Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Metabolomics analysis and chemical profiling of a decoction *Tristaniopsis Merguensis* leaf as bio supplement for antioxidant, and anti-cholesterols

Análise metabolômica e perfil químico de uma folha de decocção de *Tristaniopsis merguensis* como suplemento biológico para antioxidantes e anticolesteróis

S. Agustini[a]*, L. P. Manalu[a], N. Lestari[a], W. Purwanto[a], Nasruddin[a], H. Widodo[b], A. Asmaliyah[c], S. H. Saputra[d], Nilawati[e] and G. Cahya[f]

[a]National Research and Innovation Agency, Research Center for Agroindustry, Cibinong Bogor, Indonesia
[b]National Research and Innovation Agency, Research Center for Pharmaceutical Ingredient and Traditional Medicine, Cibinong Bogor, Indonesia
[c]National Research and Innovation Agency, Research Center for Applied Botany, Cibinong Bogor, Indonesia
[d]National Research and Innovation Agency, Research Center for Biomass and Bioproduct, Cibinong Bogor, Indonesia
[e]National Research and Innovation Agency, Research Center for Environmental and Clean Technology, Cibinong Bogor, Indonesia
[f]Politeknik Negeri Sriwijaya, Palembang. Indonesia

Abstract

Hyper cholesterol and oxidative stress are major risk factors for the onset of cardiovascular diseases. The *T. merguensis* has been stated to comprise a superior antioxidant substance that could stipulate beneficial advantages, and traditionally applied as herbal medicine for hyper cholesterols. The aim of the study was to evaluate of decoction leaf extracts from *T. merguensis* for their cholesterol reduction effect in vitro, anti-microbial activity, antioxidant activity, flavonoids and total phenolics contents and its metabolite composition. The test results show that the water decoction extracts have a high value of total phenolic content, and flavonoids. Metabolome analysis and chemical profiling by using liquid chromatography-high resolution mass spectroscopy (LC-HRMS) leads to 30 compounds are identified in the extracts. The compounds contained in the extracts are non-flavonoid polyphenols, including 18 compounds (66.75%), followed by flavonoid polyphenols (4 compounds/17.20%), alkaloids (3 compounds/5.77%), and terpenes (1 compound/5.54%), and one protein recombinant (NP-003141/4.74%).The major compounds are recognized as quinic acid, 1,6-bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose, myricitrin, citric acid, (-)-Caryophyllene oxide, NP-003141, myricetin, 2-Amino-1,3,4-octadecanetriol, ellagic acid, and sedanolide. The decoction leaf extracts show robust antioxidant activity in DPPH assays, with IC50 values of 15.64 µg/g. It also shows appreciable ferric-reducing activity and ABTS antioxidant capacity of 118.9 mM Fe (II)/mg and 7463.3 mM TE/g. It also possesses anti-cholesterols properties in vitro; antimicrobial activity on *S. aureus and S. epidermis* at concentration of 15,000 ppm. This study concludes that decoction leaf extracts of *T. merguensis* is suggested as an organic bioactive basis, with anti-cholesterol and antioxidant capabilities. In addition, in vitro and in vivo studies are needed to confirm this work.

Keywords: biological activity, decoction, metabolomics, *T. merguensis*.

Resumo

Hipercolesterolemia e estresse oxidativo são os principais fatores de risco para o surgimento de doenças cardiovasculares. O *T. merguensis* foi declarado como uma substância antioxidante superior que poderia estipular vantagens benéficas e tradicionalmente aplicada como remédio herbal para hipercolesterolemia. O objetivo do estudo foi avaliar extratos de folhas de decocção de *T. merguensis* quanto ao seu efeito de redução de colesterol in vitro, atividade antimicrobiana, atividade antioxidante, flavonoides e teores de fenólicos totais e sua composição de metabólitos. Os resultados dos testes mostram que os extratos de decocção aquosa têm alto valor de teor de fenólicos totais e flavonoides. A análise do metaboloma e o perfil químico usando cromatografia líquida-espectroscopia de massa de alta resolução (LC-HRMS) levam a 30 compostos identificados nos extratos. Os compostos contidos nos extratos são polifenóis não flavonoides, incluindo 18 compostos (66,75%), seguidos por polifenóis flavonoides (4 compostos/17,20%), alcaloides (3 compostos/5,77%) e terpenos (1 composto/5,54%) e uma proteína recombinante (NP-003141/4,74%). Os principais compostos são reconhecidos como ácido quínico, 1,6-bis-O-(3,4,5-tri-hidroxibenzoil) hexopiranose, miricitrina, ácido cítrico, óxido de (-)-cariofileno, NP-003141, miricetina, 2-amino-1,3,4-octadecanotriol, ácido elágico e sedanolida. Os extratos de folhas de decocção mostram atividade antioxidante robusta em ensaios de DPPH, com valores de IC50 de 15,64 µg/g. Também mostram atividade de redução férrica apreciável e capacidade antioxidante ABTS de 118,9 mM Fe (II)/mg e 7463 mM TE/g. Eles também possuem propriedades anticolesterol in vitro; atividade antimicrobiana em *S. aureus* e *S. epidermis* na concentração de 15.000 ppm. Este estudo conclui que extratos de folhas de decocção de *T. merguensis* são sugeridos como uma base bioativa orgânica, com capacidades anticolesterol e antioxidante. Além disso, estudos in vitro e in vivo são necessários para confirmar este trabalho.

Palavras-chave: atividade biológica, decocção, metabolômica, *T. merguensis*.

*e-mail: sria014@brin.go.id
Received: November 5, 2024 – Accepted: March 26, 2025
Editor: Takako Matsumura Tundisi

1. Introduction

Researchers think that hyperlipidemia is one of the essential triggers of death worldwide. This condition is indicated by elevated levels of lipids, total cholesterol, low density lipoprotein and decreasing levels of high-density lipoprotein in the blood. It is a risk factor for cardiovascular, coronary arteries, as well as metabolic syndrome diseases (Mahfud et al., 2023; Ibrahim et al., 2021; Pant et al., 2015). According to WHO, cardiovascular disease will turn into the primary cause of death by 2030 (Ibrahim et al., 2021). Modern drugs and synthetic antioxidants from traditional medicine join forces as several treatments to manage hyperlipidemia. Despite modern medicine, traditional medicine has been used several medicinal plants to prevent and treat various diseases, including lowering hyperlipidemia.

Plants confine a variety of active chemicals, including as phenols, flavonoids, volatile oils, fixed oils, alkaloids, steroids, tannins, and glycosides (Rasul, 2018). Several plants have been used for medicinal applications and are traditionally consumed as decoctions and infusions as they have antioxidant potential and anti-cholesterol activity (Finimundy et al., 2020; Pant et al., 2015). These plants have been used since ancient times, boasting countless benefits associated with their health-promoting properties linked to their defensive and healing qualities (Mollica et al., 2019). The WHO estimates that about 80% of the world's population relies on herbal medicines more than current modern medicine, and in most developing countries, the use of traditional medicine and medicinal plants has been widely observed (Rasul, 2018; Karunamoorthi et al., 2013).

Traditional medicine most often applies medicinal plants in the form of a decoction. The decoction process dissolves a plant's bioactive compounds into water, making it a drinkable treatment for various illnesses. Traditional Chinese Medicine (TCM) extensively documents this method (Brendler et al., 2022), as do Indian Traditional Medicine (Ayurveda) (Zhang et al., 2018) and Indonesian traditional herbal medicine known as Jamu (Elfahmi et al., 2014).

T. merguensis, an indigenous tropical plant often found in Asian nations, including Indonesia, is one of the plants that can be exploited. It is a member of the Myrtacea family. It is packed with chemicals that are advantageous secondary metabolites. Previous analysis of phytochemicals showed that its leaves contained phenols, saponins, flavonoids, alkaloids, and steroids, where the polyphenols are the most predominant (Agustini et al., 2023). Reports stated that polyphenols have beneficial effects on biological activity such as a decrease of cholesterols, decline of blood pressure both systolic and diastolic, modulation of the constituent and function of lipoproteins, decrease of oxidative stress and inflammation, controlling glycemic index and so on (Martemucci et al., 2022). Traditionally, some parts of this plant are considered medicinal and serve as a single herb or in combination with others. Usually, when served as a drink, it is prepared by water decoction/boiling. Decoction is the most common process in the preparation of herbal medicine for drinking. Moreover, it is commonly known that non-toxic, food-grade, and reusable solvents should be used in the food sector (Marinaccio et al., 2024).

This research aimed to perform metabolome analysis of the water decoction of *T. merguensis* leaves (DTML) and unveil its antioxidant activity (including ferric reducing capacity, DPPH and ABTS radical scavenging activity), antimicrobial activity, and in vitro cholesterol-lowering potential. To our knowledge, this is the first study on the metabolite composition, biological activity principally as a possible antioxidant, antibacterial capabilities, and hypo-cholesterol agent of *T. merguensis* leaf decoction as an essential tool for human health.

2. Materials and Methods

2.1. Extraction of T. merguensis

The *T. merguensis* leaves were explicitly gathered from the Kemampo woodland region (KHDTK), Banyu Asin South Sumatera, Indonesia, and verified by the Indonesian Herbarium Bogoriense. Before being milled into a dry powder ready for extraction, the sample was allowed to dry naturally and kept out of direct sunlight until its moisture content reached 8%. The leaf powder was extracted using decoction by simmering 27 g leaf powder and 700 mL demineralized water for 30 minutes, then cooled for 3 hours at room temperature. The filtrate dried after it passed through filter paper. For further investigation, the dried extract stored in the freezer.

2.2. Determination of total phenolic and flavonoid content

We evaluated phenol content (TPC) using the Folin-Ciocalteu method described by Agustini et al. (2023). A UV-VIS spectrophotometer, 1900i Shimadzu, Japan, was used to detect the absorbance at 760 nm in wavelength. Following triplicate measurements, we presented the data as gallic acid equivalent/mg GAE/g dry extract (Stefanucci et al., 2024). The colorimetric approach was used to determined flavonoid content as stated by Agustini et al. (2023). A standard curve using quercetin to express results in milligrams of quercetin equivalents per gram of sample (mg QE/g).

2.3. LC–HRMS analysis

One milligram of the decoction extract subjected to untargeted metabolomic analysis using LC–HRMS (Windarsih et al., 2022). After 70 minutes of liquid chromatography, both positive and negative ions from electrospray ionization (ESI) were used in the mass spectrometric analysis. Compound Discoverer™ 3.2 software was used to find the compounds. The filter peak extraction employs databases MzCloud and ChemSpider and annotation masses ranging from -5 to 5 ppm.

2.4. Antioxidant activity (DPPH assay)

Antioxidant activity was determined using radical scavenging activity in the 2,2-diphenyl-1-picryl hydroxyl radical (DPPH) described by Agustini et al. (2023).

2.5. Ferric Reducing-Antioxidant Power Assay (FRAP)

The FRAP assay was carried out according to Widodo et al. (2019). The absorbance was measure at 595 nm. The findings were then estimated in Fe^{+2} equivalents (Fe^{+2} mM) using the linear regression of $FeSO_4.7H_2O$ (100-800 µM).

2.6. ABTS method or Trolox Equivalent Antioxidant Capacity (TEAC)

We performed the ABTS test using the method described by Widodo et al. (2019). The ABTS (38.4 mg) was added to 10 mL of 2.5 M potassium persulfate and thoroughly mixed. The solution was kept in a dark environment for 12-16 hours at room temperature, yielding a dark blue $ABTS^{++}$ solution. We prepared the ready-to-use $ABTS^{\bullet+}$ solution by dissolving it in methanol until it reached an absorbance value of 0.7 ± 0.02 at 743 nm. We used Trolox at dilutions ranging from 25 to 50 µM to create a standard curve for antioxidant activity evaluation. The extract (10 mg) was dissolved to 1 mL with distilled water, sonicated for 15 minutes, and diluted (10-100 µg/mL). We added 50 µL of the sample to 1950 µL of ready-to-use $ABTS^{\bullet+}$ solution, incubated the mixture for 30 minutes at 37 °C, and then measured the absorbance $\alpha\tau$ 743 nm. Finally, the antioxidant activity expressed as millimolar Trolox Equivalents per gram of extract (TE/g).

2.7. In vitro anti-bacterial screening

Anti-bacterial screening in vitro conducted using the disc diffusion method with slight modifications, as described by Agustini et al. (2023). Dimethyl sulfoxide (10%) was used to reconstituted the crude extracts of the *T. merguensis* leaf decoction to concentrations of 5,000; 10,000; 15,000; 20,000; and 25,000 mg/L.

Chemicals used and statistical analysis. Analytical-grade chemicals used throughout the study. All analyses were performed in triplicates to obtain reproducibility results, and the values mentioned in the results are the means of triplicates.

3. Results and Discussion

3.1. Phytochemical screening

T. merguensis has traditionally been used as an herbal remedy by locals to treat various diseases, including hypertension, hypercholesterolemia, and antidiabetic. Building on previous reports suggesting plant tissues are rich in phytochemical compounds, particularly phenolics and flavonoids. The phenolic and flavonoid profile of the *T. merguensis* leaf (DTML) decoction was relatively high as presented in (Table 1).

Plant phenolics, including flavonoids and non-flavonoid phenols. Nonflavonoid phenols contain phenolic acids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignin. These phenolic compounds contribute to the taste both bitter and astringent taste, the color, flavor, odor, and biological properties (Gutierrez-Grijalva et al., 2020; Iftikhar et al., 2020; Kumar and Goel, 2019; Durazzo et al., 2019). Researchers report that polyphenols have antioxidant activity, including scavenging free radicals, reducing lipid peroxidation, and protecting cells from oxidative stress. They are also anti-inflammatory and antidiabetic and may regulate insulin signaling, glucose metabolism, lipid levels, and other pathways (Gutierrez-Grijalva et al., 2020; Iftikhar et al., 2020).

Table 1. Phytochemical profile of DTML.

Phytochemicals	Concentrations
Phenolic (mg/g)	234.72 ± 0.41
Flavonoids (mg/g)	43.67 ± 0.36

Values are means ± SD (n = 3).

3.2. LC-HRMS analysis

The LC-HRMS chromatogram exhibited various peaks, as shown in Figure 1. LC-HRMS analysis tentatively identified 30 metabolites in the decoction extracts (Table 2). The compounds consist of flavonoids, phenolic acids, tannins, terpenes, and alkaloids. The compounds contained in the extracts are non-flavonoid polyphenols, including 18 compounds (66.75%), followed by flavonoid polyphenols (4 compounds/17.20%), alkaloids (3 compounds/5.77%), and terpenes (1 compound/5.54%), and one protein recombinant (NP-003141/4.74%). Among polyphenols tentatively identified as quinic acid (31.12%), 1,6-bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose (18.77%), which is classified as tannin polyphenol, citric acid (6.041%), ellagic acid (2.52%), sorbic acid (1.51), 12-oxo phytodienoic Acid (0.75%), corchorifatty acid F (0.60%), resveratrol which known as stilbenes polyphenols (0.51%), and ten others compound which relative abundance less than 0.5%. The flavonoids identified include myricitrin flavone (10.92%), myricetin (4.40%), myricetin 3-O-beta-D-galactopyranoside (3.77%), and 2-Isopropylmalic acid (0.11%). The alkaloids compound consists of 2-Amino-1,3,4-octadecanetriol (2.56%), sedanolide (1.937%), and Pyrrole-2-carboxylic acid (1. 27%). The only terpenes identified in the decoction are (-)-Caryophyllene oxide (5.54%). This identification confirms the phytochemical analysis findings, which show that total phenolic is predominant compound in DTML.

The ten predominant abundant compounds contained are quinic acid, 1,6-bis-O-(3,4,5-trihydroxybenzoyl) hexopyranose, myricitrin, citric acid, (-)-Caryophyllene oxide, NP-003141, myricetin, 2-Amino-1,3,4-octadecanetriol, ellagic acid, and sedanolide. These ten compounds comprise 88.56% relative abundance in DTML.

Reports suggest that quinic acid, a metabolite of chlorogenic acid, demonstrated strong anti-bacterial effects on *S. aureus* and also acts as an antioxidant, anti-aging, anti-inflammatory agent, antidiabetic, radioprotective, anti-neuroinflammatory, antimutagenic, antiviral, and neuroprotective agent. It also inhibits glucose glycoxidation, a metal chelator, reducing cholesterol in the serum of hyper-lipidemia and anti-malarial (Jin et al., 2024; Anoor et al., 2022; Benali et al., 2024; Goda et al., 2023; Hwang and Chung, 2018).

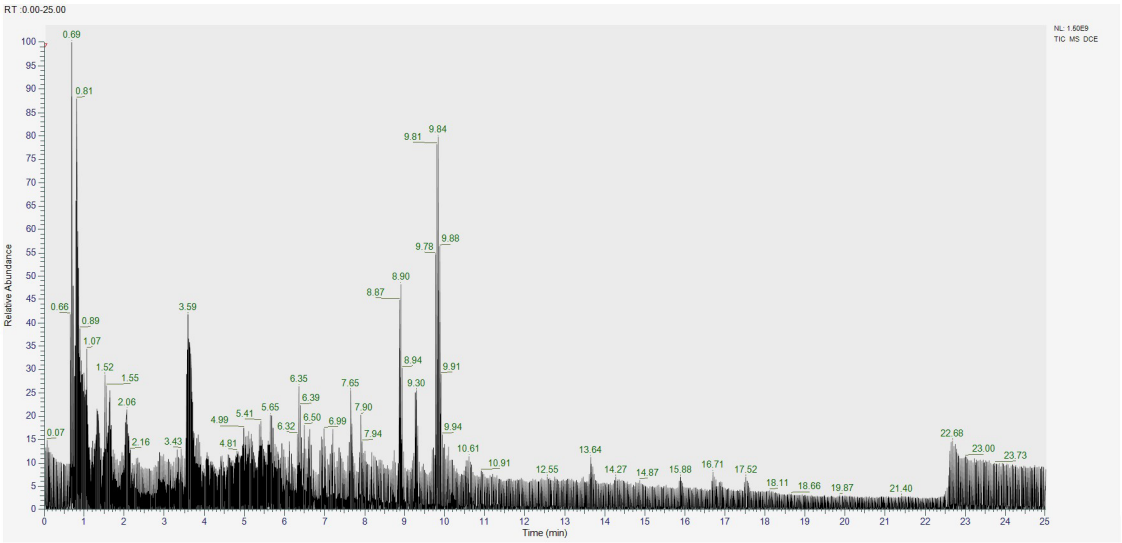

Figure 1. Total ion chromatogram of the DTML.

Table 2. Metabolite profile of DTML.

Compound	Formula	Calc. MW	RT [min]	Area (Max.)	mzCloud Best Match	% Area
D-(-)-Quinic acid	C7 H12 O6	192.06301	0.82	2.4E+09	99.7	31.12151
1,6-Bis-O-(3,4,5-trihydroxy benzoyl) hexopyranose	C20 H20 O14	484.08562	1.635	1.45E+09	85.4	18.77274
Myricitrin	C21 H20 O12	464.09589	5.685	8.43E+08	94.6	10.91666
Citric acid	C6 H8 O7	192.02675	0.934	4.67E+08	99.4	6.041602
(-)-Caryophyllene oxide	C15 H24 O	220.18255	9.707	4.28E+08	94.8	5.53893
NP-003141	C21 H20 O9	416.11093	7.005	3.66E+08	89.7	4.739092
Myricetin	C15 H10 O8	318.03722	5.663	3.4E+08	99.4	4.403181
2-Amino-1,3,4-octadecanetriol	C18 H39 N O3	317.29286	9.965	1.98E+08	84.2	2.564586
Ellagic acid	C14 H6 O8	302.0063	0.996	1.95E+08	99	2.524573
Sedanolide	C12 H18 O2	194.13063	7.431	1.49E+08	77.9	1.933049
myricetin 3-O-beta-D-galactopyranoside	C21 H20 O13	480.09114	5.155	1.37E+08	99	1.771397
Sorbic acid	C6 H8 O2	112.05271	2.774	1.17E+08	84.7	1.509269
Guanine	C5 H5 N5 O	151.04941	0.886	1.12E+08	99.5	1.454808
Pyrrole-2-carboxylic acid	C5 H5 N O2	111.03224	1.006	98126305	97.2	1.270797
12-oxo Phytodienoic Acid	C18 H28 O3	292.20398	8.733	58055708	87.6	0.751858
Corchorifatty acid F	C18 H32 O5	328.22575	8.617	46485348	84.6	0.602015
Resveratrol	C14 H12 O3	228.07864	6.618	39168028	65.7	0.507251
N1-[1-[5-(benzylthio)-4-methyl-4H-1,2,4-triazol-3-yl]-3-(methylthio)propyl]-4-(trifluoromethyl)benzamide	C22 H23 F3 N4 O S2	480.12438	6.361	39020641	69.9	0.505342
trans-Aconitic acid	C6 H6 O6	174.01591	1.005	37572959	77.3	0.486593
Pyridoxine	C8 H11 N O3	169.074	0.962	34629455	85.6	0.448473

RT = Retention Time.

Table 2. Continued...

Compound	Formula	Calc. MW	RT [min]	Area (Max.)	mzCloud Best Match	% Area
Azelaic acid	C9 H16 O4	188.10467	6.555	26121533	98.6	0.33829
Jasmonic acid	C12 H18 O3	210.12552	4.794	25951681	93.5	0.336091
Tributyl phosphate	C12 H27 O4 P	266.16491	12.554	22968985	99.8	0.297463
trans-3-Indoleacrylic acid	C11 H9 N O2	187.06342	2.324	20796932	91.5	0.269333
Penicillic acid	C8 H10 O4	170.05794	3.901	18335056	84.5	0.237451
L-Phenylalanine	C9 H11 N O2	165.07912	1.466	17873756	99.9	0.231476
OPEO	C16 H26 O2	250.19345	9.657	8718590	68.6	0.112911
2-Isopropylmalic acid	C7 H12 O5	176.06813	2.81	8689074	66.7	0.112529
Kaempferol	C15 H10 O6	286..04781	6.54	8635017	99.6	0.111829
{(1R,2R)-2-[(2Z)-5-(Hexopyranosyloxy)-2-penten-1-yl]-3-oxocyclopentyl}acetic acid	C18 H28 O9	388.17424	4.149	6864190	91.5	0.088896

RT = Retention Time.

Myricitrin demonstrated various biological effects, including an in vivo hypo glycemic effect, antidiabetic, anti-inflammatory, neuro/cardio/ hepaprotective, and anticancer effects, both in vitro and in vivo (Geng et al., 2024; Dua et al., 2021; Song et al., 2021). Studies have shown that ellagic acid can lower blood pressure and cholesterol and even minimize wrinkles caused by radiation. It also has anti-inflammatory qualities (Durazzo et al., 2019).

The presence of (-)-Caryophyllene oxide, which is terpenes in the extract, indicates that the decoction process can extract oil-soluble chemicals other than water-soluble compounds, as stated by Ghenabzia et al. (2023). It has antioxidant abilities, analgesic properties, improving the hepatic antioxidant system, anti-inflammatory, preventing lipidic oxidative damage, anticarcinogenic, and antimicrobial (Dougnon and Ito, 2021; Baldissera et al., 2017).

3.3. Antioxidant activity DPPH, ABTS, and FRAP assay

Antioxidants' primary role is to protect the body from the damaging effects of free radicals. In essence, antioxidants function by postponing or preventing the oxidation of other molecules. Antioxidant activity cannot be directly measured but is assessed by its ability to regulate the extent of oxidation. Antioxidant activity of the DTML was measured through the ability to neutralize radicals (DPPH, ABTS) and reduce ferric ions (FRAP). DPPH is a rapid, easy, and affordable way to assess an antioxidant capacity. The scavenging activity using DPPH radicals of DTML displayed concentration-dependent, as shown in Figure 2, with an IC50 of 15.64 ± 0.26 µg/mg. Compared to a positive control, a standard ascorbic acid exhibited an IC50 of 6.14 ± 0.49 µg/mg, significantly lower ($p<0,05$) than the DTML. However, since the IC50 is less than 50 µg/mg, the DTML is categorized as having robust antioxidant properties. Antioxidant properties of the extract are due to its polyphenols content as identified by LC-HRMS (83.95% relative abundance), which are dominated by D-(-)-Quinic acid, 1,6-bis-O-(3,4,5-trihydroxy benzoyl) hexopyranose, myricitrin, citric acid, myricetin, 2-Amino-1,3,4-octadecanetriol, ellagic acid, and sedanolide. Reports dictated that polyphenols work as oxidant scavengers to eliminate free radicals (Satyaeswari et al., 2018; Gutierrez-Grijalva et al., 2020; Iftikhar et al., 2020). This result means that the DTML can be consumed as a natural antioxidant crucial to preventing oxidative stress and degenerative diseases due to excess free radical levels in the body.

Table 3. FRAF and ABTS scavenging activity of the DTML.

Samples	FRAF (mM Fe (II)/mg)	ABTS (mM TE/g)
DTML	118.90 ± 7.88[a]	7463.3 ± 282.3[c]
Quercetin	196.39 ± 6.36[b]	9774.4 ± 80.3[d]

Values are means ± SD (n = 3); letters in the same column were significantly different at $p<0.05$.

The principle of ABTS assay is the capability of compounds to alleviate free radicals by giving proton radicals. ABTS testing can be performed for hydrophilic or lipophilic compounds. The ABTS assay, known as the Trolox equivalent antioxidant capacity assay (TEAC) method, uses Trolox as the standard calibration curve. Test results showed the ABTS value of DTML exhibited the activities of 7463.3 ± 28.23 mM TE/g, significantly lower ($p<0.05$) in comparison to 9774.4 ± 8.03 mM TE/g exhibited by quercetin as a positive control. The higher the ABTS value, the more active the sample is as an antioxidant. This result, in line with the scavenging activity of DPPH. FRAF and ABTS scavenging activity of the DTML is displayed in Table 3.

The FRAP test measures the degree to which the sample's antioxidant activity reduces the complex of ferric ions (Fe3+)-ligand to the ferrous complex (Fe2+) in

acidic environments. In contrast to alternative assays that identify the reduction of free radicals, the FRAP assay is the sole one that examines antioxidants (or reductants) in a specimen directly (Payne et al., 2013). Typically, it is based on the transfer of one electron. The FRAP values are expressed as mmol Fe2 +/mg calculated from a standard curve. The FRAP value of DTML is 118.9 ± 7.88 M Fe (II)/g in comparison to 196.39 ± 6.36 M Fe(II)/g exhibited by quercetin as a positive control (Table 3). A significantly lower ($p<0.05$) was observed for the DTML in reduced ferric-TPTZ than positive controls. The higher FRAP values the better activities (Jin et al., 2024). Nevertheless, the DTML shows appreciable ferric-reducing activity. This result is consistent with the DPPH assay, where phenolic acids may be responsible for ferric-reducing properties.

3.4. Anti-cholesterol activity

The development of several human illnesses, including as diabetes, cancer, and neurological disorders, is typically attributed to free radicals. In the present work, anti-cholesterol characteristics of DTML were investigated and compared to simvastatin, a standard drug to manage cholesterol levels. Amounts of cholesterol reduced at various DTML and simvastatin concentrations are illustrated in Figure 3. The decoction extract of TML displayed concentration-dependent anti-cholesterol activity, with an IC50 of 21.34 ± 0.12 µg/mg. Figure 3 demonstrated that the anti-cholesterol activity of DTML was significantly lower than a positive control of standard simvastatin (IC50 of 13.36 ± 0.56 µg/mL). However, this result reveals the potential of DTML in managing cholesterol levels, which mean it can prevent cardiovascular disease.

3.5. Antimicrobial activity

Some plant extracts have antimicrobial properties, which is because the extracts are rich in various bioactive compounds that can inhibit microbial growth (Shaaban et al., 2021). A test on the antimicrobial activity of DTML was conducted on the bacterial strains *Bacillus Subtilis* (ATCC 6633), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 25923), *Streptococcus epidermidis,* and *E. coli* (ATCC 25922). The anti-bacterial activity test results are presented in Table 4. The five series of DTML concentrations 5,000 up to 25,000 µg/mL, indicate no inhibition zone is formed in the growth of bacteria strains E. coli, B. subtilis, and S. Typhimurium tested. On the contrary, the positive control Chloramphenicol at 1,000 µg/mL showed a zone of inhibition for the bacteria tested, starting at 8.83 up to 10.96 mm (Table 4). This finding indicates that the DTML had no anti-bacterial activity on bacteria strains *E. coli*, *B. subtilis* and *S. thypimurium* at a concentration of up to 25,000 µg/mL.

On the other hand, observation of *S. epidermis* and *S. aureus* bacteria showed anti-bacterial activity which was designated by the presence of an inhibition zone on the test disc. The antimicrobial activity of DTML on *S. epidermis* bacteria began to appear at a concentration of 15,000 ppm, where the zone of inhibition was 0.71 mm and increased to 1.7 mm at a concentration of 25,000 and classified as weak. Observations also showed the anti-bacterial activity of DTML against *S. aureus* bacteria, which was demonstrated by an inhibition zone on the test disc. Anti-bacterial activity began to be seen at a of 10,000 µg/mL, where the inhibitory zone was 0.66 mm. It increased as the concentration increased to 5.15 mm at a concentration of 25,000 µg/mL. In contrast to the positive control chloramphenicol, the antimicrobial activity of DTML was lower and classified as weak. This result contradicts the composition of the bioactive component of the extract identified by LC-HRMS. A possible explanation for this case may be that the decoction contains many water-soluble impurities (Zhang et al., 2018). Another reason is that the DTML is a crude extract that contains various impurities, which may interrupt and be a barrier to the

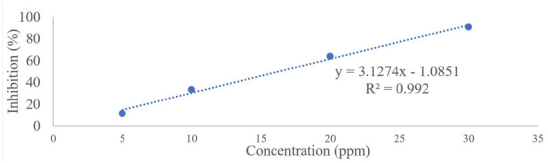

Figure 2. DPPH scavenging activity of DTML.

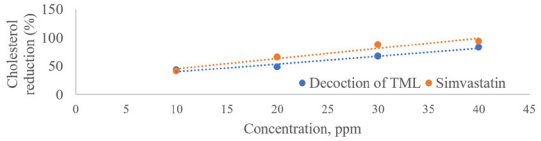

Figure 3. The anti-cholesterol activity of DTML.

Table 4. The antimicrobial test result.

Organism	DTML concentrations µg/mL					Positive control	Negative control
	5,000	10,000	15,000	20,000	25,000		
S. epidermidis	0.00	0.00	0.71 ± 0.0	1.03 ± 0.00	1.74 ± 0.03	0.81 ± 0.00	0.00
S. aureus ATCC 25923	0.00	0.66 ± 0.01	1.93 ± 0.78	3.97 ± 0.20	5.15 ± 0.29	11.99 ± 0.13	0.00
E. coli ATCC 25922	0.00	0.00	0.00	0.00	0.00	8.83 ± 0.63	0.00
B. Subtilis ATCC 6633	0.00	0.00	0.00	0.00	0.00	10.11 ± 0.03	0.00
S. thypimurium ATCC 14028	0.00	0.00	0.00	0.00	0.00	10.96 ± 0.75	0.00

n=3 (positive control, chloramphenicol 1,000 µg/mL), (negative control, DMSO).

effectiveness of bioactive components in combating the growth of bacteria.

4. Conclusions

This study implies that the decoction extract of *T. merguensis* leaf has high antioxidant activity based on DPPH, ABTS, and ferric-reducing abilities and has potential anti-cholesterol and antimicrobial activity against *S. aureus* and *S. epidermis*. The decoction in water as a single solvent can be useful for secondary metabolite extraction with many biological capacities. In this respect, the decoction extract of *T. merguensis* showed a lot of promise because they do have a high level of cholesterols inhibitory activity in addition to radical scavenging, antioxidant and antimicrobial qualities. To sum up, our analytical study is noteworthy because it is the first to report a thorough characterization of the chemical and biological activities of *T. merguensis*, with an emphasis on those characteristics associated with the therapeutic potential, particularly as natural remedy for hypercholesterolemia. Undoubtedly, further research is required to determine whether consuming *T. merguensis* as a medicine extract or as a single component mixture over an extended period of time might lessen the occurrence or severity of age-related cognitive decline.

Acknowledgements

The research is supported by scientific and technical support of the facilities of Laboratory for Biotechnology, National Research and Innovation Agency through E-Layanan Sains-BRIN.

Data Availability Statement

The entire data set that support the results of this study was published in the article itself.

References

AGUSTINI, S., PURWANTO, W., LESTARI, N., AGUSTINA, S., ARDINAL, NASRUDDIN, ASMALIYAH, HADI, E.E.W., SIAHAAN, H. and UTAMI, S., 2023. Phytochemical, GC-MS, and biological activity of extract of Pelawan Tree (*Triptaniopsis merguensis* Griff). *Rasayan Journal of Chemistry*, vol. 16, no. 4, pp. 2064-2071. http://doi.org/10.31788/RJC.2023.1648591.

ANOOR, P.K., YADAV, A.N., RAJKUMAR, K., KANDE, R., TRIPURA, C., NAIK, K.S. and BURGULA, S., 2022. Methanol extraction revealed anticancer compounds Quinic Acid, 2(5H)-Furanone and Phytol in *Andrographis paniculata. Molecular and Clinical Oncology*, vol. 17, no. 5, pp. 151. http://doi.org/10.3892/mco.2022.2584. PMid:36172002.

BALDISSERA, M.D., SOUZA, C.F., GRANDO, T.H., DOLESKI, P.H., BOLIGON, A.A., STEFANI, L.M. and MONTEIRO, S.G., 2017. Hypolipidemic effect of β-caryophyllene to treat hyperlipidemic rats. *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 390, no. 2, pp. 215-223. http://doi.org/10.1007/s00210-016-1326-3. PMid:27913825.

BENALI, T., BAKRIM, S., GHCHIME, R., BENKHAIRA, N., EL OMARI, N., BALAHBIB, A., TAHA, D., ZENGIN, G., HASAN, M.M., BIBI, S. and BOUYAHYA, A., 2024. Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnology & Genetic Engineering Reviews*, vol. 40, no. 4, pp. 3408-3437. http://doi.org/10.1080/02648725.2022.2122303. PMid:36123811.

BRENDLER, T., BRINCKMANN, J.A., DAOUST, M., HE, H., MASÉ, G., STEFFAN, K. and WILLIAMS, M., 2022. Suitability of botanical extracts as components of complex mixtures used in herbal tea infusions: challenges and opportunities. *Frontiers in Pharmacology*, vol. 13, pp. 1013340. http://doi.org/10.3389/fphar.2022.1013340. PMid:36419619.

DOUGNON, G. and ITO, M., 2021. Essential oil from the leaves of *Chromolaena odorata*, and sesquiterpene caryophyllene oxide induce sedative activity in mice. *Pharmaceuticals*, vol. 14, no. 7, pp. 651. http://doi.org/10.3390/ph14070651. PMid:34358077.

DUA, T.K., JOARDAR, S., CHAKRABORTY, P., BHOWMICK, S., SAHA, A., DE FEO, V. and DEWANJEE, S., 2021. Myricitrin, a Glycosyloxyflavone in *Myrica esculenta* bark ameliorates diabetic nephropathy via improving glycemic status, reducing oxidative stress, and suppressing inflammation. *Molecules*, vol. 26, no. 2, pp. 258. http://doi.org/10.3390/molecules26020258. PMid:33419120.

DURAZZO, A., LUCARINI, M., SOUTO, E.B., CICALA, C., CAIAZZO, E., IZZO, A.A., NOVELLINO, E. and SANTINI, A., 2019. Polyphenols: A concise overview of the chemistry, occurrence, and human health. *Phytotherapy Research*, vol. 33, no. 9, pp. 2221-2243. http://doi.org/10.1002/ptr.6419. PMid:31359516.

ELFAHMI, W., WOERDENBAG, H.J. and KAYSER, O., 2014. Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. *Journal of Herbal Medicine*, vol. 4, no. 2, pp. 51-73. http://doi.org/10.1016/j.hermed.2014.01.002.

FINIMUNDY, T.C., PEREIRA, C., DIAS, M.I., CALEJA, C., CALHELHA, R.C., SOKOVIC, M., STOJKOVIĆ, D., CARVALHO, A.M., ROSA, E., BARROS, L. and FERREIRA, I.C.F.R., 2020. Infusions of herbal blends as promising sources of phenolic compounds and bioactive properties. *Molecules*, vol. 25, no. 9, pp. 2151. http://doi.org/10.3390/molecules25092151. PMid:32375427.

GENG, Y., XIE, Y., LI, W., MOU, Y., CHEN, F., XIAO, J., LIAO, X., HU, X., JI, J. and MA, L., 2024. Toward the bioactive potential of myricitrin in food production: state-of-the-art green extraction and trends in biosynthesis. *Critical Reviews in Food Science and Nutrition*, vol. 64, no. 29, pp. 10668-10694. http://doi.org/10.1080/10408398.2023.2227262. PMid:37395263.

GHENABZIA, I., HEMMAMI, H., BEN AMOR, I.., ZEGHOUD, S., BEN SEGHIR, B. and HAMMOUDI, R., 2023. Different methods of extraction of bioactive compounds and their effect on biological activity: a review. *International Journal of Secondary Metabolite*, vol. 10, no. 4, pp. 469-494. http://doi.org/10.21448/ijsm.1225936.

GODA, M.S., EL-KATTAN, N., ABDEL-AZEEM, M.A., ALLAM, K.A.M., BADR, J.M., NASSAR, N.A., ALMALKI, A.J., ALHARBI, M., ELHADY, S.S. and ELTAMANY, E.E., 2023. Antimicrobial potential of different isolates of *Chaetomium globosum* combined with liquid chromatography-tandem mass spectrometry chemical profiling. *Biomolecules*, vol. 13, no. 12, pp. 1683. http://doi.org/10.3390/biom13121683. PMid:38136556.

GUTIÉRREZ-GRIJALVA, E., LOPEZ-MARTINEZ, L., CONTRERAS-ANGULO, L., ELIZALDE-ROMERO, C. and HEREDIA, J., 2020. Plant alkaloids: structures and bioactive properties. In: M.K. SWAMY, ed. *Plant-derived bioactive: chemistry and mode of action*. Singapore: Springer Nature, pp. 85-117. http://doi.org/10.1007/978-981-15-2361-8_5.

HWANG, I.W. and CHUNG, S.K., 2018. Isolation and Identification of myricitrin, an antioxidant flavonoid, from Daebong Persimmon

peel. *Preventive Nutrition and Food Science*, vol. 23, no. 4, pp. 341-346. http://doi.org/10.3746/pnf.2018.23.4.341. PMid:30675464.

IBRAHIM, M.R., HAMOUDA, R.A., TAYEL, A.A. and AL-SAMAN, M.A., 2021. Anti-cholesterol and antioxidant activities of independent and combined microalgae aqueous extracts in vitro. *Waste and Biomass Valorization*, vol. 12, no. 9, pp. 4845-4857. http://doi.org/10.1007/s12649-021-01349-6.

IFTIKHAR, A., ASLAM, B., IFTIKHAR, M., MAJEED, W., BATOOL, M., ZAHOOR, B., AMNA, N., GOHAR, H. and LATIF, I., 2020. Effect of *Caesalpinia bonduc* polyphenol extract on alloxan-induced diabetic rats in attenuating hyperglycemia by upregulating insulin. *Oxidative Medicine and Cellular Longevity*, vol. 2020, pp. 9020219. http://doi.org/10.1155/2020/9020219. PMid:32256963.

JIN, Q., ZHANG, C., CHEN, R., JIANG, L., LI, H., WU, P. and LI, L., 2024. Quinic acid regulated TMA/TMAO-related lipid metabolism and vascular endothelial function through gut microbiota to inhibit atherosclerotic. *Journal of Translational Medicine*, vol. 22, no. 1, pp. 352. http://doi.org/10.1186/s12967-024-05120-y. PMid:38622667.

KARUNAMOORTHI, K., JEGAJEEVANRAM, K., VIJAYALAKSHMI, J. and MENGISTIE, E., 2013. Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. *Journal of Evidence-Based Complementary & Alternative Medicine*, vol. 18, no. 1, pp. 67-74. http://doi.org/10.1177/2156587212460241.

KUMAR, N. and GOEL, N., 2019. Phenolic acids: natural versatile molecules with promising therapeutic applications. *Biotechnology Reports*, vol. 24, e00370. http://doi.org/10.1016/j.btre.2019.e00370. PMid:31516850.

MAHFUD, N., SULISTYANI, N., SARI, N., SARI, I.P., DEDI, A., OTHMAN, F. and ZAKARIA, Z.A., 2023. Antihyperlipidemic and hepatoprotective effect of *Zingiber cassumunar* rhizome extract in high-fat diet-induced hyperlipidemic rats: the role of antioxidant activity. *Sains Malaysiana*, vol. 52, no. 9, pp. 2559-2569. http://doi.org/10.17576/jsm-2023-5209-07.

MARINACCIO, L., ZENGIN, G., BENDER, O., CICHELLI, A., NOVELLINO, E., STEFANUCCI, A. and MOLLICA, A., 2024. Ultrasound assisted lycopene extraction from tomato skin waste by volatile natural deep eutectic solvent. *Food Chemistry Advances*, vol. 4, pp. 100656. http://doi.org/10.1016/j.focha.2024.100656.

MARTEMUCCI, G., PORTINCASA, P., DI CIAULA, A., MARIANO, M., CENTONZE, V. and D'ALESSANDRO, A.G., 2022. Oxidative stress, aging, antioxidant supplementation and their impact on human health: an overview. *Mechanisms of Ageing and Development*, vol. 206, pp. 111707. http://doi.org/10.1016/j.mad.2022.111707. PMid:35839856.

MOLLICA, A., STEFANUCCI, A., MACEDONIO, G., LOCATELLI, M., LUISI, G., NOVELLINO, E. and ZENGIN, G., 2019. Chemical composition and biological activity of *Capparis spinosa* L. from Lipari Island. *South African Journal of Botany*, vol. 120, pp. 135-140. http://doi.org/10.1016/j.sajb.2018.02.397.

PANT, G., SIMARIA, C., VARSI, R.A.H., BHAN, P. and SIBI, G., 2015. In vitro anti-cholesterol and antioxidant activity of methanolic extracts from flax seeds (*Linum usitatissimum* L.). *Research Journal of Medicinal Plant*, vol. 9, no. 6, pp. 300-306. http://doi.org/10.3923/rjmp.2015.300.306.

PAYNE, A.C., MAZZER, A., CLARKSON, G.J.J. and TAYLOR, G., 2013. Antioxidant assays – consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves. *Food Science & Nutrition*, vol. 1, no. 6, pp. 439-444. http://doi.org/10.1002/fsn3.71. PMid:24804054.

RASUL, M.G., 2018. Conventional extraction methods use in medicinal plants, their advantages and disadvantages. *International Journal of Basic Sciences and Applied Computing*, vol. 2, no. 6, pp. 10-14.

SATYAESWARI, J., DHAGAT, S., NAIK, S. and DIBYA, S., 2018. Phytochemical and antimicrobial studies of *Oroxylum indicum* extracts. *Pharmaceutical Bioprocessing*, vol. 6, no. 1, pp. 7-14.

SHAABAN, M.T., GHALY, M.F. and FAHMI, S.M., 2021. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. *Journal of Basic Microbiology*, vol. 61, no. 6, pp. 557-568. http://doi.org/10.1002/jobm.202100061. PMid:33871873.

SONG, X., TAN, L., WANG, M., REN, C., GUO, C., YANG, B., REN, Y., CAO, Z., LI, Y. and PEI, J., 2021. Myricetin: a review of the most recent research. *Biomedicine and Pharmacotherapy*, vol. 134, pp. 111017. http://doi.org/10.1016/j.biopha.2020.111017. PMid:33338751.

STEFANUCCI, A., MARINACCIO, L., LLORENT-MARTÍNEZ, E.J., ZENGIN, G., BENDER, O., DOGAN, R., ATALAY, A., ADEGBITE, O., OJO, F.O., ONAOLAPO, A.Y., ONAOLAPO, O.J. and MOLLICA, A., 2024. Assessment of the in-vitro toxicity and in-vivo therapeutic capabilities of *Juglans regia* on human prostate cancer and prostatic hyperplasia in rats. *Food Bioscience*, vol. 57, pp. 103539. http://doi.org/10.1016/j.fbio.2023.103539.

WIDODO, H., SISMINDARI, S., ASMARA, W. and ROHMAN, A., 2019. Antioxidant activity, total phenolic and flavonoid contents of selected medicinal plants used for liver diseases and its classification with chemometrics. *Journal of Applied Pharmaceutical Science*, vol. 9, no. 6, pp. 99-105. http://doi.org/10.7324/JAPS.2019.90614.

WINDARSIH, A., SURATNO., WARMIKO, H.D., INDRIANINGSIH, A.W., ROHMAN, A. and ULUMUDDIN, Y.I., 2022. Untargeted metabolomics and proteomics approach using liquid chromatography-Orbitrap high resolution mass spectrometry to detect pork adulteration in *Pangasius hypopthalmus* meat. *Food Chemistry*, vol. 386, pp. 132856. http://doi.org/10.1016/j.foodchem.2022.132856. PMid:35367799.

ZHANG, Q., LIN, L. and YE, W., 2018. Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, vol. 13, no. 1, pp. 20. http://doi.org/10.1186/s13020-018-0177-x. PMid:29692864.