ORIGINAL ARTICLE

Arch AHS

Archives of Allied Health Sciences 2024; 36(3): 41-51.

Inhibitory effects of methanolic extracts of *Shorea roxburghii* flowers on gastrointestinal cancer and their antioxidant properties

Sutthiwan Janthamala[1], Saranporn Pornpiphat[2], Malinee Thanee[3], Kunyarat Duenngai[4], Apinya Jusakul[5], Sarinya Kongpetch[6], Hideyuki Saya[7], Anchalee Techasen[5*]

[1] Biomedical Sciences Program, Graduate School, Khon Kaen University, Khon Kaen, Thailand.
[2] Medical Science Program, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[3] Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.
[4] Department of Thai Traditional Medicine, Faculty of Science and Technology, Phetchabun Rajabhat University, Phetchabun, Thailand.
[5] Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand.
[6] Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.
[7] Division of Gene Regulation, Cancer Center, Fujita Health University, Toyoake, Japan.

KEYWORDS
Natural plant;
Phenolic;
Flavonoid;
Antioxidant;
Anticancer.

ABSTRACT

Natural plants contain distinctive secondary metabolites valuable in developing functional foods, nutraceuticals, and pharmaceuticals for preventing and treating various diseases. Many parts of *Shorea roxburghii*, a Southeast Asian plant, contain various bioactive compounds, which have garnered attention for their medicinal properties. This study aimed to extract *S. roxburghii* flowers using methanol as a solvent and to assess their phenolic and flavonoid contents, as well as their antioxidant activity through FRAP and DPPH assays. Additionally, the potential anticancer activities on gastrointestinal cancer were investigated using cytotoxicity and apoptosis assays. The flower extract's phenolic content was determined to be 161.20 ± 0.66 µg GAE/mg, with a flavonoid content of 51.93 ± 5.16 µg QE/mg. The extract exhibited moderate antioxidant activity with 57.31 ± 4.29 µg AAE/mg of FRAP value and an EC50 value of 528.10 µg/ml for DPPH scavenging activity. The methanolic extract of *S. roxburghii* showed cytotoxic effects on AGS and KKU-100 gastrointestinal cancer cell lines, with IC50 values of 57.81 µg/ml and 122.5 µg/ml, respectively. Methanolic extract inhibited cell proliferation by inducing apoptosis, suggesting its potential as an anti-cancer agent. This study highlights the medicinal potential of *S. roxburghii* flowers, emphasizing their rich phenolic content and significant antioxidant and anti-cancer properties.

***Corresponding author:** Anchalee Techasen, PhD. Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand. Email address: anchte@kku.ac.th
Received: 25 August 2024/ Revised: 1 October 2024/ Accepted: 15 October 2024

Introduction

Reactive oxygen species (ROS) are generated in cellular metabolic processes, and regulated by endogenous antioxidant mechanisms[1]. Imbalance between ROS generation and antioxidant defense mechanisms can cause oxidative stress, resulting in cellular damage and contributing to the pathogenesis of various diseases such as cancer[2]. Recent epidemiological data revealed that gastrointestinal cancers constitute over 25% of all cancer diagnoses and about 33% of cancer-related deaths worldwide. Notably, regions in East Asia demonstrate a high prevalence of gastrointestinal cancer, including gastric and liver cancers. This has a strong association with factors such as the widespread incidence of *Helicobacter pylori* and liver fluke infection within these areas[3]. Pathogen infection causing chronic inflammation with elevated levels of ROS, resulting in damage to biomolecules, initiation of mutations, and eventually leading to the development of cancer[4].

Phytochemicals, which are secondary metabolites derived from plants, have been used directly or chemically modified to create compounds used in current medicine. Flavonoids are the most well-studied subgroup of phenolic compounds, which are the largest group of phytochemicals. They are recognized for their critical functions in plant biology and extensive health benefits. The compounds contain an aromatic ring structure with one or more hydroxyl groups, giving them antioxidant activity, reduction potential, hydrogen donation capacity, and singlet oxygen quenching ability[5]. The antioxidative properties inherent in these phytochemicals play a role in protecting biomolecules and cellular structures from oxidative damage. Medicinal plants and bioactive compounds derived from them provide a novel strategy for treating cancer. Natural plants are gaining acceptance because of their lower toxicity and side effects, as compared to chemical drugs. The Food and Drug Administration (FDA) has approved more than sixty percent of the medications which are derived from natural sources and are used in cancer treatment[6,7]. Numerous classes of extracts and medicinal plant products have demonstrated potential as anti-cancer agents on both *in vitro* and *in vivo* studies[8,9]. The *Rauvolfia serpentina* leaf extract contained many phytochemicals such as stigmasterol, lupeol, campesterol, and phytol resulting in high level of antioxidant with IC_{50} = 146.67 µg/ml by ABTS, IC_{50} = 106.16 µg/ml by DPPH, IC_{50} = 1.4 mg/ml by FRAP. The extract reduced cell viability of human hepatocarcinoma HepG2 and cervical cancer HeLa with IC_{50} = 274.12 µg/ml and 231.33 µg/ml, respectively[10]. *Hibiscus sabdariffa* L. exhibited cytotoxicity effect in Caco-2 colorectal cancer cell with IC_{50} of 17.51 ± 0.07 µg/mL through increased apoptosis[11]. The extract from *Biarum bovei* showed anticancer properties against prostate cancer cells DU-145, human breast adenocarcinoma MCF-7, and HeLa cell lines with IC_{50} ranged between 22.73 and 44.24 µg/ml[12]. In addition, crude *Annona muricata* extract had anticancer activity against breast cancer cell lines by inducing apoptosis and decreased the size of the tumor and weight in a mice model[13].

A medicinal plant, *Shorea roxburghii* is usually found in Thailand, Laos, Cambodia, Myanmar, and Vietnam[14]. Various parts of *S. roxburghii* contain a range of bioactive compounds that offer numerous health benefits. For example, *S. roxburghii* leaves has antidiabetic potential by decreased fasting blood glucose and improved the body weight, food and water intake of treated diabetic rats[15]. The extract also has antioxidant and anti-inflammatory properties demonstrated by decreased renal markers including serum creatinine, blood urea nitrogen, as well as other inflammation molecules in CTX-induced nephrotoxicity rat model[16]. *S. roxburghii* barks have rich sources of antioxidant polyphenolic compounds[17]. Resveratrol-oligomers isolated from them showed potential for anti-cancer through cell cycle arrest and apoptosis induction against SK-MEL-28 melanoma cells[18]. Moreover, two of

twelve compounds isolated from the roots of *S. roxburghii* exhibited anticancer properties against human epidermoid carcinoma Hela and KB cells (IC_{50} values range of 6.5 to 10.1 µg/ml)[19]. However, there have been few recorded investigations into the flower of *S. roxburghii*. The objectives of this study were to analyze the phenolic and flavonoid contents present in methanolic extracts of *S. roxburghii* flowers, as well as to evaluate their antioxidant activity and potential for inhibiting gastrointestinal cancer cell lines.

Materials and methods

Herbal plant collection and identification

In June 2021, flowers of *S. roxburghii* had been collected from local fields in Ubon Ratchathani province, Thailand. The plant was authenticated at the herbarium of Southern Center of Thai Medicinal Plants, located at the Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand. It was assigned the herbarium number SKP 064 19 18 01.

Sample preparation and extraction

Dried *S. roxburghii* flowers were ground into a fine powder and weighed at 450 grams. This powder was macerated in 4.5 liters of methanol for three days. The liquid extract was then filtered through Whatman no. 1 filter paper. Following filtration, the liquid extract was concentrated using a rotary evaporator (Buchi, Switzerland) under vacuum conditions. The resulting methanolic extract of *S. roxburghii* was protected from light and stored at -20°C. Before being used in further experiments, the crude extract was dissolved in 100% DMSO to prepare a stock solution at a concentration of 100 mg/ml.

Total phenolic contents

Adapted from previous research, the Folin-Ciocalteu method was performed to quantify the phenolic contained in the methanolic extract[20]. Specifically, 100 µl of 10% (w/v) Folin-Ciocalteu reagent (Merck KGaA, Darmstadt, Germany) was combined with 20 µl of methanolic extract at a concentration of 1 mg/ml and allowed to incubate for 30 minutes. Following incubation period, 80 µl of 7% Na_2CO_3 was added to the mixture. The absorbance was measured at 750 nm utilizing a microplate reader. Total phenolic content was expressed as micrograms of gallic acid equivalents per milligram of dry extract (µg GAE/mg), with reference to a standard curve using gallic acid (Sigma-Aldrich, St. Louis, MO, USA).

Total flavonoid contents

Adapted from previous research, the quantification of flavonoid content in the methanolic extract was performed utilizing the aluminum chloride ($AlCl_3$) method[20]. A 30 µl aliquot of methanolic extract at concentration 1 mg/ml was combined with 10% (w/v) $AlCl_3$ solution in distilled water at volume of 10 µl, 1M potassium acetate (CH_3CO_2K) at volume of 10 µl, distilled water at volume of 30 µl, and absolute ethanol at volume of 170 µl and incubated for 30 minutes. After incubation at room temperature, the absorbance was measured at 415 nm using a microplate reader. Quercetin, obtained from Sigma-Aldrich (St. Louis, MO, USA), was measured for setting the standard curves. Flavonoid content was expressed as micrograms of quercetin equivalents per milligram of dry extract (µg QE/mg).

Antioxidant assessments by ferric reducing antioxidant power (FRAP) analysis

The antioxidant capacity of the methanolic extract was evaluated using the FRAP assay, following a modified protocol from previous research[20]. The FRAP reagent was freshly prepared by mixing 0.25 M acetate buffer (pH 3.6), 20 mM ferric chloride from Merck (Darmstadt, Germany), and 10 mM 2,4,6-tripyridyltriazine (TPTZ) from Sigma-Aldrich (St. Louis, MO, USA) in a 10:1:1 ratio. The methanolic extract (18 µl) was combined with 182 µl of the FRAP reagent and incubated at 37°C for 30 minutes. The absorbance was measured at 593 nm using a microplate reader. Ascorbic acid, obtained from Solarbio

(Solarbio Science & Technology, Beijing, China), was used for the standard calibration curve. Micrograms of ascorbic acid equivalents per milligram of dry extract (µg AAE/mg) were used to express the antioxidant activity.

Radical scavenging activity by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

The antioxidant scavenging activity was assessed using the DPPH assay. The methanolic extract (ranging 250-5,000 µg/ml) in volume of 20 µl was mixed with 180 µl of DPPH reagent (Sigma-Aldrich, St. Louis, MO, USA). After gentle shaking for two minutes, the mixture was incubated at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a microplate reader. Using the following formula, the percentage of radical scavenging activity was determined.

$$\text{Percentage of scavenging effect (\%)} = (A_b - A_s)/A_b \times 100$$

A_b represents the absorbance of the blank reagent, while A_s denotes the absorbance of the extract reaction.

Antiradical curves were produced using the range concentrations of extract. Plotting of these data showed scavenging ability on the y-axis and concentration on the x-axis. EC_{50} values, representing the concentration required to achieve 50% antioxidant activity were calculated and reported using GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA).

Cell lines and cell culture

This study involved two cancer cell types including gastric cancer and cholangiocarcinoma cell lines. Gastric cancer (GC) cell line, AGS (CRL-1739) was obtained from the American Type Culture Collection (ATCC). For cholangiocarcinoma (CCA) cell line, KKU-100 (JCRB1568) was procured from the Japanese Collection of Research Bioresources (JCRB). The two cell types were cultivated in Ham's F-12 medium, which was supplemented with 10% fetal bovine serum and 100U/ml and 100 µg/ml of penicillin and streptomycin. Cells were maintained in a humidified incubator with 5% CO_2 at 37°C.

Cytotoxicity test

The MTT colorimetric assay was used to perform the cytotoxicity test. A total of 2,000 cells per well were plated in 96-well plates. Following a 24 h incubation, cells were exposed to methanolic extract concentrations ranging from 0 to 200 µg/ml (final DMSO concentration 0.2%) for 48 and 72 h, and then maintained at 37°C in a 5% CO_2 incubator. Following the treatment, cells were washed with PBS and incubated for 2 h at 37°C with MTT reagent. After that, the formazan crystals were dissolved by adding DMSO after the MTT reagent had been withdrawn. The percentage of cell viability was calculated by comparing with the untreated control. The half-maximal inhibitory concentration (IC_{50}), representing the levels of methanolic extract which suppressed the cell until has 50% cell viability, was determined and reported.

Apoptosis assay detected by flow cytometry

Cells were seeded at a density of 100,000 cells per well in 6-well plates. Following a 24 h incubation, the cells were treated with methanolic extract at concentrations of 160 and 320 µg/ml (final DMSO concentration 0.16 and 0.32%, respectively) for 48 h. Control was represented as 0.32% DMSO treated with the same condition. Upon completion of the treatment, the cells were trypsinized and subsequently washed with cold PBS to collect pellet cells. Then, using the Alexa Fluor® 488 annexin V/Dead Cell Apoptosis Kit (Invitrogen™, USA), the apoptotic cell distribution was evaluated. The cells were resuspended in 100 µl of 1× annexin binding buffer, 2.5 µl of Alexa Fluor® 488 annexin V, and 1 µl of 100 µg/ml PI, following the manufacturer's procedure. After staining, flow cytometry was used to evaluate the cells using a FACS Canto II device (BD Biosciences, UK).

Statistical analysis

The quantitative bar chart displaying the half maximal effective concentration (EC_{50}) values and the apoptosis effect, along with the dose-response curve illustrating the half maximal inhibitory concentration (IC_{50}), were calculated and generated using GraphPad Prism version 8.0 for Windows (GraphPad Software, San Diego, CA).

Results

Total phenolic and flavonoid content

The estimation of total phenolic content in the methanolic extract of *S. roxburghii* was conducted using gallic acid as the standard reference compound. The quantification of phenolic content was expressed as micrograms of gallic acid equivalent (GAE) per milligram of extract. The result revealed that each milligram of the methanolic extract contained approximately 161.20 ± 0.66 micrograms of phenolic compounds, as compared to the standard gallic acid. In addition, the determination of flavonoid content was expressed as micrograms of quercetin equivalents per milligram of extract. The analytical data clearly indicated that methanolic extract contain 51.93 ± 5.16 µg QE/ mg dry extract, represent in table 1.

Antioxidant activity assessments

To evaluate the antioxidant activity of the methanolic extract, two well-established assays, the ferric reducing power assay (FRAP) and the DPPH radical-scavenging capacity assay, were performed. In the FRAP, the reducing power of the extract was assessed by measuring its capacity to reduce ferric ions to ferrous ions. The results were expressed as micrograms of ascorbic acid equivalents per milligram of extract, providing a quantitative assessment of the extract's antioxidant capacity. The FRAP values of methanolic extract was 57.31 ± 4.29 µg AAE/ mg dry extract as shown in table 1.

DPPH radical-scavenging capacity assay provides a reliable method for evaluating the ability of extract to quench free radicals. The concentration of antioxidant required for 50% scavenging of DPPH radicals (EC_{50}) was determined for both the methanolic extract and standard ascorbic acid. The EC_{50} value for the methanolic extract was 528.10 µg/ml, which was comparatively higher than that of ascorbic acid as shown in figure 1.

Table 1 Total phenolic, flavonoid content and antioxidant assessed by ferric reducing power method

Shorea roxburghii extract	Total phenolic content (µg GAE/ mg dry wt)	Total flavonoid content (µg QE/ mg dry wt)	Ferric reducing power assay (µg AAE/ mg dry wt)
Methanol	161.20 ± 0.66	51.93 ± 5.16	57.31 ± 4.29

Note: The data are presented by mean ± SD.
Abbreviations: GAE, gallic acid; QE, quercetin; AAE, ascorbic acid; dry wt, dry weight of methanolic extract.

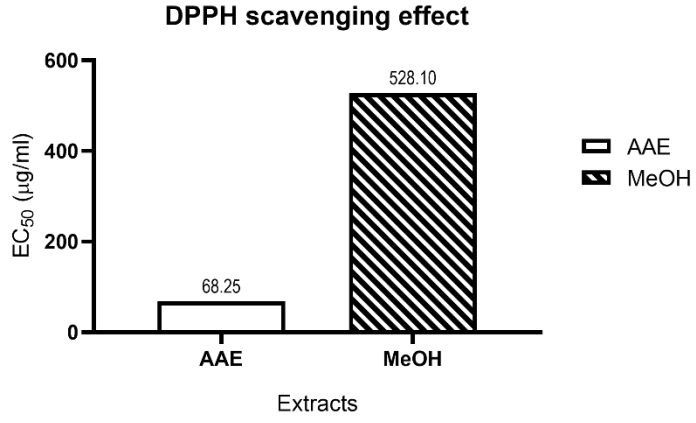

Figure 1 Radical scavenging activity of methanolic *Shorea roxburghii* extract.
EC_{50} values of DPPH scavenging effect in methanolic *S. roxburghii* extract and the standard ascorbic acid.
Abbreviations: MeOH, methanolic extract; AAE, ascorbic acid.

Methanolic extract of S. roxburghii inhibits cell proliferation and induces apoptosis

The cytotoxic effects of the methanolic extract of *S. roxburghii* were investigated on two different cancer cell lines: AGS and KKU-100 representative of GC and CCA, respectively. Following exposure periods of 48 and 72 h, cell viability assessments were conducted utilizing the MTT assay method. Our results demonstrated that AGS cells showed inhibited cancer cell growth with IC_{50} value of 161.6 µg/ml at the 48 h, as shown in figure 2A. The viability of KKU-100 cells was reduced to less than half of the control (untreated with extract) in the presence of 200 µg/ml extract at 48 h, as shown in figure 2B. After 72 h of treatment, AGS cells exhibited the greatest inhibition, displaying an IC_{50} value of 57.81 µg/ml, whereas KKU-100 cells demonstrated an IC_{50} value of 122.5 µg/ml. Our results demonstrated that methanolic extract of *S. roxburghii* had the potential to inhibit gastrointestinal cancer cell growth and gastric cancer was more sensitized compared with CCA. To identify whether methanolic extract inhibited cancer cell growth by inducing apoptosis, AGS cells were selected and treated with crude methanolic extract at concentration 160, and 320 µg/ml. After 48 h of treatment, cells were stained with Annexin V-FITC/PI for flow cytometry analysis. The result showed that the apoptosis rates were (7.0 ± 4.20) %, and (6.9 ± 3.60) %, respectively. On the other hand, the control cells exposed to 0.32% DMSO showed few cells death (0.6 ± 0.05%) in AGS. Our results indicated that methanolic extract of *S. roxburghii* slightly induced AGS cell apoptosis, as shown in figure 3.

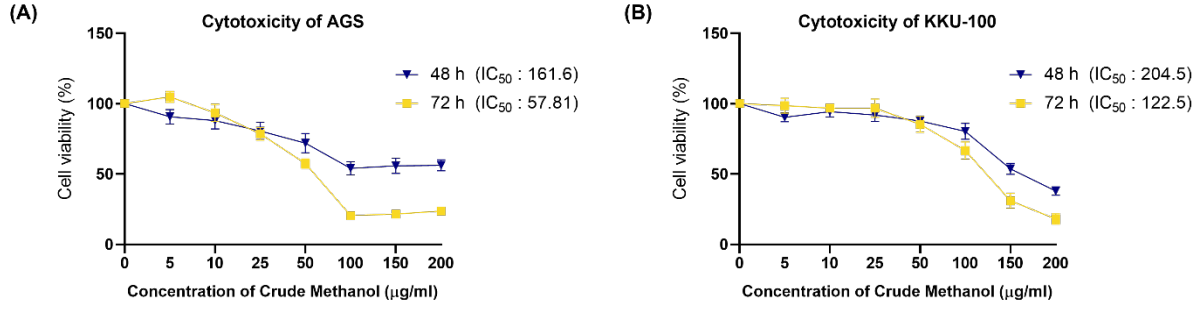

Figure 2 Cytotoxicity of methanolic *Shorea roxburghii* extract with a series concentration 0-200 µg/ml on gastrointestinal cancer cell lines.
(A) AGS treated with methanolic *S. roxburghii* extract in 48 and 72 h
(B) KKU-100 treated with methanolic *S. roxburghii* extract in 48 and 72 h

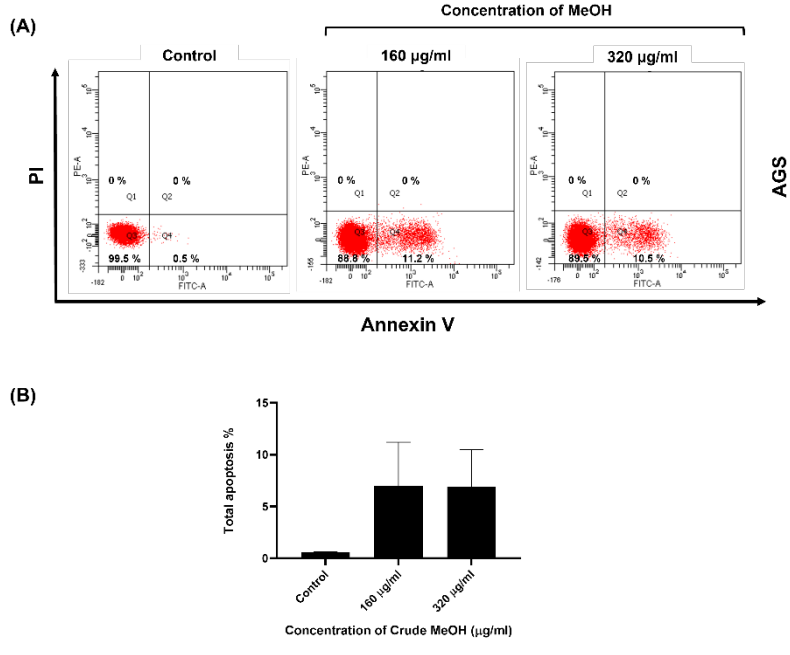

Figure 3 Apoptosis effect of methanolic *Shorea roxburghii* extract.
(A) AGS cells were treated with methanolic *S. roxburghii* extract for 48 h, and apoptosis was determined by flow cytometry using Annexin V/PI double staining
(B) Quantitative analysis of apoptosis is presented by mean ± SEM

Discussion

Plants are organisms that synthesize unique secondary metabolites to survive in various challenging environments. These molecules play crucial roles in the plant's defense mechanisms, as well as provide benefits to other organisms[21]. Phenolics are one of the largest and most diverse groups of secondary metabolites, consisting of compounds with phenol units that play essential roles in plant defense, UV protection, and structural support[22]. Due to their bioactive properties, phenolics are valuable compounds in the

development of functional foods, nutraceuticals, and pharmaceuticals aimed at preventing and treating various diseases. Phenolics are also applied in various defense mechanisms in humans, including as antioxidants, anti-cancer, anti-inflammatory, and cardiovascular protective effects[23].

S. roxburghii is a Southeast Asian plant. Various parts of it contains a variety of bioactive compounds, particularly phenolics, which have garnered attention for its medicinal properties. In our study, we focused on the methanol extract of flowers of *S. roxburghii* to assess their phenolic content and flavonoid contents. The phenolic content in the flower extract was measured at 161.20 ± 0.66 µg GAE/mg, while the stem bark extract reported by Subramanian et al[24] was estimated to contain a total phenolic content of 67.67 ± 4.90 µg/ml, expressed in gallic acid equivalents. Our result indicated that the phenolic content in the methanolic extract of *S. roxburghii* flowers was higher compared to that in the methanol extract of the stem bark. Additionally, flavonoids, one of the phenolic compounds, were found to be 51.93 ± 5.16 µg QE/mg. Supporting our findings, ampelopsin A and H, flavonols belonging to a subclass of flavonoids, were discovered in the methanolic extract of the bark and wood parts of *S. roxburghii*[17]. Other phenolic compounds were also reported in methanolic extract of the *S. roxburghii* such as hopeaphenol, 3-ethyl-4-phenyl-3,4-dihydroisocoumarins, balanocarpol, vaticanols A, and trans-resveratrol[17,25]. This highlights the potential of *S. roxburghii* flowers as a rich source of phenolic compounds that could contribute to their medicinal properties. Phenolic compounds are well-known for their antioxidant properties. They are able to donate hydrogen atoms or electrons to free radicals, thereby neutralizing them and preventing oxidative damage to cells and tissues[26]. Antioxidants play a crucial role in protecting the body against various diseases and aging processes by combating oxidative stress[2]. Our study also investigated antioxidant activity in *S. roxburghii* extract. The result showed that methanolic *S. roxburghii* extract had moderate antioxidant activity with both different methods of detection (FRAP values 57.31 ± 4.29 µg AAE/ mg and EC_{50} value 528.10 µg/ml of DPPH scavenging activity). The result revealed that antioxidant in methanolic *S. roxburghii* extract was higher than that in other medicinal plants such as *Amaranthus viridis* (EC_{50} value 2413.91 µg/ml), *Cordia dichotoma* (EC_{50} value 1145.40 µg/ml), *Sonchus oleraceus* (EC_{50} value 846.75 µg/ml), and *Citrus limon* (EC_{50} value 0.618 mg/ml)[27,28]. This study suggested that the methanolic extract of *S. roxburghii* had substantial antioxidant properties, as evidenced by its ability to reduce ferric ions and scavenge free radicals.

Cancer is a multifactorial disease, with one of the key factors being the accumulation of oxidants. High amounts of oxidants, such as ROS, are known to harm DNA, proteins, and lipids. This damage caused alterations in critical genes, which might lead to the development of cancer cells. Maintaining a balance between oxidants and antioxidants is crucial for preventing oxidative stress and its associated risks[29]. A quarter of all cancer cases worldwide and one-third of cancer-related fatalities are caused by gastrointestinal cancers. Eastern Asians had the highest lifetime risk stomach and liver cancer. This was due to the long-standing high prevalence of certain major risk factors such as smoking, excessive alcohol consumption, *Helicobacter pylori* infection, and liver flukes *Opisthorchis viverrini* infection[30]. Our study investigated the anti-cancer potential of methanolic *S. roxburghii* extract on two different gastrointestinal cancer cell lines. The result demonstrated that methanolic extract of *S. roxburghii* exhibited cytotoxic effects on both AGS and KKU-100 cancer cell lines. AGS cells are more sensitive to the extract compared to KKU-100 with IC_{50} value of 161.6 µg/ml and 204.5 µg/ml, respectively. Based on the observed sensitivity of AGS cells to the methanolic extract

of *S. roxburghii*, AGS cells were selected to investigate the apoptotic effects using flow cytometry. According to Annexin V-FITC/PI double staining, the rates of apoptosis at 160 and 320 µg/ml of crude methanolic extract in AGS were (7.0 ± 4.2) %, and (6.9 ± 3.6) %, respectively. The results suggested that crude methanolic extract of *S. roxburghii* inhibited gastrointestinal cancer cell growth via inducing apoptosis in the cells.

Conclusion

Methanolic extract of *S. roxburghii* flowers is a rich source of phenolic and flavonoid compounds with significant antioxidant and anti-cancer properties. The extract demonstrated moderate antioxidant activity with both FRAP and DPPH scavenging activity. Additionally, the methanolic extract exhibited cytotoxic effects on AGS and KKU-100 gastrointestinal cancer cell lines by induced apoptosis. These findings highlight the potential of *S. roxburghii* as a valuable resource for developing therapeutic agents against oxidative stress and cancer. Its safety, effectiveness, and mechanisms of action in humans, including clinical trial should all be further investigated in more thorough research.

Take home messages

The methanolic extract of *S. roxburghii* showed promising antioxidant and anticancer activities, making it a potential candidate for further research in antioxidant and cancer therapy.

Conflicts of interest

The authors declare no potential conflicts of interest.

Acknowledgements

We would like to acknowledge Faculty of Associated Medical Sciences, Khon Kaen University, Thailand and the Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on His Expert Program Year 2021 from Graduate School, Khon Kaen University, Thailand.

References

1. Krumova K, Cosa G. Overview of reactive oxygen species. In: Nonell S, and Flors C editor. Singlet Oxygen: Applications in Biosciences and Nanosciences. The Royal Society of Chemistry; 2016. p. 1-21.
2. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2010; 4(8): 118.
3. Huang J, Lucero-Prisno III DE, Zhang L, Xu W, Wong SH, Ng SC, et al. Updated epidemiology of gastrointestinal cancers in East Asia. Nat Rev Gastroenterol Hepatol 2023: 1-17.
4. Kay J, Thadhani E, Samson L, Engelward B. Inflammation-induced DNA damage, mutations and cancer. DNA Repair (Amst) 2019; 83: 102673.
5. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines (Basel) 2018; 5(3): 93.
6. Aliarab A, Abroon S, Rasmi Y, Aziz SG-G. Application of sesquiterpene lactone: A new promising way for cancer therapy based on anticancer activity. Biomed Pharmacother 2018; 106: 239-46.
7. Lopes CM, Dourado A, Oliveira R. Phytotherapy and nutritional supplements on breast cancer. Biomed Res Int 2017; 2017.
8. Habtemariam S, Lentini G. Plant-derived anticancer agents: Lessons from the pharmacology of geniposide and its aglycone, genipin. Biomedicines 2018; 6(2): 39.
9. Dehelean CA, Marcovici I, Soica C, Mioc M, Coricovac D, Iurciuc S, et al. Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. Molecules 2021; 26(4): 1109.

10. Roy AC, Prasad A, Priya K, Das P, Singh S, Ghosh C, et al. Anticancer effect of antioxidant-rich methanolic extract of Rauvolfia serpentina (L.) Benth. ex Kurz leaves in HepG2 and HeLa cells: A mechanistic insight. Biocatal Agric Biotechnol 2023; 50: 102674.
11. Sehim AE, Amin BH, Yosri M, Salama HM, Alkhalifah DH, Alwaili MA, et al. GC-MS Analysis, Antibacterial, and Anticancer Activities of Hibiscus sabdariffa L. Methanolic Extract: In Vitro and In Silico Studies. Microorganisms 2023; 11(6): 1601.
12. Wahab BAA, Alamri ZZ, Jabbar AA, Ibrahim IAA, Almaimani RA, Almasmoum HA, et al. Phytochemistry, antioxidant, anticancer, and acute toxicity of traditional medicinal food Biarum bovei (Kardeh). BMC Complement Med Ther 2023; 23(1): 283.
13. Syed Najmuddin SUF, Romli MF, Hamid M, Alitheen NB, Nik Abd Rahman NMA. Anti-cancer effect of Annona Muricata Linn Leaves Crude Extract (AMCE) on breast cancer cell line. BMC Complement Altern Med 2016; 16: 1-20.
14. Morikawa T, Chaipech S, Matsuda H, Hamao M, Umeda Y, Sato H, et al. Anti-hyperlipidemic constituents from the bark of Shorea roxburghii. J Nat Med 2012; 66: 516-24.
15. Zhang W, Meng J, Liu Q, Makinde EA, Lin Q, Olatunji OJ. Shorea roxburghii leaf extract ameliorates hyperglycemia induced abnormalities in high fat/fructose and streptozotocin induced diabetic rats. Chem Biodivers 2020; 17(3): e1900661.
16. Hu J, Tong C, Zhou J, Gao C, Olatunji OJ. Protective Effects of Shorea roxburghii Phenolic Extract on Nephrotoxicity Induced by Cyclophosphamide: Impact on Oxidative Stress, Biochemical and Histopathological Alterations. Chem Biodivers 2022; 19(5): e202200053.
17. Ninomiya K, Chaipech S, Kunikata Y, Yagi R, Pongpiriyadacha Y, Muraoka O, et al. Quantitative determination of stilbenoids and dihydroiso-coumarins in Shorea roxburghii and evaluation of their hepatoprotective activity. Int J Mol Sci 2017; 18(2): 451.
18. Moriyama H, Moriyama M, Ninomiya K, Morikawa T, Hayakawa T. Inhibitory effects of oligostilbenoids from the bark of Shorea roxburghii on malignant melanoma cell growth: Implications for novel topical anticancer candidates. Biol Pharm Bull 2016; 39(10): 1675-82.
19. Patcharamun W, Sichaem J, Siripong P, Khumkratok S, Jong-aramruang J, Tip-pyang S. A new dimeric resveratrol from the roots of Shorea roxburghii. Fitoterapia 2011; 82(3): 489-92.
20. Thitipramote N, Maisakun T, Chomchuen C, Pradmeeteekul P, Nimkamnerd J, Vongnititorn P, et al. Bioactive compounds and antioxidant activities from pomegranate peel and seed extracts. Food Appl Biosci J 2019; 7(3): 152-61.
21. Teoh ES, Teoh ES. Secondary metabolites of plants. Medicinal orchids of Asia 2016: 59-73.
22. Guerriero G, Berni R, Muñoz-Sanchez JA, Apone F, Abdel-Salam EM, Qahtan AA, et al. Production of plant secondary metabolites: Examples, tips and suggestions for biotechnologists. Genes 2018; 9(6): 309.
23. Rahman MM, Rahaman MS, Islam MR, Rahman F, Mithi FM, Alqahtani T, et al. Role of phenolic compounds in human disease: current knowledge and future prospects. Molecules 2021; 27(1): 233.
24. Subramanian R, Subbramaniyan P, Raj V. Antioxidant activity of the stem bark of Shorea roxburghii and its silver reducing power. SpringerPlus 2013; 2: 1-11.

25. Morikawa T, Chaipech S, Matsuda H, Hamao M, Umeda Y, Sato H, et al. Antidiabetogenic oligostilbenoids and 3-ethyl-4-phenyl-3, 4-dihydroisocoumarins from the bark of Shorea roxburghii. Bioorg Med Chem 2012; 20(2): 832-40.
26. Kruk J, Aboul-Enein BH, Duchnik E, Marchlewicz M. Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. J Physiol Sci 2022; 72(1): 19.
27. Lin K, Yeh H, Lin S-Y, Yang C, Tsai H-J, Tsai J, et al. Antioxidant activities of methanol extracts from selected Taiwanese herbaceous plants. J Food Nutr Res 2014; 2(8): 435-42.
28. Acheampong A, Okyem S, Akoto CO, Baah KA. Antioxidant, antimicrobial and FTIR analysis of methanol root extract of Cnestis ferruginea and ethanol root extract of Citrus limon. J Pharmacogn Phytochem 2018; 7(4): 2938-46.
29. Zhao Y, Ye X, Xiong Z, Ihsan A, Ares I, Martínez M, et al. Cancer metabolism: the role of ROS in DNA damage and induction of apoptosis in Cancer Cells. Metabolites 2023; 13(7): 796.
30. Wang S, Zheng R, Li J, Zeng H, Li L, Chen R, et al. Global, regional, and national lifetime risks of developing and dying from gastrointestinal cancers in 185 countries: a population-based systematic analysis of GLOBOCAN. Lancet Gastroenterol Hepatol 2024; 9(3): 229-37.