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GC-MS and ADMET Profiling of *Bruguiera gymnorrhiza* Mangrove Leaf Extract Origin Sulawesi with Antioxidant Properties

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ABSTRACT

Mangrove plants, particularly *Bruguiera gymnorrhiza*, are recognized as sources of bioactive compounds. This study analyzed the chemical profiles, safety, and antioxidant activity of *B. gymnorrhiza* leaf extract from Sulawesi, a biodiversity-rich Wallacean region. Research on *B. gymnorrhiza* leaves from Sulawesi remains scarce. GC-MS analysis identified inositol, fatty acid, ester, and terpenoid derivatives as major chemical profiles related to stress tolerance and remedial properties. ADMET predictions showed good intestinal absorption but suggested potential hepatotoxicity at high doses. Brine shrimp lethality tests revealed low acute toxicity with an LD_{50} of 873.381 µg/mL, supporting in silico findings. The extract exhibited strong antioxidant activity (IC_{50} 49.78 µg/mL), comparable to Vitamin E, and higher than reports from other regions. These results indicate that Sulawesi mangroves store valuable chemical compounds and serve as environmental health indicators. Combining chemical profiling, computational prediction, and experimental validation highlights their pharmaceutical potential while emphasizing the need for careful dose optimization and ecological monitoring. This study reinforces the importance of conserving Wallacean biodiversity and offers a foundation for safe, natural antioxidant commercialization. Future work should assess in vivo effectiveness, long-term toxicity, and the ecological impacts of pollutant immobilization in mangrove ecosystems.

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INTRODUCTION

Mangroves, thriving in highly saline and dynamic coastal ecosystems, have evolved robust chemical defense mechanisms, including antioxidant systems [1], [2], to mitigate oxidative stress induced by environmental extremes [3]. These adaptive responses position mangrove-derived compounds as promising candidates for nutraceutical and pharmaceutical applications [4]. Among mangrove species,

Bruguiera gymnorrhiza has garnered attention for its antioxidant potential.

Previous studies on *B. gymnorrhiza* leaf extracts from India, Malaysia, and South Sumatra have identified bioactive compounds with varying antioxidant capacities, such as flavonoids, phenolics, and terpenoids. For instance, Indian Sundarbans samples exhibited potent radical scavenging activity (IC_{50} 0.355 µg/mL) linked to high phenolic content [5], while Malaysian extracts showed moderate activity (IC_{50} 29–270 µg/mL) correlated with fatty acid derivatives [6]. South Sumatran leaves demonstrated lower efficacy (IC_{50} 105.09 µg/mL), suggesting regional environmental factors significantly influence phytochemical composition and bioactivity [7]. However, these studies predominantly focus on antioxidant efficacy, with limited emphasis on comprehensive chemical profiling or safety assessments, leaving critical gaps in understanding their therapeutic potential and risks. In addition, there is very little previous research on the antioxidant activity of *B. gymnorrhiza* leaf extract collected from Sulawesi. The only literature found reports the antioxidant potential of *B. gymnorrhiza* fruit extract [8]. However, differences in sampling locations can affect the antioxidant capacity of mangrove leaf extract, especially from the Wallacea region [9].

Sulawesi, situated within the Wallacea biogeographical region—a transitional zone between Asia and Australia, renowned for its unparalleled biodiversity and endemic species—presents a unique ecological niche for mangrove adaptation [10], [11]. The island's mangroves endure distinct environmental stressors, including tectonic-driven salinity gradients [12], volcanic sediment deposition [13], and hybridization of Indo-Pacific and Australasian biotic influences [14], [15]. These conditions likely drive the evolution of novel chemical profiles, as Wallacean species often develop specialized biochemical traits to thrive in isolated, dynamic ecosystems [16]. Despite this, *the origin of B. gymnorrhiza from* Sulawesi remains understudied, with prior research limited to fruit extracts. Leaves, as perennial organs directly exposed to these biogeographical pressures, may harbor unique chemical profiles shaped by Wallacea's evolutionary distinctiveness. However, no study has holistically characterized their composition, pharmacokinetic safety (ADMET), or toxicity, hindering their translation into safe nutraceuticals.

This study addresses this gap by integrating GC-MS-based chemical profiling with computational ADMET prediction and experimental toxicity validation to evaluate *B. gymnorrhiza* leaf extract from Tomini Bay, Sulawesi. By elucidating Wallacea-specific chemical compounds and their safety profiles, this study advances mangrove bioprospecting while underscoring the untapped potential of this region in the discovery of biodiversity-based drugs. Thus, this study aims to identify and evaluate the chemical profiles of GC-MS-based extracts of *B. gymnorrhiza* mangrove leaves from Sulawesi. In addition, this study also characterizes the ADMET profile and antioxidant capacity of *B. gymnorrhiza* mangrove leaf extract computationally.

These findings provide an important foundation for developing sustainable and safe antioxidant products and emphasize the importance of conservation-based research in ecosystems that are not well known but have an important biogeographical role, such as Wallacea.

METHODS

1. Sampling Location

The leaves of *B. gymnorrhiza* were collected in the coastal region of Tomini Bay at Laemanta Village, Parigi Moutong Regency, Central Sulawesi, Indonesia, located at 0°11′05.6″ S, 120°00′32.9″ E. Sampling took place in April 2020 on sunny and bright days. Samples were randomly selected from several *B-Gymnorrhiza* mangrove trees in one community (Figure 1). Environmental factors in the mangrove stand were as follows: temperature (28–30°C), pH (water 7.1–8, substrate 6.8–8), substrate (muddy), and salinity (21–22 ppt). Mangroves were identified by studying the tree shape, root type, fruit shape, flowers, and leaves according to [17].

Figure 1. *Bruguiera gymnorrhiza* leaves origin Sulawesi

2. Extraction

Air-dried leaves of *B. gymnorrhiza* were ground into fine granules and dried in an oven (Finco OV50) at 60–70°C. Then, 100 g of *B. gymnorrhiza* fine granules was macerated in 300 mL of MeOH: DCM (1:1) solvent mixture for 48 h [18]. Maceration was performed automatically, and the mixture was stirred constantly by a magnetic stirrer at room temperature. After that, the extract was filtered and evaporated using an evaporator (EYELA N-1100). The extraction was repeated three times, and the dried extract was weighed and kept at a temperature of 4°C.

3. Chemical Profile Screening by GC-MS

The chemical composition of the leaf extracts of *B. gymnorrhiza* was analyzed by a Hewlett-Packard 6890 GC-MS system fitted with an Agilent 190915-433 HP-5MS capillary column (30 m length × 250 µm diameter × 0.25 µm film thickness). The splitless mode was employed with an injector temperature of 280°C to ensure maximum transfer of samples to the column. Helium was the carrier gas with a 1 mL/min flow rate. The oven temperature program was: 150°C initial for 1 minute; 2°C/min to 155°C and 10°C/min to a final temperature of 240°C, held for 11 minutes. The total duration was 22 minutes. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV, in a range of m/z 50–550, and at an ion source temperature of 230°C. 1 µL of the extract (dissolved in methanol) was injected. The compounds were identified by comparing their mass spectra fragmentation data with the NIST and Wiley libraries [19].

4. ADMET Profile Characterization

The ADMET profile of the chemical compounds in the *B. gymnorrhiza* leaf extract

was virtually screened. The computational analysis was limited to GC-MS major compounds (absolute percentage peak area >1%). Predictions were performed with the ADMETlab 3.0 web server (https://admetlab3.scbdd.com/server/evaluation) [20] and ProTox-II (https://tox-new.charite.de/protox_II/) [21]. The Canonical SMILES strings for each compound were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The computational analysis was performed between December 2024 and January 2025.

5. Toxicity Assay (BSLT Method)

The nauplii were obtained from hatched *Artemia salina* eggs incubated in seawater (32–34 ppt) under continuous aeration and light for 48 h. *B. gymnorrhiza* leaf extracts were dissolved in DMSO (1%) and diluted in seawaters to final concentrations of 0, 20, 40, 60, 80, 100, 200, and 500 µg/mL. Each test tube (5 mL extract solution) received 10 larvae of *A. salina* (48 h old). Untreated controls contained seawater with 1% DMSO. Each treatment was conducted in triplicate under continuous light at 25°C. Mortality was recorded after 24 h, using immobility as the endpoint [22]. LD_{50} values were estimated using probit analysis with 95% confidence intervals (SPSS 20.0). Percentage mortality was calculated by:

$$Mortality\ (\%) = \frac{Number\ of\ dead\ larvae}{Total\ number\ of\ larvae} \times 100\% \quad (1)$$

6. Antioxidant Capacity Analysis

The antioxidant activity of the *B. gymnorrhiza* leaf extract and Vitamin E (as standard) was determined by the DPPH procedure [23]. The dissolved extract was prepared using ethanol at 20, 40, 60, 80, and 100 µg/mL concentrations. Then, 50 µM (19.72 µg/mL) of DPPH was added to each extract concentration and incubated in the dark at room temperature for 30 minutes. The absorbance of the DPPH solution (A) and each extract concentration (B) was determined at λ = 517 nm using a UV-VIS spectrophotometer (Shimadzu, 1800). IC_{50} values were calculated using the regression equation. The antioxidant power was obtained using equation (2):

$$Antioxidant\ capacity\ (\%) = \frac{A-B}{A} \times 100\% \quad (2)$$

RESULTS AND DISCUSSION

1. Chemical Profile of *Bruguiera gymnorrhiza* Leaves Extract

GC-MS analyzes the relative quantification of compounds in an extract [24]. GC–MS is a gaseous separation technique used to identify the compounds in natural extracts; these include organic acids, phenolic compounds, alkaloids, terpenoids, phytosterols, esters, and ketones [25]. Accordingly, GC-MS facilities can be applied for the preliminary screening of the chemical fingerprints to identify the possible pharmacological effects of any natural extract. Among 21 compounds detected from GC-MS analysis of *B. gymnorrhiza* leaf extract (Table 1 and Figure 2), mome inositol (43.77%), bis(2-ethylhexyl) adipate (24.79%), hexanedioic acid dioctyl ester (12.82%), 4,5-dimethylisothiazole (6.44%), 3-ethyl-2-(3-butenyl)-cyclohex-2-en-1-one (3.66%), and 1,3-dioxolane-2-propanal, 2-methyl- (3.22%) were the major compounds.

Table 1. Chemical profile of *Bruguiera gymnorrhiza* leaves extracts from Sulawesi by GC-MS

RT (min)	Chemical profiles	Formula	Relative Abundance (%)
2.756	Propane, 2-fluoro-2-methyl-	C_4H_9F	0.23
3.565	(5-Methyl-2-furyl)-methyl sulfur	C_6H_8OS	0.55
4.078	Dihydrothymine	$C_5H_8N_2O_2$	0.29
4.565	3-Pyridinecarboxamide	$C_{19}H_{15}N_5O$	0.18
7.074	1-borabicyclo[4.3.0]nonane	$C_{10}H_{16}$	0.42
7.785	Phenol, 2-propyl-	$C_9H_{12}O$	0.39
8.575	Cyclopentanemethylamine, 2-isopropylidene-N,N,5-trimethyl-, (1R,5R)- (-)-	$C_{12}H_{23}N$	0.33
9.055	Cyclodecane	$C_{10}H_{20}$	0.30
9.227	Heptanoic acid	$C_7H_{14}O_2$	0.15
10.27	Dodecanoic acid	$C_{12}H_{24}O_2$	0.24
11.687	Methanone, diphenyl-	$C_{13}H_{10}O$	0.10
13.293	4,5-Dimethylisothiazole	C_5H_7NS	6.44
13.517	Mome Inositol	$C_6H_{12}O_6$	7.07
14.047			36.70
14.310	3-Ethyl-2-(3-butenyl)-cyclohex-2-en-1-one	$C_{12}H_{18}O$	3.66
14.413	1,3-Dioxolane-2-propanal, 2-methyl-	$C_7H_{12}O_3$	3.22
14.573			7.68
14.749	Bis(2-ethylhexyl) adipate	$C_{22}H_{42}O_4$	2.07
21.914			15.04
14.834	Hexanedioic acid, dioctyl ester	$C_{22}H_{42}O_4$	12.82
15.838	Vomifoliol	$C_{13}H_{20}O_3$	0.46
17.522	Neophytadiene	$C_{20}H_{38}$	0.95
17.834	Spiro[2.3]hexan-4-one, 5,5-diethyl-6-methyl-	$C_{11}H_{18}O$	0.42
19.477	Citronellyl valerate	$C_{15}H_{28}O_2$	0.27

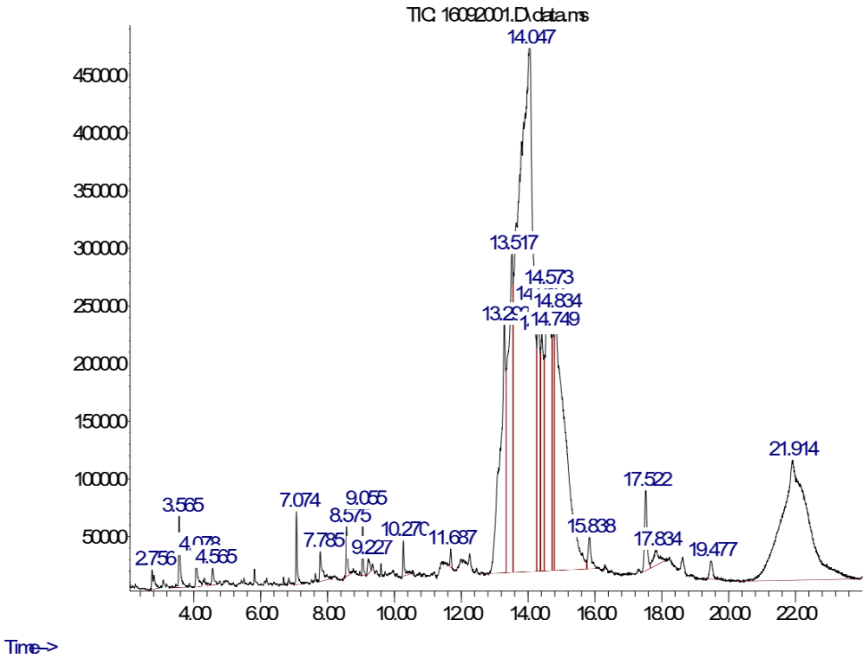

Figure 2. GC-MS Chromatogram of *B. gymnorrhiza* leaf extracts from Sulawesi

Mome inositol (MOME-) is a sugar alcohol found in stress-tolerant plants such as *Euchresta horsfieldii* [26], even though it has been newly detected in *B. gymnorrhiza*. It has been previously reported that inositol-containing compounds isolated from *Rhizophora mucronata* and *Rhizophora stylosa* mangroves possess antidiabetic, antiviral, and antiulcer effects [27]–[30]. From Table 1, some reported compounds from mangroves were also found, for example, 3-pyridinecarboxamide (which was reported to function as an adaptational protein of the mangrove *Bruguiera sexangula* to the environment) was found to play roles in: (1) NAD/NADP production for cellular redox regulation; (2) involvement in the formation of secondary metabolites of mangroves with defensive functions; and (3) participation in salt stress tolerance [31].

Fatty acid-derived compounds like heptanoic acid have previously been identified in an *Avicennia marina* leaf extract [32], and dodecanoic acid was identified in *R. stylosa* [33] and *A. marina* [34]. Fatty acid-based compositions play multiple roles in mangrove plants such as: (1) being an integral part of cell membranes; (2) involvement in plant metabolism and energy storage; (3) acting as precursors for the synthesis of other important compounds; and (4) assisting in the up-regulation of mangrove plants to adapt to extreme conditions, particularly high salinity [35].

The occurrence of terpenoids (e.g., vomifoliol, neophytadiene) follows previous observations in *Ceriops tagal* and *Avicennia alba* [36], [37], confirming the diversity of mangrove metabolism.

2. Environmental and Biological Interpretation of Detected Compounds

The phytochemistry of the extract (Table 1) captures environmental adaptation and therapeutic value. Mome inositol has antibacterial and antioxidant properties [38]–[40]. Dodecanoic acid has been reported to exhibit antimicrobial activities [41]. Bis(2-ethylhexyl) adipate, a water contaminant ester compound and plasticizer derivative, is widely used in the flexible PVC industry and in producing plastic films for food packaging. Bis(2-ethylhexyl) adipate is said to be of low toxicity (LD_{50} estimated to be above 900 mg/kg in rats) and is not classifiable as to its carcinogenicity to humans (IARC, 1990). Bis(2-ethylhexyl) adipates are not highly soluble in water and preferentially distribute to sediments and biota in aquatic systems [42]. The presence of such compounds in mangroves is attributed to their capability to serve as sinks of anthropogenic and industrial contaminants [43]. Bis(2-ethylhexyl) adipate has also been found to be degraded by rhizospheric microbial communities, altering the physicochemical characteristics of sediment and microbial composition in mangroves [44].

4,5-Dimethylisothiazole and dioctyl ester are also derivatives of ester compounds used as plasticizers. These substances can reach mangrove systems and might be preserved due to several reasons: (1) Persistence, as hexanedioic acid dioctyl ester is a synthetic organic compound that is environmentally persistent and difficult to degrade [45]; (2) Adsorption characteristics of pollutants by mangroves, which can absorb and accumulate various pollutants,

including microplastics and organic matter through roots, stems, and leaves; (3) Mangrove habitat proximity to estuaries and coastal zones where pollutants accumulate and are continuously deposited [46]; and (4) Lipophilicity, as hexanedioic acid dioctyl ester can associate with fatty tissues of plants [47]. Nevertheless, its toxicity is lower and biodegrades faster than phthalate plasticizers [48].

4,5-Dimethylisothiazole is an aromatic heterocyclic compound documented to possess anticancer [49], protease inhibitory [50], antimicrobial [51], and anti-inflammatory [50], [51] properties. 3-Ethyl-2-(3-butenyl)-cyclohex-2-en-1-one is a cyclohexenone that has been reported to exhibit anti-inflammatory and anticancer activities [52], [53]. 1,3-Dioxolane-2-propanal, 2-methyl- is an aldehyde derivative with anticancer and antimicrobial activities against *Staphylococcus aureus* and *Candida albicans* [54]. Propane, 2-fluoro-2-methyl- belongs to the group of alkyl halide compounds and shows antimicrobial activity against *Bacillus subtilis*, *S. aureus*, *Pseudomonas aeruginosa*, *E. coli*, and *C. albicans* [55].

(5-Methyl-2-furyl)-Methyl sulfur is a methyl sulfide derivative with antioxidant activity [56] and antibacterial properties against *E. coli*, *B. subtilis*, and *S. aureus* [57]. Dihydrothymine compounds are hydropyrimidine derivatives known as cancer metabolism markers [58].

1-Borabicyclo[4.3.0]nonane is part of a family of anticancer-active organoborane compounds [59]. Phenol, 2-propyl- is a phenylpropane derivative and an antioxidant [60]. Cyclopentane methylamine, 2-isopropylidene-N, N,5-trimethyl-,(1R,5R)-(-)- is an amine derivative with reported anti-inflammatory activity [61]. Cyclodecane, a class of cycloalkane compounds, is recognized as a novel cancer cell replication inhibitor [62]. Methanone, diphenyl- is an aromatic ketone compound reported to have antimicrobial, antiviral, anti-inflammatory, anticancer [63], [64], and antioxidant [65] properties. 5,5-Diethyl-6-methyl-spiro[2.3]hexan-4-one is a carbocyclic spiro compound with documented antiviral and anticancer actions [66], [67]. Citronellyl valerate is a labdane monoterpenoid derivative reported to possess antimicrobial and tumor-inhibiting properties [68].

The comparison of the chemical composition of *B. gymnorrhiza* presented in Table 1 offers strong evidence of its dual potential: acting as a source of chemical compounds with broad nutraceutical and pharmaceutical applications, and serving as an environmental health indicator through its capacity to absorb and accumulate contaminants from surrounding environments.

3. ADMET Prediction of Major Compounds

Moreover, the six major compounds determined by GC-MS were subjected to in-silico approaches to predict ADMET profiles based on ADMETlab 3.0 as described in Table 2. Prediction of toxicity excretion followed the guidelines provided by Banerjee et al., where a predicted value close to 1 indicates that the prediction model is highly confident in the ADMET prediction results.

Furthermore, probabilities close to 1 suggest that the tested compounds are structurally similar to those in the database used to develop the ProTox-III 3.0 prediction model [21].

The profiles of computational ADMET (Table 2) showed positive gastrointestinal HIA values (>0.8) and low BBB permeability (BBB ≈ 0.5) predicted for mome inositol and bis(2-ethylhexyl) adipate, which are consistent with ProTox 3.0 predictions. Although ADMETlab 3.0 considered the extract to be orally safe (FDAMDD > 1000.00 mg/kg), there remains a risk of hepatotoxicity, and thus, dose optimization is necessary during nutraceutical and pharmaceutical formulation development.

Human intestinal absorption (HIA) indicates drug absorption from the gastrointestinal system into the human bloodstream after oral administration. The HIA indicator is an important parameter in the ADMET evaluation of a drug candidate. HIA measures drug absorption from the gastrointestinal tract to the bloodstream following oral administration. HIA is an important index in the ADMET assessment of a drug candidate [69]. Its permeability is well correlated with human intestinal permeability and has been employed in studies of drug efflux [70]. P-glycoproteins are responsible for the cellular efflux of compounds across various organs. According to the ADMET prediction, the main compounds possess low bioavailability; thus, dissolution will be necessary for oral administration.

The BBB indicator measures a molecule's ability to permeate the blood-brain barrier to access its central nervous system target. A predicted value close to 1 suggests potential CNS activity, whereas a value close to 0 suggests activity in the peripheral nervous system [71]. Plasma protein binding refers to how much a drug binds to proteins in blood plasma. More importantly, only the unbound species are pharmacologically active and subject to metabolism or excretion [72]. Cytochrome P450 (CYP450) metabolism is crucial in cell homeostasis [73].

Table 2 also indicates that the Sulawesi-origin *B. gymnorrhiza* leaf extract could be metabolized in the human liver. However, Table 2 also revealed that two compounds were predicted to be unstable with liver metabolizing enzymes, suggesting that further in vivo examination is necessary.

4. Toxicity Evaluation and Experimental Confirmation (BSLT Assay)

Differences between in silico and in vitro toxicity underscore the need to combine the two approaches to obtain an overall estimate of risk. Thus, to validate the in silico predictions of ADMET, in vitro toxicity testing was performed using the BSLT method. Results are shown in Table 3 for the BSLT assay of *B. gymnorrhiza* leaf extracts.

Table 2. ADMET profiles from *Bruguiera gymnorrhiza* leaves extract by GC-MS from Sulawesi

ADMET Profiles	Major chemical profiles from *Bruguiera gymnorrhiza* leaves extract detected by GC-MS.											
	Mome Inositol		Bis(2-ethylhexyl) adipate		Hexanedioic acid, dioctyl ester		4,5-Dimethyliso-thiazole		3-Ethyl-2-(3-butenyl)-cyclohex-2-en-1-one		1,3-Dioxolane-2-propanal, 2-methyl-	
Absorption												
Human intestinal absorption	0.671	●	0.016	●	0.065	●	0.029	●	0.003	●	0.023	●
Caco-2 permeability	–6.071	●	–4.949	●	–5.05	●	–4.788	●	–4.591	●	–4.748	●
P-glycoprotein substrate	0.49	●	0.072	●	0.021	●	0.353	●	0.001	●	0.177	●
P-glycoprotein inhibitor	0.013	●	0.253	●	0.007	●	0.022	●	0.864	●	0.382	●
$F_{20\%}$	0.396	●	0.835	●	0.9	●	0.062	●	0.01	●	0.057	●
$F_{30\%}$	0.697	●	0.68	●	0.799	●	0.157	●	0.035	●	0.021	●
Distribution												
Blood-Brain Barrier	0.7	●	0.247	●	0.015	●	0.793	●	0.994	●	0.88	●
Plasma protein binding	23.335	●	96.865	●	97.603	●	81.616	●	93.695	●	29.461	●
Metabolism												
CYP450 2C9 substrate	0.999	●	0.006	●	0.403	●	0.418	●	0.662	●	0.71	●
CYP450 2D6 substrate	0	●	0	●	0.001	●	0.803	●	0.105	●	0.081	●
CYP450 3A4 substrate	0	●	1.0	●	0.988	●	0.992	●	0.388	●	0.129	●
CYP450 2C9 inhibitor	0	●	1.0	●	0.926	●	0.298	●	0.246	●	0.044	●
CYP450 2D6 inhibitor	0	●	0.04	●	0.899	●	0.051	●	0.032	●	0.03	●
CYP450 2C19 inhibitor	0	●	1.0	●	1.0	●	0.939	●	0.966	●	0.033	●
CYP450 3A4 inhibitor	0	●	1.0	●	0.92	●	0.56	●	0.166	●	0.11	●
Human liver microsomal stability	0.001	●	0.996	●	0.352	●	0.412	●	0.957	●	0.148	●
Excretion-Toxicity												
Hepatoxicity	0.87	Inactive	0.84	Inactive	0.84	Inactive	0.60	Inactive	0.65	Inactive	0.84	Inactive
Neurotoxicity	0.91	Inactive	0.91	Inactive	0.93	Inactive	0.56	Inactive	0.62	Active	0.87	Inactive
Nephrotoxicity	0.58	Active	0.52	Active	0.50	Active	0.80	Inactive	0.81	Inactive	0.59	Inactive
Respiratory toxicity	0.66	Inactive	0.99	Inactive	0.99	Inactive	0.59	Inactive	0.96	Inactive	0.92	Inactive
Cardiotoxicity	0.83	Active	0.83	Inactive	0.86	Inactive	0.79	Inactive	0.62	Inactive	0.72	Inactive
Drug-induced liver injury (DILI)	0.017	●	0.052	●	0.148	●	0.931	●	0.153	●	0.264	●
FDA Maksimum Daily Dose (FDAMDD)	0.089	●	0.348	●	0.11	●	0.165	●	0.485	●	0.124	●
Predicted LD_{50} mg/kg (Accuracy)	10000	100%	5000	100%	5000	100%	500	54.26%	1720	72.9%	5000	68.07%
LIPINSKI Rules	Accepted	●	Accepted	●	Accepted	●	Accepted	●	Accepted	●	Accepted	●

Table 3. Toxicity assay (BSLT) of *B. gymnorrhiza* leaf extracts from Sulawesi.

Concentration (µg/mL)	Number of larvae that died after 24 hours			Mortality (%)
	1	2	3	
500	1	2	1	13.33
200	1	1	1	10.00
100	0	0	1	3.33
80	0	1	0	3.33
60	0	0	0	0
40	0	0	0	0
20	0	0	0	0
0	0	0	0	0

BSLT tests showed low acute toxicity, revealing an LD_{50} = 873.381 µg/mL. Therefore, the extract is classified as "low-toxic" according to Clarkson criteria: highly toxic when LD_{50} between 0 and 100 µg/mL, medium-toxic when LD_{50} between 100 and 500 µg/mL, low-toxic when LD_{50} between 500 and 1000 µg/mL, and nontoxic when LD_{50} > 1000 µg/mL [74].

The results of the BSLT assay are consistent with the ADMET prediction in Table 2, indicating that the main compounds identified by GC-MS may exhibit toxicity at high doses. The low toxicity results support the safe use of effective doses as antioxidants.

5. Antioxidant Capacity Assessment

The antioxidant activity of the mangrove leaf extracts of *B. gymnorrhiza* was determined using the DPPH method. The antioxidant activity of *B. gymnorrhiza* leaf extract is shown in Figure 3.

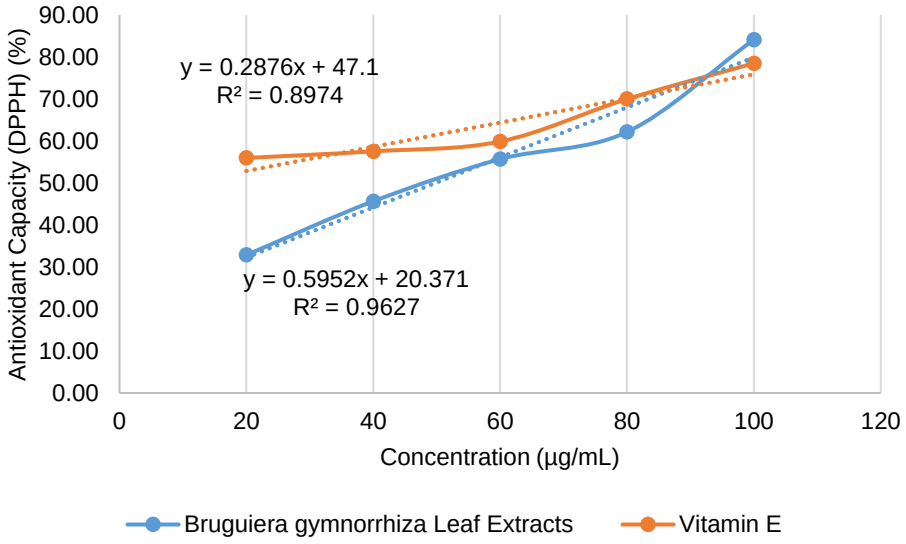

Figure 3. Antioxidant capacity of *B. gymnorrhiza* leaf extracts originating from Sulawesi and Vitamin E.

The radical-scavenging activity of *B. gymnorrhiza* leaf extract was significant (IC_{50} = 49.78 ± 0.35 µg/mL), statistically comparable to Vitamin E (IC_{50} = 10.63 ± 1.39 µg/mL) at a DPPH concentration of 19.72 µg/mL. The antioxidant strength is "powerful" [75] compared to South Sumatran *B. gymnorrhiza* (DPPH IC_{50} = 105.09 µg/mL

with DPPH 39.432 µg/mL) [7], and similar to Malaysian samples (DPPH IC_{50} between 29–270 µg/mL with DPPH 8000 µg/mL) [6], possibly due to unique environmental stressors in Sulawesi. The high phenolic and inositol content could interact synergistically to scavenge ROS, resembling the antioxidant pattern of Indian Sundarbans mangroves [5].

The DPPH assay for antioxidant measurement strongly depends on the DPPH concentration used. Lower DPPH concentrations (<118 µg/mL) are appropriate for testing extracts with high antioxidant activity [76], while higher DPPH concentrations are required for extracts with lower activity to ensure complete reaction [77]. Therefore, antioxidant measurements using DPPH must be validated by positive controls, such as Vitamin C or Vitamin E [78]. This research has effectively unveiled the distinct chemistry and medicinal properties of Sulawesi leaf extracts of *Bruguiera gymnorrhiza* growing in the Wallacea region. GC-MS profiling identified constituents, including inositol, esters, fatty acids, and terpenoid derivatives, revealing biotechnological and nutraceutical value in the under-studied Wallacea mangroves. The strong antioxidant power (higher than other geographic locations and equivalent to Vitamin E) highlights the potential for nutraceutical and pharmaceutical applications.

While the BSLT assay confirmed the absence of severe acute toxicity, computational ADMET predictions indicated a potential risk of hepatotoxicity and drug interaction for several major chemical constituents, reaffirming the necessity of an integrated (in silico–in vitro) approach for safety evaluation. Thus, this study not only emphasizes the potential of Sulawesi *B. gymnorrhiza* as a source of pharmacologically important compounds but also highlights the importance of comprehensive toxicity screening and environmental consideration in bioprospecting natural products from ecologically unique regions like Wallacea.

CONCLUSION

This study highlights Sulawesi *Bruguiera gymnorrhiza* leaf extracts as a rich source of nutraceutical and pharmaceutical compounds (e.g., derivatives of inositol, ester, fatty acid, and terpenoid) with therapeutic potential, shaped by Wallacea's unique environment. GC-MS and ADMET analyses suggest a favorable safety profile and antioxidant efficacy, though hepatotoxicity risks at high doses require caution. BSLT tests confirmed low toxicity. The findings underscore Wallacea's untapped biodiversity and advocate integrated chemical, computational, and experimental approaches for sustainable bioprospecting. To ensure safe applications, further research should explore in vivo effects, optimal dosing, and mangrove pollutant monitoring. This work positions *B. gymnorrhiza* as a promising natural antioxidant source, reinforcing Wallacea's ecological and bio-nutra-pharmaceutical significance.

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