant stirring and fluorescence was determined using an Varioskan LUX multimode microplate reader at $\lambda_{ex} = 370$ and $\lambda_{em} = 440$ nm. Equation (2) was used to quantify the antiglycation activities.

2.8. Statistical analysis

SPSS version 25 was used for all statistical analyses. One-way analysis of variance (ANOVA) was performed for statistical comparison of the extracts, and the Tukey's HSD test was used to assess differences between means, with p values less than 0.05 considered significant. Pearson correlation was used to evaluate the relationship between the biological activities and polyphenolic content at two statistical levels ($p < 0.05$ and $p < 0.01$). Each figure reflects three separate experiments, and results are reported as the mean ± standard deviation (SD). The variable slope nonlinear and linear regression methods was used to determine IC_{50} values (GraphPad Prism 5.0.1, GraphPad Software, San Diego, California USA).

Table 2
The total phenolic and flavonoid contents of *T. boivinii* extracts.

Extract	Total Phenolic Content (TPC, mg GAE/g DW)	Total Flavonoid Content (TFC, mg QE/g DW)	TFC/TPC ratio
FME	0.96 ± 0.03^{j}	0.32 ± 0.05^{jk}	0.33
FEF	0.74 ± 0.05^{j}	0.43 ± 0.06^{j}	0.58
FBF	1.89 ± 0.17^{i}	0.95 ± 0.05^{i}	0.50
FWF	0.45 ± 0.06^{k}	0.21 ± 0.07^{k}	0.47
LME	29.40 ± 1.85^{c}	14.09 ± 1.36^{c}	0.73
LEF	40.58 ± 2.94^{a}	20.32 ± 1.62^{a}	0.50
LBF	31.41 ± 2.77^{b}	16.38 ± 1.86^{b}	0.52
LWF	16.11 ± 1.35^{d}	9.82 ± 1.35^{e}	0.63
SME	4.69 ± 0.21^{g}	2.57 ± 0.22^{h}	0.55
SEF	6.32 ± 0.53^{f}	3.89 ± 0.72^{gh}	0.62
SBF	5.32 ± 0.34^{g}	2.98 ± 0.30^{h}	0.56
SWF	4.10 ± 0.25^{h}	0.37 ± 0.12^{k}	0.09
BME	15.09 ± 1.21^{e}	8.14 ± 0.48^{f}	0.67
BEF	18.29 ± 1.03^{c}	11.41 ± 0.16^{d}	0.64
BBF	14.85 ± 1.27^{d}	10.12 ± 0.28^{de}	0.74
BWF	12.05 ± 1.69^{e}	4.96 ± 0.26^{g}	0.41

TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalent; QE, quercetin equivalent; FME, fruit methanol extract; FEF, fruit ethyl acetate fraction; FBF, fruit *n*-butanol fraction; FWF, fruit water fraction; LME, leaves methanol extract; LEF, leaves ethyl acetate fraction; LBF, leaves *n*-butanol fraction; LWF, leaves water fraction; SME, stem methanol extract; SEF, stem ethyl acetate fraction; SBF, stem *n*-butanol fraction; SWF, stem water fraction; BME, bark methanol extract; BEF, bark ethyl acetate fraction; BBF, bark *n*-butanol fraction; BWF, bark water fraction. Values expressed are means ± standard deviation (SD) of three parallel measurements. Different letters (a-k) in the same column indicate significant differences ($p < 0.05$) by Tukey's HSD test.

3. Results and discussion

3.1. Phytochemical content

Polyphenols, particularly phenolics and flavonoids, have potent antiradical properties, which underpin their ability to reduce the risk of numerous chronic diseases such as diabetes (Lin et al., 2016). In this study, various extracts from the fruits, leaves, stem and bark of *T. boivinii* were analyzed for their extraction yield and collective bioactive constituents. As shown in Table 1, the extraction yield of the extracts and partitions varied from 0.6% to 26.1%, with the highest yield for FME (26.1%) and the lowest yield for SBF (0.6%). The yield of the extracts was different because of the composition of the individual parts of the plant, while the yield of the fractions varied because of the different polarity of the solvents used for the separation (Naima et al., 2015). Preliminary phytochemical evaluation of the extracts showed that the LEF fraction contained the highest TPC content (40.58 ± 2.94 mg GAE/g DW) and TFC content (20.32 ± 1.62 mg QE/g DW), while the FWF extract had the lowest TPC content (0.45 ± 0.06 mg GEA/g DW) and TFC content (0.21 ± 0.07 mg QE/g DW) among the extracts (Table 2). The TPC content of the extracts varied significantly from 0.45 to 40.58 mg GEA/g DW, while the TFC content ranged from 0.21 to 20.32 mg QE/g DW. The TFC/TPC ratio of plant extracts showed that the extracts contained more than 50% flavonoids except the extract FME (33%). The partitions also contained more than 50% flavonoids, except for the water partitions (FWF, SWF and BWF), and the highest flavonoid contents were observed in BBF (74%) and LME (73%). Considering the selectivity of methanol for phenolics (Zhao and Hall, 2008), all plant parts were extracted with this solvent to maximize such yields. We observed that the low polar fractions (ethyl acetate partition) had higher phenolic and flavonoid contents, while the high polar fractions (water partition) had low phenolic and flavonoid contents. These observations were in agreement with the findings of Saravanakumar et al. (2021) that contents of phenols and flavonoids varies in the extracts of different polarity. The results indicate that the phenols and flavonoids of the extract are mainly distributed in the ethyl acetate and butanol fractions.

3.2. Chemical constituents of *T. boivinii* based on LC-MS analysis

The ethyl acetate fractions (FEF, LEF, SEF and BEF) of *T. boivinii* were analyzed for their chemical constituents using LC-MS. The results of the analysis revealed the presence of several chemical constituents mainly belonging to tannins, lignans, triterpenoids and flavonoids. Tannins and lignans have been found to be a characteristic class of compounds in the genus *Terminalia* (Amalraj and Gopi, 2017; Zhang et al., 2019). Thirty-eight compounds were identified from the ethyl acetate fractions and their relative base peak chromatograms are shown in Figs. S1–4 (Supplementary Material). Table S1 shows detailed information on the identified compounds based on high-resolution mass spectrometry values obtained in positive and negative ionization modes. The compounds were tentatively identified by comparing the MS spectra with information from the literature. All the identified compounds were previously isolated from the genus *Terminalia* using various methods (Zhang et al., 2019). However, to our knowledge, this is the first report on the phytochemical study of *T. boivinii*. Gallic acid, methyl gallate, quercetin, and ellagic acid were found in all fractions studied. Similarly, Zhang et al. (2019) reported that gallic acid and ellagic acid were present in several species of the genus *Terminalia*. Researchers have shown that a majority of simple phenolic compounds have antioxidant, antibacterial, hypoglycemic, liver and kidney protective properties (Singh et al., 2016). Tannins, lignans and triterpenoids not only possess the above activities but also have beneficial effects on immune regulation, cardiovascular diseases and diabetes (Cock, 2015).

Table 3
Antioxidants activities of *T. boivinii* extracts.

Extract	Radical scavenging assays IC_{50} values (µg/mL)		Reducing power FRAP (µg AAE/mL)	Ferrous ion Chelating IC_{50} values (µg/mL)
	DPPH	Superoxide ion		
FME	528.41 ± 16.52^{a}	350.60 ± 9.61^{c}	2.50 ± 0.28^{i}	851.63 ± 28.34^{ab}
FEF	88.83 ± 5.64^{b}	289.49 ± 8.74^{e}	6.62 ± 1.03^{gh}	669.87 ± 17.23^{c}
FBF	84.32 ± 4.32^{b}	465.75 ± 11.25^{b}	5.80 ± 1.21^{h}	>1000
FWF	>1000	605.43 ± 14.64^{a}	1.55 ± 0.56^{i}	478.92 ± 14.36^{d}
LME	33.10 ± 3.23^{d}	142.27 ± 5.45^{h}	9.17 ± 0.24^{ef}	973.44 ± 24.78^{a}
LEF	9.55 ± 0.98^{h}	55.64 ± 6.82^{p}	16.70 ± 0.42^{a}	>1000
LBF	31.14 ± 1.57^{d}	81.31 ± 4.28^{n}	12.64 ± 1.05^{c}	>1000
LWF	27.62 ± 1.14^{de}	135.72 ± 5.58^{i}	10.52 ± 0.57^{de}	433.41 ± 9.25^{e}
SME	85.91 ± 3.79^{b}	174.60 ± 4.89^{f}	7.29 ± 0.43^{gh}	487.60 ± 11.86^{d}
SEF	25.27 ± 2.46^{e}	105.08 ± 4.25^{l}	12.77 ± 0.86^{c}	>1000
SBF	60.31 ± 2.75^{c}	116.16 ± 5.68^{k}	9.83 ± 1.28^{e}	>1000
SWF	80.70 ± 3.64^{b}	156.70 ± 6.19^{g}	8.08 ± 0.82^{fg}	301.47 ± 9.54^{f}
BME	12.29 ± 1.33^{g}	115.93 ± 4.17^{k}	11.82 ± 1.17^{d}	802.40 ± 21.4^{b}
BEF	10.82 ± 0.89^{gh}	80.34 ± 5.82^{n}	14.65 ± 1.24^{b}	>1000
BBF	15.53 ± 1.28^{f}	96.11 ± 4.21^{l}	12.24 ± 1.08^{c}	>1000
BWF	15.04 ± 1.02^{f}	124.62 ± 3.52^{j}	10.18 ± 0.85^{e}	480.84 ± 8.98^{d}
BHT	15.17 ± 0.56^{f}	311.05 ± 8.18^{d}	–	–
Quercetin	69.73 ± 4.25^{c}	50.90 ± 2.01^{p}	–	–
EDTA	–	–	–	120.72 ± 2.68^{g}

DPPH, 1,1-Diphenyl-2-trinitrophenylhydrazine; FRAP, ferric reducing antioxidant power; AAE, ascorbic acid equivalent; FME, fruit methanol extract; FEF, fruit ethyl acetate fraction; FBF, fruit *n*-butanol fraction; FWF, fruit water fraction; LME, leaves methanol extract; LEF, leaves ethyl acetate fraction; LBF, leaves *n*-butanol fraction; LWF, leaves water fraction; SME, stem methanol extract; SEF, stem ethyl acetate fraction; SBF, stem *n*-butanol fraction; SWF, stem water fraction; BME, bark methanol extract; BEF, bark ethyl acetate fraction; BBF, bark *n*-butanol fraction; BWF, bark water fraction. Values expressed are means ± standard deviation (SD) of three parallel measurements. Different letters (a-p) in the same column indicate significant differences ($p < 0.05$) by Tukey's HSD test.

3.3. Antioxidant activities

3.3.1. Radical scavenging activities

The extracts and fractions of *T. boivinii* were assessed for their ability to scavenge DPPH and superoxide anion radicals. Although DPPH and superoxide anion are not physiologically relevant, they are commonly used as indicators for evaluating the antiradical activity of several plant extracts via hydrogen donating and electron delocalizing antioxidants (Dorman et al., 2003). As shown in Table 3, the plant extracts and partitions showed considerable antiradical activities against both DPPH and superoxide anion radical. The antiradical activities of the extracts and partitions varied considerably, with the LEF fraction showing the lowest IC_{50} values for DPPH (9.55 ± 0.98 μg/mL) and superoxide anion (55.64 ± 6.82 μg/mL) compared to the extracts, partitions and standards (BHT and quercetin), indicating significant radical scavenging activities. The FWF fraction showed the lowest radical scavenging effects among the extracts for both DPPH ($IC_{50} > 1000$ μg/mL) and superoxide anion ($IC_{50} = 605.43 \pm 14.64$ μg/mL). Pearson coefficients (Table 5) revealed strong correlations ($p < 0.01$) between polyphenol content and antiradical activities of the extracts, suggesting that phenolic components (especially flavonoids) are responsible for the plant activities. This observation is consistent with the literature in which several flavonoid compounds were described for their scavenging properties, and the amount of hydroxyl groups in the structure was closely related to the antiradical activity (Rice-Evans et al., 1996).

3.3.2. Ferrous reducing antioxidant power activities

The ability of plant extracts to reduce antioxidant power was evaluated by their electron donating capacity. The reducing power activities of the extracts of *T. boivinii* followed a similar pattern to the antiradical activity (Table 3). All the samples exhibited reducing activity; the ethyl acetate fractions showed high activity (LEF: 16.70 ± 0.42 μg AAE/mL; BEF: 14.65 ± 1.24 μg AAE/mL; SEF: 12.77 ± 0.86 μg AAE/mL) followed by the *n*-butanol fractions (LBF: 12.64 ± 1.05 μg AAE/mL; BBF: 12.24 ± 1.08 μg AAE/mL). The water fractions of the plant part exhibited the lowest radical scavenging activities. Moreover, the ethyl acetate and *n*-butanol fractions exhibited the strongest reduction capacities and radical scavenging activities, which might be related to the higher bioactive content and phytochemical composition of these extracts. The results of these antioxidant assays are consistent with previous studies that also showed a significant relationship between phytochemical content and antioxidant capacity (Wang et al., 2017; Zengin et al., 2018a). Pearson coefficients showed a high correlation ($p < 0.01$) between TPC ($r = 0.757$), TFC ($r = 0.756$) and reduction power activities of the studied samples (Table 5). Similarly, a positive correlation was observed between polyphenol content and antioxidant activity in different parts of *E. lathyris* (Zhang et al., 2017).

3.3.3. Ferrous ion-chelating activities

The metal chelating activity of the extracts of *T. boivinii* was assessed and the IC_{50} values are presented in Table 3. The results of metal chelation properties were in contrast to the scavenging and reducing power activities of the extracts. The SWF fraction (301.47 ± 9.54 μg/mL) followed by the water fractions (LWF > FWF > BWF) showed the highest metal chelating activity, while in the other antioxidant assays, the water fractions showed the least activity compared to the other extracts. Similarly, all ethyl acetate and *n*-butanol fractions showed lower metal chelating activity while they were the most active in the other antioxidant assays. Nevertheless, all extracts were less active than the reference compound EDTA ($IC_{50} = 120.72 \pm 2.68$ μg/mL). These results were similar to the findings of Saleem et al. (2021) in the methanol extracts of *Emex australis* Steinh.

Table 4
Enzyme inhibitory activity of the extracts of *T. boivinii*.

Fractions	IC_{50} values (μg/mL)		
	α-Glucosidase	α-Amylase	Lipase
FME	533.32 ± 7.65^{b}	627.32 ± 9.78^{a}	>1000
FEF	70.38 ± 3.34^{d}	128.73 ± 4.24^{c}	167.78 ± 5.75^{g}
FBF	317.21 ± 8.18^{c}	489.39 ± 7.59^{b}	679.65 ± 7.64^{b}
FWF	>1000	>1000	878.06 ± 12.20^{a}
LME	10.72 ± 1.21^{hi}	78.41 ± 5.61^{f}	197.31 ± 7.96^{f}
LEF	6.29 ± 0.78^{jk}	56.20 ± 4.91^{g}	177.76 ± 5.82^{fg}
LBF	8.83 ± 1.07^{i}	62.13 ± 6.23^{g}	248.72 ± 5.53^{e}
LWF	14.75 ± 1.54^{gh}	97.2 ± 7.67^{e}	364.80 ± 8.12^{c}
SME	20.21 ± 1.28^{f}	108.68 ± 8.17^{d}	347.27 ± 8.29^{c}
SEF	15.68 ± 1.43^{g}	92.84 ± 4.38^{e}	189.34 ± 6.18^{f}
SBF	12.23 ± 0.98^{h}	112.30 ± 7.46^{d}	298.70 ± 5.47^{d}
SWF	27.16 ± 1.04^{e}	128.53 ± 9.28^{c}	370.85 ± 5.69^{c}
BME	4.37 ± 0.48^{l}	62.41 ± 2.51^{g}	105.76 ± 4.30^{i}
BEF	2.65 ± 0.45^{m}	43.74 ± 3.71^{h}	94.43 ± 3.49^{j}
BBF	3.68 ± 0.21^{l}	54.86 ± 4.36^{g}	128.41 ± 5.99^{h}
BWF	5.78 ± 0.56^{k}	70.31 ± 4.13^{f}	288.64 ± 9.36^{d}
Acarbose	942.58 ± 19.02^{a}	10.72 ± 0.42^{i}	–
Ursolic acid	–	–	89.20 ± 2.49^{j}

FME, fruit methanol extract; FEF, fruit ethyl acetate fraction; FBF, fruit *n*-butanol fraction; FWF, fruit water fraction; LME, leaves methanol extract; LEF, leaves ethyl acetate fraction; LBF, leaves *n*-butanol fraction; LWF, leaves water fraction; SME, stem methanol extract; SEF, stem ethyl acetate fraction; SBF, stem *n*-butanol fraction; SWF, stem water fraction; BME, bark methanol extract; BEF, bark ethyl acetate fraction; BBF, bark *n*-butanol fraction; BWF, bark water fraction. Values expressed are means ± standard deviation (SD) of three parallel measurements. Different letters (a-m) in the same column indicate significant differences ($p < 0.05$) by Tukey's HSD test.

Table 5
Pearson's correlation coefficients between phytochemical contents, antioxidant and enzyme inhibitory activities of *T. boivinii*.

	TPC	TFC	DPPH	SAR	FRAP	FIC
TPC	1	0.960**	-0.720**	-0.612**	0.757**	0.203
TFC	0.960**	1	-0.829**	-0.623**	0.756**	0.291*
AG	-0.597**	-0.629**	0.848**	0.902**	-0.848**	-0.262
α-Glucosidase	-0.642**	-0.655**	0.970**	0.905**	-0.754**	-0.200
α-Amylase	-0.593**	-0.612**	0.939**	0.933**	-0.728**	-0.191
Lipase	-0.482*	-0.536**	0.351*	0.475**	-0.579**	-0.085
HMG-CoA	0.546**	0.561**	-0.668**	-0.835**	0.749**	-0.151

TPC, total phenolic content; TFC, total flavonoid content; DPPH, 1,1-Diphenyl-2-trinitrophenylhydrazine; SAR, superoxide anion radical; FIC, ferrous ion chelating; FRAP, ferric reducing antioxidant power; AG, antiglycation; HMG-CoA, 3-Hydroxy-3-methylglutaryl-coenzyme A reductase; *correlation significant at $p < 0.05$; **correlation significant at $p < 0.01$.

3.4. Antiglycation activities

Non-enzymatic glycation of proteins is caused by the formation of unstable Schiff bases, which are transformed to Amadori products by the interaction of free amino acids with reducing sugars. The degradation of Amadori products to α-dicarbonyl components such as methylglyoxal before cyclic reactions, dehydration and oxidation, leads to the production of stable and irreversible advanced glycation end products (AGEs) (Ma et al., 2016). In the present work, the extracts of *T. boivinii* were tested for their potential to inhibit the production of AGEs (Fig. 1). All fractions showed moderate antiglycation activities with IC_{50} values ranging from 61.20 to 867.74 μg/mL, which were significantly lower than that of the reference compound, rutin ($IC_{50} = 37.42 \pm 1.25$ μg/mL). Compared to the other extracts, the fraction BEF exhibited the highest antiglycation activity with an IC_{50} value of 61.20 ± 1.22 μg/mL, while the FWF fraction had the lowest activity with an IC_{50} value of 867.74 ± 10.85 μg/mL. Pearson correlation ($p < 0.01$) showed a strong relationship between TPC ($r = -0.597$), TFC ($r = -0.629$) and antiglycation activities. The inhibition of the extracts on the formation of AGEs could be due to a combination of reactive carbonyl species trapping, metal chelating and free radical scavenging properties (Ramonaitytė et al., 2009).

3.5. Enzymatic activities

3.5.1. Alpha-glucosidase and α-amylase inhibition activities

Natural products are believed to be an important means of treating serious health disorders such as diabetes mellitus by inhibiting important key enzymes. (Zengin et al., 2018b). Two major enzymes (α-glucosidase and α-amylase) are thought to play important roles in regulating plasma glucose levels and controlling postprandial glycemia. One of the most important strategies to control glycemic levels is to inhibit these antidiabetic enzymes, which delay the breakdown of carbohydrates in the intestine. (Uysal et al., 2018). In this work, the extracts of *T. boivinii* were tested for their α-glucosidase and α-amylase inhibitory activities. All extracts showed inhibitory effects against both enzymes (Table 4). All samples (except for FWF) inhibited α-glucosidase more efficiently than the reference acarbose. However, in inhibiting amylase, acarbose was more effective than the extracts. The fraction BEF was most active against both α-glucosidase ($IC_{50} = 2.65 \pm 0.45$ μg/mL) and α-amylase ($IC_{50} = 43.74 \pm 3.71$ μg/mL), while the FWF fraction had the least activity against both enzymes, with an IC_{50} value greater than 1000 μg/mL. The inhibitory effects of the extracts were significantly ($p < 0.01$) correlated with TPC and TFC ($-0.642 \le r \ge -0.655$ for α-glucosidase and $-0.593 \le r \ge -0.612$ for α-amylase) (Table 5), suggesting that flavonoids contribute to the enzymatic activity of the extracts. These results are in agreement with a previous report (de Sousa Gomes et al., 2021), which confirmed that flavonoids were responsible for the enzymatic inhibition of *Terminalia phaeocarpa* extracts. The ethyl acetate and *n*-butanol fractions exhibited higher inhibitory activity compared to the water fractions and extracts.

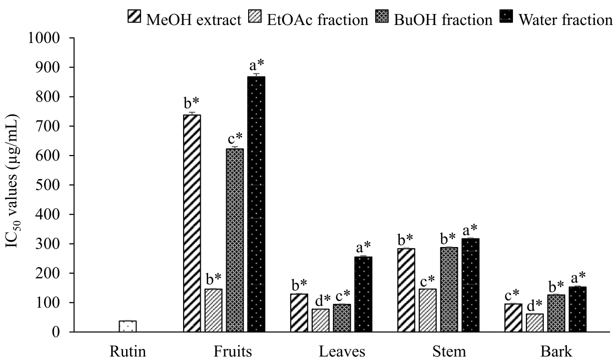

Fig. 1. Antiglycation activities of *T. boivinii* extracts and partitions. Results are expressed as the mean ± standard deviation (n = 3). Asterisk (*) indicate a significant difference ($p < 0.05$) vs standard. Different letters (a–d) represent a significant difference ($p < 0.05$) within a cluster by Tukey's HSD test.

3.5.2. Pancreatic lipase and HMG-CoA reductase inhibition activities

Inhibition of lipase in combination with other key digestive enzymes has been found to lower postprandial glucose levels and appetite, enhancing the efficacy of treatment in obese patients (Holmbäck et al., 2020). Moreover, downregulation of lipase along with HMG-CoA reductase inhibitory activity was determined to be effective in inducing hypolipidemic effect of therapeutic agents (Talawar et al., 2017). In this work, *in vitro* inhibitory activities of pancreatic lipase and HMG-CoA reductase were also conducted, and the results showed considerable strong activities associated with the BEF fraction as compared to other extracts. The fraction BEF effectively inhibited lipase activity with an IC_{50} value of 94.43 ± 3.49 μg/mL which was not significantly different from the reference compound ursolic acid (IC_{50} value 89.20 ± 2.49 μg/mL). As shown in Table 4, all other fractions showed good inhibitory activities against lipase, although statistically lower than that of the ursolic acid. All samples and lovastatin (positive control) were tested for their HMG-CoA reductase inhibitory activity at the same concentration (10 μg/mL) (Fig. 2). The LEF fraction showed the highest inhibitory activity (96.3%), while lovastatin (91.0%), LEM (92.8%) and LBF (89.4%) had similar inhibitory activity and were significant compared to the other extracts. Pearson correlation analysis showed a significant negative relationship between TPC ($r = -0.482$, $p < 0.05$), TFC ($r = -0.536$, $p < 0.01$) and lipase inhibitory activities, while a positive association ($p < 0.01$) was observed for HMG-CoA reductase inhibitory activities TPC ($r = 0.546$) and TFC ($r = 0.561$). These results indicate that the phytochemical content of the tested samples contributes to the inhibitory activities of the extracts. The hypolipidemic activity of the extracts may be attributed to their bioactive constituents, either individually or mutually with synergistic effect on the therapeutic properties (Talawar et al., 2017).

3.6. Relationship between antioxidant, antiglycation and enzyme inhibition activity

Pearson correlation was performed to understand the relationships between the extract and fractions of *T. boivinii*, polyphenol content and respective bioassays (antioxidant, antiglycation and enzyme inhibitory activity. As indicated in Table 5, strong positive correlations ($p < 0.01$) were found between α-amylase ($r = 0.939$ DPPH, $r = 0.933$ SAR), α-glucosidase ($r = 0.970$ DPPH, $r = 0.905$ SAR), antiglycation ($r = 0.848$ DPPH, $r = 0.902$ SAR) and radical scavenging activities, while lipase showed moderate correlation with DPPH ($r = 0.351$, $p < 0.05$) and SAR ($r = 0.475$, $p < 0.01$). In addition, Pearson correlation analysis showed a significantly high negative correlation ($p < 0.01$) between DPPH ($r = -0.668$), SAR ($r = -0.835$) and inhibition of HMG-CoA reductase. The considerable correlations between antioxidants and enzyme inhibition suggest that the group of bioactive chemicals that have antioxidant activity could also be responsible for the enzyme inhibitory effect of the extracts. According to the correlation data, the antiradical and metal chelating abilities of the extracts could serve as a starting point for the search for enzyme inhibitory compounds. It seems that excellent radical scavengers and metal chelators also have good enzyme inhibitory properties. Therefore, the identification of antioxidants from natural sources can be a starting point for the search for natural substances that can prevent metabolic disorders. Moreover, antioxidants have the potential to reduce oxidative stress-induced abnormalities and possess various biological effects under different conditions (Talawar et al., 2017). There is growing evidence that antioxidants from natural sources may be beneficial in the prevention and treatment of metabolic disorders (Bahadoran et al., 2012). Several studies have indicated that increased oxidative stress is relatively strongly associated with metabolic disorders (Ceriello and Motz, 2004; Grattagliano et al., 2008). Oxidative stress is a double-edged sword as it can be both the cause and the consequence of metabolic syndrome. It has been suggested that an imbalance between free radical production and an impaired antioxidant defense system, leading to accumulation of oxidative damage, may play an important role in pathological conditions such as insulin resistance, altered energy production, and endothelial dysfunction, which are important risk factors for metabolic syndrome (Bahadoran et al., 2012). Glycoxidation is increased in metabolic syndrome leading to altered enzyme activities, altered binding of ligands to their receptors and altered protein functionality and immunogenicity. Hyperglycemia-induced oxidative stress leads to the accumulation of advanced glycation end products (AGEs), which cause further cellular damage (Rani et al., 2016), supporting the close relationship between metabolic disorders and oxidative stress.

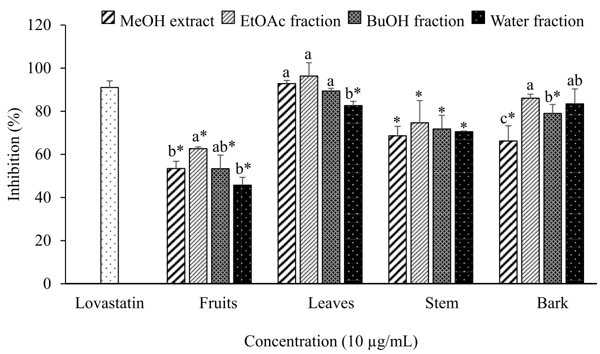

Fig. 2. HMG-CoA reductase inhibition activities of *T. boivinii* extracts and partitions. Results are expressed as the mean ± standard deviation (n = 3). Asterisk (*) indicate a significant difference ($p < 0.05$) vs standard. Different letters (a–d) represent a significant difference ($p < 0.05$) within a cluster by Tukey's HSD test.

4. Conclusions

This is the first study to comprehensively evaluate the antioxidant, enzyme inhibitory and non-enzymatic activities of *T. boivinii* extracts. It was found that the ethyl acetate fractions from all parts exhibited higher phenolic and flavonoid content, superior anti-radical, reducing, antiglycation and enzyme inhibitory activities. However, the water fractions exhibited better metal chelating activities compared to the other extracts. Pearson correlation analysis showed a strong relationship between phytochemical content, antioxidant (DPPH, SAR and FRAP) and enzyme inhibitory properties. According to the current results, the fruits, leaves, stem and bark of *T. boivinii* have high potential as a source of antioxidants and enzyme inhibitors and can be used as an alternative remedy for a variety of public health problems. However, further studies on toxicity are required.

Funding

Ministry of Science and Technology, Taiwan (MOST 105-2320-B-020-002-MY3, MOST 108-2320-B-020-003, and MOST 109-2320-B-020-001).

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.

Acknowledgement

The liquid chromatography-mass spectrometry (LC-MS) analysis was conducted in the Precision Instruments Centre of National Pingtung University of Science and Technology.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2021.102235.

References

Alwhaibi, M., Balkhi, B., Alhawassi, T.M., Alkofide, H., Alduhaim, N., Alabdulali, R., Drweesh, H., Sambamoorthi, U., 2018. Polypharmacy among patients with diabetes: a cross-sectional retrospective study in a tertiary hospital in Saudi Arabia. BMJ Open 8, e020852. https://doi.org/10.1136/bmjopen-2017-020852.

Amalraj, A., Gopi, S., 2017. Medicinal properties of Terminalia arjuna (Roxb.) Wight & Arn.:A review. J. Tradit. Complement. Med. 7, 65–78. https://doi.org/10.1016/j.jtcme.2016.02.003.

Artasensi, A., Pedretti, A., Vistoli, G., Fumagalli, L., 2020. Type 2 diabetes mellitus: a review of multi-target drugs. Molecules 25, 1987. https://doi.org/10.3390/molecules25081987.

Bahadoran, Z., Golzarand, M., Mirmiran, P., Shiva, N., Azizi, F., 2012. Dietary total antioxidant capacity and the occurrence of metabolic syndrome and its components after a 3-year follow-up in adults: Tehran Lipid and Glucose Study. Nutr. Metab. 9, 1–9. https://doi.org/10.1186/1743-7075-9-70.

Baskaran, G., Salvamani, S., Ahmad, S.A., Shaharuddin, N.A., Pattiram, P.D., Shukor, M.Y., 2015. HMG-CoA reductase inhibitory activity and phytocomponent investigation of Basella alba leaf extract as a treatment for hypercholesterolemia. Drug Des. Dev. Ther. 9, 509. https://doi.org/10.2147/Dddt.S75056.

Basu, T., Panja, S., Ghate, N.B., Chaudhuri, D., Mandal, N., 2017. Antioxidant and antiproliferative effects of different solvent fractions from Terminalia belerica Roxb. fruit on various cancer cells. Cytotechnology 69, 201–216. https://doi.org/10.1007/s10616-016-0051-6.

Ceriello, A., Motz, E., 2004. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler. Thromb. Vasc. Biol. 24, 816–823. https://doi.org/10.1161/01.ATV.0000122852.22604.78.

Chang, S.T., Wu, J.H., Wang, S.Y., Kang, P.L., Yang, N.S., Shyur, L.F., 2001. Antioxidant activity of extracts from Acacia confusa bark and heartwood. J. Agric. Food Chem. 49, 3420–3424. https://doi.org/10.1021/jf0100907.

Chen, Z., Zhang, D., Guo, J.J., Tao, W., Gong, R.X., Yao, L., Zhang, X.L., Cao, W.-G., 2019. Active components, antioxidant, inhibition on metabolic syndrome related enzymes, and monthly variations in mature leaf hawk tea. Molecules 24, 657. https://doi.org/10.3390/molecules24040657.

Cock, I., 2015. The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae). Inflammopharmacology 23, 203–229. https://doi.org/10.1007/s10787-015-0246-z.

Datta, S., Pal, N.K., Nandy, A.K., 2017. In vitro antibacterial activity of bioactive potent compounds from Terminalia chebula against some common human pathogens. Pharmacol. Pharm. 8, 283–291. https://doi.org/10.4236/pp.2017.89021.

de Araújo, A.R., Iles, B., de Melo Nogueira, K., do Nascimento Dias, J., Plácido, A., Rodrigues, A., Albuquerque, P., Silva-Pereira, I., Socodatto, R., Portugal, C.C., 2019. Antifungal and anti-inflammatory potential of eschweilenol C-rich fraction derived from Terminalia fagifolia Mart. J. Ethnopharmacol. 240, 111941 https://doi.org/10.1016/j.jep.2019.111941.

De la Iglesia, R., Loria-Kohen, V., Zulet, M.A., Martinez, J.A., Reglero, G., Ramirez de Molina, A., 2016. Dietary strategies implicated in the prevention and treatment of metabolic syndrome. Int. J. Mol. Sci. 17, 1877. https://doi.org/10.3390/ijms17111877.

de Sousa Gomes, J.H., Mbiakop, U.C., Oliveira, R.L., Stehmann, J.R., de Pádua, R.M., Cortes, S.F., Braga, F.C., 2021. Polyphenol-rich extract and fractions of Terminalia phaeocarpa Eichler possess hypoglycemic effect, reduce the release of cytokines, and inhibit lipase, α-glucosidase, and α-amilase enzymes. J. Ethnopharmacol. 271, 113847 https://doi.org/10.1016/j.jep.2021.113847.

Dinis, T.C., Madeira, V.M., Almeida, L.M., 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315, 161–169. https://doi.org/10.1006/abbi.1994.1485.

Dorman, H., Peltoketo, A., Hiltunen, R., Tikkanen, M., 2003. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chem. 83, 255–262. https://doi.org/10.1016/S0308-8146(03)00088-8.

Fyhrquist, P., Mwasumbi, L., Hæggström, C.A., Vuorela, H., Hiltunen, R., Vuorela, P., 2002. Ethnobotanical and antimicrobial investigation on some species of Terminalia and Combretum (Combretaceae) growing in Tanzania. J. Ethnopharmacol. 79, 169–177. https://doi.org/10.1016/S0378-8741(01)00375-0.

Grattagliano, I., Palmieri, V.O., Portincasa, P., Moschetta, A., Palasciano, G., 2008. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. J. Nutr. Biochem. 19, 491–504. https://doi.org/10.1016/j.jnutbio.2007.06.011.

Gulati, V., Harding, I.H., Palombo, E.A., 2012. Enzyme inhibitory and antioxidant activities of traditional medicinal plants: potential application in the management of hyperglycemia. BMC Compl. Alternative Med. 12, 1–9. https://doi.org/10.1186/1472-6882-12-77.

Handelsman, Y., Jellinger, P.S., 2011. Overcoming obstacles in risk factor management in type 2 diabetes mellitus. J. Clin. Hypertens. 13, 613–620. https://doi.org/10.1111/j.1751-7176.2011.00490.x.

Holmbäck, U., Forslund, A., Grudén, S., Alderborn, G., Söderhäll, A., Hellström, P.M., Lennernäs, H., 2020. Effects of a novel combination of orlistat and acarbose on tolerability, appetite, and glucose metabolism in persons with obesity. Obes. Sci. Pract. 6, 313–323. https://doi.org/10.1002/osp4.405.

Hou, X.D., Song, L.L., Cao, Y.F., Wang, Y.N., Zhou, Q., Fang, S.Q., Wu, D.C., Zang, S.Z., Chen, L., Bai, Y., Ge, G.B., Hou, J., 2020. Pancreatic lipase inhibitory constituents from Fructus Psoraleae. Chin. J. Nat. Med. 18, 369–378. https://doi.org/10.1016/S1875-5364(20)30043-1.

Kesharwani, A., Polachira, S.K., Nair, R., Agarwal, A., Mishra, N.N., Gupta, S.K., 2017. Anti-HSV-2 activity of Terminalia chebula Retz extract and its constituents, chebulagic and chebulinic acids. BMC Compl. Alternative Med. 17, 110. https://doi.org/10.1186/s12906-017-1620-8.

Khan, M.S.A., Ahmed, N., Arifuddin, M., Zakaria, Z.A., Al-Sanea, M.M., Khundmiri, S.U.K., Ahmed, I., Ahmed, S., Mok, P.L., 2018. Anti-nociceptive mechanisms of flavonoids-rich methanolic extract from Terminalia coriacea (Roxb.) Wight and Arn. leaves. Food Chem. Toxicol. 115, 523–531. https://doi.org/10.1016/j.fct.2018.03.021.

Kouassi, E.K., Coulibaly, I., Pop, R., Adela, P., Allico, J.D., 2019. In vitro antioxidant potency and antifungal efficiency of four local Terminalia species against Fusarium strains. J. Exp. Agric. Int. 29, 1–7. https://doi.org/10.9734/JEAI/2019/45047.

Lillich, F.F., Imig, J.D., Proschak, E., 2020. Multi-target approaches in metabolic syndrome. Front. Pharmacol. 11 https://doi.org/10.3389/fphar.2020.554961, 1996.

Lim, S., Eckel, R.H., 2014. Pharmacological treatment and therapeutic perspectives of metabolic syndrome. Rev. Endocr. Metab. Disord. 15, 329–341. https://doi.org/10.1007/s11154-014-9298-4.

Lin, Y.S., Chen, C.R., Wu, W.H., Wen, C.L., Chang, C.I., Hou, W.C., 2015. Anti-α-glucosidase and anti-dipeptidyl peptidase-IV activities of extracts and purified compounds from Vitis thunbergii var. taiwaniana. J. Agric. Food Chem. 63, 6393–6401. https://doi.org/10.1021/acs.jafc.5b02069.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., 2016. An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules 21, 1374. https://doi.org/10.3390/molecules21101374.

Liu, G., Shi, M., Mosley, J.D., Weng, C., Zhang, Y., Lee, M.T.M., Jarvik, G.P., Hakonarson, H., Namjou-Khales, B., Sleiman, P., 2021. A Mendelian randomization approach using 3-HMG-coenzyme-A reductase gene variation to evaluate the association of statin-induced low-density lipoprotein cholesterol lowering with noncardiovascular disease phenotypes. JAMA Netw. Open 4, e2112820. https://doi.org/10.1001/jamanetworkopen.2021.12820 e2112820.

Ma, H., Liu, W., Frost, L., Kirschenbaum, L.J., Dain, J.A., Seeram, N.P., 2016. Glucitol-core containing gallotannins inhibit the formation of advanced glycation end-products mediated by their antioxidant potential. Food Funct. 7, 2213–2222. https://doi.org/10.1039/c6fo00169f.

McGaw, L., Rabe, T., Sparg, S., Jäger, A., Eloff, J., Van Staden, J., 2001. An investigation on the biological activity of Combretum species. J. Ethnopharmacol. 75, 45–50. https://doi.org/10.1016/s0378-8741(00)00405-0.

Mongraw-Chaffin, M., Foster, M.C., Anderson, C.A., Burke, G.L., Haq, N., Kalyani, R.R., Ouyang, P., Sibley, C.T., Tracy, R., Woodward, M., 2018. Metabolically healthy obesity, transition to metabolic syndrome, and cardiovascular risk. J. Am. Coll. Cardiol. 71, 1857–1865. https://doi.org/10.1016/j.jacc.2018.02.055.

Morocho, V., Valle, A., Garcia, J., Gilardoni, G., Cartuche, L., Suarez, A.I., 2018. Alpha-glucosidase inhibition and antibacterial activity of secondary metabolites from the Ecuadorian species Clinopodium taxifolium (Kunth) Govaerts. Molecules 23, 146. https://doi.org/10.3390/molecules23010146.

Naima, R., Oumam, M., Hannache, H., Sesbou, A., Charrier, B., Pizzi, A., Charrier-El Bouhtoury, F., 2015. Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan Acacia mollissima barks. Ind. Crop. Prod. 70, 245–252. https://doi.org/10.1016/j.indcrop.2015.03.016.

Okatch, H., Ngwenya, B., Raletamo, K.M., Andrae-Marobela, K., 2012. Determination of potentially toxic heavy metals in traditionally used medicinal plants for HIV/AIDS opportunistic infections in Ngamiland District in Northern Botswana. Anal. Chim. Acta 730, 42–48. https://doi.org/10.1016/j.aca.2011.11.067.

Okutan, L., Kongstad, K.T., Jäger, A.K., Staerk, D., 2014. High-resolution α-amylase assay combined with high-performance liquid chromatography-solid-phase extraction-nuclear magnetic resonance spectroscopy for expedited identification of α-amylase inhibitors: proof of concept and α-amylase inhibitor in cinnamon. J. Agric. Food Chem. 62, 11465–11471. https://doi.org/10.1021/jf5047283.

Oyaizu, M., 1986. Studies on products of browning reactions: antioxidative activities of product of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307.

Oyenihi, A.B., Brooks, N.L., Oguntibeju, O.O., Aboua, G., 2014. Antioxidant-rich natural products and diabetes mellitus. In: Oguntibeju, O. (Ed.), Antioxidant-antidiabetic Agents and Human Health. IntechOpen, Jeneza Trdine 9, 51000 Rijeka, Croatia, pp. 317–345. https://doi.org/10.5772/57192.

Philip, C.J., Carrol, L.D., Longdet, I.Y., Chinyere, A.J., 2017. Antidiabetic activities of the aqueous root bark and flower extracts of Terminalia catappa on streptozotocin-induced diabetes in male Wister Rats. Eur. J. Med. Plants 20, 1–9. https://doi.org/10.9734/EJMP/2017/34702.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J.-C., Bailleul, F., Trotin, F., 2000. Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J. Ethnopharmacol. 72, 35–42. https://doi.org/10.1016/S0378-8741(00)00196-3.

Ramonaitytė, D.T., Keršienė, M., Adams, A., Tehrani, K.A., De Kimpe, N., 2009. The interaction of metal ions with Maillard reaction products in a lactose-glycine model system. Food Res. Int. 42, 331–336. https://doi.org/10.1016/j.foodres.2008.12.008.

Ramsay, R.R., Tipton, K.F., 2017. Assessment of enzyme inhibition: a review with examples from the development of monoamine oxidase and cholinesterase inhibitory drugs. Molecules 22, 1192. https://doi.org/10.3390/molecules22071192.

Rani, V., Deep, G., Singh, R.K., Palle, K., Yadav, U.C., 2016. Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies. Life Sci. 148, 183–193. https://doi.org/10.1016/j.lfs.2016.02.002.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956. https://doi.org/10.1016/0891-5849(95)02227-9.

Saleem, A., Javeed, A., Ashraf, M., Akhtar, M.F., Akhtar, B., Sharif, A., Akhtar, K., Raza, M., Hamid, I., Peerzada, S., 2017. Anti-inflammatory, anti-nociceptive and antipyretic potential of Terminalia citrina fruit extracts. Afr. J. Tradit., Complementary Altern. Med. 14, 24–30. https://doi.org/10.21010/ajtcam.v14i5.4.

Saleem, H., Zengin, G., Sarfraz, M., Alafnan, A., Locatelli, M., Tartaglia, A., Ahmad, I., Mahomoodally, M.F., Khurshid, U., Ahemad, N., 2021. Phytochemical composition and in-vitro pharmacological evaluation of Emex australis Steinh: a natural source of enzyme inhibitors. South Afr. J. Bot. https://doi.org/10.1016/j.sajb.2021.02.023.

Saravanakumar, K., Park, S., Mariadoss, A.V.A., Sathiyaseelan, A., Veeraraghavan, V.P., Kim, S., Wang, M.-H., 2021. Chemical composition, antioxidant, and anti-diabetic activities of ethyl acetate fraction of Stachys riederi var. japonica (Miq.) in streptozotocin-induced type 2 diabetic mice. Food Chem. Toxicol. 155, 112374 https://doi.org/10.1016/j.fct.2021.112374.

Semenya, S., Potgieter, M., Tshisikhawe, M., 2013. Use, conservation and present availability status of ethnomedicinal plants of Matebele-Village in the Limpopo Province, South Africa. Afr. J. Biotechnol. 12, 2392–2405. https://doi.org/10.5897/AJB12.2572.

Senkoro, A.M., Barbosa, F.M., Moiane, S.F., Albano, G., de Barros, A.I.R., 2014. Bark Stripping from forest tree species in Madjadjane, Southern Mozambique: medicinal uses and implications for conservation. Nat. Resour. 5, 192–199. https://doi.org/10.4236/nr.2014.55018.

Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40, 945–948. https://doi.org/10.1021/jf00018a005.

Siddiqui, M.A., Rasheed, S., Saquib, Q., Al-Khedhairy, A.A., Al-Said, M.S., Musarrat, J., Choudhary, M.I., 2016. In vitro dual inhibition of protein glycation, and oxidation by some Arabian plants. BMC Compl. Alternative Med. 16, 1–10. https://doi.org/10.1186/s12906-016-1225-7.

Singh, A., Bajpai, V., Kumar, S., Kumar, B., Srivastava, M., Rameshkumar, K.B., 2016. Comparative profiling of phenolic compounds from different plant parts of six Terminalia species by liquid chromatography–tandem mass spectrometry with chemometric analysis. Ind. Crop. Prod. 87, 236–246. https://doi.org/10.1016/j.indcrop.2016.04.048.

Talawar, S.T., Harohally, N.V., Ramakrishna, C., Suresh Kumar, G., 2017. Development of wheat bran oil concentrates rich in bioactives with antioxidant and hypolipidemic properties. J. Agric. Food Chem. 65, 9838–9848. https://doi.org/10.1021/acs.jafc.7b03440.

Talevi, A., 2015. Multi-target pharmacology: possibilities and limitations of the "skeleton key approach" from a medicinal chemist perspective. Front. Pharmacol. 6, 205. https://doi.org/10.3389/fphar.2015.00205.

Uysal, S., Ugurlu, A., Zengin, G., Baloglu, M.C., Altunoglu, Y.C., Mollica, A., Custodio, L., Neng, N.R., Nogueira, J.M., Mahomoodally, M.F., 2018. Novel in vitro and in silico insights of the multi-biological activities and chemical composition of Bidens tripartita L. Food Chem. Toxicol. 111, 525–536. https://doi.org/10.1016/j.fct.2017.11.058.

Wang, H., Guo, X., Hu, X., Li, T., Fu, X., Liu, R.H., 2017. Comparison of phytochemical profiles, antioxidant and cellular antioxidant activities of different varieties of blueberry (Vaccinium spp.). Food Chem. 217, 773–781. https://doi.org/10.1016/j.foodchem.2016.09.002.

York, T., Van Vuuren, S., De Wet, H., 2012. An antimicrobial evaluation of plants used for the treatment of respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. J. Ethnopharmacol. 144, 118–127. https://doi.org/10.1016/j.jep.2012.08.038.

Zengin, G., Llorent-Martínez, E.J., Fernández-de Córdova, M.L., Bahadori, M.B., Mocan, A., Locatelli, M., Aktumsek, A., 2018a. Chemical composition and biological activities of extracts from three Salvia species: S. blepharochlaena, S. euphratica var. leiocalycina, and S. verticillata subsp. amasiaca. Ind. Crop. Prod. 111, 11–21. https://doi.org/10.1016/j.indcrop.2017.09.065.

Zengin, G., Zheleva-Dimitrova, D., Gevrenova, R., Nedialkov, P., Mocan, A., Ćirić, A., Glamočlija, J., Soković, M., Aktumsek, A., Mahomoodally, M.F., 2018b. Identification of phenolic components via LC-MS analysis and biological activities of two Centaurea species: C. drabifolia subsp. drabifolia and C. lycopifolia. J. Pharmaceut. Biomed. Anal. 149, 436–441. https://doi.org/10.1016/j.jpba.2017.11.045.

Zhang, L., Wang, C., Meng, Q., Tian, Q., Niu, Y., Niu, W., 2017. Phytochemicals of Euphorbia lathyris L. and their antioxidant activities. Molecules 22, 1335. https://doi.org/10.3390/molecules22081335.

Zhang, X.R., Kaunda, J.S., Zhu, H.T., Wang, D., Yang, C.R., Zhang, Y.J., 2019. The genus Terminalia (Combretaceae): an ethnopharmacological, phytochemical and pharmacological review. Nat. Prod. Bioprospect. 9, 357–392.

Zhao, B., Hall III, C.A., 2008. Composition and antioxidant activity of raisin extracts obtained from various solvents. Food Chem. 108, 511–518. https://doi.org/10.1016/j.foodchem.2007.11.003.

Zimmermann, G.R., Lehar, J., Keith, C.T., 2007. Multi-target therapeutics: when the whole is greater than the sum of the parts. Drug Discov. Today 12, 34–42. https://doi.org/10.1016/j.drudis.2006.11.008.