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Publisher: Taylor & Francis

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Subject area: Engineering: Industrial and Manufacturing Engineering | Chemistry: General Chemistry | Chemical Engineering: General Chemical Engineering

Source type: Journal

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Engineering
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Publisher

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56

Publication type

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2009-2026

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chiefeditor.cyta@gmail.com

Scope

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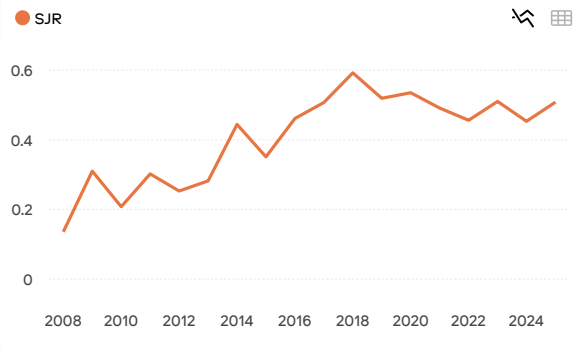

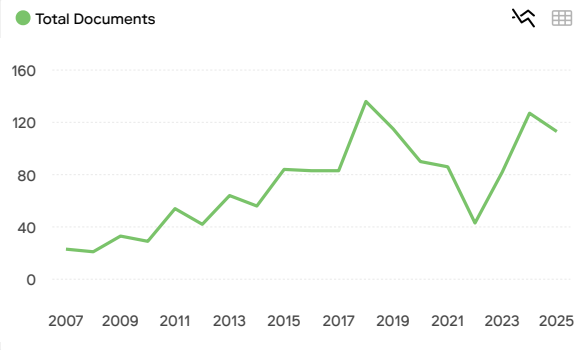

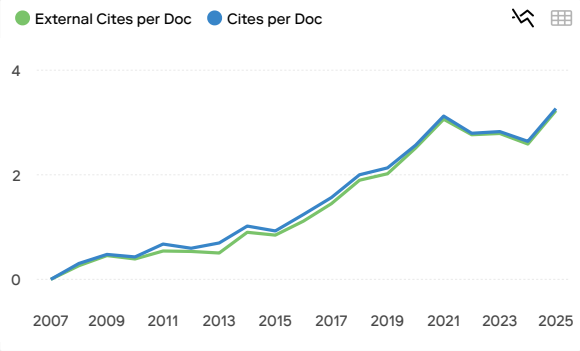

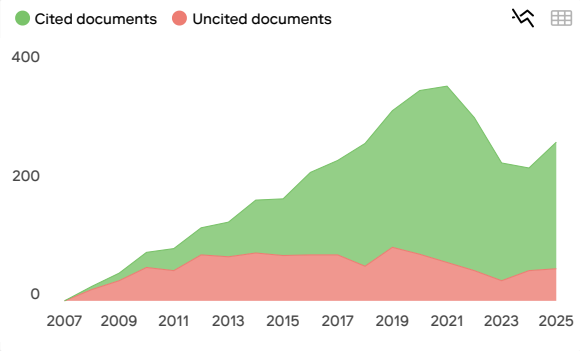

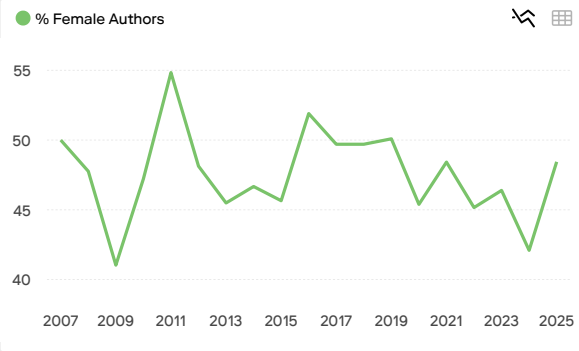

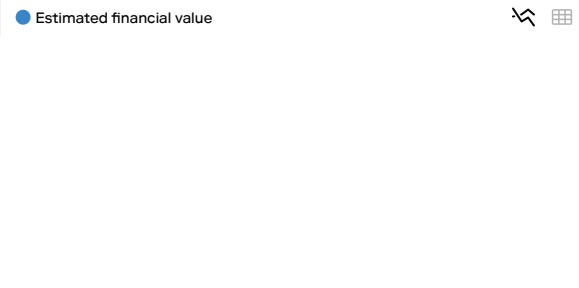

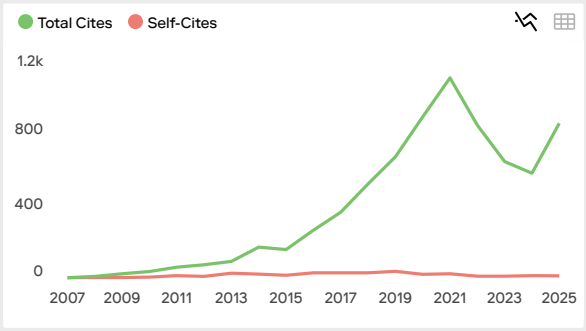

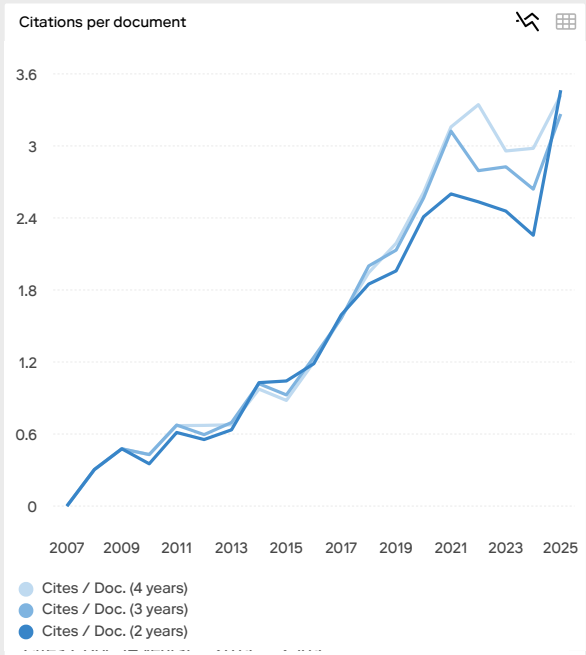

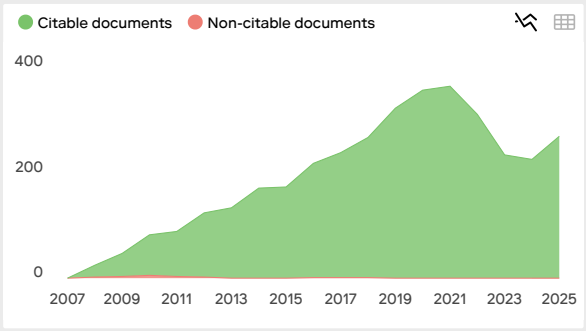

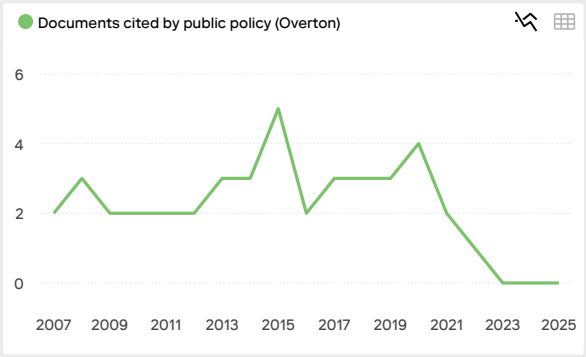

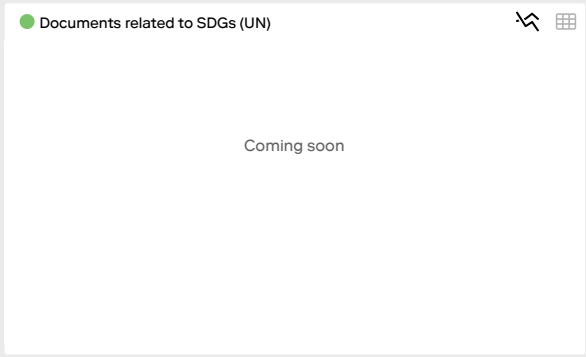

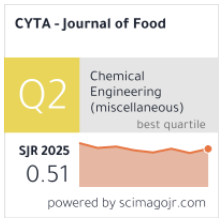

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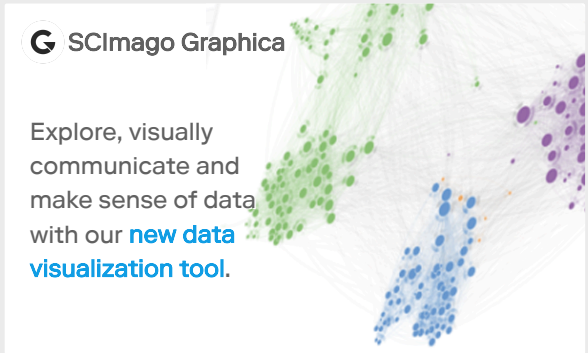

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Sir, what is the quartile for food science?

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3 messages

CyTA - Journal of Food <onbehalfof@manuscriptcentral.com> Thu, May 7, 2026 at 2:20 AM
Reply-To: dr.j.antonio.torres@gmail.com
To: kunyarat.d@pcru.ac.th
Cc: anchte@kku.ac.th, j.sutthiwan@kkumail.com, bam.srp@kkumail.com, promraksabundit@gmail.com, sumalin.dee@pcru.ac.th, kunyarat.d@pcru.ac.th

06-May-2026

Dear Dr Kunyarat Duenngai:

Ref: Phytochemical Composition, Antioxidant, and Anticancer Activities of Pikat Trichintalamaka, a Traditional Thai Herbal Formula

CONGRATULATIONS!

We are pleased to accept your paper in its current form which will now be forwarded to the publisher for copy editing and typesetting. The reviewer comments are included at the bottom of this letter, along with those of the editor who coordinated the review of your paper.

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Sincerely,

Dr J. Antonio Torres
Distinguished Professor, Tecnologico de Monterrey &
Chief Editor, CyTA - Journal of Food
Email: dr.j.antonio.torres@gmail.com
==========

Kunyarat Duenngai <kunyarat.d@pcru.ac.th> Thu, May 7, 2026 at 10:01 AM
To: dr.j.antonio.torres@gmail.com

Dear Dr. J. Antonio Torres,

Thank you very much for your email and for the acceptance of our manuscript entitled “Phytochemical Composition, Antioxidant, and Anticancer Activities of Pikat Trichintalamaka, a Traditional Thai Herbal Formula” for publication in CyTA - Journal of Food.

We greatly appreciate the valuable comments and support from the editor and reviewers throughout the review process.

As requested, we will submit the clean editable version of the manuscript (without tracked changes/highlighted text) to the provided email address: TCYT-peerreview@journals.tandf.co.uk.

We look forward to receiving the proofs and further instructions regarding the publication process.

Thank you again for your consideration and support.

Sincerely,

Dr. Kunyarat Duenngai
On behalf of all authors

Asst. Prof. Dr. Kunyarat Duenngai
Department of Thai Traditional Medicine,
Faculty of Science and Technology,
Phetchabun Rajabhat University,
Phetchabun, Thailand, 67000
Tel.056-717122:8243
Mobile. 089-8427905
Fax.056-717123

[Quoted text hidden]

J. Antonio Torres <dr.j.antonio.torres@gmail.com> Thu, May 7, 2026 at 9:51 PM
To: Kunyarat Duenngai <kunyarat.d@pcru.ac.th>

Dear Dr Duenngai,

I am delighted to congratulate you once again. I take this opportunity to share that we started a new Early Career Researcher Board to attract and support promising early career scientists. This new Taylor & Francis initiative offers ECB members will help you build a global network while gaining valuable experience in preparing and assessing research publications. Once we reach a critical mass, I will organize video conferences to develop an Action Plan shaped by member input (see the attached document for more information).

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Best regards from Space CIty USA, Houston

PS: In my former position at Oregon State University I mentored many undergraduates and supervised the graduate programs of Thai students, which was one of the top three largest international groups. I have been to Thailand and hopefully will visit it again soon with my family. Xulei, my spouse, is also a food engineer and currently the Manager of the NASA Space Food Program for ISS and the Artemis Mission. Our Antonella (7 yrs old) is already a world traveler since has already visited six continents.

J. Antonio Torres, PhD (DSV-Chile, MIT'84)
cell: +1-541-207-2903
Distinguished International Professor
Tecnologico de Monterrey, México

Chief Editor, CYTA-Journal of Food, Taylor & Francis

Editor, Innovative Food Science & Emerging Technologies (IFSET), Elsevier

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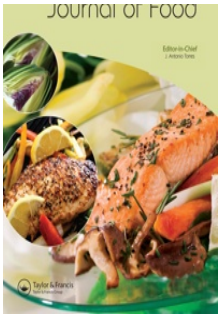

An open access journal

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Anchalee Techasen et al.

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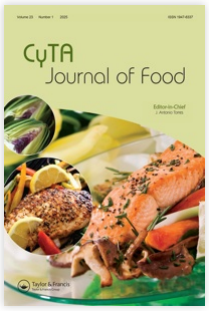

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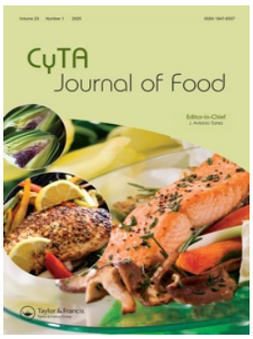

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ISSN: 1947-6337 (Print) 1947-6345 (Online) Journal homepage: www.tandfonline.com/journals/tcyt20

Phytochemical composition, antioxidant, and anticancer activities of Pikat Trichintalamaka, a traditional Thai herbal formula

Anchalee Techasen , Sutthiwan Janthamala , Saranporn Pornpiphat , Bundit Promraksa , Sumalin Deechan & Kunyarat Duenngai

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2026, VOL. 24, NO. 1, 2672213
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RESEARCH ARTICLE

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Phytochemical composition, antioxidant, and anticancer activities of Pikat Trichintalamaka, a traditional Thai herbal formula

Anchalee Techasen[a], Sutthiwan Janthamala[a], Saranporn Pornpiphat[a], Bundit Promraksa[b], Sumalin Deechan[c] and Kunyarat Duenngai[c]

[a]Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand; [b]Regional Medical Sciences Center 2 Phitsanulok, Department of Medical Sciences, Ministry of Public Health, Phitsanulok, Thailand; [c]Department of Thai Traditional Medicine, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun, Thailand

ABSTRACT

Gastrointestinal cancers remain major health burdens due to poor survival and limited treatment options. Traditional Thai herbal remedies contain bioactive compounds with antioxidant and anticancer potential. This study evaluated the phytochemical profile, antioxidant capacity, and in vitro anticancer potential of Pikat Trichintalamaka (PTL), a traditional polyherbal formulation. PTL exhibited high flavonoid (474.17 ± 0.32 mg QE/g dry wt) and phenolic contents (399.54 ± 0.06 mg GAE/g dry wt), along with strong antioxidant activity via DPPH and FRAP assays. ^{1}H-NMR analysis identified key metabolites, including amino acids, vitamins, short-chain fatty acids, and polyphenols. PTL demonstrated significant time-dependent antiproliferative effects against KKU-100 cholangiocarcinoma and AGS gastric cancer cell lines. The anticancer activity likely stems from combination interactions among its phytochemical constituents and antioxidant properties. These findings position PTL as a promising natural candidate for gastrointestinal cancer treatment, though further in vivo and clinical studies are required to confirm safety and therapeutic efficacy.

ARTICLE HISTORY
Received 13 January 2026
Accepted 6 May 2026

KEYWORDS
Pikat Trichintalamaka; phytochemicals; antioxidant; anticancer; gastric cancer; cholangiocarcinoma

Introduction

Gastrointestinal cancers (GIC) are a group of malignant diseases that originate in the organs of the digestive system (Guo et al., 2022). GIC are remain a significant global health challenge due to their high mortality rates (Ali et al., 2024). The causes of GIC are multifactorial. Lifestyle factors such as diet, smoking, excessive alcohol consumption, and obesity are known contributors (Jardim et al., 2023). Chronic inflammation is one of the risk factors of cancer, leading to the release of reactive oxygen species (ROS). These ROS can cause DNA damage, leading to mutations, genomic instability, and altered cellular pathways. Gastric cancer continues to be a significant health concern globally, especially in East Asia (Sung et al., 2021) and ranks as the fifth most prevalent cancer globally (Thrift & Nguyen, 2021). *Helicobacter pylori* infection, through chronic inflammation contributes to the development of gastric cancer by inducing oxidative stress and promoting an environment conducive to tumorigenesis (Salvatori et al., 2023). Cholangiocarcinoma (CCA) exhibits notable geographic variation, with markedly elevated rates in Southeast Asia, particularly in northeastern Thailand (Sriamporn et al., 2004). Among the known risk factors for CCA, *Opisthorchis viverrini* plays a significant role in CCA development (Tyson & El-Serag, 2011). Chronic inflammation caused by *O. viverrini* damages the bile ducts, potentially leading to malignant transformation and contributes to the progression of CCA (Kaewpitoon et al., 2008). Conventional chemotherapy for GIC demonstrates limited efficacy and substantial toxicity (Aslam et al., 2014), highlighting the urgent need for novel therapeutic strategies with improved safety profiles.

CONTACT Kunyarat Duenngai Kunyarat.d@pcru.ac.th Department of Thai Traditional Medicine, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun, Thailand

Thai herbal medicine offers a promising avenue for anticancer drug discovery (Pradubyat et al., 2024). Antioxidants play a crucial role in cancer prevention and therapy by neutralizing ROS (Iqbal et al., 2024). *Zingiber officinale* exhibited the highest antioxidant properties as evidenced by *in vitro* evaluations. Its bioactive compounds exhibit anticancer effects by inhibiting proliferation and inducing apoptosis (Mao et al., 2019). The synergistic effects of Belog Plus (BP), a polyherbal formulation demonstrated high levels of total phenolic and total flavonoid contents, which enhances its potent antioxidant properties (Pradubyat et al., 2024). Phytochemical compounds and antioxidants are critical in reducing ROS-induced DNA damage, a key factor in cancer development (Muchtaridi et al., 2024). Quercetin, which is high content in *Allium cepa* L. (onion), has been shown to significantly reduce the risk of gastric cancer (Ekström et al., 2011; Scalbert et al., 2005). Licochalcone, a compound from *Glycyrrhiza uralensis* root, inhibited HepG2 cell growth by inducing morphological changes and disrupting the G2/M cell cycle progression (Wang et al., 2018). The combination of plant extracts can lead to potentially effects, potentially enhancing their overall effectiveness and reducing the need for high doses of individual extracts (Ren et al., 2025).

Pikat Trichintalamaka (PTL), a traditional Thai polyherbal formulation, comprises plants with documented pharmacological activities relevant to cancer therapy. (PTL) consists of three herbal plants: *Rheum officinale* Baill, *Terminalia chebula* Retz, and *Garcinia hanburyi* Hook F. Previous studies have reported that *R. officinale* extracts exhibit anticancer properties through several types of cancers (Li et al., 2009; Wei et al., 2011). *T. chebula* extracts demonstrated high phenolic and flavonoid content, which is related to their cytotoxicity effect on human breast and lung cancer cell lines (Ravi Shankara et al., 2016). *G. hanburyi* extract inhibited colorectal cancer cell proliferation and induced apoptosis by reducing mitochondrial membrane permeability and activating caspase-3 (Vichitsakul et al., 2023). The combination of these botanicals may provide enhanced antioxidant and antiproliferative effects against GIC through multiple complementary mechanisms, including ROS scavenging, anti-inflammatory activity, and direct cytotoxic actions.

To characterize PTL's bioactive constituents, this study employs nuclear magnetic resonance (NMR) spectroscopy, a high-throughput, non-destructive technique that enables rapid structural identification and quantification of complex plant metabolites (Salem et al., 2020) (Rolin et al., 2013). Janthamala et al. reported potential compounds with anticancer properties derived from the flowers of *Shorea roxburghii* using NMR techniques. Their study revealed a correlation between cinnamic acid and its derivative, ferulic acid, and the reduction in cell viability in gastrointestinal cancer cell lines. These findings highlight the ability of NMR spectroscopy to link metabolite profiling with relevant biological activity (Janthamala et al., 2024; Wang et al., 2021).

Therefore, this study hypothesizes that PTL exerts anticancer effects through combined antioxidant and antiproliferative mechanisms. We aim to: (1) evaluate PTL's antioxidant activity, (2) identify its metabolites compounds via NMR spectroscopy, and (3) assess its antiproliferative effects on GIC cell lines.

Materials and methods

Sample collection

Rheum officinale Baill., *Terminalia chebula* Retz., and *Garcinia hanburyi* Hook.f. were purchased from Chakkrawat Herb Store, Bangkok, Thailand. *Terminalia chebula* Retz. was authenticated based on morphological characteristics according to the Thai Herbal Pharmacopoeia (THP) online e-book published by the Bureau of Drug and Narcotic, Department of Medical Sciences, Ministry of Public Health (Ministry of Public Health, 2021), ensuring correct botanical identification. *Rheum officinale* Baill. was authenticated based on morphological characteristics according to *Monographs of Selected Thai Materia Medica* (Vol. 1) published by the Department of Medical Sciences (1995). *Garcinia hanburyi* Hook.f. was authenticated based on morphological characteristics according to the Thai Herbal Pharmacopoeia (THP) published by the Department of Medical Sciences, Ministry of Public Health, Thailand.

Sample preparation

The PTL formulation consists of an equal proportion (1:1:1 weight ratio) of three plants: *Rheum officinale* Baill., *Terminalia chebula* Retz., and *Garcinia hanburyi* Hook.f. Specifically, 50 g of each plant material were

combined (yielding a total of 150 g) and ground into a fine powder. The resulting powder was extracted by maceration in 95% ethanol at a 1:10 plant-to-solvent ratio (w/v, using 1500 mL of ethanol) for three days at room temperature with manual stirring twice daily. This extraction process was repeated for a total of three cycles, with the plant residues being air-dried between each cycle. The liquid extracts from all three cycles were pooled, filtered through gauze and Whatman No. 1 filter paper, and then concentrated using a rotary evaporator under vacuum. The final extraction yield of the crude extract was 35.39% (w/w). To preserve its integrity for subsequent analyses, the extract was stored in amber glass containers at −20 °C.

Analysis of total phenolic content using the Folin–Ciocalteu colorimetric method

A solution of PTL was prepared at a concentration of 1 mg/ml and diluted to 100 µg/ml. Then, 100 µl of 10% (v/v) Folin–Ciocalteu reagent was added to a 96-well plate and mixed with 20 µl of PTL. The mixture was incubated at room temperature for 30 min. After incubation, 80 µl of 7% Na_2CO_3 solution was added, the absorbance was measured at a wavelength of 750 nm using a microplate reader (EZ Read 2000 Microplate Reader). All measurements were performed in duplicate. The results were presented as the phenolic compound concentration of the PTL relative to the standard gallic acid calibration curve, expressed in milligrams of gallic acid equivalent per gram dry weight of PTL (mg GAE/g dry wt of PTL).

Analysis of total flavonoid content using the aluminum chloride method

A solution of PTL was prepared at a concentration of 1 mg/ml and diluted to 100 µg/ml. The solution of ethanol, distilled water, 10% (w/v) $AlCl_3$, and 1M CH_3CO_2K were added to a 96-well plate in volumes of 170, 30, 10, and 10 µl, respectively. Next, 30 µl of the diluted PTL was added, mixed, and incubated at room temperature for 30 min. The absorbance was measured at a wavelength of 415 nm. The findings were presented as the flavonoid compound concentration of PTL relative to the standard quercetin calibration curve, expressed in milligrams of quercetin equivalent per gram dry weight of PTL (mg QE/g dry wt of PTL).

Antioxidant activity using the ferric reducing antioxidant power (FRAP) assay

A solution of PTL was prepared at a concentration of 1 mg/ml and diluted to 100 µg/ml. The FRAP working reagent was freshly prepared by mixing 0.25M acetate buffer (pH 3.6), 10 mM 2,4,6-Tripyridyltriazine (TPTZ), and 20 mM Ferric chloride at a ratio of 10:1:1. The reagent (182 µl) was added to a 96-well plate and mixed with 18 µl of the diluted PTL solution. After incubation at 37 °C for 30 min, the absorbance was measured at a wavelength of 593 nm. The results were reported in the antioxidant capacity of the PTL compared to the standard of ascorbic acid, in units of milligrams of ascorbic acid equivalent per gram dry weight of PTL (mg AAE/g dry wt of PTL).

Antioxidant activity using the DPPH radical scavenging assay

A stock solution of PTL was prepared at a concentration of 50 mg/ml and diluted to 0.5, 1, 2, 5, and 10 mg/ml, respectively. Then, 20 µl of each diluted PTL solution was mixed with 180 µl of 0.1 mM DPPH in a 96-well plate. The 0.1 mM DPPH solution was prepared by 0.001 g of DPPH and dissolved in 25 ml of ethanol. After mixing, the solution was incubated at room temperature for 30 min, and the absorbance was measured at a wavelength of 517 nm. The results were reported as the percentage of DPPH radical scavenging activity. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH Scavenging (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100.$$

$\text{Abs}_{\text{control}}$ is the absorbance of the control (DPPH solution without sample), and $\text{Abs}_{\text{sample}}$ is the absorbance of the reaction mixture containing the extract.

The concentration of PTL required to scavenge 50% of DPPH free radicals (EC_{50}) was determined by nonlinear regression analysis using GraphPad Prism version 8.0.1 with a log(agonist) vs. normalized response model. Ascorbic acid served as the positive control.

^{1}H-NMR spectral analysis

In brief, the ethanolic PTL extract was prepared by diluting it with dimethyl sulfoxide-d6 (DMSO-d6) containing 0.1 vol% TMS (Merck KGaA, Cat no.103587), then vortexed for 1 min at room temperature. The mixture was ultrasonicated for 1 h at room temperature and then filtered through a 0.20 µm filter (Corning, USA) to remove any remaining particulates. The clear supernatant was subsequently transferred into a 5 mm NMR tube for analysis. The sample characterization was performed using ^{1}H NMR spectroscopy at 400 MHz on a Bruker Avance 400 NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany). A total of 32 scans were acquired at 27 °C using the zg30 pulse sequence with water signal suppression. For data processing, spectral regions below 0.5 ppm and above 10.0 ppm were excluded to eliminate interference from the residual solvent/TMS peaks and downfield noise, respectively. The NMR spectral data was analyzed using the Metabom8 package (v. 0.4.4) to investigate the resonances of interest. The metabolite identification was conducted using statistical total correlation spectroscopy (STOCSY) and cross-referenced with metabolite databases, including the Biological Magnetic Resonance Data Bank (BMRD), The Natural Product Magnetic Resonance Database (NPMRD) and the Human Metabolome Database (HMDB).

Anticancer activity of PTL using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

AGS were used as gastric cancer cells (purchased from the American Type Culture Collection), and KKU-100 (JCRB1568) were used as CCA cells. The method involved culturing AGS and KKU-100 cells in a 96-well plate at a density of 2000 cells per well and incubating the cells at 37 °C. Cells were allowed to adhere for 24 h prior to treatment. Then, a diluted PTL solution with concentrations of 3.125, 6.25, 12.5, 25, and 50 ng/ml, 100 µl per well, and a maximum percentage of DMSO is 0.005% was added and incubated for 48 and 72 h, respectively. Cells treated with DMSO served as vehicle control. After the incubation period, the cells were washed with 1× Phosphate-Buffered Saline (PBS), and the PBS was removed. MTT reagent was prepared as a 5 mg/mL stock solution in PBS and diluted to a working solution before use. Then, 100 µl of MTT solution was added to each well and incubated at 37 °C for 2 h. After incubation, MTT solution was removed, and Dimethyl Sulfoxide (DMSO) was added to dissolve the formazan crystals in each well. The absorbance was measured at a wavelength of 540 nm. The results were reported as the percentage of cell survival and the concentration of PTL that inhibited the growth of cancer cells by 50% (Inhibitory concentration: IC_{50}), which was reported in nanograms per milliliter (ng/ml). Analysis was conducted using GraphPad Prism V.8.0.1 software.

Results

Analysis of total phenolic content, flavonoid content

The total phenolic content (TPC) and total flavonoid content (TFC) of the PTL are presented in Table 1. Quantitative analysis revealed that the TPC of PTL was 399.54 ± 0.06 mg GAE/g dry wt of PTL, while the TFC was determined to be 474.17 ± 0.32 mg QE/g dry wt of PTL.

Antioxidant activity testing by using FRAP and DPPH radical scavenging assay

The antioxidant activity of PTL was evaluated using two complementary methods: the Ferric Reducing Antioxidant Power (FRAP) assay and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. As presented in Table 1, the FRAP assay demonstrated that PTL possessed an antioxidant activity of 475.47 ± 0.09 mg AAE/g dry wt of PTL.

For the DPPH radical scavenging assay, PTL exhibited the highest radical scavenging activity of 83.92% ± 0.22% at a concentration of 10 mg/mL, as illustrated in Figure 1a. The effective concentration

Table 1. Phytochemical contents and antioxidant activity in Pikat Trichintalamaka.

Compound	Phenolic contents (mg GAE/g dry wt of PTL)	Flavonoid contents (mg QE/g dry wt of PTL)	FRAP (mg AAE/g dry wt of PTL)
PTL	399.54 ± 0.06	474.17 ± 0.32	475.47 ± 0.09

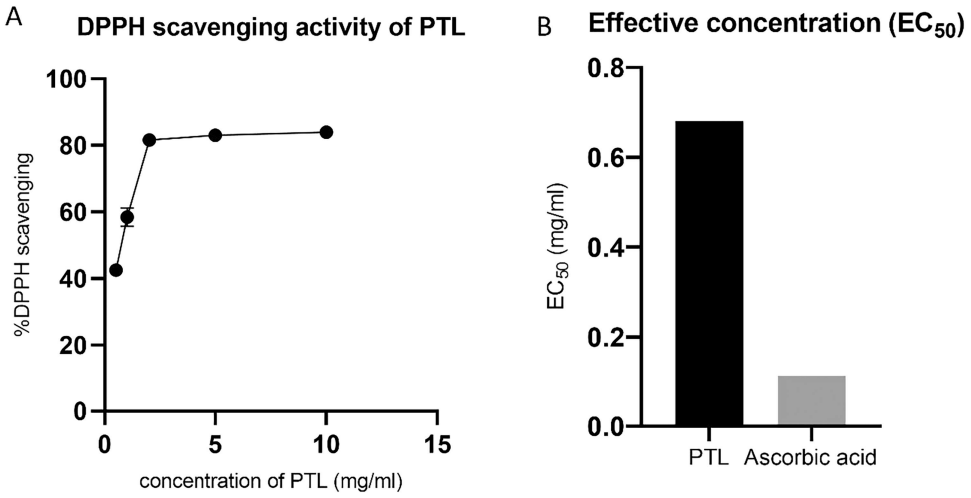

Figure 1. The percentage of DPPH free radical scavenging of Pikat Trichintalamaka extract (A). Effective concentration (EC_{50}) of PTL with ascorbic acid used as a control (B).

required to scavenge 50% of DPPH radicals (EC_{50}) was subsequently calculated from the dose-response curve. As shown in Figure 1b, the EC_{50} value of PTL was determined to be 0.6802 mg/mL.

Metabolite composition of PTL

The main metabolite composition of PTL was investigated using ^{1}H-NMR-based metabolic profiling, as shown in Figure 2 and Table 2. The identified peaks verified by matching chemical shifts and peak patterns with databases. The ^{1}H-NMR analysis of the herbal extract revealed a complex metabolic profile, predominantly characterized by phenolic acids and flavonoids (e.g. gallic, ellagic, and chlorogenic acids, along with kaempferol), which represent the major bioactive constituents. Additionally, the extract contained significant amounts of primary metabolites, including organic acids (butyrate, acetate, succinate), amino acids (valine, phenylalanine), and carbohydrates (glucose). The presence of water-soluble vitamins, specifically ascorbic acid and pyridoxine, further contributes to the nutritional value of the extract as summarized in Table 2.

Anticancer activity of Pikat Trichintalamaka using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

To assess anticancer properties of PTL on AGS gastric cancer and KKU-100 CCA cells, the cells were treated with varying concentrations of PTL (3.125, 6.25, 12.5, 25, and 50 ng/ml) for 48 and 72 h. The concentration required to achieve a 50% reduction in cancer cell growth (Inhibitory concentration: IC_{50}) was evaluated. The study revealed that PTL exhibited anti-cancer properties against both AGS and KKU-100 cell lines. The IC_{50} values for AGS cells were 15.29 and 9.51 ng/ml after 48 and 72 h, respectively, as illustrated in Figure 3a. Meanwhile, the IC_{50} values observed in KKU-100 cells were 21.38 and 16.99 ng/ml at 48 and 72-h, respectively, as shown in Figure 3b.

Discussion

Pikat Trichintalamaka (PTL) is a traditional Thai polyherbal formulation consisting of three medicinal plants: *Rheum officinale* Baill, *Terminalia chebula* Retz., and *Garcinia hanburyi* Hook. F. These botanicals have long been employed in traditional medicine systems for various therapeutic purposes. Previous studies have reported the pharmacological properties of the individual constituent herbs and their isolated bioactive compounds (Clementi & Misiti, 2010; Vuong, 2021). *T. chebula* Retz. is rich in tannins, polyphenols, and flavonoids, which contribute to its potent antioxidant and antimicrobial properties (Mandeville & Cock, 2018). Gambogic acid, a

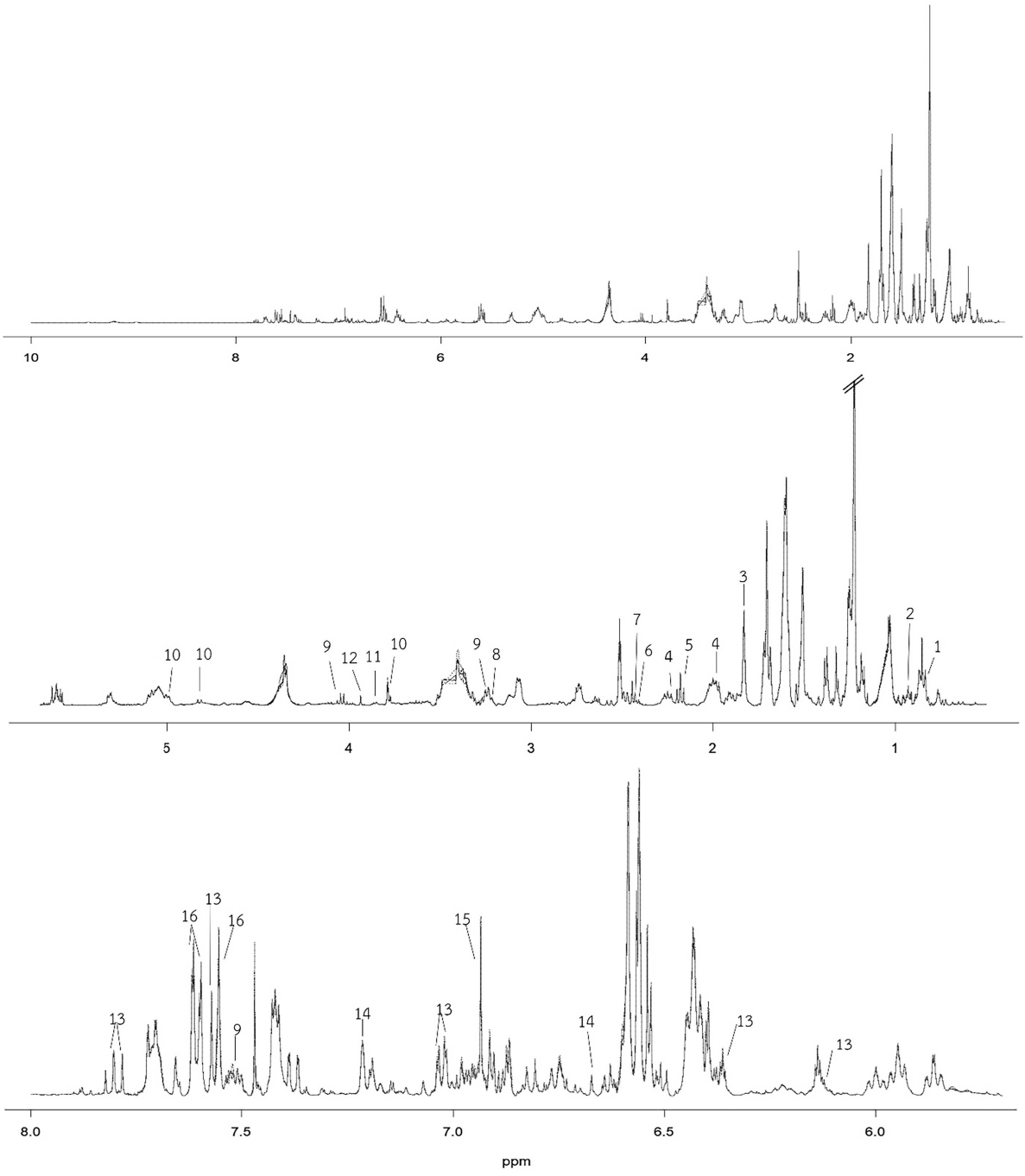

Figure 2. 400 MHz ^{1}H NMR spectrum of alcoholic extract from Pikat Trichintalamaka. (1 = butyrate, 2 = valine, 3 = acetic acid, 4 = chlorogenic acid, 5 = valerate, 6 = succinate, 7 = pyridoxine, 8 = taurine, 9 = glucose, 10 = ascorbic acid, 11 = syringic acid, 12 = kaempferol, 13 = ellagic acid, 14 = phenylalanine, 15 = gallic acid, 16 = vanillic acid).

bioactive compound derived from *G. hanburyi*, has been demonstrated to exhibit considerable anticancer and anti-inflammatory activities (Liu et al., 2020). However, research on the pharmacological effects of their combination remains limited. This study demonstrates that the three-herb combination form may enhance antioxidant and anti-cancer activities. The PTL revealed a flavonoid content of 474.17 ± 0.32 mg QE/g dry wt of PTL and a phenolic content of 399.54 ± 0.06 mg GAE/g dry wt of PTL. These values are notably higher than those reported for some other Thai polyherbal formulations; for instance, the ethanolic extract of Belog Plus (BP), which only provides 80.6 mg GAE/g BP of phenolic components. The increased levels of antioxidant capacity shown in this investigation could be explained by the larger amounts of these phytochemicals in PTL (Pradubyat

Table 2. [1]NMR chemical shift assignments and identification of metabolites in Pikat Trichintalamaka extract.

Metabolite group	Metabolites	Correspondence peak (ppm)	Reference
Phenolic acids and flavonoids	Chlorogenic acid	1.95 (m), 2.22 (m), 4.00 (m), 4.33 (m), 5.28 (m), 6.35 (d), 7.00 (dd), 7.69 (dd)	NP0000578
	Ellagic acid	6.67 (s), 7.21 (s)	NP0001478
	Gallic acid	6.93 (s)	NP0000524
	Kaempferol	6.12 (d), 6.39 (d), 7.01 (dd), 7.78 (dd)	NP0000460
	Syringic acid	3.93 (s), 7.57 (s)	NP0000022, bmse010206
	Vanillic acid	7.54 (s), 7.55 (s), 7.58 (dd)	NP0000013
Organic acids/	Acetic acid	1.82 (s)	NP0000174
	Butyrate	0.83 (t)	NP0000892
SCFAs	Succinate	2.41 (s)	NP0000496
	Valerate	2.15 (t), 0.85 (t)	NP0000153
Amino acids	Phenylalanine	7.37 (m)	HMDB0000159
	Taurine	3.22 (m)	NP0000908
	Valine	0.91 (dd), 3.60 (d)	NP0000609
Carbohydrates/sugars	Glucose	3.76 (dd), 4.80 (d)	NP0000072
Vitamins and antioxidants	Ascorbic acid	3.77 (dd), 3.85 (td), 4.01 (dd), 4.98 (d)	NP0044244, bmse000182
	Pyridoxine	2.43 (s)	NP0001128

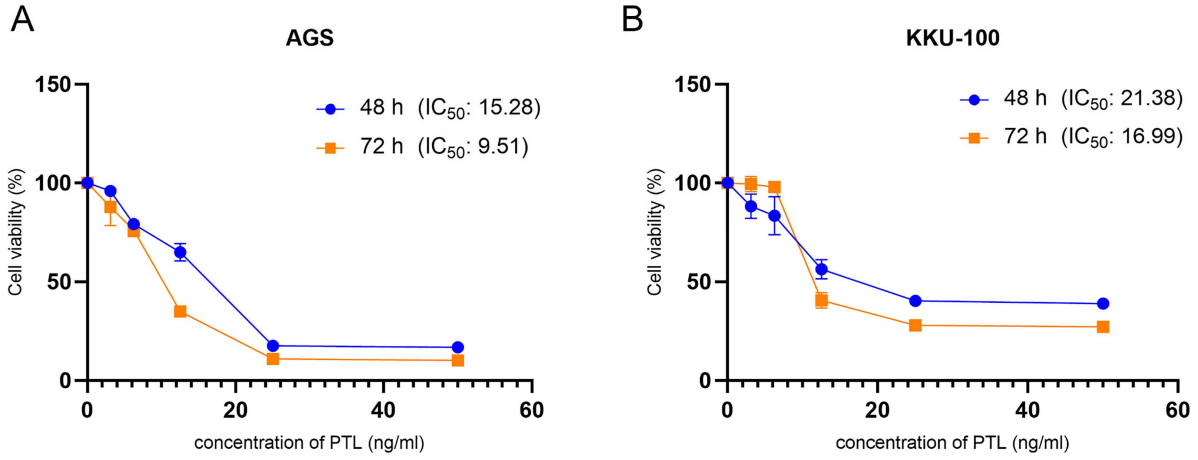

Figure 3. The anticancer effect of Pikat Trichintalamaka on AGS (A) KKU-100 (B) at 48 and 72 hours of treatment.

et al., 2024). The combination of the three medicinal herbs and potential combination interactions that enhance the extractability and total concentration of polyphenolic compounds may be the cause of these elevated levels (Ravi Shankara et al., 2016). This comparison underscores the remarkably elevated phenolic content of the PTL formulation, indicating its significant potential as a natural antioxidant source. The PTL extract, in contrast, had a phenolic content of 399.54 mg GAE/g dry wt of PTL, which was significantly greater than the amounts found in previous studies. Pomegranate peel has been reported to have between 84 and 134 mg/g of flavonoids, while PTL showed a significantly greater content of 474.17 mg QE/g dry wt of PTL. These results show that PTL is remarkably rich in polyphenolic chemicals, indicating a strong bioactive potential, while the PTL extract demonstrated a flavonoid concentration of 474.17 mg QE/g dry wt of PTL. It is plausible that the co-extraction of multiple plant materials may influence the total polyphenolic yield; however, whether this represents true synergistic enhancement or simply an additive effect remains to be determined through fractionation and combination index studies.

The antioxidant capacity of PTL was evaluated using both the FRAP and DPPH assays, which together provide complementary perspectives on its electron-donating and radical scavenging capacities. The FRAP test showed that PTL has a strong ability to reduce ferric iron (475.47 ± 0.09 mg AAE/g dry wt of PTL). The DPPH radical scavenging assay demonstrated the antioxidant effectiveness of PTL, achieving a maximum scavenging activity of 83.92% ± 0.22% at a concentration of 10 mg/ml of PTL. The EC_{50} value for DPPH scavenging was established at 0.6802 mg/ml. PTL demonstrated slightly higher antioxidant efficacy than aqueous extracts of *Curcuma longa* ($IC_{50} \approx 0.77$ mg/ml) (Boudou et al., 2025) and was approximately six times more potent than *Hibiscus sabdariffa* (roselle) extract ($IC_{50} \approx 4.06$ mg/ml) (Wu et al., 2018). These

comparisons should be interpreted cautiously, however, as differences in extraction solvents, plant matrices, and assay conditions may affect the comparability of results across studies. Collectively, the antioxidant data suggest that PTL is a relatively rich source of antioxidant compounds; however, further mechanistic studies, including cellular antioxidant assays and reactive oxygen species (ROS) quantification, are needed to confirm these findings in biologically relevant systems.

The 400 MHz ^{1}H NMR spectrum of the alcoholic extract of PTL exhibited an extensive profile of biologically active metabolites. *T. chebula* Retz. was recognized as a major contributor to the rejuvenating and health-enhancing characteristics usually associated with Rasayana formulations in Ayurveda. The data substantiates the hypothesis that the therapeutic effects of PTL could originate from the combination interaction of its many phytochemical components (Bulbul et al., 2022). The identified metabolites were categorized into several functional groups, including short-chain fatty acids (butyrate, acetic acid, and valerate), amino acids (valine and phenylalanine), organic acids (succinate), vitamins (pyridoxine and ascorbic acid), sulfur-containing metabolites (taurine), carbohydrates (glucose), and polyphenolic compounds such as chlorogenic acid, syringic acid, gallic acid, vanillic acid, ellagic acid, and the flavonoid kaempferol. The presence of butyrate, acetic acid, and valerate short-chain fatty acids (SCFAs) typically formed during microbial fermentation indicates possible plant–microbe interactions that may enhance the biological activity of the PTL extract. Also, finding valine, which is an important amino acid, suggests that the extract could assist with generating proteins and perform other metabolic functions in cells. The evidence of succinate and glucose indicates that primary energy metabolic pathways are involved, since succinate is fundamental to the citric acid cycle, the essential factor for the maintenance of cellular energy homeostasis. The important antioxidant potential, however, of the PTL extract is evident in the presence of chlorogenic acid and several other types of polyphenolic metabolites. These metabolites are known to be capable of enhancing cellular integrity by protecting the cells against inflammatory processes and damage suffered by free radical oxidation (Dongmo et al., 2025).

Among the identified metabolites, gallic acid chlorogenic acid and kaempferol are of particular significance, as both have been previously reported to possess antioxidant, anti-inflammatory, and antiproliferative properties in various experimental models. Gallic acid has previously been detected in the fruit extracts of *Terminalia chebula*. This phenolic compound is known for its strong antioxidant ability. Gallic acid has been widely documented to demonstrate antibacterial, anti-inflammatory, and anticancer activity, highlighting its possible enhancement of the total bioactivity of the PTL formulation (Kumar et al., 2020). Furthermore, a number of phytochemicals included in the PTL extract, such as ascorbic acid and chlorogenic acid, have been emphasized for their potent antioxidant qualities. In addition to its antioxidant properties, chlorogenic acid has been linked to anti-inflammatory and anticancer properties, underscoring its pharmacological significance in the PTL formulation (Naveed et al., 2018). Ascorbic acid (vitamin C), a potent water-soluble antioxidant, is essential for preserving redox equilibrium and restoring other antioxidant compounds. This dual functionality contributes to the overall antioxidant defense capacity of biological systems by protecting cellular components from oxidative damage (Padayatty et al., 2003). The combined bioactive compounds present in the formulation may collectively contribute to its enhanced antioxidant and therapeutic activities. While several identified metabolites have been previously reported to exhibit anticancer properties, their specific contributions in the PTL extract remain to be experimentally validated.

The identification of pyridoxine (vitamin B6) and ascorbic acid (vitamin C) in the PTL extract suggests its potential role in supporting immune regulation, collagen biosynthesis, and metabolic processes. The presence of these micronutrients is consistent with the traditional therapeutic applications of the formulation; however, functional validation through further experimental studies is required to substantiate these properties.

The phytochemicals identified in the present study demonstrated notable antioxidant activity and have been previously reported to possess anticancer properties, suggesting a potential dual mechanism of action underlying the therapeutic activity of the PTL formulation. The lower IC_{50} value observed in AGS cells compared to that reported for kaempferol alone (Song et al., 2015) may indicate that the PTL extract possesses enhanced cytotoxic potential. This effect could be associated with the presence of multiple bioactive compounds within the extract; however, whether this reflects additive or interactive effects cannot be established in the present study. In addition, variations in experimental conditions across studies

should be considered when interpreting such comparisons. The significant herb in PTL *T. chebula* has been shown by Shankara et al. to have an IC_{50} of 208.16 ± 3.7 µg/ml in A-549 human lung cancer cells, which is markedly higher than the IC_{50} obtained in the present study (Ravi Shankara et al., 2016). This comparison would indicate that a much greater anticancer effect may be obtained in the combination. PTL had a significant inhibitory effect on the growth of the KKU-100 CCA cells. It had an IC_{50} of 16.99 ng/ml, which is lower than the IC_{50} of 22.57 µg/ml established for *T. chebula* extract in CCA cells (Chekdaengphanao et al., 2022). The IC_{50} values obtained in this study suggest that AGS gastric cancer cells exhibited a greater sensitivity to PTL treatment compared to KKU-100 CCA cells, as reflected by consistently lower IC_{50} values at both 48 and 72 h. This trend may indicate differential cellular responsiveness between the two cell lines. However, as no statistical comparison between cell lines was performed, these observations should be interpreted descriptively. The present study demonstrates that PTL exhibits significant cytotoxic activity against gastric cancer and CCA cell lines. Notably, kaempferol, a bioactive compound previously reported in *T. chebula* with well-documented anticancer properties, was not detected in the PTL extract, thereby confirming that the observed cytotoxicity is attributable to the overall phytochemical composition of the PTL formulation rather than to this single constituent (Jokar et al., 2016).

These data suggest that the combination of the three herbal components in the PTL formulation may enhance anticancer activity. The lower IC_{50} values observed for the PTL formulation, compared to the individual extracts, indicate a potential combination interaction among their bioactive constituents. This finding is consistent with the principles of polyherbal therapy, as exemplified by the Benjakul formulation. Previous metabolic profiling and bioactivity studies of Benjakul have demonstrated that the integration of multiple medicinal plants provides a diverse range of secondary metabolites, including phenolic acids and alkaloids, which collectively contribute to stronger antioxidant and anticancer activities than those observed in single-herb extracts (Duenngai et al., 2024). In contrast to individual herb extracts, these findings further support the therapeutic potential of multi-component herbal formulations in cancer treatment. PTL exhibited remarkably different levels of cellular toxicity toward AGS gastric cancer cells than KKU-100 CCA cells, which suggests that different cancer cell lines possess varying degrees of sensitivity to it. This selectivity likely arises from different absorbed levels of the various bioactive compounds, differences in intracellular metabolic pathways. This variety suggests that a thorough understanding of the type-specific profiles of cancers is indispensable when assessing the therapeutic value of such complex herbal mixtures. However, as variations in extraction methods, experimental conditions, and cell models may significantly influence the observed activity.

Although the present study demonstrates encouraging and consistent findings, it should be acknowledged that it is exploratory in nature, with several experiments conducted at limited replication. Consequently, the results should be interpreted with caution, and additional studies with greater replication are warranted to strengthen statistical robustness and generalizability.

Regarding the claimed combination interactions among the constituent herbs, it is important to note that the present study did not perform fractionation studies or formal combination index analyses. Therefore, any enhancement in biological activity relative to individual herb extracts should be interpreted as a hypothesis supported indirectly by the observed pharmacological data, rather than as experimentally demonstrated synergism. Future studies employing systematic fractionation, bioassay-guided isolation, and combination index methodologies would be necessary to confirm this hypothesis.

Conclusions

This study demonstrates that PTL possesses measurable antioxidant and cytotoxic activities *in vitro*. The formulation's biological effects are attributed to its rich content of phenolic and flavonoid compounds, alongside a diverse profile of bioactive metabolites identified through NMR. The cytotoxic effects of PTL against AGS gastric and CCA cell lines are likely mediated by the presence of key compounds such as kaempferol, gallic acid, and ellagic acid. The high phytochemical richness correlates with potent antioxidant activity, which collectively contributes to the observed inhibition of cancer cell proliferation. These findings provide a preliminary pharmacological basis for the study of PTL in the context of gastrointestinal cancer research. While these results are promising, several limitations must be acknowledged. This study was conducted exclusively using *in vitro* models, and the observed effects may not fully recapitulate the

complexity of biological interactions *in vivo*. Furthermore, the specific compounds responsible for the combination cytotoxic effects have not been isolated or quantified, and the precise signaling pathways, pharmacokinetic properties, and bioavailability profiles remain uncharacterized. Future studies incorporating *in vivo* models and detailed mechanistic investigations are therefore essential to validate these findings and establish the clinical relevance of PTL as a candidate for gastrointestinal cancer therapy.

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Author contributions

CRediT: **Anchalee Techasen:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft; **Sutthiwan Janthamala:** Data curation, Investigation, Visualization; **Saranporn Pornpiphat:** Data curation, Formal analysis, Investigation, Visualization; **Bundit Promraksa:** Data curation, Formal analysis, Investigation, Validation, Visualization; **Sumalin Deechan:** Resources; **Kunyarat Duenngai:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Validation, Writing – review & editing.

Disclosure statement

The authors declare no potential conflicts of interest in relation to this study.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval statement

Regarding human subjects, the research is exempt from ethical considerations. All authors declare that there was no human specimen used in this study.

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