Phytochemical Constituents and Antioxidant Potential of the Flower Extract of Bruguiera gymnorrhiza

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Abstract

This study investigated the phytochemical composition and antioxidant potential of sequential solvent extracts (hexane, ethyl acetate, and methanol) obtained from the flowers of *Bruguiera gymnorrhiza*. The methanol extract yielded the highest amount of crude material, reflecting the abundance of polar constituents, whereas the ethyl acetate and hexane extracts produced substantially lower yields. GC–MS analysis revealed diverse chemical profiles across the three fractions, including sugars, furans, terpenoids, sterols, long-chain hydrocarbons, and several unidentified compounds, particularly in the semi-polar and polar extracts. Antioxidant activity assessed using DPPH and ABTS assays demonstrated a clear polarity-dependent trend: the methanol extract exhibited the strongest radical-scavenging activity (IC_{50} = 52.25 ± 0.35 and 28.07 ± 0.85 µg/mL), followed by the ethyl acetate extract, whereas the hexane extract showed minimal activity. These findings highlight *B. gymnorrhiza* flowers as a promising natural source of antioxidant compounds, especially within the methanol fraction, and suggest that further structural elucidation of the unidentified metabolites could reveal additional bioactive molecules with potential therapeutic value.

Keywords: *Bruguiera gymnorrhiza*, Antioxidant, Phytochemical, Flower extract

1. Introduction

Bruguiera gymnorhiza (L.) Savigny, commonly known as the large-leafed orange mangrove, is an important medicinal plant within Southeast Asian mangrove ecosystems, including the coastal regions of Thailand. Belonging to the Rhizophoraceae family, this species plays a vital ecological role by stabilizing shorelines, enhancing biodiversity, and supporting coastal resilience (Ruang-areerate, 2023). Traditionally, various parts of the plant have been used to treat ailments such as diarrhea, inflammation, and skin disorders (Bandaranayake, 2002; Kathiresan & Bingham, 2001), highlighting its potential as a rich source of bioactive compounds. Phytochemicals are naturally occurring plant metabolites produced in response to environmental stress and biological threats. These compounds—comprising phenolics, flavonoids, tannins, triterpenoids, and alkaloids—are widely recognized for their diverse biological activities. Among them, antioxidant activity is particularly significant, as antioxidants mitigate oxidative damage associated with chronic diseases such as cardiovascular disorders, diabetes, and cellular degeneration (Prior et al., 2005; Shahidi & Ambigaipalan, 2015). Consequently, the determination of total phenolic and flavonoid contents is central to evaluating the biological potential of medicinal plants. Although previous studies have

examined the leaves, bark, and roots of *B. gymnorrhiza*, demonstrating antioxidant, anti-inflammatory, and antidiabetic properties (Musara et al., 2020; Shilpi et al., 2012), detailed information on the phytochemical composition of the flowers remains scarce (Kalasuba et al., 2023). This gap in knowledge is notable, as floral tissues of many plant species often contain high levels of phenolics and flavonoids, making them promising sources of natural antioxidants suitable for the development of cosmetic ingredients, dietary supplements, and health-related innovations. Therefore, the present study aimed to investigate the extraction of *B. gymnorrhiza* flowers, characterize their phytochemical constituents, and evaluate their antioxidant potential using standard assays such as DPPH and ABTS. The findings generated from this work are expected to contribute new scientific insights into the chemical value of this mangrove species and provide a foundation for future natural product development in the health and beauty industries.

2. Research Objective

1. To extract the flowers of *Bruguiera gymnorrhiza* using various solvents and to systematically characterize their phytochemical constituents by employing standard analytical methods.

2. To evaluate the antioxidant potential of the flower extracts of *Bruguiera gymnorrhiza* through standardized radical scavenging assays.

3. Materials and Methods

3.1 Preparation of Extracts

The flowers of *B. gymnorrhiza* were collected from Samut Songkhram Province, Thailand, in February 2025. The plant material was air-dried to a final weight of 341.76 g and subsequently ground into a fine powder. The powdered flowers were subjected to sequential extraction using hexane, ethyl acetate, and methanol in accordance with increasing solvent polarity. At each stage, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator to obtain the corresponding crude extracts.

3.2 Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical profiles of the flower extracts of *B. gymnorrhiza* were analysed using a Shimadzu GC–MS QP2020 instrument equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. A 1-µL aliquot of each extract was injected in split mode with a 1:20 split ratio. The temperature programme for the GC oven commenced at 70 °C with an initial hold of 2 min, followed by heating at 5 °C/min to 200 °C and maintained for 20 min. The temperature was then raised to 230 °C and held for 15 min, further increased to 250 °C for an additional 15 min, and finally elevated to 320 °C with a final hold of 20 min. The ion source was kept at 250 °C and operated under electron ionisation (EI). Total ion chromatograms (TIC) were obtained across an m/z range of 35–500. Compounds were tentatively identified by matching their mass spectra with those available in the NIST17 reference library.

3.3 Evaluation of Antioxidant Activity

3.3.1 DPPH Radical Scavenging Assay

The antioxidant activity of the flower extracts of *B. gymnorrhiza* was assessed using the DPPH radical scavenging assay, following the method adapted from Alzagameem et al.

Extract solutions at various concentrations (5, 10, 25, 50, 100, 250, and 500 µg/mL) were mixed with an equal volume of DPPH working solution in a 96-well microplate. The reaction mixture was incubated in the dark for 30 minutes to prevent photodegradation. Absorbance was subsequently measured at 517 nm using a microplate reader, with Trolox serving as the reference antioxidant. The percentage of radical scavenging activity (% inhibition) was calculated for each concentration, and the IC_{50} value was obtained from the concentration–response curve. All experiments were performed in triplicate to ensure accuracy and reproducibility (Kakatum et al., 2024).

3.3.2 ABTS Radical Cation Decolorization Assay

The ABTS assay was performed according to the procedure described by García et al., with slight modifications. The $ABTS^{\bullet+}$ stock solution was prepared by reacting ABTS diammonium salt (Sigma-Aldrich, USA) with potassium persulfate ($K_2S_2O_8$, Sigma-Aldrich, USA) in distilled water and allowing the mixture to stand in the dark for 18 hours to generate the radical cation. Prior to analysis, the $ABTS^{\bullet+}$ solution was diluted with distilled water to achieve a stable absorbance. In a 96-well microplate, aliquots of the diluted $ABTS^{\bullet+}$ solution were mixed with flower extracts of *B. gymnorrhiza* at concentrations of 5, 10, 25, 50, 100, 250, 500, and 1000 µg/mL. After a 10-minute incubation period in the dark, absorbance was measured at 734 nm. Trolox (≥97%, Sigma-Aldrich, USA) was used as the positive control (Chokchaisiri, et.al., 2025). All measurements were conducted in triplicate. The percentage of inhibition was calculated using Equation (1):

equation 1: $\%\ \text{Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$

where $A_{control}$ is the absorbance of the control

A_{sample} is the absorbance of the sample

The half-maximal inhibitory concentration (IC_{50}) was determined by analyzing the linear graph that shows the relationship between the sample concentration and % inhibition.

4. Results and discussion

The sequential extraction of *Bruguiera gymnorrhiza* flowers using hexane, ethyl acetate, and methanol yielded 8.90 g, 2.85 g, and 81.57 g of crude extract, respectively. The markedly higher yield obtained from the methanol fraction indicates the predominance of polar phytochemicals such as sugars, phenolic derivatives, and glycosides, which are widely reported as major constituents of mangrove flowers and leaves (Bandaranayake, 2002; Kathiresan & Bingham, 2001). This extraction pattern aligns with previous studies on Rhizophoraceae plants, which tend to accumulate high amounts of water-soluble metabolites.

GC–MS profiling (Table 1. and Figure 1.) of the hexane extract revealed a composition dominated by non-polar hydrocarbons, including tetracontane and hexatriacontane, together with triterpenoids (lupeol, β-amyrin, friedoolean derivatives), sterols (stigmasterol, γ-sitosterol), and tocopherol-type compounds. Although certain terpenoids and sterols possess known antioxidant or anti-inflammatory activities, the overall chemical profile of this fraction is largely composed of long-chain hydrocarbons, which exhibit minimal radical-scavenging capacity (Habib et al, 2018). This explains the relatively weak antioxidant activity observed for the hexane extract.

Table 1. Summary of GC–MS Identified Compounds from *Bruguiera gymnorrhiza* Flower Extracts.

Extract	R.Time (min)	Area%	Similarity	Base m/z	Compound Name
Hexane	6.580	2.40	94	68.05	D-Limonene
	10.091	0.24	96	95.10	Isoborneol
	10.324	0.29	96	95.10	endo-Borneol
	41.142	0.34	96	57.10	Docosane
	56.092	1.37	96	57.05	Pentacosane
	59.742	1.45	96	69.05	Squalene
	63.520	32.67	96	57.10	Tetracontane
	66.791	0.98	94	57.10	Hexatriacontane
	67.619	1.38	96	151.10	γ-Tocopherol
	68.105	0.86	88	147.10	β-Sitosterol acetate
	69.459	5.66	96	165.10	Vitamin E
	71.670	1.87	91	55.05	Stigmasterol
	72.392	2.62	-	204.15	(No hit)
	72.783	7.20	92	43.05	γ-Sitosterol
	72.987	2.98	75	204.15	9,19- Cyclo- 27- norlanostan- 25- one derivative
	73.299	0.56	92	218.15	β-Amyrin
	73.630	4.07	92	95.10	Lup-20(29)-en-3-one
	73.943	1.26	79	109.10	2,2,4- Trimethyl- 3- (3,8,12,16- tetramethyl- heptadecyl) - pentane-like compound
	74.112	6.64	93	95.10	Lupeol
	75.071	3.06	76	150.10	Spiro[2.5]octane derivative
	75.479	1.15	77	98.10	(-)-Globulol
	76.727	16.72	92	109.10	Humulane-1,6-dien-3-ol
	94.302	4.23	76	93.05	1,1,3,3- Tetramethyl- 1,3- bis[(5- methyl- 2- (1- methylethyl) - cyclohexyl)]disiloxane
EtOAc	16.322	0.89	98	57.10	Pentadecane
	17.519	4.69	74	58.05	1-Propanol, 3-mercapto-
	18.717	2.48	85	44.00	3-Thietanol
	21.233	1.10	98	57.10	Hexadecane
	23.915	3.79	82	87.05	4-O-Methylmannose
	25.529	0.50	82	124.05	Trimethyl- oxobutyl cyclohexenone
	25.671	0.96	97	57.10	Octadecane
	29.938	0.62	97	57.10	Heneicosane
	48.492	0.73	96	57.10	Eicosanal
	63.501	4.74	96	57.10	Tetracontane
	67.408	0.80	89	95.10	Lupeol, trifluoroacetate
	67.621	0.78	96	151.10	γ-Tocopherol

Table 1. (Continues)

Extract	R.Time (min)	Area%	Similarity	Base m/z	Compound Name
EtOAc	68.105	0.79	88	147.15	β-Sitosterol acetate
	69.247	2.31	96	57.10	Tetracontane
	69.457	1.44	95	165.10	Vitamin E
	71.149	0.60	86	43.05	Campesterol
	71.666	1.64	—	55.05	(No hit)
	72.386	0.70	—	204.15	(No hit)
	72.784	6.14	—	43.05	(No hit)
	72.985	1.22	—	204.15	(No hit)
	73.638	0.45	—	109.10	(No hit)
	74.121	2.80	—	95.10	(No hit)
	78.729	6.86	—	189.15	(No hit)
	78.984	0.58	—	109.10	(No hit)
	79.350	0.75	—	109.10	(No hit)
	79.415	1.64	—	109.10	(No hit)
	79.500	3.57	—	109.10	(No hit)
	80.079	6.70	—	93.10	(No hit)
	93.550	4.41	—	109.15	(No hit)
	95.108	17.26	—	109.10	(No hit)
	95.620	6.66	—	93.10	(No hit)
MeOH	3.095	0.07	93	61.00	Glyceraldehyde
	3.142	0.28	98	96.05	Furfural
	9.589	0.62	95	43.00	Pyranone derivative
	12.002	5.14	91	97.05	5-Hydroxymethylfurfural
	14.143	0.78	73	58.05	1-Propanol, 3-mercapto-
	17.611	1.62	75	58.05	1-Propanol, 3-mercapto-
	18.867	0.85	95	60.00	1,6-anhydro-β-D-glucopyranose
	21.466	0.57	94	73.05	1,6-Anhydro-β-D-glucofuranose
	21.859	1.25	78	60.00	Butanoic acid, octyl ester
	26.558	59.30	82	87.05	4-O-Methylmannose
	29.071	0.62	96	73.05	n-Hexadecanoic acid
	67.406	0.67	88	189.15	Lupeol, trifluoroacetate
	68.111	0.33	88	147.15	β-Sitosterol acetate
	71.672	0.36	92	55.05	Stigmasterol
	72.782	0.69	93	43.05	γ-Sitosterol
	74.117	0.95	93	95.10	Lupeol
	87.345	1.61	84	93.10	Lup-20(29)-en-3-ol, acetate
	89.886	6.08	86	109.10	Lup-20(29)-en-3-ol, acetate
	92.758	14.45	—	109.10	(No hit)
	94.945	3.76	—	218.15	(No hit)

The ethyl acetate extract displayed greater chemical diversity, featuring a mixture of mid-polar metabolites such as pentadecane, hexadecane, tocopherols, lupeol derivatives, and sterol esters including β- sitosterol acetate. A notable characteristic of this fraction was the large

number of peaks classified as "No hit," suggesting the presence of semi-polar compounds—possibly phenolic derivatives, oxidized terpenoids, or glycoside-linked metabolites—not commonly found in mass spectral libraries. This observation is consistent with earlier reports highlighting that mangrove species often contain unique or under-characterized secondary metabolites with strong antioxidant activity (Botosoa et al., 2025). Accordingly, the semi-polar nature of this extract likely contributes to its moderate antioxidant properties.

The methanol extract, which yielded the largest amount of crude material, exhibited a distinct phytochemical pattern dominated by highly polar compounds. The major peak, 4-O-methylmannose (59.30%), was accompanied by 5-hydroxymethylfurfural (HMF), 1,6-anhydro-glucose derivatives, and several triterpenoid acetates such as Lup-20(29)-en-3-ol acetate. Two large peaks with no spectral match were also detected, which may represent polar polyphenols, sugar conjugates, or novel glycosylated structures that are often poorly detected by GC–MS. Such compounds have been widely implicated in the antioxidant capacity of mangrove species (Zhou et al., 2025; Pandey et al., 2014). The chemical composition of the methanol extract therefore strongly supports its superior antioxidant performance relative to the other fractions. When the GC–MS profiles were compared with the antioxidant assay results, a clear polarity-dependent relationship became evident. The methanol extract demonstrated the strongest radical-scavenging activity, followed by the ethyl acetate extract, whereas the hexane fraction exhibited only modest activity. This pattern reflects the chemical nature of each extract: polar phenolics, glycosides, and oxygenated terpenoids—abundant in the methanol fraction—are recognized as major contributors to antioxidant activity (Shahidi & Ambigaipalan, 2015), while long-chain hydrocarbons, which dominate the hexane extract, are largely inert toward reactive oxygen species.

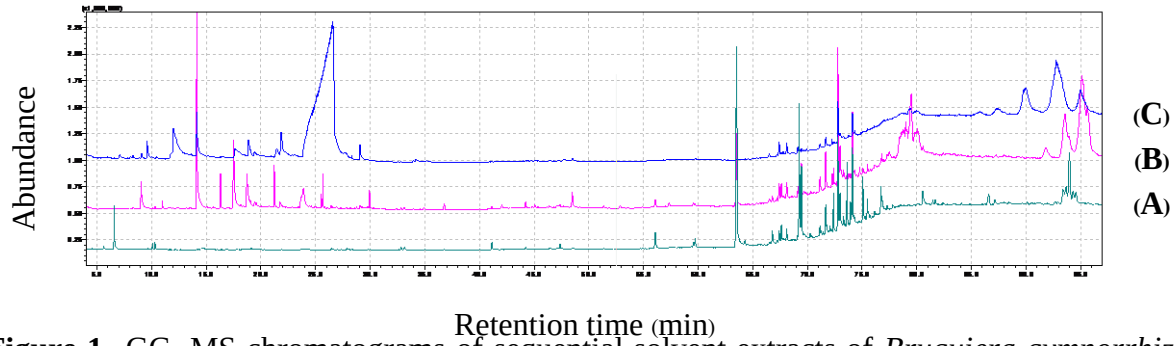

Figure 1. GC–MS chromatograms of sequential solvent extracts of *Bruguiera gymnorrhiza* flowers: (A) BG-F-Hexane extract, (B) BG-F-EtOAc extract, and (C) BG-F-MeOH extract.

The antioxidant activity of the three solvent extractions of *B. gymnorrhiza* flowers was evaluated using DPPH and ABTS assays (Table X). The methanol extract showed the strongest activity (IC_{50} = 52.25 ± 0.35 µg/mL for DPPH; 28.07 ± 0.85 µg/mL for ABTS), followed by the ethyl acetate extract (73.03 ± 3.14 and 48.73 ± 0.80 µg/mL). The hexane extract exhibited very weak activity (IC_{50} >1000 and 373.05 ± 1.41 µg/mL). Trolox demonstrated the highest radical-scavenging capacity as expected.

The superior antioxidant activity of the methanol fraction corresponds with its GC–MS profile, which revealed abundant polar constituents—such as 4-O-methylmannose, HMF, anhydro-sugars, and triterpenoid acetates—along with several unidentified peaks that may represent phenolic- or glycoside-type antioxidants commonly reported in mangrove plants (Zhou et al., 2012; Pandey et al., 2014). In comparison, the ethyl acetate extract, containing

tocopherols, sterol esters, and semi-polar terpenoids, exhibited moderate activity, consistent with previous reports showing that mid-polarity plant metabolites contribute modestly to radical quenching (Bandaranayake, 2002).

The hexane extract showed minimal activity due to its high content of long-chain hydrocarbons (e.g., tetracontane, hexatriacontane), which generally lack hydrogen-donating or electron-transfer capability (Habib et al, 2018). Although small amounts of tocopherols and triterpenoids were detected, their concentrations were insufficient to enhance activity.

Overall, the results demonstrate a clear polarity-dependent trend—methanol > ethyl acetate > hexane—which reflects the well-established role of polar phenolics, sugars, and oxygenated terpenoids as the main contributors to antioxidant activity in plant systems (Shahidi & Ambigaipalan, 2015). The methanol extract of *B. gymnorrhiza* flowers therefore represents the most promising source of antioxidant compounds for further phytochemical study.

Table 2. Antioxidant Activities of *Bruguiera gymnorrhiza* Flower Extracts Determined by DPPH and ABTS Assays

Sample	Antioxidant Activity IC_{50} (µg/mL)	
	DPPH	**ABTS**
BG-F-Hexane	>1000	373.05 ± 1.41
BG-F-EtOAc	73.03 ± 3.14	48.73 ± 0.80
BG-F-MeOH	52.25 ± 0.35	28.07 ± 0.85
Trolox	**6.47 ± 0.12**	**7.87 ± 0.11**

5. Conclusion

This study demonstrates that the flowers of *Bruguiera gymnorrhiza* contain a diverse range of phytochemicals, with the methanol extract yielding the highest amount of polar constituents, followed by ethyl acetate and hexane extracts. GC–MS profiling revealed the presence of sugars, furans, terpenoids, sterols, and several unidentified compounds that may represent novel or under-reported metabolites typically found in mangrove species. Consistent with their chemical composition, the methanol extract exhibited the strongest antioxidant activity in both DPPH and ABTS assays, while the ethyl acetate extract showed moderate activity and the hexane extract displayed minimal radical-scavenging potential. Overall, the findings highlight *B. gymnorrhiza* flowers as a promising natural source of antioxidant compounds, particularly those enriched in the methanol fraction. The presence of numerous unidentified peaks suggests that further structural elucidation using advanced analytical techniques (LC–MS/MS, NMR) is warranted to uncover additional bioactive constituents with potential pharmacological applications.

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