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Metabolite profiling, in vitro antioxidant and α-glucosidase inhibitory properties of *Timonius ternifolius* leaves

MAINGELLINE B. VIVIT[1,♥], RAMJEE G. BATUYONG[2], ALONDRA FAE T. DAMIAN[2], CECILE A. GAOAT[1,2], KRISTIAN GAY D. BELTRAN[1,3], ANABELLE B. ALEJO[1,3], MICHAEL A. CALARAMO[4], FRANKLIN V. IBANA[1,3], MAE ANN R. BATUYONG[1,2,5]

[1]Tuklas Lunas Development Center, Research Directorate, Mariano Marcos State University, City of Batac 2906, Ilocos Norte, Philippines. Tel.: +63-777-923133, ♥email: mbvivit@mmsu.edu.ph
[2]Department of Biological Sciences, College of Arts and Sciences, Mariano Marcos State University, City of Batac 2906, Ilocos Norte, Philippines
[3]Department of Pharmacy, College of Health Sciences, Mariano Marcos State University, City of Batac, 2906, Ilocos Norte, Philippines
[4]Northwestern University Ecological Park and Botanic Gardens, Airport Avenue, Bengcag, Laoag City, 2900, Ilocos Norte, Philippines
[5]Natural Resources Conservation and Management Center, Research Directorate, Mariano Marcos State University, City of Batac, 2906, Ilocos Norte, Philippines

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Abstract. *Vivit MB, Batuyong RG, Damian AFT, Gaoat CA, Beltran KGD, Alejo AB, Calaramo MA, Ibana FV, Batuyong MAR. 2024. Metabolite profiling, in vitro antioxidant and α-glucosidase inhibitory properties of* Timonius ternifolius *leaves. Biodiversitas 25: 3027-3034.* The genus *Timonius* (Rubiaceae) possesses different pharmacological properties. One understudied endemic species under this genus from the Philippines is the *Timonius ternifolius* (Bartl. ex DC.) Fern.-Vill. Hence, this study investigates the physicochemical properties and phytochemical constituents of the leaf ethanolic extract (EE) and subjected to solvent partitioning to yield the following subfractions: hexane (HE), ethyl acetate (EA), and methanol (ME) extracts, which were utilized for the in vitro antioxidant, α-glucosidase and α-amylase assays. Quantitative tests of *T. ternifolius* leaves revealed the following physicochemical properties: herbal tea-like odor, fibrous and fluffy appearance, 50.07% moisture content, 3.10% total ash content, and a slightly acidic pH value of 5.13. The liquid chromatography-mass spectrometry (LC-MS) analysis has identified eight putative compounds namely glabrolide, aloinoside B, 2-benzyl octanal, geumonoid, (±) gomisin M1, 25-O-Acetyl-7,8-didehydrocimigenol-3-O-β-D-xylopyranoside, lucialdehyde B, and kadsuric acid. The extracts' concentrations of flavonoids, phenols, and terpenoids were 1.23±0.19 mg QE/g, 20.77±0.77 mg GAE/g, and 6.07±0.53 mg/g, respectively. Antioxidant profiling among the subfractions showed EE exhibiting the highest at 59.15±2.08 µg/mL (IC_{50} value). Moreover, significant α-glucosidase inhibition was noted in the EE extract at 99.74%, EA at 99.71%, and ME at 99.45%. The results showed that *T. ternifolius* leaves are a promising lead source of pharmacologically active compounds with antioxidant and antidiabetic activities, expanding information on plant-derived alternative treatments and developing medicinal products.

Keywords: α-glucosidase, liquid chromatography-mass spectrophotometry, LC-MS, Rubiaceae, *Timonius ternifolius*

INTRODUCTION

The Rubiaceae family comprises more than 13,000 species of trees, shrubs, herbs, geofrutices, myrmecophiles, epiphytes, and lianas, making it one of the largest among the flowering plants (Bremer and Eriksson 2009; Davis et al. 2009). Most studies on the Rubiaceae family are based on taxonomy; however, there is an increasing interest in exploring its medicinal properties. In addition, species of this family have been utilized in traditional medicine (Igustita et al. 2023) and screened to produce bioactive chemical constituents with pharmacological activities, such as anthraquinones, iridoids, indole alkaloids, terpenoids, flavonoids, tannins, saponins, aliphatic and aromatic compounds, chologenic acid (Gandamalla et al. 2017), giving rise to potential new nature-oriented drugs and management for antimalarial, antimicrobial, antihypertension, antidiabetic, antioxidant, anti-inflammatory, mutagenic, antiviral, analgesic, anti-amyloidogenic, neuroprotective activities and muscle relaxant (Martins and Nunez 2015; Sadino et al. 2018; Adewole et al. 2021). Moreover, several species have isolated and identified new compounds such as vomifoliol, p-coumaric acid, stigmasterol, and hexenoic acid glycoside (Tan et al. 2020; Ramil et al. 2021).

In the Philippines, most of the phytochemical and pharmacological analyses that have been done are from the genera *Hedyotis*, *Lasianthus*, *Ophiorrhiza*, *Psychotria*, *Psydrax, Tarenna, Uncaria*, and *Villaria* (Castro et al. 2016; Olivar et al. 2017; Tan and An 2020; Aureada et al. 2023; Tan and Ishikawa 2023). Considering that the country ranks among the top 20 regions with the highest diversity of Rubiaceae species, with four endemic genera (Alejandro et al. 2010, Arriola et al. 2016), several compounds from these plants are still waiting to be described since there are limited investigations for their biochemical compositions. Thus, plants of interest are assessed as additional resources that can be used for research into potential pharmacological applications.

The genus *Timonius* DC. has the most species under the tribe Guettardeae. It consists of hundreds of species distributed in the Paleotropics, excluding Africa, and extends to tropical Australia and the Pacific Islands (Darwin 2010). One of the reported endemic species under this genus that thrives in the lowland forests and thickets, specifically at the

Kalbario Patapat Natural Park (KPNP) and Metropolitan Ilocos Norte Watershed Forest Reserve (WINWFR) in the Northwestern part of the Philippines is the *Timonius ternifolius* (Bartl. ex DC.) Fern.-Vill (Batuyong et al. 2020; Batuyong et al. 2021). It is a shrub or small tree with axillary inflorescences that bear few to numerous flowers, possessing a multi-locular ovary and drupaceous fruits with several pyrenes. There are no other reports on the chemical composition as well as biological activities of the extracts of *T. ternifolius* aside from the recent study of Esguerra et al. (2024), which described *T. ternifolius* to have a comparable hydroxyl radical scavenging antioxidant of 81.28% ±4.69 with the control in terms of antioxidant activity. However, studies have been conducted on several species of the same genus, such as *T. timon,* with the following triterpenes identified to be present, such as 3β,6β,23-trihydroxy-olean-12-en-28-oic acid (Martins and Nunez 2015). While *T. flavescens* (Jack) Baker leaves contain (3β)-Stigmast-5-en-3-ol,3β.-(Acetyloxy)-15α.-hydroxy-5.α.-cholesta-8(14),9(11) -dien-7-one,alpha-tocopherol, hexadecanoic acid, nonanoic acid, phytol, 2,3-dihydrobenzofuran, heptanoic acid, neophytadiene, and campesterol, that have been described to possess antidiabetic property (Sipahutar et al. 2023). The same species also showed anti-inflammatory activity by inhibiting the lipoxygenase enzyme (Chung et al. 2009), antidiabetic activity, and immunostimulatory activity (Gaol et al. 2021). Moreover, *T. celebicus* Koord. leaves and stems exhibited strong antioxidant and weak to moderate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Praptiwi et al. 2021).

Moreover, there are around 16,690 recorded medicinal plants in the Philippines, but the Department of Health has approved only 10 of them as Herbal Medicinal Plants (Cordero et al. 2020). Thus, in addition to the Rubiaceae plants from the Philippines considered for their biological activities, this study was carried out to evaluate the total flavonoid, phenol, and terpenoid content along with the in vitro antioxidant and α-glucosidase inhibitory properties of the ethanolic leaf extracts of *T. ternifolius,* which will serve as a basis for future research.

MATERIALS AND METHODS

Chemical reagents and equipment

Analytical-grade chemicals were used in the study. The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Folin-Ciocalteu's reagent, and quercetin were bought from Sigma-Aldrich (USA). Sodium carbonate was purchased from Merck (Germany); ethanol, methanol, hexane, and ethyl acetate from Chem Supply Pty Ltd. (Australia); sodium carbonate from Merck Germany); ascorbic acid from Himedia Lab. Pvt. Ltd. (India); potassium acetate from Loba Chemie (India); and aluminum chloride from Techno Pharmchem Haryana (India). Acarbose, α-amylase, α-glucosidase, dinitro salicylic acid (DNSA), sodium phosphate buffer, *p*-nitrophenyl-α-D-glucopyranoside, and dimethylsulfoxide (DMSO) used in the bioassays were Sigma-Aldrich (USA). Formic acid, acetonitrile, and Leucine enkephalin were HPLC grade.

Plant collection and sample preparation

Before plant collection, a certification/permit from concerned local government units was secured. The leaf samples of *T. ternifolius* were acquired from Mt. Pao Range in the Municipality of Adams, Ilocos Norte. The collected fresh leaves (500-1000 g) were cleaned, and oven-dried (Binder Model E28, USA) at 50°C for 2 to 3 days. The dried leaves were homogenized using a grinder (Osterizer, USA). The powdered samples were placed in screw-capped bottles and refrigerated before extraction.

Plant extract preparation

Powdered leaves (600 g) were macerated in 80% ethanol (1:3 w/v). After 48 hours, the extracts were filtered, and the process was repeated twice. The extracts were concentrated using a rotary evaporator (Heidolph Model HEI-VAP Advantage, Germany). The crude ethanolic extract (EE) weighing 7.5 g was dissolved in methanol (ME) at a 90:10 ratio. Then, it was exhaustively extracted with n-hexane (HE), followed by ethyl acetate (EA) at a ratio of 1:2 (v/v). The subfractions were concentrated using a speed vacuum concentrator (Eppendorf Model Concentrator Plus, USA) and stored in an amber-colored bottle at -20°C (Evermed Model LF140W, Italy).

Physicochemical and organoleptic evaluation

The physicochemical characteristics of *T. ternifolius* leaf and crude extract, such as organoleptic characteristics, moisture content, total ash, pH, and extractive values, were determined according to the WHO quality control methods for herbal materials guidelines (WHO 2011).

Phytochemical analysis and LC-MS profiling

The LC-MS analysis was conducted at the University of the Philippines Manila. The crude extract was dissolved in methanol and diluted it to 1,000 µg/mL. After which, a 0.2 µm PTFE syringe filter passed through the solution, and then 1µL was injected into the Ultra-performance liquid chromatography (UPLC, Waters UPLC Class I, USA). The separation was performed on the ACQUITY HSS T# C18 column (100 mm × 2.1 mm × 1.8 µm) at 40°C. The mobile phase consisted of solvent (A) water with 0.1% formic acid and solvent (B) acetonitrile with 0.1% formic acid. Gradient elution was performed at a flow rate of 0.5 mL/min with an elution profile of [95% (A), 5% (B)] from 0 to 0.5 min, and [5% (A), 95% (B)] from 0.5 to 15 min.

The mass spectrometric analysis used Waters Xevo G2-XS QTof, MS^E mode. The parameters were set at a capillary voltage of 1.0kV (ESI^+), 120°C source temperature, 550°C desolvation temperature, 40V cone voltage, 50 L/h cone gas flow, and 950 L/h desolvation gas flow. The mass analyzer was scanned from 500-1,200 m/z for 0.150 s. The collision energy was set to a high ramp of 15 to 50 eV with Leucine enkephalin compound used as a reference for mass correction.

The mass screening was carried out using the UNIFI data analysis software. The base peak ions of distinct peaks were subjected to library matching using the Waters Traditional Chinese Medicine (TCM) library. Annotation of the candidate masses was based on the accurate mass

match, isotopic ratio match, and precursor ion intensity counts. The data acquisition mode implements an unbiased data collection of all spectral features or signals detected.

Determination of total flavonoid content (TFC)

The method of Phong et al. (2022) was used with slight modifications to determine the total flavonoid content. About 5 g of powdered dried plant material was macerated with 80% ethanol for 24 hours with frequent shaking. The filtered extracts were evaporated using a rotary evaporator (Sagbo et al. 2017). The dried crude extracts were used for TFC and TPC determination.

About 1 mg of extract was dissolved in ethanol and diluted to 1,000 µg/mL. Briefly, 30 µL of the extract/ standard was added with 90 µL of 95% ethanol, 6 µL of 10% aluminum chloride, and 6 µL of 1M potassium acetate solution. The resulting mixture was made up to 300 µL and incubated for 30 minutes using an incubator shaker (FinePCR Model BAE07-H300, Korea). Next, using a microplate reader, the absorbance was measured at 415 nm (BMG Labtech Model Clariostar, Germany). The quercetin equivalents were used to express the flavonoid content and calculated as mg/g DW basis.

Determination of total phenol content (TPC)

About 30 µL of extract and the standard were separately added with 150 µL of 10% Folin-Ciocalteu's reagent. The mixture was incubated for 3-8 minutes, and then 120 µL of sodium carbonate solution (7.5%, w/v) was added. An incubation for 1 hour followed by measuring the absorbance at 765 nm using a microplate reader. Phenolic content was expressed in gallic acid equivalents and then calculated as mg/g DW basis (Phong et al. 2022).

Determination of total terpenoid content

Dried plant material (2 g) was soaked in 50 mL of 97% ethanol for 24 h with frequent stirring. The extracts were filtered, and the filtrate was placed into a separatory funnel. The solution was extracted with 50 mL petroleum ether, shaken, and allowed to rest until a layer was formed. The petroleum ether extract, the top layer, was collected and eventually concentrated to dryness at 40°C. The weight of the dried extract, considered a crude terpenoid, was measured. The total terpenoid was expressed as mg/g DW sample powder (Oncho et al. 2021).

In vitro antioxidant activity

With some modifications, the antioxidant assay was done using the DPPH radical scavenging method (La et al. 2021). The extracts and ascorbic acid were diluted to different concentrations (10 to 300 µg/mL). Consequently, 75 µL of the extract/standard was reacted with 225 µL of DPPH (0.01 mM) and kept in the darkroom for 30 minutes. The absorbance was measured at 517 nm using a microplate reader. The radical scavenging activity (RSA) was computed using the formula:

$$RSA(\%) = \frac{Absorbance\ of\ Control\ -\ Absorbance\ of\ Sample}{Absorbance\ of\ Control}\ X\ 100$$

The half-maximal inhibitory concentration (IC_{50}), which indicates the needed amount of sample to scavenge or inhibit the action of DPPH by 50%, was determined using the 4PL non-parametric regression of dose inhibition curve plot between concentrations of extracts versus radical scavenging activity (AAT Bioquest Inc. 2023).

In vitro enzyme inhibitory activities

The detection of the subfractions through α-glucosidase and α-amylase inhibitory activities was conducted at the Terrestrial Natural Products Laboratory (TNPL) of the University of the Philippines-Diliman, Quezon City, Philippines.

α-amylase inhibitory activity

The inhibition of α-amylase was determined using the 3,5-dinitrosalicylic acid (DNSA) colorimetric method. The enzymatic reaction was initiated by dispensing 75 µL of the enzyme (2,000 mU/mL) to the test sample analysis tube containing 10 µL sample (300 µg/mL), 110 µL sodium phosphate buffer (50 mM, pH 6.9), and 105 µL of the starch solution (10,000 µg/mL). The mixture was incubated for 10 minutes at 37°C. After incubation, 200 µL of DNSA (0.03M) was immediately added, and the sample tube was placed in a boiling water bath for 15 minutes. Then, the contents of the test tubes were diluted with 1000 µL of buffer and mixed by shaking. Next, 300 µL aliquots were transferred in a 96-well microplate for each analysis. The absorbance was measured at 540 nm using a UV/VIS Spectrophotometer (Multiskan Go®, USA). Acarbose was used as the positive control, while DMSO was the negative control. The blank was prepared as the samples/control but did not contain the enzyme.

The percent inhibition of the samples and the positive control were determined based on the following:

$$\%\ \text{Inhibition activity} = \frac{\text{Absorbance } uninhibited - \text{Absorbance } inhibited}{\text{Absorbance } uninhibited} \times 100$$

α-glucosidase inhibitory activity

The assay is based on the action of the α-glucosidase enzyme on *p*-nitrophenyl-α-D-glucopyranoside as the substrate. A 10 µL aliquot from the sample working stock solution (300 µg/mL) was dispensed into the designated replicate wells of a 96-well quartz microplate. Then, 190 µL sodium phosphate buffer (50 mM, pH 6.80) was added to each sample well. Meanwhile, 200 µL DMSO in PBS (5%) was dispensed into negative control wells, while 200 µL Acarbose (1,500 µg/mL) was placed into positive control wells. Each well was added with 50 µL enzyme (120 mU/mL) and incubated for 10 minutes at 37°C. After incubation, 50 µL of the substrate (1.86 mM) was added to each well to initiate the enzymatic reaction. The absorbance was measured at 405 nm every 30 seconds for 30 minutes using a UV/VIS microplate spectrophotometer. The positive control was Acarbose, while DMSO was used for the negative control.

Therefore, to determine the inhibitory activity of each sample, it was based on the average slope of the negative control replicates and the individual slope of each sample replicate:

$$\% \text{ Inhibition activity} = \frac{\text{Absorbance } uninhibited - \text{Absorbance } inhibited}{\text{Absorbance } uninhibited} \times 100$$

Statistical analysis

The physicochemical, quantitative phytochemical analysis, and antioxidant activity data experiments were done in three replicates. The data were shown as means±standard deviations (SD) and analyzed using Analysis of Variance (ANOVA) in Completely Randomized Design (CRD) using STAR 2.0.1 (IRRI 2013).

RESULTS AND DISCUSSION

Physicochemical and organoleptic characteristics

The physicochemical and organoleptic determinations are prerequisites to establishing raw materials' identity, quality, and purity. They serve as vital benchmarks to ensure consistency (WHO 2011; Hait 2021) and provide information for pharmaceutical applications. Table 1 presents the characteristics of the *T. ternifolius* dried leaves and extracts. The results show that dried leaves have a distinctive olive color and a fibrous and fluffy texture. The aroma resembles that of herbal tea. The fresh leaves have a moisture content of 50.07±0.35%. Next, 1% and 10% solutions of the dried powdered leaves have a pH of 5.13±0.06 and 4.60±0.10, respectively.

Conversely, the crude extract displays a dark brown to black color and a sweet aroma with a coarse texture in its dried state. It has a pH of 3.83±0.06, a moisture content of 2.76±0.68%, and is practically insoluble in water. Moreover, the obtained ash value of the dried leaves is 3.10±0.29 for the total ash, 0.09±0.08% for water-soluble ash, and 0.62±0.03% for acid-insoluble ash. The ash values would indicate the purity and estimate the inorganic residues present in plant materials (Hait 2021).

The extractive values show an estimated quantity of phytochemicals that can be extracted from plant material, dependent on the solvent's polarity. Thus, the extractive values in Table 1 give information on the nature of phytoconstituents in the sample (WHO 2011; Hait 2021). The cold maceration was employed using water, ethanol (95 and 80%), methanol (95 and 80%), ethyl acetate, and hexane. Results show that *T. ternifolius* leaves extracted with methanol (80%) have the highest extractive value of 217.87±3.90 mg/g among the other solvents. This was followed by 80% ethanol (209.05±2.9), 95% methanol (205.33±2.0), 95% ethanol (167.63±2.90), and water (138.57±0.70) (Table 1).

Phytochemical content and metabolite profiling of *T. ternifolius* leaves

Phytochemicals have been known for their function in the survival and adaptation of plants, including their role in each species' physical and sensory attributes. Interestingly, the pharmaceutical applications of these compounds are an emerging trend, and studies have shown remarkable therapeutic activities in vitro and *in vivo* (Afzal et al. 2015; Velu et al. 2018). The total flavonoid, phenolic, and terpenoids were estimated using the Folin-Ciocalteu, aluminum chloride, and gravimetric methods. The phenolic compounds in the *T. ternifolius* leaves had the highest concentration at about 20.77±0.77 mg/g of dried extract. The terpenoid content is about 6.07±0.53 mg/g of dried powder, while total flavonoid is 1.23±0.19 mg/g of dried extract.

A total of 36 distinct peaks were observed from the sample (Figure 1) using LC-MS analysis. Each peak corresponds to one component in the sample. Moreover, 8 of these peaks were putatively identified as glabrolide, aloinoside B, 2-Benzyl octanal, geumonoid, (±)-gomisin M1, 25-O-Acetyl-7,8-didehydrocimigenol-3-O-β-D-xylopyranoside, lucialdehyde B, and kadsuric acid, using the Traditional Chinese Medicine library (Table 2). Most of these compounds were terpenoids. The other compounds elucidated were phenol, anthracene, and an aldehyde. The presence of these compounds with reported activities in the literature may explain the biological activities exhibited by the extracts in this study. Moreover, it suggests a wide range of pharmacological activities of *T. ternifolius* leaves, such as neuroprotective, antiviral, anti-cancer, cytotoxic, and laxative (Table 3), that can be explored further.

Table 1. Organoleptic and physicochemical characteristics of *Timonius ternifolius* leaf and ethanolic crude extract

Parameters	Leaves	Ethanolic crude extract
Color	Olive	Dark brown to black
Odor	Herbal tea-like	Sweet
Appearance	Fibrous and fluffy	Hard, coarse
Moisture content (%)	50.07±0.35	2.76±0.68
pH (1%)	5.13±0.06	3.83±0.06
pH (10%)	4.60±0.10	-
Solubility in water	-	Practically insoluble
Ash content (%)		
Total ash	3.10±0.29	-
Water soluble ash	0.09±0.08	-
Acid insoluble ash	0.62±0.03	-
Extractive values (mg/g)**		
Water	138.57±0.70^{d}	-
Methanol (95%)	205.33±2.00^{b}	-
Methanol (80%)	217.87±3.90^{a}	-
Ethanol (95%)	167.63±2.90^{c}	-
Ethanol (80%)	209.05±2.90^{b}	-
Ethyl acetate	56.00±0.80^{e}	-
Hexane	38.57±0.20^{f}	-

Note: **Highly significant at p>0.01. Means with the same letter are not significantly different at LSD 0.01

Antioxidant activity

Moreover, evaluating the antioxidant activity of various *T. ternifolius* extracts provides valuable insight into the plant's capacity to reduce oxidative stress caused by free radicals, which cause the development of various diseases, including diabetes. The study used the DPPH free radical scavenging activity assay to assess the potential of *T. ternifolius* leaf extracts to neutralize or reduce the effects of free radicals.

Among the extracts, as revealed in Figure 2, ethanolic extracts (EE) exhibited the highest antioxidant activity, ranging from 4.03 to 94.82% at 9.375 to 300 µg/mL. The EE also demonstrated the most potent activity at 59.15 µg/mL, although with lower potency than ascorbic acid (14.87 µg/mL). The highest activity for methanolic extract (ME) was 57.44%, with a potency of 478.39 µg/mL. The remaining extracts exhibited lower (<50%) antioxidant activity.

The observed antioxidant properties are attributed to bioactive compounds in *T. ternifolius* leaves, particularly phenolics, flavonoids, and terpenoids. Numerous studies have highlighted the significant antioxidant activities of phenolic compounds. The hydroxyl group of these compounds is where the reducing or inhibiting capability occurs through hydrogen atom donation (HAT) or single electron transfer (SET) to free radicals (Santos-Sánchez et al. 2019; Gutiérrez-Del-Río et al. 2021). The effectiveness and strength of phenolics as antioxidants are associated with the quantity and positioning of these hydroxyl groups. Similarly, flavonoid's hydroxyl groups in the catechol B-ring and pyran C-ring contribute significantly to the overall antioxidant capacity (Dias et al. 2021; Gutiérrez-Del-Río et al. 2021).

Terpenoids, another class of compounds identified in *T. ternifolius*, are reported to act as primary or synergistic antioxidants. Aside from the HAT or SET mechanism, terpenoids can quench free radicals via singlet oxygen transfer (SOT). The collaborative interplay of terpenoids with other antioxidants could hold the potential for heightened efficacy (Yang et al. 2020).

Table 2. Chemical compounds putatively identified from *Timonius ternifolius* leaves

Name	Retention time (RT)	Chemical formula	Observed m/z	Molecular class
Glabrolide	5.43	$C_{30}H_{44}O_4$	469.3313	Terpenoid
Aloinoside B	6.20	$C_{27}H_{32}O_{13}$	587.1735	Anthracene
2-Benzyl octanal	6.53	$C_{15}H_{22}O$	219.1742	Aldehyde
Geumonoid	7.99	$C_{30}H_{46}O_5$	487.3410	Terpenoid
(±)-Gomisin M1	8.31	$C_{22}H_{26}O_6$	409.1623	Phenol
25-O-Acetyl-7,8-didehydrocimigenol-3-O-β-D-xylopyranoside	9.52	$C_{37}H_{56}O_{10}$	699.3565	Terpenoid
Lucialdehyde B	9.62	$C_{30}H_{44}O_3$	453.3359	Terpenoid
Kadsuric acid	11.25	$C_{30}H_{46}O_4$	471.3468	Terpenoid

Table 3. Reported biological activities of putative compounds found in *Timonius ternifolius* leaves

Compound	Biological activity	Further information	References
Glabrolide	BACE-1 inhibitor	β-secretase novel inhibitor	Arif et al. (2020)
Alongside B	Laxative	Rat intestinal bacteria metabolized alongside B into barbaloin, iso barbaloin, and a hydroxyl	Gao et al. (2005)
2-benzyl octanal	-	-	-
Geumonoid	Anti-HIV	Inhibits HIV-1 protease	Xu et al. (2000)
(±) Gomisin M1	Anti-HIV	Most potent with <0.65 microM EC_{50} and >68 therapeutic index	Chen et al. (2006)
25-O-Acetyl-7,8-didehydrocimigenol-3-O-β-D-xylopyranoside	Reduce autophagic degradation	Suppressed the expression of lysosomal cathepsin B (CTSB) in multidrug-resistant liver cancer HepG2/ADM cells	Sun et al. (2017)
	Anti-cancer	Inhibited breast cancer cell growth	Einbond et al. (2008)
Lucialdehyde B	Antiviral	Potent inhibitor of Herpes simplex virus	Niedermeyer et al. (2005)
	Antiproliferative	Suppress proliferation and induce apoptosis in nasopharyngeal carcinoma CNE2 cells.	Liu et al. (2023)
Kadsuric acid	Anti-cancer	Inhibited human liver cell growth; induced autophagy in lung fibroblast	Biosynth (2024)

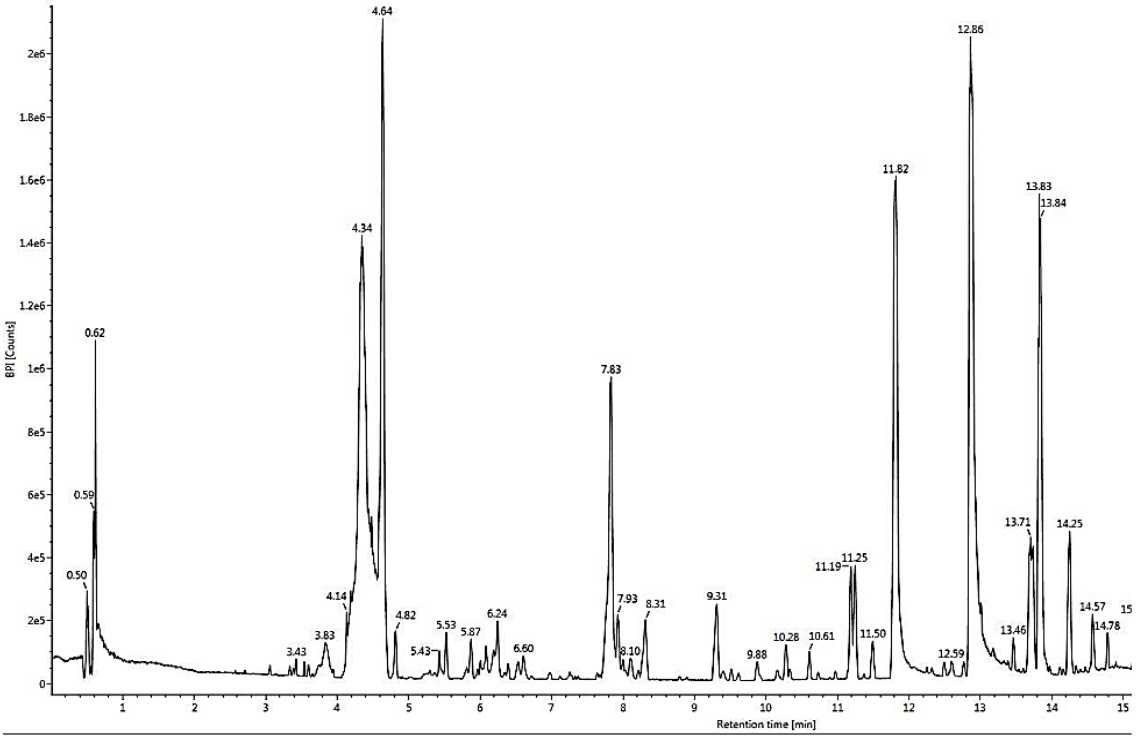

Figure 1. Chromatogram of *Timonius ternifolius* leaf crude extract

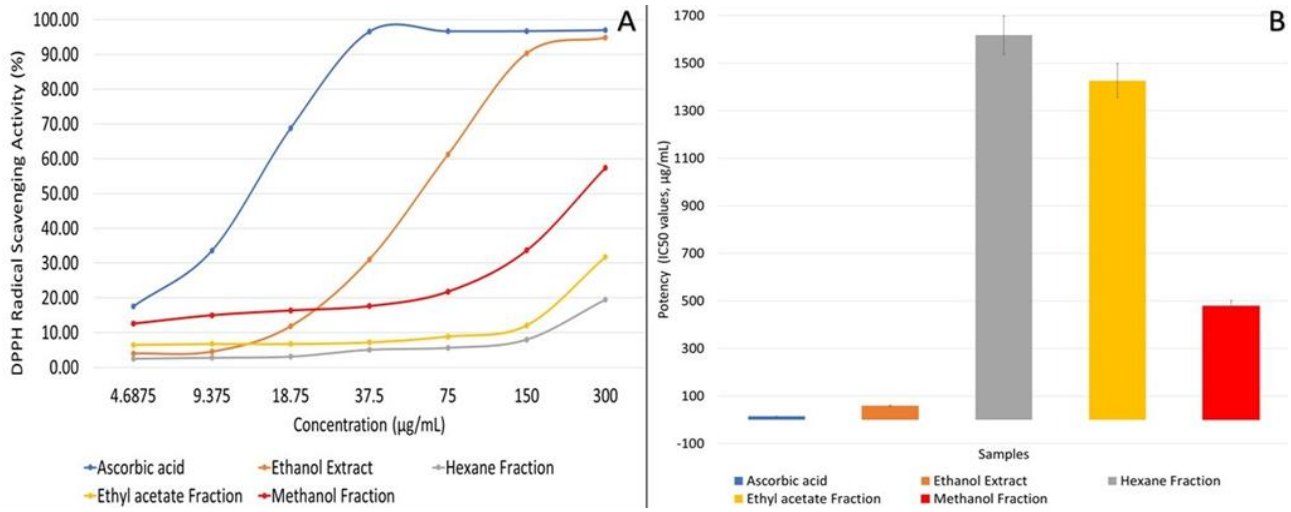

Figure 2. A. Antioxidant activity; B. Potency of the different extracts from *Timonius ternifolius* leaves

Antidiabetic activity

The antidiabetic activity of a substance is achieved through the modulation of postprandial hyperglycemia. Postprandial hyperglycemia is characterized by elevated blood glucose levels after meals and is critical to diabetes management. The α-amylase and α-glucosidase enzymes are key enzymes in the absorption of glucose and the digestion of carbohydrates. Thus, inhibiting these enzymes is essential to impede the hydrolysis and absorption of carbohydrates, preventing the surge in blood glucose levels and mitigating the risk of hyperglycemia (Gong et al. 2020). This study subjected the different *T. ternifolius* leaves (10 ppm) extracts from *T. ternifolius* leaves (10 ppm) to the α-amylase and α-glucosidase inhibition assay, as shown in Figure 3.

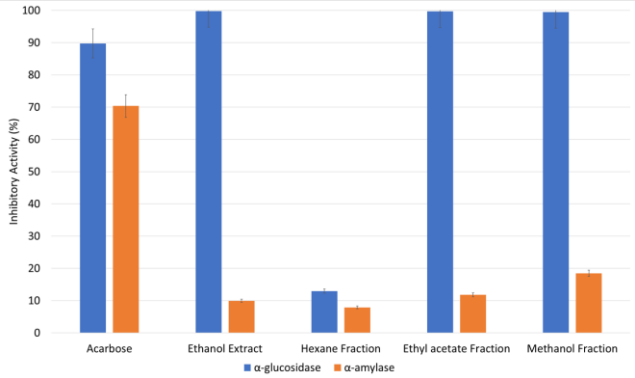

Figure 3. Antidiabetic activity: α-glucosidase of the different crude extracts

Based on the results, the EE, EA, and ME extracts showed promising inhibition against the α-glucosidase enzyme at 99.74%, 99.71%, and 99.45%, respectively. Interestingly, the activity of the extracts was higher than the standard drug acarbose at 89.76%. This is contrary to the α-amylase inhibition assay that showed weak inhibitory activity. Therefore, these results imply that *T. ternifolius* crude extract mainly exerts antidiabetic properties by targeting the enzyme α-glucosidase, which affects the postprandial glucose metabolism pathway and inhibits glucose absorption.

This study described the characteristics of the *T. ternifolius* leaves and crude extracts. The results of untargeted LC-MC metabolite profiling indicated the presence of several compounds that may be pharmacologically significant. Thus, this warrants further study of the chemistry and pharmacological aspects of *T. tenifolius*. Also, the observed in vitro antioxidant and α-glucosidase inhibitory activities of the plant revealed significant prospects for developing plant-based biologically active material with attributed properties.

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