Research Square

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Preliminary phytochemical, antibacterial and cytotoxic screening of Baliospermum solanifolium (Burm.) Suresh extracts

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Abstract

Background

Baliospermum solanifolium (Burm.) Suresh is a medicinal plant belonging to the family Euphorbiaceae and has long been used in Southeast Asian traditional medicine for the treatment of various ailments, including inflammatory disorders and infections. Despite its traditional use, scientific evidence supporting its antimicrobial and cytotoxic activities remains limited. Therefore, this study aimed to investigate the phytochemical composition, antibacterial activity, and cytotoxic potential of *B. solanifolium* (Burm.) Suresh extracts prepared using different ethanol concentrations.

Methods

Crude extracts of *B. solanifolium* (Burm.) Suresh stems were prepared using ethanol at concentrations of 95%, 80%, and 60%, designated as BS-95, BS-80, and BS-60, respectively. Preliminary phytochemical screening was performed using standard qualitative tests. Total phenolic content (TPC) and total flavonoid content (TFC) were determined using spectrophotometric methods. Antibacterial activity was evaluated using the disc diffusion method against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Bacillus subtilis*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the broth microdilution method. Cytotoxic activity was assessed using the MTT assay against several human cancer and normal cell lines.

Results

Phytochemical screening revealed the presence of flavonoids and compounds containing unsaturated lactone rings in all extracts, whereas alkaloids were not detected. The BS-95 extract showed the highest phenolic content (516.16 mg GAE/g extract), while the BS-80 extract exhibited the highest flavonoid content (481.79 mg QE/g extract). Antibacterial testing demonstrated inhibitory activity against several bacterial strains, particularly *Staphylococcus aureus*. The BS-80 extract showed antibacterial activity against *S. aureus* and *B. subtilis*, with MIC and MBC values of 3.13 mg/mL. Cytotoxicity was observed against both cancer and normal cell lines, with the BS-80 extract showing IC_{50} values of 2.81–2.91 mg/mL against HT29, MCF7, and HepG2 cells

Conclusion

These findings demonstrate that *B. solanifolium* (Burm.) Suresh extracts possess antibacterial and cytotoxic activities, which may be associated with their phenolic and flavonoid constituents. The results highlight the potential of this medicinal plant as a promising source of bioactive compounds for further pharmaceutical and biomedical research.

1. Background

Baliospermum solanifolium (Burm.) Suresh is a medicinal plant belonging to the family Euphorbiaceae and is widely distributed throughout Southeast Asia. The plant has been traditionally used in folk medicine for the treatment of various ailments, including inflammatory disorders, skin infections, and gastrointestinal diseases [1, 2]. Several medicinal plants have been reported to contain bioactive phytochemicals such as flavonoids, phenolic compounds, alkaloids, and terpenoids, which exhibit antimicrobial and anticancer activities [3–7].

Despite its traditional use, scientific evidence supporting the biological activities of *B. solanifolium* (Burm.) Suresh remains limited. In particular, information regarding the antimicrobial and cytotoxic properties of extracts obtained using different ethanol concentrations is still scarce [8]. Ethanol is commonly used as an extraction solvent because its polarity can be adjusted by varying the concentration, which may influence the extraction efficiency of bioactive compounds [9, 10].

2. Methods

Plant material and botanical authentication

Fresh stems of *Baliospermum solanifolium* (Burm.) Suresh were collected from naturally occurring plants growing along a public roadside area in Ban Lat District, Phetchaburi Province, Thailand. The plant material was collected by Asst. Prof. Surapong Kengtong, Faculty of Pharmacy, Eastern Asia University, Thailand. The plants were naturally occurring and were not cultivated or intentionally planted at the collection site. The vegetation at the collection site was routinely cut or removed as part of roadside maintenance.

The plant material was formally identified as *Baliospermum solanifolium* (Burm.) Suresh by Asst. Prof. Surapong Kengtong. A voucher specimen was prepared and deposited in the medicinal plant herbarium collection of the School of Pharmacy, Eastern Asia University, Thailand, under voucher number BS-EAU-001. The voucher specimen is retained by the School of Pharmacy for botanical verification of the plant material used in this study.

The collection was conducted solely for academic research purposes and was not intended for commercial utilization or commercial exploitation of the plant genetic resource. The collection site was not privately owned land; therefore, permission from a private landowner was not applicable. No specific plant-collection permit was obtained for the collection described in this study.

Botanical characteristics

Baliospermum solanifolium (Burm.) Suresh, commonly known as "Tong Taek" in Thai, is a perennial shrub belonging to the family Euphorbiaceae. The plant typically grows to a height of 1–3 meters and is characterized by erect, branched stems with smooth or slightly hairy surfaces. The leaves are simple, alternate, and ovate to elliptic in shape, measuring approximately 5–15 cm in length and 3–8 cm in

width. The leaf margins are serrate or occasionally entire, with a pointed apex and rounded to cuneate base. The upper leaf surface is dark green and glabrous, while the lower surface is paler with prominent venation.

The inflorescence consists of terminal or axillary racemes. Male flowers are small, yellowish-green, and located in the upper part of the raceme, whereas female flowers are fewer and positioned at the base. The fruit is a small, three-lobed capsule containing oval-shaped seeds. The stem, when cut, exudes a milky latex, which is characteristic of many plants in the Euphorbiaceae family.

The plant material used in this study was collected from Phetchaburi Province, Thailand. Macroscopic characteristics of the collected specimens are shown in Figure 1A–F, including the whole plant, stems, leaves, and reproductive structures.

Preparation of plant extracts

Fresh stems of *B. solanifolium* (Burm.) Suresh. were washed, cut into small pieces, and air-dried at room temperature before being ground into coarse powder. The powdered material was extracted by maceration with 95%, 80%, or 60% ethanol at a plant-to-solvent ratio of 1:10 (w/v) for 5 days at room temperature with occasional stirring. The filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C and dried to obtain crude extracts. The extraction yields (%) were calculated based on the dry weight of plant material.

Preliminary phytochemical screening

Preliminary phytochemical screening of *B. solanifolium* (Burm.) Suresh extracts was performed using standard qualitative tests **[11].**

Preliminary phytochemical screening of *B. solanifolium* (Burm.) Suresh extracts revealed the presence of flavonoids and compounds containing unsaturated lactone rings in all tested extracts (BS-95, BS-80, and BS-60). Positive reactions were observed in both the Shinoda and ferric chloride tests, indicating the presence of flavonoids. In addition, all extracts showed positive responses to Kedde and Raymond reagents, suggesting the presence of compounds containing unsaturated lactone rings. In contrast, alkaloids were not detected in any of the extracts, as indicated by negative results obtained from both Dragendorff's and Mayer's reagent tests.

Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) of *B. solanifolium* (Burm.) Suresh extracts was determined using the Folin–Ciocalteu method [5, 7]. Briefly, the plant extract was mixed with Folin–Ciocalteu reagent followed by sodium carbonate solution. After incubation at room temperature, the absorbance was measured at 765

nm using a UV–Vis spectrophotometer. Gallic acid was used as a standard, and the results were expressed as milligrams of Gallic acid equivalents per gram of extract (mg GAE/g extract).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method [4]. The extract was mixed with aluminum chloride solution and incubated at room temperature. The absorbance was measured at 415 nm. Quercetin was used as a standard, and the results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

Antibacterial activity (Disc diffusion method)

Antibacterial activity of *B. solanifolium* (Burm.) Suresh extracts was evaluated using the disc diffusion method with slight modifications [12, 13]. The bacterial strains used in this study included *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilis* ATCC 6633.

Bacterial cultures were grown on Mueller–Hinton agar (MHA) at 37 °C for 24 h. A bacterial suspension was prepared in sterile 0.85% NaCl solution and adjusted to the turbidity of a 0.5 McFarland standard (approximately 1×10^8 CFU/mL). The bacterial suspension was then evenly spread onto MHA plates using a sterile cotton swab. Each extract was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 250 mg/mL. Sterile paper discs (6 mm in diameter) were impregnated with 20 µL of each extract solution and placed on the surface of the inoculated agar plates. The plates were incubated at 37 °C for 18 h. After incubation, the diameter of the inhibition zone around each disc was measured in millimeters using a vernier caliper.

Gentamicin was used as a positive control, while the solvent was used as a negative control.

Determination of MIC and MBC

The MIC and MBC were determined using the broth microdilution method [14]. Each well contained 50 µL of plant extract solution and 50 µL of bacterial suspension. The microplates were incubated at 37 °C for 18 h. The MIC was defined as the lowest concentration of the extract that showed no visible bacterial growth compared with the growth control. To determine the MBC, an aliquot from wells showing no visible growth was subcultured onto Mueller–Hinton agar plates and incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration of extract that resulted in no bacterial colony growth on agar plates.

Cytotoxicity assay

Cytotoxic activity was evaluated using the MTT assay against human cancer cell lines (HT29, MCF7, and HepG2) and normal cell lines (Vero and HaCaT)[15, 16]. Cells were treated with serial two-fold dilutions of each extract, starting at 5 mg/mL, for 24 h. Six concentrations were prepared (5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 mg/mL). After treatment, MTT solution (5 mg/mL) was added and the cells were incubated for 4 h. Formazan crystals were then dissolved in DMSO/SDS (9:1, v/v), and absorbance was measured at 570 nm. Cell viability was calculated, and IC_{50} values were determined from the concentration–response curves

Statistical analysis

Data are expressed as mean ± SD from three independent experiments.

3. Results

Table 1. Extraction yield of *Baliospermum solanifolium* stem extracts prepared using different ethanol concentrations.

Extract	Yield (%)
BS-95	1.66
BS-80	3.33
BS-60	3.33

Values are expressed as percentage of dry weight.

Preliminary phytochemical screening of *B. solanifolium* (Burm.) Suresh extracts revealed the presence of flavonoids and compounds containing unsaturated lactone rings in all extracts (BS-95, BS-80, and BS-60). Positive reactions were observed in the Shinoda and ferric chloride tests, indicating the presence of flavonoids. In addition, positive responses to Kedde and Raymond reagents suggested the presence of compounds containing unsaturated lactone rings. However, alkaloids were not detected in any of the extracts, as evidenced by negative results obtained with Dragendorff's and Mayer's reagents (Table 2).

Table 2 Preliminary phytochemical screening of *B. solanifolium* (Burm.) Suresh extracts prepared with different ethanol concentrations

Phytochemical test	BS-95	BS-80	BS-60
Flavonoids			
Shinoda test	+	+	+
Ferric chloride test	+	+	+
Unsaturated lactone ring			
Kedde reagent	+	+	+
Raymond reagent	+	+	+
Alkaloids			
Dragendorff's reagent	–	–	–
Mayer's reagent	–	–	–

Table footnote: (+) = presence detected; (–) = not detected

Total Phenolic and Flavonoid Contents

The total phenolic content (TPC) and total flavonoid content (TFC) of *B. solanifolium* (Burm.) Suresh extracts are presented in Table 3. Among the tested extracts, BS-95 exhibited the highest phenolic content (516.16 mg GAE/g extract), followed by BS-80 (391.81 mg GAE/g extract) and BS-60 (279.27 mg GAE/g extract). In contrast, the highest flavonoid content was observed in the BS-80 extract (481.79 mg QE/g extract), followed by BS-95 (448.72 mg QE/g extract) and BS-60 (376.65 mg QE/g extract).

Table 3 Total phenolic content (TPC) and total flavonoid content (TFC) of *Baliospermum solanifolium* (Burm.) Suresh extracts

Plant extract	TPC (mg GAE/g extract)	TFC (mg QE/g extract)
BS-95	516.16	448.72
BS-80	391.81	481.79
BS-60	279.27	376.65

Table footnote: Values represent means of three independent experiments. GAE = gallic acid equivalent; QE = quercetin equivalent. Data are presented as mean ± SD (n=3)

Antibacterial activity (disc diffusion)

The antibacterial activity of *B. solanifolium* (Burm.) Suresh extracts was initially evaluated using the disc diffusion assay. The extracts showed inhibitory activity against several bacterial strains, particularly

Staphylococcus aureus, with inhibition zones ranging from approximately 7 to 10 mm (Table 4).

Table 4 Antibacterial activity of *Baliospermum solanifolium* (Burm.) Suresh extracts (inhibition zone diameter, mm)

Extract	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
BS-95	8.19	9.98	7.26	8.13
BS-80	–[a]	–[a]	7.13	6.93
BS-60	–[a]	–[a]	–[a]	6.71
Gentamicin	23.00	24.03	23.00	28.00
DMSO	–[a]	–[a]	–[a]	–[a]

Table footnote: –[a] No inhibition zone detected
Values represent mean diameter of inhibition zones (mm) from three independent experiments.

Determination of MIC and MBC

To further confirm the antibacterial activity observed in the disc diffusion assay, MIC and MBC values were determined against all bacterial strains (Table 5). The BS-95 and BS-80 extracts showed inhibitory activity against *S. aureus* with identical MIC values of 3.13 mg/mL. However, The BS-80 extract showed a lower MBC than BS-95 against *S. aureus*, with an MBC equal to its MIC (3.13 mg/mL), whereas BS-95 showed an MBC of 6.25 mg/mL. An MBC/MIC ratio of ≤4 was observed for all extract–bacterium combinations with measurable MIC and MBC values.

Table 5 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Baliospermum solanifolium* (Burm.) Suresh extracts (mg/mL)

Bacterial strain	Extract	MIC	MBC	MBC/MIC
E. coli	BS-95	12.50	12.50	1
	BS-80	–[a]	–[a]	–
	BS-60	–[a]	–[a]	–
P. aeruginosa	BS-95	6.25	12.50	2
	BS-80	–[a]	–[a]	–
	BS-60	–[a]	–[a]	–
S. aureus	BS-95	3.13	6.25	2
	BS-80	3.13	3.13	1
	BS-60	–[a]	–[a]	–
B. subtilis	BS-95	3.13	3.13	1
	BS-80	3.13	3.13	1
	BS-60	6.25	6.25	1

Table footnote: –[a] Not determined (no inhibition zone in disc diffusion assay or MIC >12.50 mg/mL)
MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration

Values represent means of three independent experiments.

Cytotoxicity assay

The cytotoxic activity of *B. solanifolium* (Burm.) Suresh extracts was evaluated using the MTT assay against several human cancer cell lines (HT29, MCF7, and HepG2) and normal cell lines (Vero and HaCaT). The results indicated that BS-80 exhibited strong cytotoxic activity against several cancer cell lines, whereas BS-60 showed relatively lower activity (Table 6).

Table 6 Cytotoxic activity (% cytotoxicity) of *Baliospermum solanifolium* (Burm.) Suresh extracts at 5 mg/mL

Extract	HT29	MCF7	HepG2	Vero	HaCaT
BS-95	20.89	98.84	61.99	30.95	57.95
BS-80	99.47	98.64	94.01	93.50	98.48
BS-60	11.50	97.58	23.69	18.69	27.00

Table footnote: Values represent mean percentage of cytotoxicity from three independent experiments (n=3). Cells were treated with extracts at 5 mg/mL for 24 h. Standard deviations were <10% of the mean values (data not shown).

Table 7 Half-maximal inhibitory concentration (IC_{50}) values (mg/mL) of *Baliospermum solanifolium* (Burm.) Suresh extracts against cancer and normal cell lines determined by the MTT assay

Extract	HT29	MCF7	HepG2	Vero	HaCaT
BS-95	–□	2.703 ± 0.05	4.216 ± 0.03	–□	1.039 ± 0.01
BS-80	2.907 ± 0.04	2.880 ± 0.04	2.808 ± 0.02	4.276 ± 0.01	3.933 ± 0.04
BS-60	–□	2.543 ± 0.01	–□	–□	–□

Table footnote: –□ IC_{50} could not be determined because 50% inhibition was not achieved at the highest concentration tested (5 mg/mL).

Values are presented as mean ± SD from three independent experiments (n=3)

Table 8 Selectivity index (SI) of *Baliospermum solanifolium* (Burm.) Suresh extracts against cancer cell lines using Vero cells as normal reference

Extract	HT29	MCF7	HepG2
BS-95	–□	1.58	1.01
BS-80	1.47	1.48	1.52
BS-60	–□	1.68	–□

Table footnote:–□ Not determined (IC_{50} could not be calculated due to <50% cytotoxicity at the highest concentration tested)
SI = IC_{50} (Vero) / IC_{50} (cancer cell line). Values were calculated from mean IC_{50} shown in Table 7.

SI > 1 indicates selectivity towards cancer cells.

4. Discussion

The presence of flavonoids detected in the extracts may contribute to the antimicrobial and cytotoxic activities observed in this study. Flavonoids have been widely reported to possess antibacterial properties through several mechanisms, including disruption of microbial cell membranes and inhibition of nucleic acid synthesis [4, 17]. In addition, compounds containing unsaturated lactone rings have also been associated with various biological activities, including antimicrobial and cytotoxic effects [18].

The relatively high phenolic and flavonoid contents detected in the extracts may contribute to the biological activities observed in this study. Phenolic compounds and flavonoids are widely reported to exhibit antimicrobial and cytotoxic activities through several mechanisms, including disruption of

bacterial cell membranes, inhibition of enzymatic activity, and induction of apoptosis in cancer cells [5, 7].

The present findings are consistent with previous reports demonstrating that phenolic and flavonoid-rich plant extracts exhibit antibacterial activity against Gram-positive bacteria, particularly *Staphylococcus aureus* [4, 11]. Furthermore, the results demonstrated that ethanol concentration influenced the biological activity of the extracts. The BS-80 extract showed bactericidal activity against *S. aureus* and *B. subtilis*, with MBC/MIC ratios of 1.00 for both organisms, whereas BS-95 showed broader antibacterial activity across the tested bacterial strain, suggesting that intermediate ethanol concentrations may enhance the extraction efficiency of certain bioactive compounds responsible for antimicrobial activity [9]. The MBC/MIC ratio (≤ 4) suggested that the extract exerted bactericidal rather than bacteriostatic effects [14].

Interestingly, the BS-80 extract showed notable antibacterial and cytotoxic activities than the BS-95 extract despite having lower total phenolic content. This finding suggests that the biological activity may depend not only on the total amount of phenolic compounds but also on the composition and polarity of individual phytochemicals extracted at intermediate ethanol concentrations. In addition to antimicrobial effects, the extracts exhibited cytotoxic activity against several human cancer cell lines [15, 16]. Medicinal plants have long been recognized as valuable sources of anticancer agents due to their ability to induce apoptosis, inhibit cell proliferation, and modulate cellular signaling pathways [6, 19–21]. The high cytotoxic activity observed for the BS-80 extract indicates that *B. solanifolium* (Burm.) Suresh may contain compounds with potential anticancer properties. Selectivity index analysis provides additional information regarding the relative safety of plant extracts by comparing their cytotoxicity toward cancer and normal cells [10]. In the present study, using Vero cells as normal reference, BS-80 exhibited SI values of 1.47–1.52 across all three cancer cell lines, indicating low but consistent selectivity toward cancer cells. BS-60 showed an SI of 1.68 against MCF7 cells, while BS-95 demonstrated modest SI values of 1.58 (MCF7) and 1.01 (HepG2). Although several extracts exhibited strong cytotoxic effects, the SI values suggested only low to moderate selectivity overall, indicating that further studies are needed to isolate the active constituents and improve cancer cell selectivity.

Previous studies have reported that flavonoids and phenolic compounds can induce apoptosis through mitochondrial dysfunction, activation of caspases, and cell-cycle arrest [4, 17]. Therefore, the cytotoxic effects observed in the present study may be associated with these mechanisms, although further mechanistic studies are required. Similar antimicrobial activity has been reported for other medicinal plant extracts [21]. Plant secondary metabolites are known to exhibit diverse biological activities [22]. Essential oils and other volatile compounds have also been shown to possess biological effects [23]. Overall, the results of this study support the traditional use of *B. solanifolium* (Burm.) Suresh and highlight its potential as a promising source of natural bioactive compounds. Further studies involving phytochemical characterization and mechanism-of-action investigations are necessary to identify the active constituents responsible for the observed biological activities.

Limitations of the study

The present study has several limitations that should be considered. First, the phytochemical analysis performed in this study was limited to preliminary qualitative screening and determination of total phenolic and flavonoid contents. Detailed chemical characterization and identification of the specific bioactive compounds responsible for the observed biological activities were not performed.

Second, the antimicrobial activity was evaluated against a limited number of bacterial strains. Therefore, the spectrum of antibacterial activity of *B. solanifolium* (Burm.) Suresh extracts may not fully represent their potential effects against other clinically relevant microorganisms.

Third, the cytotoxicity assessment was conducted using in vitro cell culture models only. Although the results demonstrated cytotoxic effects against several cancer cell lines, the mechanisms underlying these effects were not investigated. In addition, in vivo studies were not performed to evaluate the pharmacological efficacy, toxicity, and safety of the extracts in biological systems.

Finally, the study focused on crude extracts prepared using different ethanol concentrations. Further studies involving fractionation, purification, and structural identification of active compounds are necessary to better understand the pharmacological potential of *B. solanifolium* (Burm.) Suresh.

Future perspectives

Future studies should focus on the detailed phytochemical characterization of *B. solanifolium* (Burm.) Suresh extracts in order to identify the specific bioactive compounds responsible for the observed antibacterial and cytotoxic activities. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS), and nuclear magnetic resonance (NMR) spectroscopy may be employed to isolate and characterize the active constituents.

In addition, further investigations are required to elucidate the mechanisms of action underlying the antimicrobial and cytotoxic effects of the extracts. Such studies may include the evaluation of bacterial cell membrane disruption, inhibition of key metabolic pathways, and induction of apoptosis or cell cycle arrest in cancer cells.

Moreover, additional studies involving a broader range of clinically relevant microbial strains and cancer cell lines would provide a more comprehensive understanding of the biological potential of *B. solanifolium* (Burm.) Suresh. Finally, in vivo studies are necessary to evaluate the pharmacological efficacy, safety, and toxicity of the extracts in animal models, which would provide important information for future therapeutic development.

Although the extracts exhibited promising biological activities, the relatively low selectivity indices suggest that purification of active constituents may improve anticancer selectivity while reducing toxicity toward normal cells.

5. Conclusion

The results of this study demonstrate that *B. solanifolium* (Burm.) Suresh extracts possess antimicrobial and cytotoxic activities. Among the tested extracts, BS-80 showed notable antibacterial and cytotoxic activities, including bactericidal effects against *Staphylococcus aureus*. These findings provide scientific evidence supporting the potential use of *B. solanifolium* (Burm.) Suresh as a source of bioactive compounds for further pharmaceutical and biomedical applications.

Declarations

Funding

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Ethics approval and consent to participate

Ethical approval was not required because this study did not involve human participants or experimental animals. The plant material was collected from naturally occurring Baliospermum solanifolium (Burm.) Suresh plants growing along a public roadside area in Ban Lat District, Phetchaburi Province, Thailand. The plants were not cultivated or intentionally planted, and the collection was conducted solely for academic research purposes without an intention for commercial utilization or commercial exploitation of the plant genetic resource. The collection site was not privately owned land; therefore, permission from a private landowner was not applicable. No specific plant-collection permit was obtained for this collection. A voucher specimen (BS-EAU-001) was prepared and deposited in the medicinal plant herbarium collection of the School of Pharmacy, Eastern Asia University, Thailand.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' Contributions

Anong Teeravanichpong: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Supervision, Project administration.

Apakorn Songsumanas: Investigation, Methodology, Data curation.

Tanatip Thamacharoensuk: Investigation, Methodology, Data curation.

Thitaree Theerachayanan: Funding acquisition, Conceptualization, Methodology.

All authors contributed to the study and approved the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence (AI) tools

The authors used artificial intelligence (AI) tools to assist in language editing and improving the clarity of the manuscript. The AI tool was used only for grammatical correction and language refinement. All scientific content, data analysis, interpretation, and conclusions were developed and verified by the authors. The authors take full responsibility for the content of this manuscript.

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Figures

Figure 1A



Whole plant habit

Figure 1B



Stem morphology

Figure 1C



Branch and leaf arrangement

Figure 1D



Figure 1E



Figure 1F



Figure 1

Botanical characteristics of *Baliospermum solanifolium*(Burm.) Suresh.

(A) Whole plant habit. (B) Stem morphology. (C) Branch and leaf arrangement. (D) Serrate leaf margin. (E). Fruit (F) Representative plant specimen collected from Phetchaburi Province, Thailand.

Source: Photographs taken by the authors.

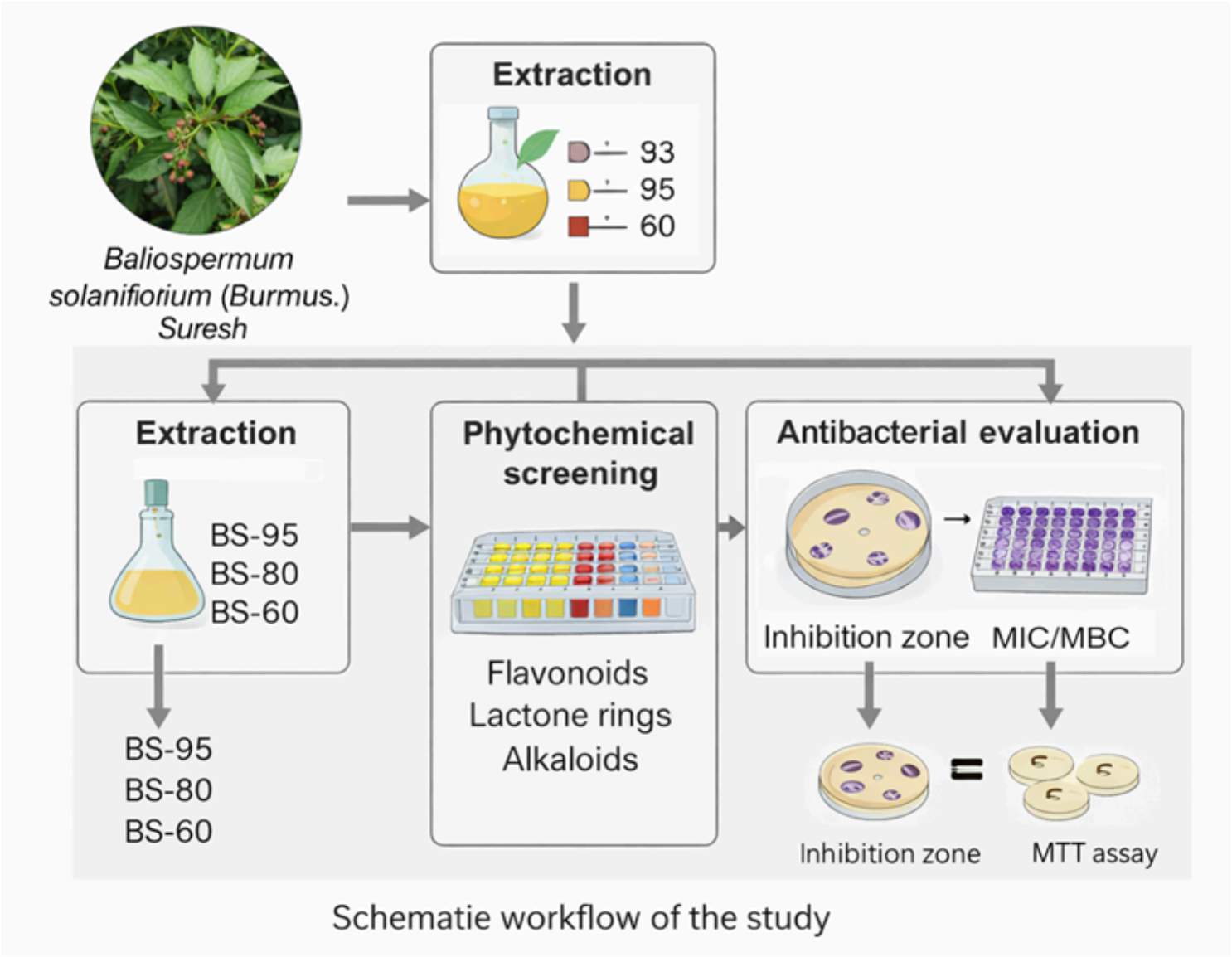


Figure 2

Schematic workflow of the study

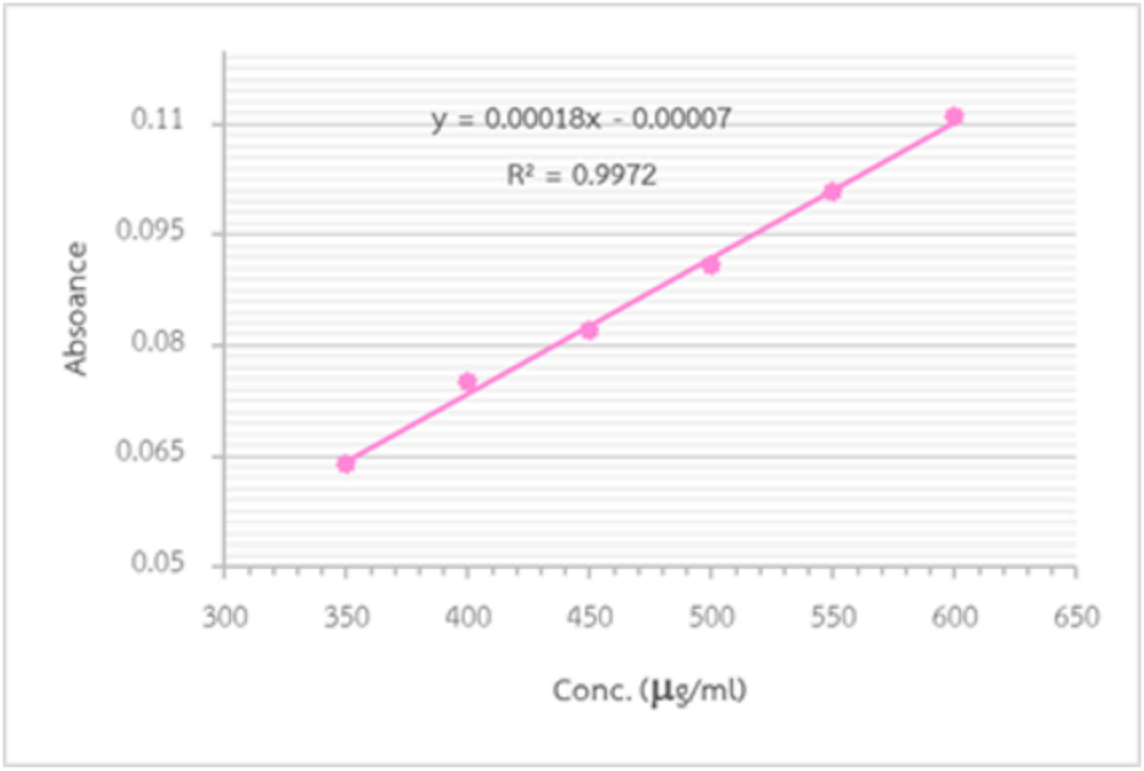


Figure 3

Standard curve of Quercetin used for determination of total flavonoid content (TFC).

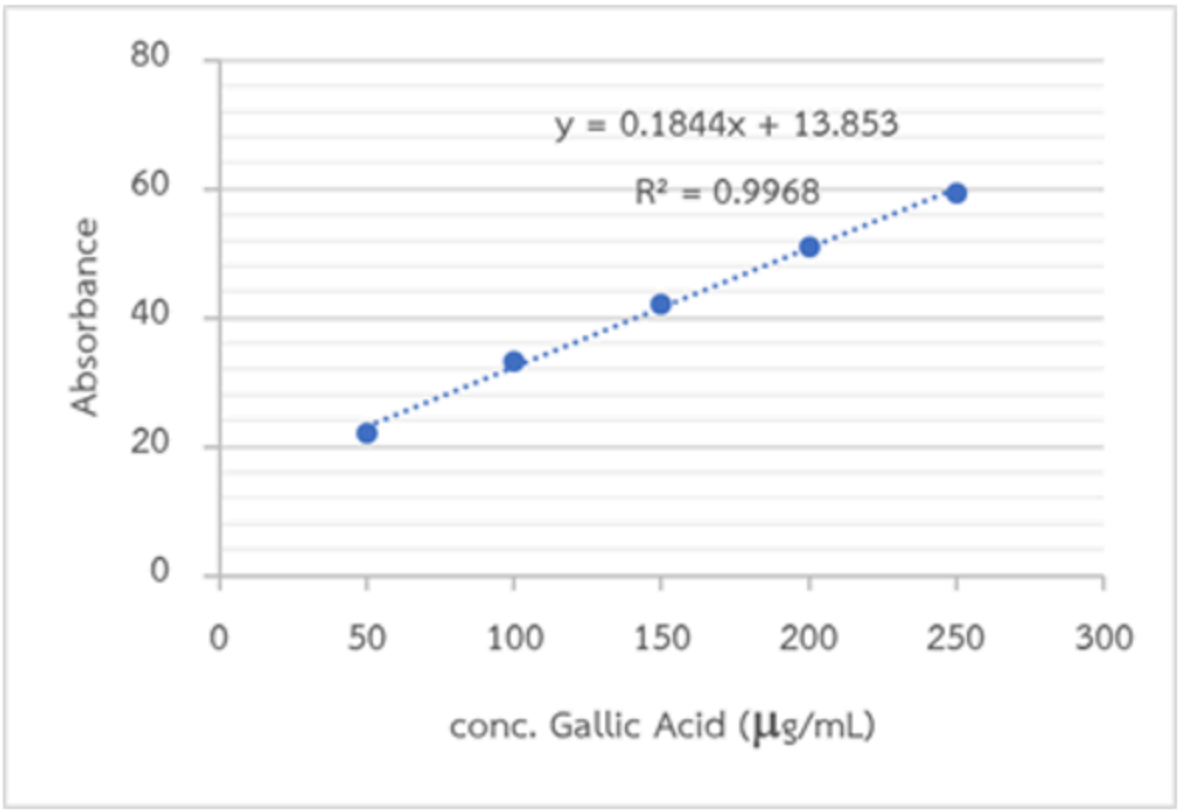


Figure 4

Standard curve of Gallic Acid used for determination of total phenolic content (TPC).