

Anticancer Activity of the Thai Herbal Formula “Kerra” Versus Afatinib and Doxorubicin *In Vitro*

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Abstract: - One of the greatest challenges impeding the achievement of global health goals and afflicting millions of people each year is cancer. Prevailing cancer treatment options come with inherent risks, and significant adverse effects, and can result in financial burdens. Nowadays, many turn to alternative medicine, which have played an integral part in the treatment of a myriad of diseases especially when other approaches are ineffective or unable to be deployed. However, an opulent number of herbs seldom have reliable evidence derived from research to support their claims of phytochemical properties contributing to the alleviation of illnesses. Kerra is a Thai herbal formula that possesses immunomodulatory properties and anti-tumor activities which provides a safe, efficacious, and holistic treatment for cancer. Kerra offers therapeutic benefits without resulting in severe adverse effects, ameliorates overall welfare and quality of life for advanced care patients, and is cost-effective, allowing them to be vastly accessible for widespread use, particularly in areas facing disparities due to insufficient economic stability. This study explores the *in vitro* anticancer effect of the Kerra formula utilizing cytotoxicity assays on six cancer cell lines, compared to afatinib, a targeted therapy for non-small cell lung carcinoma, and doxorubicin, a chemotherapeutic agent. Anticancer activity was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay and the half-maximal inhibitory concentrations (IC₅₀) were calculated for the A431, A549, HepG2, HCT-116, NCI-H1975, MCF-7, and HeLa cell lines. Kerra showed the best effectiveness against HCT-116 and A431. The IC₅₀ values showing inhibition of the growth of colorectal carcinoma (HCT-116 cell line) by Kerra, afatinib, and doxorubicin are 72.96, 3.574, and 63.39 µg/ml, respectively. The IC₅₀ values for inhibition of epidermoid carcinoma (A431 cell line) by Kerra and afatinib are 96.1 and 2.449 µg/ml, respectively. The findings revealed the Kerra extract can inhibit the growth of different types of cancer cells. From our results, Kerra may be advantageous to use in conjunction with conventional cancer interventions, offer an economical solution, and improve the quality of life remarkably for end-stage cancer patients by mitigating severe adverse events.

Key-Words: - Thai herb, Anticancer, Cytotoxic, Cell culture, Phytochemistry, Traditional medicine, Cancer cell

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1 Introduction

In today's time, modern medicine has alleviated the quality of life of people around the globe and has provided accessible healthcare services. Research efforts have yielded a plethora of medical breakthroughs which have presented innovative treatments and vaccinations for diseases that in the past may have had a worse prognosis, leading to morbidity and fatal illness. Nevertheless, cancer remains an adversity where the key to optimum interventions is still unknown, and is the second leading cause of death worldwide accounting for 9.3 million deaths annually, [1] and the trend is still unfortunately increasing. The global cancer burden is predicted to escalate to 28.4 million cases in 2040, [2], instigating a threat to achieving global health goals and welfare amelioration. Devastatingly, the incidence of early-onset cancers which includes breast, tracheal, prostate cancer, etc. has risen by 79% from 1990 to 2019, [3].

Therefore, this exacerbates the impending need to enforce primary prevention, awareness of risks, screening, and search for efficient and accessible treatment approaches to deter repercussions from the burden of cancer. The latest medical practices fail to overcome many challenges despite research endeavors including high expenses and adversities associated with treatment. A new approach is needed to help modern medicine, the answer is herbal medicine. Thai herbs have been used since ancient times to ameliorate illness and symptoms. Especially since the COVID-19 pandemic paved the way for the common use of herbs to alleviate illnesses ranging from mild to severe cases, in prevailing times, an abundance of people turn to ancient herbal remedies that are deemed to be safe and are appealing because their prices are within reach for frequent utilization.

A study conducted in a Thai Buddhist temple herbal medicine hospice included cancer patients who received an herbal remedy for cancer treatment from the hospice and reported a statistically significant improvement in symptoms such as pain, fatigue, and insomnia which correlates with a better quality of life, [4].

However, in Thailand, some traditional herbal medicines are allowed to be placed on the market without official registration as a drug, and people still take these herbs albeit they may not have enough conclusive evidence to prove their efficacy and safety profiles. Conversely, Kerra is a Thai traditional herbal formulation that proved to be efficacious in tackling many diseases through a holistic approach, for people of all ages, including chronic inflammatory conditions, and has had

extensive research conducted to discover the mechanism of action behind the medical properties of the phytochemical compounds found in the formula. It originally surfaced to widespread use during the unprecedented crisis brought upon by the COVID-19 pandemic as hospitals filled to the brim with severe cases, so the redundant patients were denied hospitalization. Many desperately searched for treatment options and turned to traditional herbs, Kerra, in particular during self-isolation. Self-isolated SARS-CoV-2 patients taking Kerra capsules had no increase in the severity of illness with better treatment outcomes.

In addition to evidence shown from self-use by the general population, Kerra's mechanisms for stopping replication of the virus are known. It can inhibit inflammatory response and viral replication enzymes in SARS-CoV-2. The medicinal properties in the herbs that constitute Kerra for example are relief of fever, suppression of inflammation, and stimulation of macrophages then boosting the immune system contributing to its holistic effects.

Furthermore, research shows that Kerra possesses anti-inflammatory properties, immunomodulatory actions, and anti-tumor activities. The Kerra extract can reduce inflammation by suppressing IL-6, activate apoptosis and cell death in colorectal cancer cell lines, suppress cell proliferation of adenocarcinoma cell lines via the EIF2 signaling pathway, and may induce apoptosis in HCT116 cells through the regulation of caspase-8 and caspase-9, [5].

The Takkasila Scripture is what Kerra medicine is derived from. It has medical expertise in treating epidemics and fevers and is a highly valuable resource for Kerra. The Takkasila scripture is 1 of 14 scriptures in the Thai royal textbook of traditional medicine. Kerra has been used to treat patients with chronic diseases who have been through countless medical interventions with no hope left. It has helped patients with infectious diseases such as COVID-19, & influenza, cancer, autoimmune and inflammatory conditions such as rheumatoid arthritis, psoriasis, systemic lupus erythematosus, inflammatory bowel disease, irritable bowel syndrome, and patients with diabetes and its complications. Currently, there are limited treatment options for many of these conditions also entailing severe side effects and fees. *Tinospora crispa* (L.) Hook. f. & Thomson, an extract found in Kerra was found to be insulinotropic in cell culture and animal studies, so it can be inferred that it can be a potential drug for diabetes mellitus, [6].

An investigation of some Thai herbal medicine extracts on inhibition of cholangiocarcinoma cell

lines proved to be successful, [7]. Cholangiocarcinoma has a high incidence, especially in Northeastern Thailand and is aggressive. This shows that phytochemicals in herbs show potential benefits for investment in cancer research as potent drug options. Proteomics studies on Kerra analyzed its apoptotic pathways in colorectal cancer cell lines and revealed dose-dependent inhibition, instigation of apoptotic activity, and regulation of key proteins involved in cell death and proliferation pathways such as MYC and CDKN1A, [5]. There is sufficiently strong evidence that Kerra delivers an efficacious measure to tackle a diverse amalgamation of diseases, most significantly cancer, without severe side effects, alleviates quality of life, especially in advanced care patients, and is inexpensive, which can become extremely accessible for use on a widescale to pertain to the scarcity of vital resources. Kerra has 11 bioactive constituents as shown in Table 1.

Table 1. List of herbal extracts in the Kerra formulation

Names of the herbal extracts
<i>Dracaena loureiroi</i> Gagnep. (Jan Dang)
<i>Tarenna hoensis</i> Pit. (Jan Khao)
<i>Schumannianthus dichotomus</i> (Roxb.) Gagnep. (Kla)
<i>Caryota mitis</i> Lour. (Tao Rung)
<i>Homalomena aromatica</i> (Spreng.) Schott (Tao Giad)
<i>Momordica cochinchinensis</i> (Lour.) Spreng. (Fug Khao)
<i>Citrus aurantiifolia</i> (Lime)
<i>Combretum quadrangulare</i> Kurz. (Sa Gae)
<i>Dregea volubilis</i> (L.f.) Benth. ex Hook.f. (Gra Toong Ma Ba)
<i>Tiliacora triandra</i> (Colebr.) Diels (Ya Nang)
<i>Tinospora crispa</i> (L.) Hook. f. & Thomson (Boraped)

2 Materials and Methods

2.1 Compound Preparation

The Kerra powder from the capsules was dissolved with 100% DMSO to reach 100 mg/mL, then 2-fold serial dilution was used to get 9 concentrations. The freshly prepared solution was diluted to the final concentrations of 1250, 625, 312.5, 156.25, 78.125,

39.06, 19.53, 9.77, and 4.88 µg/mL in the culture medium with a DMSO concentration of no more than 1% (v/v).

2.2 Cell Culture

The A431, A549, HepG2, HCT-116, NCI-H1975, MCF-7, and HeLa cell lines were acquired from The American Type Culture Collection (ATCC). The A431, A549, MCF-7, and HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM). The HepG2 cells were cultured in the Eagle's Minimum Essential Medium. The HCT-116 cells were cultured in McCoy's 5A Medium. The NCI-H1975 cells were cultured in the Roswell Park Memorial Institute (RPMI) 1640 Medium. All cell types included 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The incubator conditions for cell culture were a humidified atmosphere of 5% CO₂ at a temperature of 37°C. The respective media and cell density (cells/well) of each cell line are shown in Table 2.

Table 2. Cell type, media, and cell density (cells/well) of cancer cell lines

Cell lines	Cell Types	Media	Cell Density (cells/well)
A431	Epidermoid carcinoma	Dulbecco's modified Eagle's medium	6,000
A549	Adenocarcinoma of human alveolar basal epithelial cell	Dulbecco's modified Eagle's medium	6,000
HepG2	Hepatocellular carcinoma	Eagle's Minimum Essential Medium	15,000
HCT-116	Colorectal carcinoma	McCoy's 5A Medium	6,000
NCI-H1975	Non-small cell lung carcinoma	Roswell Park Memorial Institute (RPMI) 1640 Medium	6,000
MCF-7	Human breast cancer with positive estrogen, progesterone, and glucocorticoid receptors	Dulbecco's modified Eagle's medium	6,000
HeLa	Cervical cancer	Dulbecco's modified Eagle's medium	6,000

2.3 Cytotoxicity Assay

The MTT test was used to determine cancer cell growth inhibition. The change of a tetrazolium dye to an insoluble formazan compound by viable cells was measured. Cancer cell lines were placed in 96-well plates at a density of 6×10^3 cells per well except for HepG2 which was 15×10^3 cells per well. The plates were incubated overnight at 37 °C with 5% CO₂. Then the cells were treated with 100 µL of varying concentrations of the Kerra in ethanol solution and incubated for 72 hours. Anticancer drugs, Afatinib and Lapatinib (Santa Cruz Biotechnology, USA) were the reference compounds. The culture medium was changed to 100 µL of 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) (Thermo Fisher Scientific, USA) solution (0.5 mg MTT in 1 mL

culture medium) and incubated at 37 °C for 3 hours. After that, the medium was discarded and 50 µL of DMSO was placed in each well for solubilization of formazan. The absorbance of each well was measured at 570 nm using a microplate spectrophotometer (Synergy HTX Multi-Mode reader, BioTek, USA). The half-maximal inhibitory concentration (IC₅₀) values were determined using the absorbance of Kerra-treated wells to the untreated control. All experiments were done in triplicate.

2.4 Statistical Analysis

The IC₅₀ values and coefficient of determination (R²) were calculated using the concentration of Kerra and the respective percent inhibition of each cell line from triplicate experiments using GraphPad Prism Software Version 10.0.0.

3 Results

3.1 Anticancer Activity on Cell Lines

Table 3. Cancer cytotoxic effects comparison in different cell lines

IC ₅₀ values (µg/mL)			
Cancer Cytotoxicity	Kerra	Afatinib (485.937 g/mol)	Doxorubicin (543.52 g/mol)
A431	96.1	2.449	-
HCT-116	72.96	3.574	63.39
HepG2	106.1	3.642	1032.47
MCF-7	127.8	-	350.03
A549	166.2	6.200	29
NCI-H1975	101.6	0.0777	-
HeLa	109.3	-	-

The in vitro investigation reveals that Kerra is efficacious at inhibiting the proliferation of cancer cells shown by its toxic impact on the A431, A549, HepG2, HCT-116, NCI-H1975, MCF-7, and HeLa cell lines, as shown in Table 3. Its low IC₅₀ values, lack of discrimination against cell types, and dose-dependent response support Kerra as a potential treatment method. Comparisons to afatinib and doxorubicin show that Kerra has effective anticancer activities and benefits as a natural phytochemical that plays a role as an alternative to conventional treatment. The experiment was not conducted for the MCF-7 cell line for afatinib, and the A431 and NCI-H1975 cell lines for doxorubicin. The dose-dependent inhibition activity of the Kerra solution with a 0.5% DMSO concentration on all the cell lines is shown in Figure 1, Figure 2, Figure 3,

Figure 4, Figure 5, Figure 6 and Figure 7. The IC₅₀ is represented as the mean ±SD of triplicate experiments.

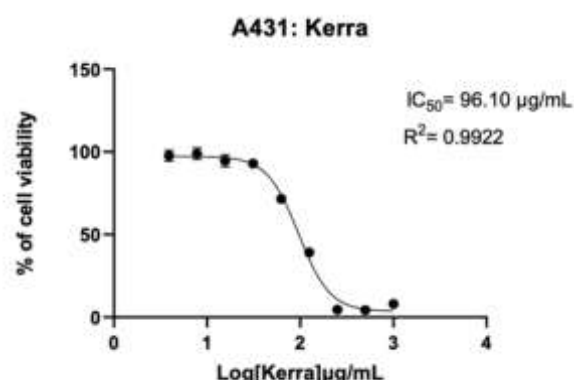


Fig. 1: Kerra on A431 cell line growth inhibition

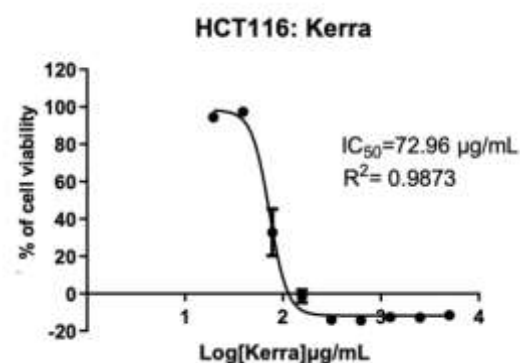


Fig. 2: Kerra on HCT116 cell line growth inhibition

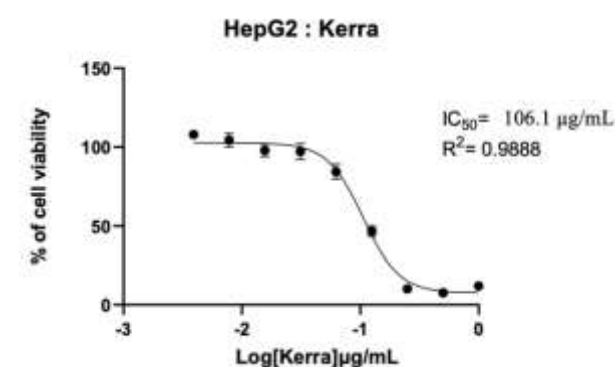


Fig. 3: Kerra on HepG2 cell line growth inhibition

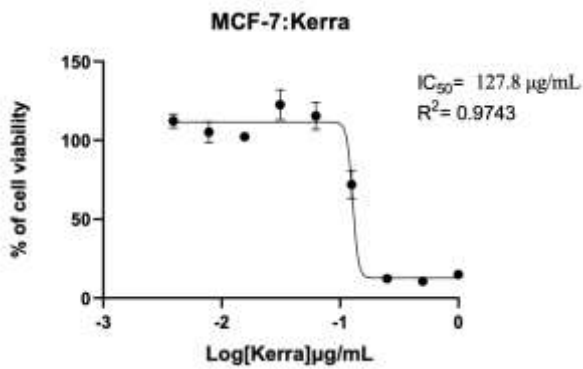


Fig. 4: Kerra on MCF-7 cell line growth inhibition

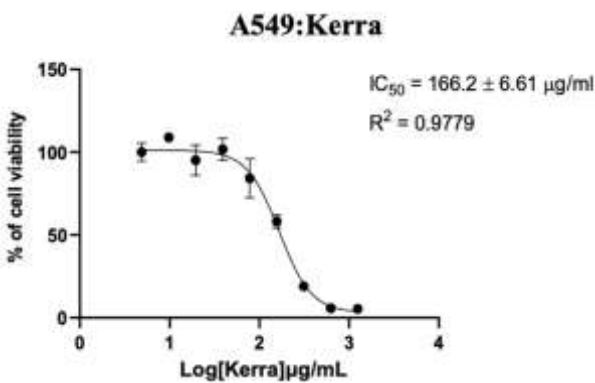


Fig. 5: Kerra on A549 cell line growth inhibition

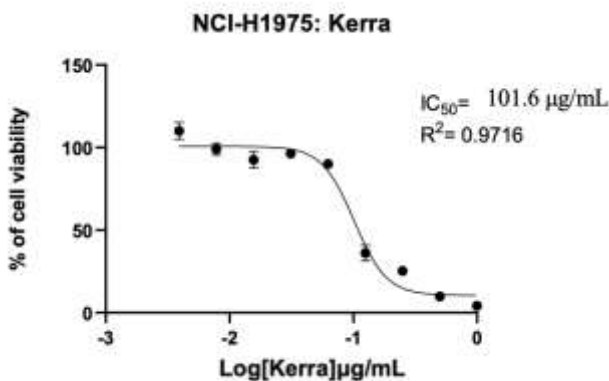


Fig. 6: Kerra on NCI-H1975 cell line growth inhibition

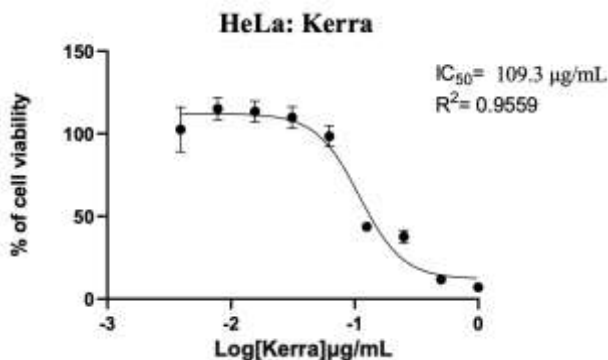


Fig. 7: Kerra on HeLa cell line growth inhibition

The IC₅₀ curves show that Kerra is effective at inhibiting the proliferation of cancer cells. Comparing between cell lines, we can see that Kerra does not discriminate inhibition between each cancer type.

4 Discussion

This study's findings open a new door towards a new way of cancer treatment, without detrimental repercussions, highlighting the anti-tumor potency of Kerra through an *in vitro* approach. Kerra may be comparable to conventional therapeutic agents in terms of its ability to kill a diverse range of cancer types. The herbal formula has identified bioactive compounds that contribute to its anti-inflammatory effects, immunomodulatory actions, and anti-tumor activities that are all beneficial to cancer cell growth inhibition. Kerra is 500mg per capsule, and many capsules can be taken orally per day along with other herbal formulas to fight cancer, which is more convenient than chemotherapy sessions requiring hospitalization.

A cohort investigating the use of traditional Chinese medicine in gastric cancer patients after receiving surgery reported a statistically significant decrease in deaths compared to patients who did not use herbs and they concluded that the medicine may be possible to use in gastric cancer patients after surgery and adjuvant chemotherapy, [8]. This enhances the strength of support behind phytochemicals as a successful measure against diverse diseases. Kerra has also been taken in self-isolated SARS-CoV-2 patients and showed improved treatment outcomes not requiring hospitalization as a result of the herbs' anti-inflammatory effects which can benefit cancer treatment via modulation of tumor environment. Adversely, some safety profiles remain unknown when it comes to using herbs in conjunction with conventional cancer therapy. A review reported potential anticancer drug-herb interactions with herbs from the 2020 Thai Herbal Pharmacopoeia, [9]. Clinical studies on Kerra are still ongoing so its efficacy and safety may be available in the future and benefit modern cancer treatment, but current preclinical studies and general use in the population have not brought about any adverse events and have drastically improved the well-being of stage IV cancer patients due to the synergistic effects of each herbal ingredient in the formula.

Kerra is derived from ancient medicinal practices that focuses on a holistic approach to allow the body to maintain in a condition which the immune system can act effectively and be protected

from impairment, to fight against diseases or pathogenic agents. In addition, ancient medicine from a myriad of herbal extracts calls for synergistic effects from the combination of its bioactive constituents, increasing potency and lowering adverse effects to a minimum. Modern medicine is based on a different mechanism, using a single chemical compound to inhibit or agonize a specific target, which inevitably leads to severe side effects impacting well-being.

An oral pill form targeted therapy that irreversibly inhibits the ErbB family of tyrosine kinases for non-small cell lung cancer with non-resistant epidermal growth factor receptor (EGFR) mutations is Afatinib, [10]. Since it is a targeted therapy, fewer side effects occur, such as diarrhea, acneiform eruptions, and stomatitis, but may vary in severity, [11], [12]. Doxorubicin is an anthracycline chemotherapeutic agent used in a variety of different cancer types but can bring along many systemic side effects especially cardiotoxicity, [13], [14], [15]. Current therapeutic options are effective but seldom come without drastic adverse events, so herbs with anti-tumor activities like Kerra should be further investigated as an alternative that can lead to potential medical breakthroughs in cancer treatment.

Moreover, in Thailand, incidences of cholangiocarcinoma, an aggressive type of cancer, are highly prevalent in the north-eastern region. Pollution from fine particulate matter that is 2.5 microns or less in diameter (PM_{2.5}) has exceeded the standards of safety for many years and has been correlated to an increase in the incidence of lung cancer. With the rise in the trend of cancer and a known cancer distribution pattern within the population, the discovery of novel interventions is warranted, and the key may be herbal drugs like Kerra.

Prevailing traditional medicine usage and ethnomedicine are not strictly monitored, leading to false information, dangerous adversities, and unsuccessful treatment. However, if competent herbs with strong evidence from research can be pushed for legal registration and commercialization, more people can look for affordable, safe, and effective treatment. Further research is warranted to investigate the efficacy and safety profiles of the herbs in larger clinical studies and its potential to become an effective and economical cancer cure with improved quality of life for frail patients in the real world. Kerra has high potential and supporting evidence for utilization as an amalgamation with conventional or other herbal approaches, or as a successful alternative for those who do not want to

risk undesirable side effects and can have improved well-being despite illness.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

C. N. wrote the manuscript with input from all authors. K. C. conceived and planned the experiments. K.P. carried out the experiments. P.S. and C.T. supervised the project.

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Conflict of Interest

The author has no conflicts of interest to declare.

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