Antioxidant Activity and Cytotoxicity Against HepG2 Liver Cancer Cells in vitro of Phitsawat, a Thai Traditional Medicine Formula

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Abstract

This study aimed to analyze the chemical composition, antioxidant activity, and cytotoxicity against HepG2 liver cancer cells of the Thai Traditional medicine formula Phitsawat. Phitsawat extract demonstrated antioxidant capacity in DPPH, ABTS, and FRAP assays, although lower than the positive control Trolox. The ethanol extract of the sample had a total phenolic content (TPC) of 39.65 ± 0.39 mg GAE/g extract, a total flavonoid content (TFC) of 9.96 ± 0.19 mg QE/g extract, and a total tannin content (TTC) of 39.43 ± 2.98 mg TAE/g extract. Phytochemical analysis revealed the presence of cannabinoids, monoterpenes, sesquiterpenes, and phytosterols. The extract exhibited a concentration-dependent inhibitory effect on HepG2 cell proliferation, with significant cytotoxicity observed at 10 μg/mL. Microscopic evaluation showed that the extract caused morphological changes in HepG2 cells, including cell rounding, shrinkage, and loss of attachment. These findings provide fundamental data on the phytochemical profile and anti-cancer potential of the Phitsawat herbal formula, supporting its further development as an effective and safe Thai traditional medicine.

Keywords: Thai Traditional medicine, Phytochemicals, Antioxidant, Cytotoxicity, HepG2 cells

1. Introduction

Cancer is a major global public health problem. The World Health Organization reported in 2020 that there were 19.3 million new cancer cases and 10 million cancer deaths worldwide, with a projected increase to 28.4 million new cases by 2040 [1]. In Thailand, cancer has been the leading cause of death since 1999, with mortality rates increasing from 78.9 per 100,000 population in 2003 to 91.1 per 100,000 in 2010 [2].

Cancer is caused by genetic mutations that lead to uncontrolled cell division, invasion of surrounding tissues, and metastasis to other organs [3].While chemotherapy and radiation therapy are the main treatment modalities, they often have severe side effects on normal cells. Therefore, there is growing interest in studying natural compounds, particularly phenolic and

flavonoid compounds, which possess antioxidants and various other bioactivities, including anti-cancer properties [4].

The human hepatocellular carcinoma cell line HepG2 is commonly used to study the anti-cancer effects of natural extracts, as it closely resembles human liver cancer cells. Moreover, more than 50% of the drugs used to treat cancer patients are derived from natural sources [5].

Phitsawat, Thai Traditional medicine formula, contains several medicinal plants, including *Myristica fragrans*, *Elettaria cardamomum*, *Syzygium aromaticum*, and *Cannabis sativa*, all of which have reported pharmacological activities, including antioxidant and anti-cancer effects [6-7]. However, there is a lack of scientific evidence on the chemical composition and pharmacological properties of the Phitsawat formula [8]. This study aims to analyze the chemical composition, antioxidant activity, and cytotoxicity against HepG2 cells of the Phitsawat herbal extract, providing fundamental data for the development of an effective and safe Thai traditional medicine.

Research Objective

The objectives of this study were to analyze the phytochemical composition, antioxidant properties, and cytotoxic effects against liver cancer cells of the traditional Thai herbal formula Phitsawat.

2. Materials and methods

1. Sample Preparation and Extraction

The Phitsawat herbal formula used in this study contained 8 herbs in varying proportions: cardamom 3.70%, nutmeg flowers 3.70%, Borneo camphor 3.70%, aloe vera 3.70%, nutmeg 3.70%, cloves 14.81%, camphor 14.81% and cannabis 51.85%. Dried and ground Phitsawat herbal powder was extracted with 95% ethanol solvents using sonication-assisted extraction. The extracts were filtered, concentrated under vacuum, and stored in amber glass bottles for further analysis.

2. Determination of antioxidant activity

The antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays, with Trolox employed as the positive control. These methodological approaches were described in the study conducted by Yongram et al., 2025 [9].

3. Phytochemical analysis

The phytochemical composition of the cannabis extract was investigated via GC-MS analysis, adopting the instrumental conditions described by Palmieri et al. in their 2021 publication [10]. Additionally, the total phenolic and tannin contents were determined using the Folin-Ciocalteu assay, while the total flavonoid content was quantified via the aluminum chloride colorimetric method, as outlined in the studies by Sripan et al. (2022) [11] and Champatasi et al. (2022) [12], respectively.

4 Cytotoxicity assay

The cytotoxicity of Phitsawat extracts against HepG2 cells was determined using the MTT assay. HepG2 cells were exposed to various concentrations (0.5-10 μg/mL) of the treatments for 24 hours. Cell viability was measured by absorbance at 490 nm and expressed as a percentage compared to the Negative control (DMSO). Morphological changes of HepG2 cells were also observed under an inverted microscope after 24 hours of treatment. The results indicate that the extracts exhibit concentration-dependent cytotoxicity against HepG2 cells, and the treatments caused morphological alterations in the cells [13].

3. Results

3.1 Antioxidant activity

The Phitsawat ethanol extract demonstrated antioxidant activity, with DPPH, ABTS, and FRAP IC_{50} values of 134.64±1.85 μg/mL, 48.09±1.41 μg/mL, and 60.24±2.16 mmol/100g extract, respectively. However, the antioxidant potency was lower compared to the Trolox positive control.

Table 1 Antioxidant activity of the Phisawat formulas extracted.

Solvent	antioxidant activity		
	DPPH; IC_{50} (μg/ml)	**ABTS; IC_{50} (μg/ml)**	**FRAP; IC_{50} (mmol/100g extract)**
EtOH	134.64±1.85^{e}	48.09±1.41^{g}	60.24±2.16^{d}
Trolox	7.35±0.04^{a}	6.06±0.05^{a}	1642.08±34.39^{a}

Note: Data are presented as mean ± SD of three replicates from three independent experiments. Values within a column with different subscript letters are significantly different at $p < 0.05$.

3.2 Phytochemical analysis

The phytochemical analysis results presented in Table 2 show that the ethanol extract of the sample had a total phenolic content (TPC) of 39.65 ± 0.39 mg GAE/g extract, a total flavonoid content (TFC) of 9.96 ± 0.19 mg QE/g extract, and a total tannin content (TTC) of 39.43 ± 2.98 mg TAE/g extract.

Table 2 Results of total phenolic determination Total flavonoids and total tannins**.**

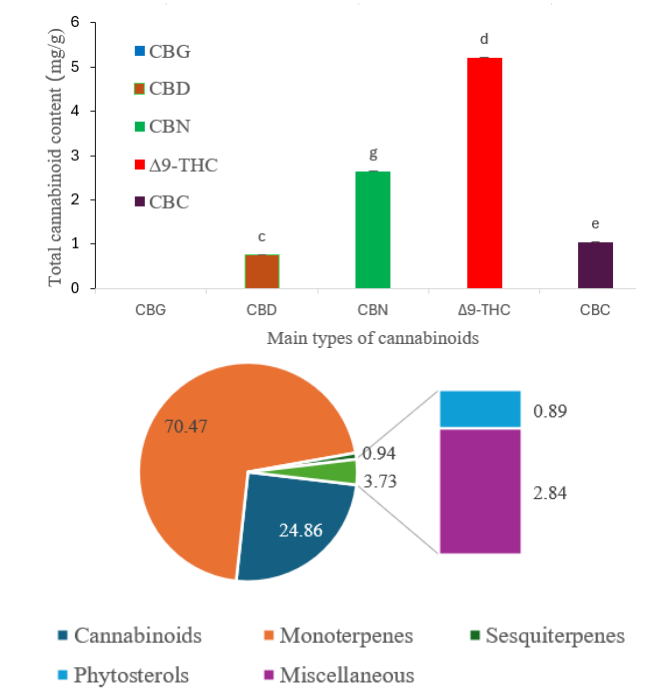
Solvent	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	TTC (mg TAE/g extract)
EtOH	39.65±0.39^{b,c}	9.96±0.19^{f}	39.43±2.98^{c}

Note: Values are expressed as mean ± SD (n = 3) from triplicate experiments. Means with different letters in a column were significantly different at the level of $p < 0.05$.

A B

Figure 1 shows the total cannabinoid content of Phisawat formulations, with Δ9-THC having the highest concentration, followed by CBN, CBD, and CBG. The phytochemical analysis revealed the presence of various compounds in the extract**,** including cannabinoids, monoterpenes, sesquiterpenes, and phytosterols. The major cannabinoids identified were Δ9-THC (16.97%). Among the monoterpenes, (+)-2-Bornanone was the most abundant compound, accounting for 46.63% of the total peak area. The presence of these diverse phytochemicals suggests the potential therapeutic properties of the Phisawat formulations.

Figure 1 Total cannabinoid content (A) and Chemical composition of the Pisawat herbal formula analyzed by GC-MS technique (B).



A **B**

3.3 Inhibition of HepG2 cell proliferation

Microscopic analysis revealed significant morphological changes in HepG2 cells when treated with Phitsawat ethanol extract for 24 hours. The control group treated with DMSO showed normal epithelial-like HepG2 cell morphology and adherence. However, increasing concentrations of the ethanol extract (0.5-10 µg/mL) led to progressive cell death characteristics, including rounding, shrinkage, and loss of surface attachment, resulting in decreased cell density, particularly at concentrations ≥5 µg/mL presented in Figure 3-4**.**

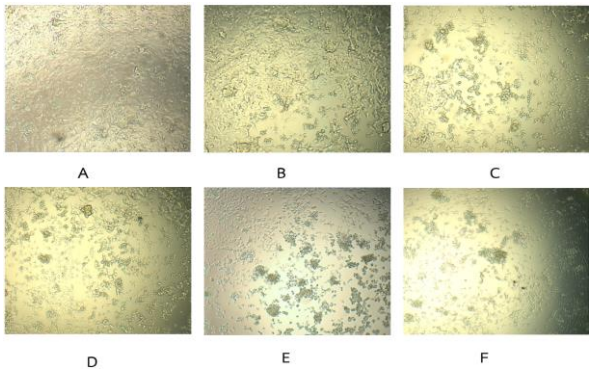
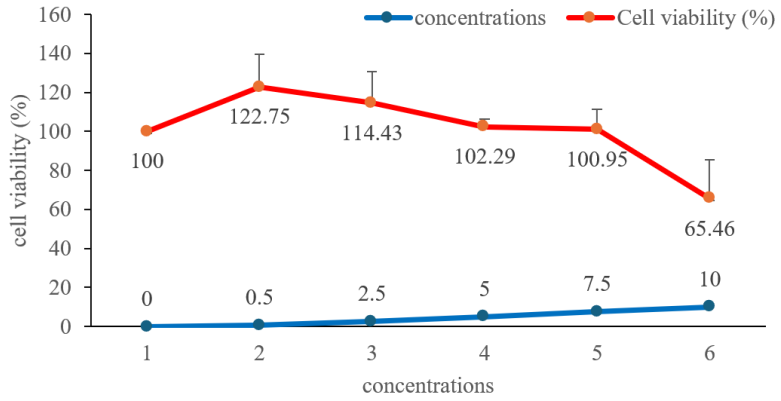


Figure 3 Inverted phase contrast microscopy showing the effects of ethanol extract of Phitsawat on HepG2 cells at 24 h. (A) Negative control (DMSO), (B) 0.5 µg/mL, (C) 2.5 µg/mL, (D) 5 µg/mL, (E) 7.5 µg/mL, (F) 10 µg/mL.

4. Inhibition of HepG2 cell proliferation

The Phitsawat ethanol extract exhibited a dose-dependent effect on HepG2 cell proliferation, with a slight proliferative effect at lower concentrations (0.5-2.5 µg/mL) and a significant inhibition of cell viability by 65.46±19.79% at 10 µg/mL.

Figure 4 The effects of Phitsawat ethanol extract on HepG2 cell viability



Note: % cell viability was measured by MTT assay. Data are presented as mean ± SD of three replicates from three independent experiments.

CONCLUSION AND FUTURE WORK

The study demonstrated that Phitsawat herbal formula possesses notable antioxidant properties and cytotoxic effects against HepG2 liver cancer cells. Chemical analysis revealed significant levels of bioactive compounds, including cannabinoids (primarily Δ9-THC at 16.97%) and (+)-2-Bornanone (46.63%), along with phenolics, flavonoids, and tannins. The extract showed dose-dependent cytotoxicity against HepG2 cells, with significant inhibition at 10 µg/mL. Future research should investigate the molecular mechanisms of action, potential synergistic effects between compounds, and conduct in vivo studies to validate its therapeutic potential. Clinical trials would be necessary to establish safety and efficacy for potential development as an anti-cancer treatment.

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