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Comparative antioxidant and antibacterial activities of ethanol extract from different parts of *Syzygium gratum*

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Abstract

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Herbal extracts are widely recognized as valuable sources of bioactive compounds with antioxidant and antimicrobial properties. However, variations in biological activity among different plant parts remain insufficiently explored for many medicinal plants. This study aimed to comparatively evaluate the antioxidant and antimicrobial activities of extracts obtained from four different parts including young leaves (YL), mature leaves (ML), branches (BR), and twigs (TW) of *Syzygium gratum*. Plant materials were extracted using ethanol with maceration method, and antioxidant activity was determined through total phenolic content (TPC) analysis along with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, while antibacterial activity was evaluated against two selected microorganisms, *E. coli*, *S. aureus* using disc diffusion method. Among the tested extracts, the extract from young leaves exhibited the strongest antioxidant capacity with 18.01±2.25 mg gallic acid equivalents per gram of dried weight for TPC and 67.05% for DPPH assay. In addition, the extract showed the most antimicrobial effects with 10.8±0.1 and 11.1±0.1 mm for *E. coli* and *S. aureus*, respectively. The YL extract was further investigated the chemical constituents by using GC–MS technique, and the result revealed that the four major constituents were Dihydrooroxylin A, neophytadiene, tetrapentacontane, and g-tocopherol. The observed data was likely associated with major GC–MS identification constituents, including Dihydrooroxylin A and γ–tocopherol, which have been previously reported to exhibit antioxidant and antimicrobial effects. In addition, these data highlighted the importance of plant–part selection especially for YL extract in maximizing biological activity and supported the potential application of *S. gratum* as a natural source of antioxidant and antimicrobial agents.

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1. Introduction

Herbal plants have been widely recognized as natural reservoirs of bioactive constituents exhibiting a broad range of biological functions, particularly antioxidant and antimicrobial properties. In particular, these properties are important due to increasing concerns over oxidative stress–related diseases and the global rise of antimicrobial resistance. As a result, the search for effective natural antioxidants and antimicrobial agents from plant materials has gained increasing scientific attention [1 – 3].

Secondary metabolites from plants such as phenolic compounds, flavonoids, terpenoids, and alkaloids are known to contribute significantly to antioxidant and antimicrobial activities [4]. However, the distribution and concentration of these bioactive compounds may considerably among different plant parts, including leaves, twigs, stems, and roots. Despite the widespread use of whole plants or specific organs in traditional medicine, comparative studies evaluating the biological activities of different plant parts remain limited for many medicinal species.

S. gratum (synonym: *Eugenia grata* Wight) has been traditionally employed in Thai medicine for the treatment of

gastrointestinal discomforts such as dyspepsia, indigestion, peptic ulcer, and diarrhea, with additional traditional use reported in relation to bacterial infections, asthma, and cardiovascular-related conditions and has been reported to possess various pharmacological properties [5 – 6]. Previous studies have mainly focused on young leaves which usually used as a food ingredient [6 – 10]. Traditional use of *S. gratum* for gastrointestinal disorders, including diarrhea, suggests a possible association with microbial infections. Accordingly, antibacterial assays were performed in this study to provide experimental support for these ethnomedicinal applications. However, systematic comparisons of biological activities among different plant parts have not been thoroughly investigated. To understand plant–part dependent variation in biological activities is essential for the efficient utilization of plant resources and for identifying the most promising plant parts for further development. In present study demonstrated that the comparative evaluation the antioxidant and antimicrobial activities of extracts obtained from four different parts of *S. gratum*. The findings expected to provide scientific evidence supporting the selection of appropriate plant parts for biological applications and to contribute to the value–added utilization of medicinal plant resources.

2. Materials and Methods

Reagents and standards

1,1–Diphenyl–2–picrylhydrazyl (DPPH) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Gallic acid, sodium carbonate, and the Folin–Ciocalteu reagent were supplied by Merck (Darmstadt, Germany). Ascorbic acid served as the reference antioxidant. Analytical-grade reagents and solvents were used for all remaining experimental procedures.

Plant materials

Samples of *S. gratum* comprising young leaves, mature leaves, branches, and twigs were collected in Chachoengsao province, Thailand, during May 2025 as shown in Fig. 1 Botanical identification was carried out by Mr.Pitphiboon Thanphuthon, Department of Thai Traditional Medicine, Faculty of Science, Ramkhamhaeng, Thailand and a specimen was deposited at the Faculty of Science, Rajabhat Rajanagarindra University, Thailand.

Plant extract preparation

Fresh aerial parts of *S. gratum*, including young leaves (YL), mature leaves (ML), branches (BR), and twigs (TW). The samples were thoroughly rinsed with distilled water and allowed to air-dry, followed by oven drying at 60 °C for 72 h.

The dried plant materials were finely grounded and sieved through a 60 mesh sieve and stored in a desiccator prior to extraction. For each plant part, 5.0 g of the powdered sample was macerated with 150 mL of 95% (v v^{-1}) ethanol in closed containers at room temperature for 72 h.

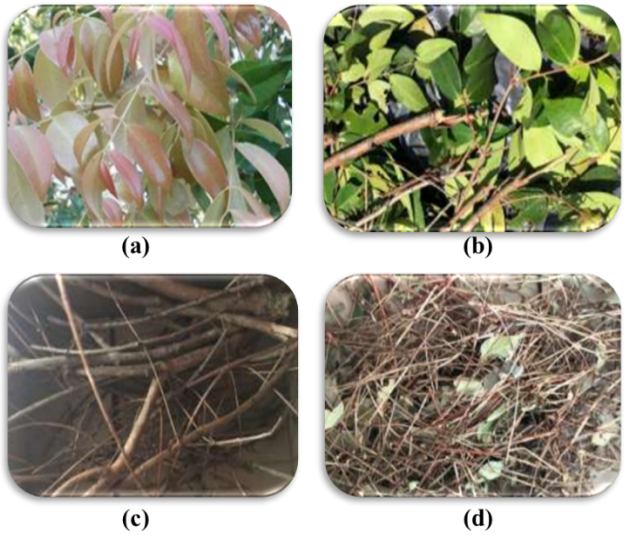

Fig. 1 Different plant parts of *S. gratum* used in this study: (a) young leaves, (b) mature leaves, (c) branches, and (d) twigs.

All extractions were carried out independently in triplicate. The reaction mixtures were filtered using Whatman No. 1 filter paper, and the resulting solutions were then concentrated under reduced pressure at 35–40 °C by rotary evaporation. The crude extracts were stored at 4 °C prior to test the biological activities and Gas chromatography–mass spectrometry (GC–MS).

Total phenolic content determination

A modified Folin–Ciocalteu colorimetric method was applied for the assessment of total phenolic content (TPC) [11]. Briefly, 5 mg of each crude extract was dissolved in 10 mL of 95% (v v^{-1}) ethanol. An aliquot of 200 µL of the resulting solution was mixed with 1 mL of 0.5 N Folin–Ciocalteu reagent, followed by neutralization of the reaction mixture using 1 mL of 7.5% (w v^{-1}) sodium carbonate.

The reaction mixtures were incubated at room temperature for 2 h, and the absorbance was measured at 750 nm using a UV–visible spectrophotometer (Shimadzu uv1900i). All measurements were carried out in triplicate. The TPC was quantified and presented as milligrams of gallic acid equivalents per gram of dry mass. (mgGAE g^{-1}DW). The concentration was calculated from a gallic acid calibration curve (Fig. 2) [12 – 13]. Thereby, gallic acid calibration solutions at concentrations of 20, 40, 60, 80 and 100 mg L^{-1}, respectively.

Method accuracy was examined using a standard addition recovery approach, adapted from previously reported procedures [14 – 15]. A gallic acid standard solution at an intermediate concentration (60 mg L^{-1}) was added to crude ethanol extracts obtained from four plant parts of *S. gratum*, while 95% (v v^{-1}) ethanol was used as the blank matrix. Both spiked and unspiked samples were analysed in triplicate using the Folin–Ciocalteu colorimetric

method under identical experimental conditions. Percentage recovery was calculated as:

$$\% \text{ Recovery} = \frac{C_{spiked} - C_{sample}}{C_{added}}$$

where C_{spiked} represents the concentration measured after standard addition, C_{sample} is the concentration of the unspiked extract, and C_{added} is the concentration of gallic acid added. The recovery results were used to indicate method accuracy for total phenolic content determination.

Antioxidant activity determination

The antioxidant activities of the crude extracts was determined using the DPPH radical scavenging assay, adapted from previously reported procedures [16 – 17], with ascorbic acid employed as the reference standard.

In brief, 5 mg of each crude extract was dissolved in 10 mL of 95% (v v^{-1}) ethanol and DPPH solution (0.1 mM) was prepared in 95% (v v^{-1}) ethanol and storred under dark condition prior to use. Ascorbic acid standard solutions in the concentration range of 0 – 0.60 mg L^{-1} were prepared to construct the calibration curve. An aliquot (0.2 mL) of each standard solution was combined with 1.8 mL of DPPH solution and allowed to react in the dark at ambient temperature for 30 min, after which the absorbance was measured at 517 nm using a UV–Vis spectrophotometer.

For sample analysis, 0.2 mL of diluted crude extract was mixed with 1.8 mL of the DPPH solution and treated under the same conditions. The DPPH radical scavenging activity was calculated from the decreasing in absorbance relative to the blank according to the following equation:

$$\%\text{Inhibition} = \left[\frac{A_0 - (A_1 - A_2)}{A_0}\right] \text{x } 100$$

where A_0 is the absorbance of the control solution (0.2 mL of 95% (v v^{-1}) ethanol mixed with 1.8 mL of DPPH solution), A_1 is the absorbance of the reaction mixture containing 0.2 mL of the sample solution and 1.8 mL of DPPH solution, and A_2 represents the absorbance of the sample blank, which was prepared through the addition of 0.2 mL of the sample solution to 1.8 mL of 95% (v v^{-1}) ethanol.

The antioxidant capacity of the extracts was subsequently quantified using an ascorbic acid calibration curve and reported as milligrams of ascorbic acid (AA) equivalents per gram dried weight (mg AA g^{-1} DW).

Antibacterial assay

The antibacterial activity of crude ethanol extracts obtained from different parts of *S. gratum* was examined against Escherichia coli (*E. coli*) and Staphylococcus aureus previously by Phinetsathian et al. [17]. (*S. aureus*) using the paper disc diffusion method descrived

Pure cultures of the test bacteria were maintained on nutrient agar and incubated at 37 °C for 24 h. A single colony of each strain was inoculated into nutrient broth and incubated under shaking conditions at 37 °C for 24 h. The bacterial suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard prior to use.

Mueller–Hinton agar plates were measured by dispensing 20 mL sterilized medium into sterile Petri dishes. The adjusted bacterial suspensions were spread over the agar surface using sterile cotton swabs. Sterile paper discs (6 mm diameter) were placed on the inoculated agar, and 20 µL of each crude ethanol extract, prepared from 5 mg of each crude extract was dissolved in 10 mL of 95% (v v^{-1}) ethanol, was applied to the discs. Discs loaded with the extraction solvent alone were included as negative controls, 20 µL of 95% (v v^{-1}) ethanol. The plates were left at room temperature for 30 min to allow diffusion and then incubated at 37 °C for 24 h.

Antibacterial activity was determined by quantifying the diameters of the inhibition zones formed around the discs, measured with a vernier caliper. All experiments were performed in triplicate, and the results were expressed as mean values. The procedure was adapted from previously reported antibacterial assays.

Preliminary phytochemical screening of extracts

Phytochemical screening was performed with major phytochemical constituents including tannins, xanthones, terpenoids, steroids, reducing sugar, flavonoids, alkaloids, and saponins. The principal methods were measure regarding the previous reports by Ayoola et al. [18] and Tiranakwit et al. [19] with some modifications as follow:

For anthraquinone screening, the plant extract (0.5 g) was treated with ethanol (1 mL) and heated in the presence of sulfuric acid (H_2SO_4) (10 mL). The hot mixture was immediately filtered through Whatman No. 1 filter paper. The resulting filtrate was shaken with chloroform (5 mL), after which the organic layer was separated and treated with 7% (v v^{-1}) ammonia solution (1 mL). The development of a characteristic color change was taken as an indication of anthraquinones.

In the flavonoid test, the plant extract (0.5 g) was first dissolved in 2 mL of ethanol, followed by the addition of sodium hydroxide solution. The mixture was shaken for 5 min and filtered by Whatman No.1. Subsequently, 1% (v v^{-1}) hydrochloric acid (HCl) was introduced into the extract solution, and a loss of the yellow coloration was used as an indicator of flavonoids.

The presence of saponins was inferred from the formation of stable foam after the plant extract was dissolved in 2 mL of water, vigorously agitated using a vortex mixer for 30 s, and subsequently maintained in a water bath at 40 °C.

For tannin detection, 0.5 g of the plant extract was heated in 10 mL of water, followed by filtration through Whatman No. 1 filter paper. Subsequently, a few drops of 0.1% (w v^{-1}) ferric chloride solution were added, and the appearance of a brownish-green or blue–black coloration was recorded as a positive indication.

Test for cardiac glycosides, 0.5 g of extract which was diluted to 5 mL in dichloromethane and then by Whatman No.1. The solution was added 2 mL of conc. sulfuring acid containing one drop of ferric chloride solution 0.1% (w v^{-1}). A greenish ring formed just above the brown ring and gradually spread throughout acid layer.

For terpenoids, 0.5 g of the extract was dissolved in 2 mL of chloroform. Concentrated H_2SO_4 (3 mL) was carefully added to form a separate layer. The formation of a reddish–brown coloration at the interface indicated the presence of terpenoids.

For steroids, 0.5 g of extract was dissolved in 1 mL of chloroform, then filter using Whatman No.1. Then, the solution was added with 1 mL of 96% sulfuric acid. The red–colored lower layer was indicated.

For alkaloid test, 0.5 g of extract was dissolved in 1 mL of ethanol then filter using Whatman No.1. The solution was treated with 1 mL of 1% (v v^{-1}) hydrochloric acid, followed by the addition of two drops of Wagner's reagent (potassium bismuth iodide). The formation of a reddish–brown precipitate accompanied by turbidity was taken as a positive indication.

Phytochemical screening was performed qualitatively by visual comparison of color intensity, precipitation, and foam formation between the extract samples and the control (without crude extract). The responses were recorded using a semi-quantitative scale as high (+++), moderate (++), low (+), or absent (–).

GC–MS determination

The GC–MS analysis was performed by an Agilent GC7890B. GC–MS analysis was performed using a ZB–5–MS Plus capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Electron ionization (EI) was operated at 70 eV, with helium employed as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. Samples were injected at a volume of 0.1 µL using a split ratio of 1:20, and the injector temperature was maintained at 250 °C. The oven temperature program followed the same conditions applied in the GC analysis. The column temperature was initially held at 50 °C for 3 min, subsequently increased to 280 °C at a rate of 10 °C min^{-1}, and maintained at this temperature for an additional 20 min. The total run time was 37.5 min under a constant pressure of 10 psi. Mass spectra were acquired over an m/z range of 30–500 with a scan interval of 0.5 s. Prior to analysis, each crude extract (2.0 mg) was dissolved in 1 mL of methanol and thoroughly filtered. Compound assignment was based on comparison of retention times and mass spectral patterns with reference data available in the NIST GC–MS library [20].

Statistical AnalysisThe statistical analysis was conducted using triplicate experiments (n = 3). Data are presented as mean ± standard deviation (SD).

3. Results and Discussion

Extraction yield from Different plat parts

The yields of crude ethanol extracts from different parts (young leaves, mature leaves, branches, and twigs) of *S. gratum* was calculated and revealed in Table 1. Among the tested plant parts, mature leaves provided the highest extraction yield (63.02%), followed by young leaves (56.97%). On the contrary, the low yield obtained from branches and twigs were 23.02% and 23.55%, respectively. These results indicated that leaf tissues of *S. gratum* are richer in extractable constituents compared to woody plant parts.

Table 1 Yield of crude extracts obtained from different parts of *S. gratum*.

Plant parts	Mass of dried plant powder (g)	Mass of crude extract (g)	Yield (%)
Young leaves	5.02	2.86	56.97
Mature leaves	5.03	3.17	63.02
Branches	5.04	1.16	23.02
Twigs	5.01	1.18	23.55

Total phenolic content (TPC) and antioxidant activities of plant extracts

The total phenolic content of the crude extracts was quantified using a gallic acid calibration. Absorbance was measured at 750 nm, and a linear response was observed over the concentration range of 20–100 mg L^{-1}, described by the regression equation as $Y = 0.0041x + 0.0207$ ($R^2 = 0.998$) (Fig. 2).

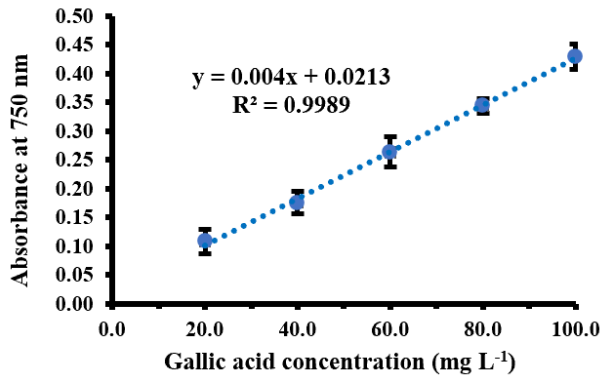

Fig. 2 Calibration curve of gallic acid obtained using the Folin–Ciocalteu method (20–100 mg L^{-1}), measured at 750 nm

Under the experimental conditions applied, the limits of detection (LOD) and quantification (LOQ), calculated as three and ten times the standard deviation of the blank, were 17.11 and 51.83 mg L^{-1}, respectively. Method reproducibility was determined at 60 mg L^{-1} using triplicate

measurements and a relative standard deviation (RSD) of 1.36% was obtained. The recovery results are presented in Table 2, where the percentage recoveries obtained for the crude extracts from all four plant parts ranging from 80.54% to 89.76%. In analytical chemistry, method accuracy is commonly reflected by the degree of agreement between the measured value and the accepted reference value, with recovery levels between 80% and 110% commonly cited as an indicative acceptance range according to AOAC SMPR 2015.009 [21]. These values fall within the range commonly reported for routine Folin–Ciocalteu spectrophotometric assays and indicate acceptable analytical reliability for total phenolic content determination in the present study [12–13].

The total phenolic content of crude extracts obtained from different parts of *S. gratum* is presented in Table 2. Among the tested plant parts, young leaves exhibited the highest TPC as 18.01±2.25 mg GAE g^{-1}DW. For mature leaves, TPC of the extract was 16.96±2.68 mg GAE g^{-1}DW, while lower TPC were observed in branches and twigs as 9.27±2.73 mg GAE g^{-1}DW and 10.87±1.03 mg GAE g^{-1} DW, respectively.

The antioxidant activity of crude extracts obtained from different parts of *S. gratum* is summarized in Table 2. For the antioxidant activity, the antioxidant capacity and %DPPH inhibition was calculated using the calibration curve as shown in Fig.3.

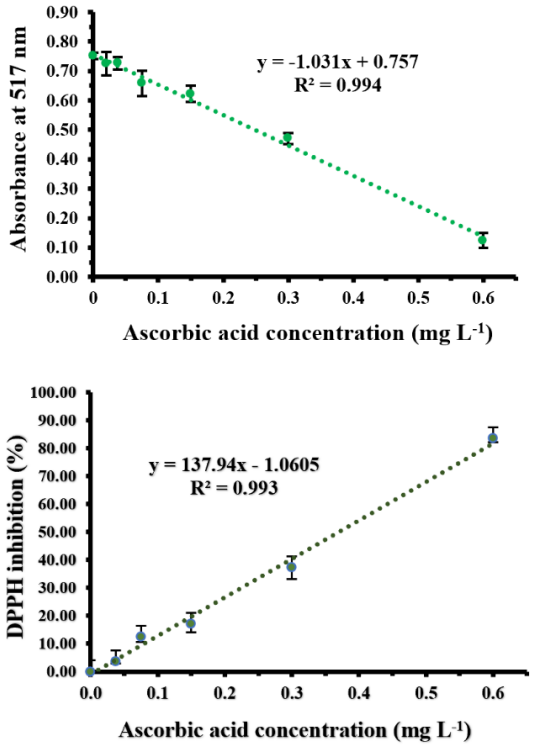

Fig. 3 (a) Calibration curve of ascorbic acid (0–0.6 mg L^{-1}) obtained from the DPPH assay, measured at 517 nm. (b) Effect of ascorbic acid concentration (0–0.6 mg L^{-1}) on DPPH radical scavenging activity.

Among the tested plant parts, young leaf extract exhibited the highest antioxidant capacity (0.31±0.04 mg ascorbic acid g^{-1} DW), corresponding to the greatest percentage inhibition relative to ascorbic acid (67.05 %). Mature leaf and twig extracts showed antioxidant activities, with inhibitory values of 66.70% and 64.80%, respectively. On the other hand, branch extract demonstrated the lowest antioxidant capacity and inhibition with 55.48%. The results indicate a clear variation in antioxidant activity of YL which was different plant parts of *S. gratum*.

Table 2 The total phenolic content (TPC) and antioxidant activities of extracts of *S. gratum*.

Plant parts	TPC		Antioxidant	
	mg GAE g^{-1} DW	Recovery %	mg AA g^{-1} DW	% I
YL	18.01±2.25[a]	80.54	0.31±0.04[a]	67.05
ML	16.96±2.68[a]	85.19	0.25±0.10[a]	66.70
BR	9.27±2.78[a]	84.29	0.16±0.07[a]	55.48
TW	10.87±1.03[a]	89.76	0.18±0.11[a]	64.80

Noted: [a]Values represent the mean of three independent replicates, with variability expressed as standard deviation (SD).

In literature, Sriket [22] showed the %inhibition using DPPH assay of *S. gratum* young left extract as 78.35%. The data was comparable to our observation data and supported the antioxidant activity of this plant. Phenolic compounds are recognized as major contributors to antioxidant activity in plant extracts. The higher total phenolic contents observed in leaf extracts of *S. gratum*, particularly young and mature leaves, may explain their stronger antioxidant potential observed in subsequent assays. In previous data, many constituents found in this plant showed in various types of compounds including flavonoids, anthraquinones, triterpenoids, and long chain fatty acid [23]. In leaves, the active metabolites were known as a rich source of phenolic compounds. In contrast, woody parts such as branches and twigs contain lower levels of phenolics, which is consistent with the lower total phenolic contents observed in this study. These findings emphasized that the importance of plant-part selection were studied when evaluating the biological potential of medicinal plants and the compounds should be further investigated in mechanistic action in various biological model.

Antibacterial activity

The antimicrobial activity of crude extracts obtained from different parts of *S. gratum* was showed in Table 3 and Fig. 4. All extracts exhibited inhibitory effects against both *E. coli* and *S. aureus*, although the degree of activity varied among plant parts. Young leaf extract showed the strongest antibacterial activity against *E. coli*, with a clear zone diameter of 10.8±0.1 mm, while mature leaf and twig extracts also demonstrated notable inhibitory effects. Against *S. aureus*, young leaf extract again exhibited the highest antibacterial activity (11.1±0.1 mm), followed by mature leaf and twig extracts. In contrast, branch extract showed the weakest antibacterial activity against both tested microorganisms. The antimicrobial activity observed in this

study demonstrated a clear dependence on plant part selection. Leaf extracts of *S. gratum*, particularly young leaves, exhibited stronger antibacterial activity against both Gram–negative (*E. coli*) and Gram–positive (*S. aureus*) bacteria compared to other parts. This trend is consistent with the relatively higher total phenolic content and antioxidant activity observed in the leaf extracts, indicating that phenolic constituents may be associated with the observed antibacterial effects. Leaves are known to accumulate secondary metabolites involved in plant defense, which may explain their enhanced antimicrobial potential. The comparatively lower activity observed in branch extracts may reflect reduced concentrations of such bioactive compounds. Overall, the results highlight the importance of selecting appropriate plant parts to maximize antimicrobial efficacy.

Table 3 Clear zone diameters of crude extracts obtained from different parts of *S. gratum*.

Plant parts	Clear zone diameter (mm)	
	E. coli	***S. aureus***
YL	10.8 ±0.1[a]	11.1 ±0.1 [a]
ML	8.3 ±0.2 [a]	10.5 ±0.2 [a]
BR	5.5 ±02 [a]	6.2 ±0.1 [a]
TW	7.6 ±0.1 [a]	8.0 ±0.1 [a]

Noted: [a]Results are reported as mean values derived from three replicates, with SD indicating data dispersion.

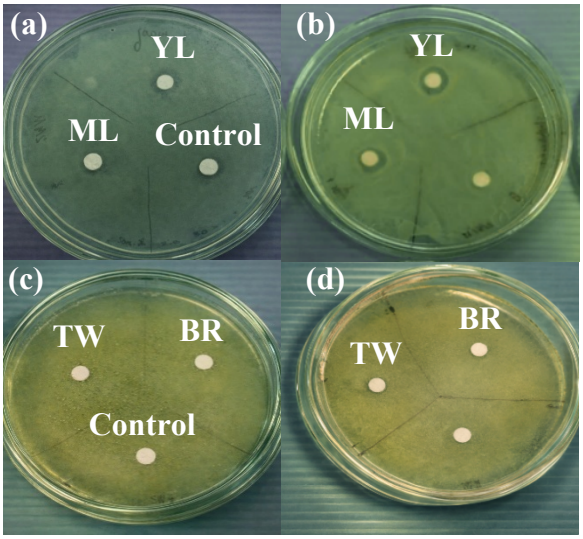

Fig. 4 Clear zone diameters of crude ethanol extracts from different parts of *S. gratum* against (a and c) *E. coli* and (b and d) *S. aureus*.

Comparative antibacterial responses between Gram–positive and Gram–negative bacteria have been consistently reported in plant-derived antibacterial studies, where leaf–based extracts are frequently observed to exhibit stronger inhibitory effects toward Gram-positive organisms such as *S. aureus* [24 – 25]. By comparison, the lower susceptibility of Gram-negative bacteria is commonly associated with structural features of the cell envelope, particularly the outer membrane enriched in lipopolysaccharides (LPS), which may restrict the penetration of certain antibacterial constituents [26 – 27].

The relatively higher antibacterial response of the young leaf extract may be associated with organ–dependent differences in phytochemical compositions. Previous comparative studies have shown that leaves generally exhibit higher phenolic and flavonoid contents than other plant parts, alongside enhanced antibacterial activity [28 – 30]. In the absence of compound-specific or mechanistic investigations, the antibacterial effects observed in this study are therefore best interpreted as arising from the combined contribution of multiple phytochemical constituents rather than from a single dominant component.

Preliminary phytochemical screening of extracts

All extracts obtained from different parts of *S. gratum* were screened the preliminary phytochemical constituents and the results was showed in Table 4.

Table 4 Preliminary phytochemical screening of extracts from different parts of *S. gratum*.

Phytochemicals	Test Results			
	YL	ML	BR	TW
Anthraquinones	–	–	++	+
Flavonoids	++	+	+	+
Saponins	–	++	+++	–
Tannins	++	+++	+++	+++
Cardiac Glycosides	–	++	++	++
Terpenoids	+	+++	++	+
Steroids	++	++	–	+
Alkaloids	+++	++	++	++

Note: (+++), moderate (++), low (+), and absent (–).

After phytochemical interpretation, the results demonstrate variaty of secondary metabolite distribution within *S. gratum*. The finding indicated that leaves, particularly YL, contain higher levels of alkaloids and flavonoids, which may contribute to enhanced antioxidant properties. In contrast, bark exhibited the greatest diversity and strongest presence of multiple phytochemical classes, including saponins, tannins, anthraquinones, and cardiac glycosides.

To the best of our knowledge, based on available database (Google Scholar, Scopus, and SciFinder), a comparative preliminary phytochemical screening across different plant parts of *S. gratum* has not been previously reported. Limited studies are available within the Syzygium genus. For example, Tambe et al. [31] reported phytochemical screening of leaf extracts from *S. cumini* and identified that alkaloids, flavonoids, steroids, and phenols was predominant constituents in methanol and aqueous extracts. These findings are consistent with the present study, where alkaloids and flavonoids were the major constituents detected in YL extract.

It should be noted that the present study provided original qualitative insight into the distribution of phytochemicals in different plant tissues of *S. gratum*. These preliminary screening results illustrated a foundation for

subsequent quantitative analysis and structural identification of bioactive constituents in specifically plant parts.

GC–MS data of young leaf extract

The chemical constituents identified by GC–MS was shown in Table 5 and Fig. 5 From GC–MS analysis, twelve compounds were found and indicated a high confidence level (≥ 90% similarity) based on the NIST library matching.

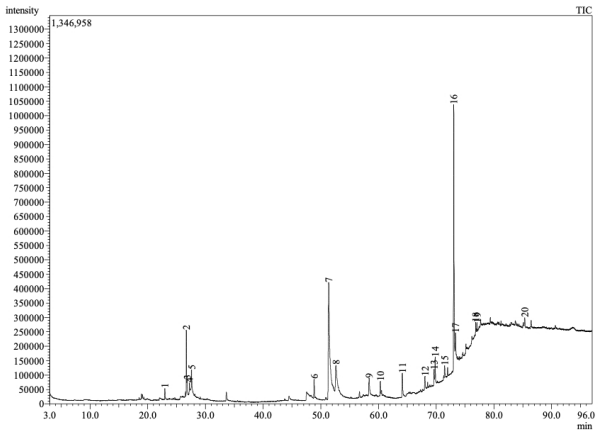

Fig. 5 TIC chromatogram of the YL extract from *S. gratum*

Table 5 Chemical constituents identified by GC–MS with similarity ≥ 90%.

Compound	Rt (min)	Similarity (%)	Area (%)
Neointermedeol	15.23	93	0.76
Neophytadiene	18.47	96	4.60
3,7,11,15–Tetramethylhexadec–2–ene	19.12	90	1.23
Neophytadiene	19.68	92	0.70
Neophytadiene	20.35	92	1.77
Dihydrooroxylin A	27.84	90	8.31
Squalene	29.47	93	1.61
Tetrapentacontane	31.26	94	3.29
Dotriacontane	32.15	92	1.70
g–Tocopherol	33.84	93	2.45
Phytol stearate	35.62	92	0.88

The identified constituents mainly belong to flavonoids, terpenoids, hydrocarbons, phytosterols, and lipophilic compounds, reflecting the non–polar to moderately non–polar nature of the extract. Among the detected compounds, dihydrooroxylin A, a flavonoid derivative, was the most abundant constituent, accounting for 8.31% of the total peak area, suggesting that it is a major component contributing to the chemical profile of the extract. This compound, dihydrooroxylin A, widely used in the field of medicine and health care has been found to have antibacterial, anti–inflammatory, antioxidant and anti–tumor activities. [32 – 33]. Therefore, in this study, it may be use as a marker for plant chemotaxonomy and qualification of the extract for the further aspect such as reducing and stabilizing agents for green synthesis as well as in environmentally friendly and naturally approaches.

In addition, neophytadiene, a diterpene hydrocarbon, was detected at multiple retention times (18.47, 19.68, and 20.35 min), with a combined area percentage of 7.07%, indicating either the presence of isomeric forms or thermal rearrangement during GC analysis. Neophytadiene is widely recognized as a plant–derived stress metabolite and has been reported to possess anti–inflammatory, antimicrobial, and antioxidant properties [34]. Its repeated detection supports its ecological relevance as a defensive compound.

Other terpenoid–related constituents such as neointermedeol,3,7,11,15–tetramethylhexadec–2–ene, squa–lene, and phytol stearate further emphasize the dominance of terpenoid biosynthetic pathways. Squalene (1.61%), a triterpene precursor, is well known for its antioxidant and membrane–stabilizing roles [35 – 36], while γ–tocopherol (2.45%), a form of vitamin E, directly contributes to antioxidant defense by scavenging reactive oxygen species. For long–chain hydrocarbons, tetrapentacontane (3.29%) and dotriacontane (1.70%) were also detected, which are commonly reported in plant waxes and are associated with protective barrier functions against environmental stress [37].

4. Conclusion

This study demonstrated that crude extracts obtained from different parts of *S. gratum* exhibited distinct extraction yields, total phenolic contents, antioxidant capacities, and antimicrobial activities. Among the tested plant parts, leaf extracts, particularly young leaves, showed relatively higher extraction yield, total phenolic content, and antioxidant activity, together with stronger observed antibacterial effects against *E. coli* and *S. aureus*. In contrast, woody parts such as branches and twigs displayed lower levels of biological activity. Based on the GC–MS data, dihydrooroxylin A played a crucial significate feature for antioxidant and antimicrobial efficacy of young leave extract of this plant. In addition, the observed variation in bioactivity highlights the importance of plant–part selection in maximizing the functional potential of *S. gratum*. Overall, the results support the use of *S. gratum* leaves as a promising natural source of antioxidant and antimicrobial agents and provide useful scientific information for the value–added utilization of this medicinal plant. Furthermore, studies investigating the synergistic interactions among multiple bioactive phytochemical constituents in plant extracts remain limited; therefore, this aspect warrants further investigation.

5. Suggestions

Based on the findings of this study, further research is recommended to identify and characterize the bioactive compounds responsible for the antioxidant and antimicrobial activities of *S. gratum*, particularly in leaf extracts, using advanced analytical techniques. In addition, future studies should investigate the influence of

environmental factors such as soil characteristics, seasonal variation, and ecological conditions on phenolic accumulation and bioactivity from an environmental chemistry perspective. Evaluating the environmental safety, stability, and biodegradability of *S. gratum* extracts is also suggested to support their sustainable use as natural antioxidant and antimicrobial agents. Such investigations would enhance understanding of plant–environment interactions and promote environmentally responsible utilization of this medicinal plant.

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7. Declaration of Generative AI in Scientific Writing

AI techniques were only used for enhancing readability and language quality improvement.

8. CRediT Author Statement

Amornrassamee Jinnarak: Conceptualization; Supervision; Design; Methodology; Validation; Writing – Original draft preparation

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9. Research Involving Human and Animals Rights

Not applicable

10. Ethics Approval and Consent to Participate

Not applicable

11. Declaration of Competing Interest

The authors confirm that no financial or personal interests exist that could have influenced the conduct or reporting of this study.

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