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Original Article

Free Radical Scavenging and Anticancer Activities of Methanolic Twig Extract of Annonaceae Plant

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ABSTRACT

Introduction: The Annonaceae family represents a promising source of bioactive compounds with potential therapeutic applications. This study aimed to comprehensively evaluate the antioxidant and anticancer potential of methanolic twig extracts from seven Annonaceae species. **Methods:** Twig specimens from *Cananga latifolia*, *Goniothalamus elegans*, *Goniothalamus tamirensis*, *Melodorum fructicosum*, *Polyalthia dubia*, *Polyalthia cerasoides*, and *Uvaria fauveliana* were subjected to standardized methanolic extraction. Antioxidant activity was assessed using DPPH radical scavenging assay. Anticancer potential was evaluated through Sulforhodamine B assay against three human cancer cell lines and normal human dermal fibroblasts at 25 µg/mL concentration. **Results:** *Goniothalamus elegans* demonstrated exceptional antioxidant activity (IC_{50} = 5.62 ± 1.21 µg/mL) comparable to ascorbic acid. In anticancer evaluation, *G. elegans* exhibited remarkable cytotoxicity against MCF-7 (95.19 ± 0.62%) and HeLa (94.46 ± 1.69%) cancer cells while demonstrating exceptional selectivity with minimal toxicity toward normal cells (19.72 ± 1.19%). *Melodorum fructicosum* showed highest activity against HT-29 colon cancer cells (75.84 ± 1.57%). **Conclusions:** *Goniothalamus elegans* demonstrated exceptional dual bioactivity with both potent antioxidant properties and selective anticancer effects. The remarkable selectivity indices and broad-spectrum anticancer activity suggest significant clinical potential. These findings provide scientific validation for traditional medicinal uses of Annonaceae species.

KEYWORDS: Annonaceae, Anticancer, Antioxidant activity, Cancer cell selectivity, DPPH assay, *Goniothalamus elegans*

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INTRODUCTION

Cancer represents one of the most significant global health challenges of the 21st century, with an estimated 20 million new cases diagnosed and 9.7 million deaths recorded worldwide in 2022[1]. The burden is projected to reach 35 million new cases by 2050, representing a 77% increase from current levels[2]. This alarming trend highlights the urgent need for innovative therapeutic approaches that are both effective and accessible.

The pathogenesis of cancer is intricately linked to oxidative stress, a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms[3]. Free radicals play crucial roles in cell signaling under physiological conditions. However, excessive accumulation leads to oxidative damage of critical biomolecules, contributing to carcinogenesis and tumor progression[4].

The limitations and adverse effects associated with conventional cancer chemotherapy have prompted intensive research into natural products as alternative or complementary therapeutic agents[5]. Plant-derived compounds offer several advantages, including structural diversity, reduced toxicity profiles, and multi-target mechanisms of action[6].

The Annonaceae family, comprising more than 2,400 species distributed across 120 genera, represents a particularly promising source of bioactive compounds with anticancer potential[7]. This family of tropical and subtropical trees has been extensively used in traditional medicine systems for treating various ailments, including cancer-related conditions[8].

Annonaceous acetogenins represent a unique class of natural products found almost exclusively in the Annonaceae family[9]. These compounds have shown potent cytotoxic effects against various cancer cell lines through inhibition of mitochondrial complex I and induction of apoptosis[10].

Despite extensive traditional use and promising preliminary studies, systematic investigations of their free radical scavenging and anticancer activities remain limited. Therefore, this study aimed to comprehensively evaluate the free radical scavenging activity and anticancer potential of methanolic twig extracts from seven Annonaceae species.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Seven Annonaceae specimens were obtained from Phu Chong-Na Yoy National Park, Na Chaluai District, Ubon Ratchathani Province, Thailand. The collected species included *Cananga latifolia*, *Goniothalamus elegans*, *Goniothalamus tamirensis*, *Melodorum fructicosum*, *Polyalthia dubia*, *Polyalthia cerasoides*, and *Uvaria fauveliana*. Botanical authentication was carried out by U-sa Thonagpairoj, and voucher specimens were deposited at the Herbarium, Department of Biology, Mahasarakham University[11].

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Plant Material Preparation and Extraction

Fresh twig specimens were thoroughly cleaned with distilled water and air-dried in shade for 7 days under controlled environmental conditions (temperature 25 ± 2°C, relative humidity 60 ± 5%).[12] Samples were further dried in a forced-air oven at 50°C until constant weight was achieved. The dried materials were ground into coarse powder and stored in airtight containers until extraction.

Extraction Procedure

Methanolic extraction was performed using standardized maceration protocol. Briefly, 200 g of powdered twig material was extracted with 1000 mL of analytical-grade methanol at room temperature for 5 consecutive days with periodic agitation. Extracts were filtered, concentrated using rotary evaporation at 40°C, and dried in vacuum oven. Extraction yields were calculated as percentage weight/weight on dry weight basis. Crude extracts were stored at -20°C until biological activity evaluation.

Antioxidant Activity Assessment

Free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay[13]. Stock solutions were prepared by dissolving crude extracts in 10% DMSO to achieve 10 mg/mL concentration. Serial dilutions were prepared to obtain working concentrations ranging from 10 to 1000 µg/mL. DPPH working solution was freshly prepared at 152 µM concentration. For the assay, 750 µL of each extract concentration was mixed with 750 µL of DPPH solution and incubated in darkness at room temperature for 20 minutes. Absorbance was measured at 517 nm. Ascorbic acid was used as positive control. IC_{50} values were determined by linear regression analysis.

Anticancer Activity Evaluation

Anticancer activity was evaluated using the Sulforhodamine B (SRB) colorimetric assay on three human cancer cell lines: MCF-7 breast adenocarcinoma (CLS No.300273), HT-29 colon carcinoma (CLS No.300215), and HeLa cervical carcinoma (ATCC CCL-2), along with normal human dermal fibroblasts (HDFa : Invitrogen C-013-5C).[14] Cells were cultured in DMEM supplemented with 10% FBS and antibiotics. For cytotoxicity assessment, cells were seeded in 96-well plates at appropriate densities and incubated for 24 hours. Test extracts were added at 25 µg/mL concentration and incubated for 72 hours. Following treatment, cells were fixed with trichloroacetic acid, stained with SRB, and absorbance measured at 540 nm. Percentage inhibition was calculated relative to untreated controls. All cell lines were obtained from authenticated sources. All experimental procedures were conducted in accordance with institutional biosafety guidelines and good laboratory practices.

Statistical Analysis

Data are expressed as mean ± standard deviation from three independent experiments. Statistical analysis employed one-way ANOVA followed by Duncan's multiple range test. Significance was set at $p < 0.05$.

RESULTS

Extraction Yields

Methanolic extraction yielded varying amounts of crude extracts, ranging from 1.96% to 11.05% (w/w). *Polyalthia dubia* demonstrated the highest extraction yield (11.05%), while *Melodorum fructicosum* produced the lowest yield at 1.96% (Table 1).

Table 1. Percentage yield of methanolic extract of Annonaceae twig species

No.	Scientific name	% yield
1	*Cananga latifolia*	5.71
2	*Goniothalamus elegans*	5.86
3	*Goniothalamus tamirensis*	5.59
4	*Melodorum fructicosum*	1.96
5	*Polyalthia dubia*	11.05
6	*Polyalthia cerasoides*	3.15
7	*Uvaria fauveliana*	4.59

Table 2. IC_{50} values of DPPH assay of methanolic extract of Annonaceae twig species

No.	Scientific name	IC_{50} (µg/mL)
1	*C. latifolia*	$46.91 \pm 3.81^{d*}$
2	*G. elegans*	5.62 ± 1.21^{b}
3	*G. tamirensis*	74.05 ± 9.01^{e}
4	*M. fructicosum*	36.43 ± 0.55^{c}
5	*P. dubia*	4595.11 ± 60.71^{g}
6	*P. cerasoides*	154.79 ± 6.17^{f}
7	*U. fauveliana*	91.43 ± 4.42^{e}
8	Ascorbic acid	3.75 ± 0.21^{a}

*According to Duncan's test, mean values ± standard error followed by different letter in the same column are significantly different at $p < 0.05$.

Table 3. Inhibitory capacity in cancer cell and normal cell of methanolic extract of Annonaceae twig species

No.	Scientific name	% inhibition at 25 µg extract/mL			
		MCF-7	HT-29	HeLa	Normal cell
1	*C. latifolia*	$25.47 \pm 3.87^{e*}$	40.73 ± 5.27^{c}	12.87 ± 2.98^{c}	nd
2	*G. elegans*	95.19 ± 0.62^{a}	50.78 ± 4.00^{b}	94.46 ± 1.69^{a}	19.72 ± 1.19^{a}
3	*G. tamirensis*	63.24 ± 4.11^{c}	24.20 ± 2.91^{d}	25.55 ± 18.16^{c}	20.09 ± 4.09^{a}
4	*M. fructicosum*	85.09 ± 1.84^{b}	75.84 ± 1.57^{a}	60.06 ± 10.58^{b}	9.76 ± 3.07^{b}
5	*P. dubia*	80.46 ± 2.37^{b}	37.89 ± 1.12^{c}	22.13 ± 3.63^{c}	26.15 ± 3.23^{a}
6	*P. cerasoides*	44.06 ± 7.76^{d}	19.25 ± 3.34^{d}	17.03 ± 7.16^{c}	nd
7	*U. fauveliana*	68.19 ± 1.37^{c}	27.48 ± 1.44^{d}	33.31 ± 5.01^{c}	25.70 ± 3.35^{a}

*According to Duncan's test, mean values ± standard error followed by the different letter in the same column are significantly different at $p < 0.05$. nd = not detected

Free Radical Scavenging Activity

The DPPH radical scavenging assay revealed substantial variation in antioxidant potential, with IC_{50} values ranging from 5.62 to 4595.11 µg/mL (Table 2). *Goniothalamus elegans* exhibited exceptional antioxidant activity (IC_{50} = 5.62 ± 1.21 µg/mL), comparable to ascorbic acid (IC_{50} = 3.75 ± 0.21 µg/mL). *Polyalthia dubia* showed the poorest antioxidant potential (IC_{50} = 4595.11 ± 60.71 µg/mL).

Anticancer Activity

Evaluation of anticancer activity against three human cancer cell lines revealed significant cytotoxic potential (Table 3). *Goniothalamus elegans* demonstrated exceptional anticancer activity against MCF-7 breast cancer cells (95.19 ± 0.62% inhibition) (Figure 1.) and HeLa cervical cancer cells (94.46 ± 1.69% inhibition). Remarkably, *G. elegans* showed exceptional selectivity with minimal toxicity toward normal cells (19.72 ± 1.19% inhibition), representing a selectivity ratio of approximately 4.8:1. *Melodorum fructicosum* displayed potent activity against HT-29 colon cancer cells (75.84 ± 1.57%) and MCF-7 cells (85.09 ± 1.84%).

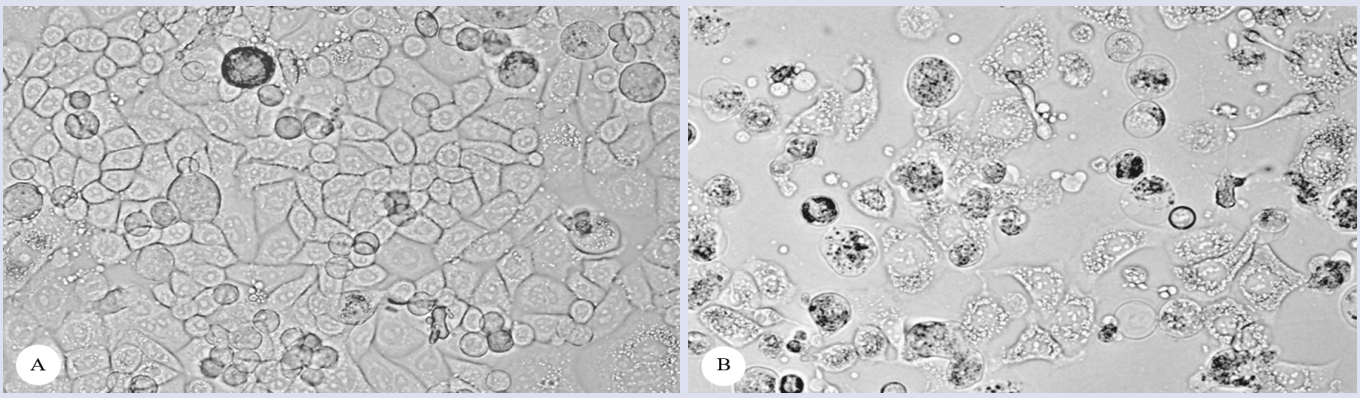

Figure 1. Morphological changes in MCF-7 cells. A: Non-cytotoxic MCF-7 cells (control). B: MCF-7 cells treated with methanolic *G. elegans* twig extract at 25 µg/mL showing significant cell death and morphological alterations.

DISCUSSION

This study represents the first comprehensive evaluation of the free radical scavenging and anticancer activities of methanolic twig extracts from seven Annonaceae species, providing valuable insights into their therapeutic potential. The substantial variation in extraction yields (1.96% to 11.05%) reflects inherent phytochemical diversity within this family[15].

The exceptional antioxidant activity demonstrated by *Goniothalamus elegans* represents a significant finding in natural product research. Its IC_{50} value (5.62 µg/mL) was comparable to ascorbic acid, suggesting the presence of potent phenolic compounds and alkaloids. It should be noted that compound attribution in this study is based on known phytochemistry of the *Goniothalamus* genus reported in the literature, rather than direct chemical identification in this study. Comprehensive phytochemical analysis is planned as part of future bioassay-guided fractionation work to identify and characterize the specific bioactive constituents. The observed activities align with compounds known to occur in *Goniothalamus* species[16].

The anticancer activities observed align with the known presence of annonaceous acetogenins in these species. These unique compounds have demonstrated remarkable cytotoxic effects through inhibition of mitochondrial complex I[9]. The exceptional selectivity of *G. elegans* (selectivity ratio approximately 4.8:1) is particularly noteworthy, as many conventional chemotherapeutic agents lack discrimination between cancer and normal cells.

The morphological changes observed in MCF-7 cells treated with *G. elegans* extract provide visual confirmation of cytotoxic effects. Cell shrinkage, membrane blebbing, and loss of cellular adhesion are characteristic features of apoptotic cell death, suggesting induction of programmed cell death rather than necrotic cell death[17].

Study limitations include evaluation of crude extracts at a single concentration for anticancer screening. This screening-level design was deliberately chosen to efficiently identify species with promising bioactivity for further investigation. Comprehensive IC_{50} determination and detailed dose-response analyses are planned for future work with the most active candidates. Additionally, the study evaluated crude extracts without bioassay-guided fractionation. Future studies should include comprehensive dose-response analyses, bioassay-guided fractionation to identify specific bioactive compounds, and *in vivo* validation using appropriate animal models. The development of standardized extraction protocols and sustainable cultivation approaches will be essential for pharmaceutical development.

CONCLUSION

This comprehensive study demonstrates the significant therapeutic potential of Annonaceae twig extracts, particularly *Goniothalamus elegans* and *Melodorum fructicosum*, as sources of novel anticancer agents with favorable selectivity profiles. The remarkable cancer cell selectivity exhibited by *G. elegans*, combined with its potent antioxidant activity, positions it as a priority candidate for further development. The species-specific activity patterns observed underscore the importance of systematic bioprospecting approaches and validate the ethnopharmacological significance of the Annonaceae family. Future research priorities should focus on bioassay-guided isolation of active compounds, mechanistic studies, and *in vivo* validation of therapeutic potential. The findings contribute significantly to evidence supporting the therapeutic potential of Annonaceae species and provide a strong foundation for continued investigation as a source of novel anticancer agents.

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