REVIEW ARTICLE

Anti-cancer Activity of *Strobilanthes crispus* Against Colon Cancer Cell Lines: A Scoping Review of Literature

Muhammad Arif Hakimi Salihuddin, Abdul Aziz Azlan, Siti Aisyah Abd Ghafar, Haslinda Ramli, Rohazila Mohamad Hanafiah

Department of Basic Science, Faculty of Dentistry, Universiti Sains Islam Malaysia, Menara B, Persiaran MPAJ, Jalan Pandan Utama, Ampang, 55100, Kuala Lumpur.

ABSTRACT

Being the third most common cancer case in men and the second in women, colon cancer affecting approximately 1.36 million people worldwide. *Strobilanthes crispus*, herb native to Madagascar, Malaysia, and Indonesia, has been studied for its medicinal properties, including anticancer effects. This study aims to review the anti-proliferative activity of *S. crispus* against colon cancer cell lines through systematic review of data and literature. From a number of 515 articles gathered from the search, 221 were passed for final analysis. The results indicate that *S. crispus* effectively induces colon cancer cells death over time while sparing healthy cells. While existing studies suggest the herb's anticancer potential, the findings are not yet conclusive and further clinical research is recommended to explore its effectiveness in treating colon cancer patients.
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Keywords: Anti-proliferative, Anticancer, Colon cancer, Colorectal cancer, *Strobilanthes crispus*

Corresponding Author:
Rohazila Mohamad Hanafiah, PhD
Email: rohazila@usim.edu.my
Tel : +603-4289 2400

INTRODUCTION

The final segment of the human digestive tract which is consisting of colon and rectum, begin at the ileocecal valve, which signifies the terminal of the small intestine and terminate at the anus. Together, they span approximately one yard. Colonic epithelial cells that border the organ's lumen are the origin of colon cancers. Every five days, they regenerate from a stem cell niche situated at the base of colonic epithelial cell crypts. The ultimate consequence of a multiphase colon neoplasia manner that lasts for a number of years could be colon cancer. Neoplastic tubular colon adenomas initially appear as pedunculated polypoid forms that grow into the colon's lumen. They evolved over time into dysplastic cellular cytology and increasingly disordered villous histology, and it is only the moment the intrusive cells rupture the base of the epithelial basement membrane when they are recognized as true malignancies(1).

Colon cancer, also referred to as bowel cancer or colorectal cancer, is a malignancy that initiates its uncontrollable cell proliferation in the colon or rectum part of the large intestine. It has been recorded to be the third most frequent cancer case that happened in men, and the second most frequently happening cancer case among women globally(2). The World Health Organization (WHO) reports that 1.93 million new instances of colorectal cancer (CRC) were recorded around the globe in 2020, and a total of 916,000 patients succumbed to the malignancy(3).

While there are variations in the occurrence and fatality rates across the globe, colon cancer ranks second among cancer-related causes of death worldwide. Notably, Asia plays a significant role, contributing the highest number of incident cases (957,896 or 51.8%) and deaths (461,422 or 52.4%) across all genders and age groups worldwide(3). It has been categorized as the second prevalent cancer in Malaysia which affects around 12.3% of the total population, trailing breast cancer which represents around 18.1% of the population(4). Malaysia is going through development of its population with rising prosperity and proliferated familiarity of causative factors for colon cancer, such

as smoking, westernized diet, and obesity(5). Most of the colon cancer patients seeking treatment in Malaysia are already at an advance stage with poor prognosis, and hence, more expensive treatment. This can clearly cause an increase in health burden to the patients. Currently, a prescribed and organized national colon cancer screening program is not present in Malaysia, and colon cancer patients can only rely on surgery as the best option of cure(6).

In the last few decades, advancement in curative programs for developed colon cancer has been accompanied by a significant improvement in endurance, depletion of fatal rates, and increase in effectiveness. Upon identification, 20% of just identified colon cancer patients present with advanced cancer stages has no therapeutic alternatives presently accessible. *Irinotecan or Oxaliplatin* in synthesis with 5-Fluorouracil treatments are typical mainstays of present structural therapy as they are deemed to be potent in palliative therapy amidst chemotherapy treatments(7).

However, in clinical practice, there is a need for dose reduction due to several complication of chemotherapy(8). This is caused by neutropenia as the most frequent reason for dose reduction of chemotherapy (30%). Other frequent complications resulting in the reduction of dosage are polyneuropathy (16%) and diarrhea (14%). Less frequent reasons for dose reduction comprised mainly mucositis, hand foot syndrome, hyperemesis, worsening of general condition and symptom-related causes(7).

Strobilanthes crispus (*S. crispus*) is a herb indigenous to nations ranging from Madagascar to Malaysia and Indonesia that belongs to the family of *Acantheceae*. It is also domestically called as daun "picah beling" in Jakarta and "jin batu" in Malaysia(9). It has been used conventionally as laxative, antidiabetic, antilytic and a diuretic agent. Samuel et al. (10) noted that orang Asli in Malaysia used the fresh leaves of *S. crispus* to boost their immune system(10).

Due to its various phytochemical groups such as polyphenols, catechins, alkaloids, caffeine, tannins, and vitamins (C, B1, B2), stigmasterol and β-sitosterol, many researchers have studied its pharmacological characteristics such as anti-diabetic, wound healing, antimicrobial, and its anticancer actions(11).

It has been reported that *S. crispus* juice significantly decreased blood glucose level in male and female treated and untreated rats(9). Fadzelly et al. (12) stated that fermented and unfermented tea from the old leaves of *S. crispus* can reduce serum glucose level and improve lipid profile(12). *S. crispus* is also known for its wound healing properties. Al-Henhena et al. (13) found that external application of *S. crispus* ethanol extract leaves extensively enhance acceleration of wound closure in rats(13). *S. crispus* has also been reported to have antibacterial properties against *Bacillus cereus, Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa*.

S. crispus also has been said to have anticancer properties. Muslim et al. (14) noted that aqueous extract of *S. crispus* do not have any cytotoxic effect towards various cell lines while methanolic extract of *S. crispus* have cytotoxic effect towards MCF-7 and T-47D cell lines with its IC_{50} is 160.16 and 121.53 µg/mL(14). Endrini, Rahmat, & Ismail, (15) found that *S. crispus* have cytotoxic activity towards CaCo-2 and HepG2 cell lines with IC_{50} of 25.1 and 28 µg/mL which mediated by c-*myc* downregulation(15). Chong et al. (16) found that *S. crispus* ethanolic extract induced apoptosis towards MF-7 cell lines by inducing Sub-G1 cell cycles phase, caspase 3/7 and p53(16).

Aims

The purpose of this analysis is to systematically review the publications on *Strobilanthes crispus's* ability to arrest the growth of colon cancer cell lines. Specifically, the present paper focused on the cytotoxicity action of *S. crispus* against colon cancer cell lines, morphological changes and cell regulation of colon cancer after treatments with *S. crispus* and mode of cell death of colon cancer cell lines after treatments with *S. crispus*.

MATERIAL AND METHODS

Scoping Review Design

The initiation of this review procedure was according to the implementation of the scoping review methodology, which aligns with a framework outlined by the Joanna Briggs Institute (JBI) and is conducted by the revised procedure of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses module for Scoping Reviews, known as the PRISMAScR. This methodology was used to make it easier to investigate huge-scale research questions and clarify data from a variety of evidence sources(17). Systematic analysis is performed on data obtained from diverse range of study types and procedures that are pertinent to the intervention/concept and outcome/context of the subject matter.

Information Sources

The general review process comprised data extraction from the publications, synthesis of findings, and systematic literature search and screening. The terms or keywords of "*Strobilanthes crispus*" relative to the intervention and "colon cancer or colorectal cancer" for context, were utilized in the literatures inquire procedure. These key terms were pinpointed from the preliminary scoping of the publications and key terms. The query engines in this review were ScienceDirect, PubMed Central, SCOPUS and Google Scholar databases. The query publication was refined for academic journals and articles, written in English language and published

within the years 2000 to 2024. Supplemental applicable literatures were retrieved through a non-automated query of the reference lists of the comprised researches.

Selection of Sources of Evidence and Eligibility Criteria
Two researchers conducted the reviewing separately, and they came to the following conclusions: i) studies that attempted to assess alternatives for cancer therapy in their populace were comprised for the subsequent qualification analysis because it's possible that *Strobilanthes crispus* is one of the most assessed alternatives for cancer therapy and that colon cancer is one of the highest cancer cases reported globally; ii) studies that focused to scrutinize alternatives for cancer therapy in their population were considered relevant. Correspondingly, studies which published on the effect of *Strobilanthes crispus* on colon cancer, and explained how *Strobilanthes crispus* inhibits colon cancer were comprised for complete literature analysis. Any disputes arising between the two researchers were settled by consulting a third researcher.

Data Charting Process and Data Items
The articles included in the review underwent extraction and synthesis of information, which were then summarized in table categorized into descriptive, methodological, and thematic divisions. These classifications matched the goals and inquiries of the analysis. During the protocol stage, a charting table was created to make reporting easier and to summarize the findings, references, and author information. All of the updates to this table came from the review process itself. In order to make sure the data was in line with the study questions, two researchers piloted the data extraction process using the charting form in a few experiments. A thorough assessment was conducted on literature concerning the impact of *Strobilanthes crispus* on colon cancer cells, with the intention of addressing the specified review questions:
1. Does *Strobilanthes crispus* possess anti-proliferative properties against colon cancer cells?
2. How *Strobilanthes crispus* effects colon cancer cells upon exposure?

RESULTS

Synthesis of Result
A total of 515 articles were identified from the initial search. 26 articles were written in different language instead of English and were excluded from analysis. Then, a total of 268 duplicates were also removed from analysis. Finally, a total of 221 articles were selected for further analysis. The total also included 11 articles retrieved from the reference list from some articles. The summary of the screening process is shown in Figure 1.

The objective of this scoping review was to compile the findings and provide a summary of the research, focusing on presenting an overview rather than evaluating the individual studies' quality. Consequently, the overall evaluation took a narrative approach rather than a quantitative one. The summary of the descriptive outcome was presented concerning the impact of *Strobilanthes crispus* on cells associated with colon cancer.

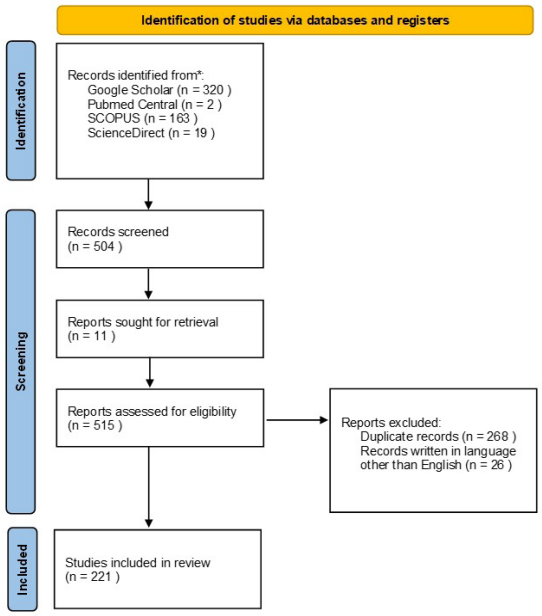

Figure 1: Summary of screening process of literature.

Anti-Proliferative Activity of *Strobilanthes crispus* Against Colon Cancer Cell
Colon cancer is the term used for cancer originating in the colon. Common indications of colon cancer encompass alterations in gut patterns, such as bowel irregularity or diarrhea, presence of blood in the stool, anaemia, weight loss, and more. A review by Ng et al. (18) assessed the growth inhibitory effect of *S. crispus* on Caco-2 cancer cells utilizing several extracts, including ethanol, methanol, chloroform, hexane, and ethyl acetate. Furthermore, two distinct bioactive substances—stigasterol and β-sitosterol—from the leaves were employed. MTT results showed that extracts with both methanolic and chloroform showed tumor growth suppressing effects on Caco-2 cells. The anti-proliferative effects of β-sitosterol and stigmasterol, two beneficial substances, were also observed in the cancer cells. Strong anti-proliferative properties were demonstrated by the chemical, which had an IC_{50} value of 8.3 µg/mL. IC_{50} value is defined as half-maximal inhibitory concentration, a measurement of how much of a drug or inhibitor is required to reduce a biological process by half. In this case, the biological process that needs to be reduced or inhibited is the colon cancer cells' biological process. IC_{50} is a common way to measure a drug's potency. Additionally, the researchers noticed that the substance suppressed the production of the c-*myc* gene, which activated apoptosis(18).

The growth of aberrant crypt foci (ACF) in the colon

is successfully inhibited by *S. crispus* extract, leading to a substantial decline in the full count of ACF. Rats administered with Azoxymethane (AOM) and given 250 or 500 mg/kg of *S. crispus* extract presented a markedly reduce net ACF/colon count than rats treated with Azoxymethane alone. When comparing the *S. crispus* administered groups to the fluorouracil-administered group, the reported inhibition of aberrant crypt foci (ACF) formation—which serves as a signal of tumor initiation—ranged from 70.6% to 71.3%. In contrast, the Azoxymethane-treated group exhibited a 72.6% inhibition. The occurrence and multiplicity decreased significantly in the *S. crispus* treated groups compared to both fluorouracil and Azoxymethane treated groups(19). In another study, scientists conducted the MTT and BrdU assays to assess the effect of the leaf ethanol extract on HT-29 cells. With an IC_{50} value of 52 ± 6.3 µg/mL, the results showed that the ethanol extract was effective in causing cancer cells to die. Nevertheless, when contrasted with the positive control, doxorubicin, which had an IC_{50} value of 52.2 ± 2.9 µg/mL, there was no statistically significant difference ($p > 0.05$)(16).

Ismail et al. (11) evaluated the effects of varied *S. crispus* extracts, obtained from both leaves and flowers, on the proliferation of HT-29 cells. These extracts included hexane, dichloromethane, ethyl acetate, and methanol. It was observed that the leaf extracts in ethyl acetate and methanolic form have anticancer characteristics; they showed IC_{50} values of 70.2 ± 1.4 and 59.0 ± 0.8 µg/mL, respectively. Conversely, the hexane and dichloromethane extracts showed no anticancer activity. In the case of flower extracts, the dichloromethane and ethyl acetate extracts demonstrated tumor-suppressing effects, with IC_{50} values of 90.3 ± 1.1 and 42.0 ± 1.8 µg/mL, respectively, while the hexane and methanolic extracts had no discernible impact(11).

In a different investigation, γ-sitosterol was isolated from fractions 97-102 as white needle-shaped crystals obtained from the crude chloroform extract of *S. crispus*. Following a purification process involving washing with n-hexane and recrystallization from methanol (MeOH), the mixture exhibited a singular blot on the TLC plate. Structural configuration was achieved through infrared and mass spectrometry spectra. The cytotoxic impact of γ-sitosterol was assessed, revealing its most potent cytotoxicity against colon carcinoma cell lines (Caco-2) with IC_{50} values of 8.3, 21.8, and 28.8 mg/mL, respectively. The research showed that untreated Caco-2 cell lines articulated the c-*myc* gene (218 bp). The articulation of c-*myc* genes in both cell lines and the suppressive effects of γ-sitosterol on Caco-2 cell lines were shown by analyzing PCR results on a 1.5% agarose gel. When cells were administered with 30 mg/mL of γ-sitosterol, the c-*myc* gene was not expressed. Confocal micrographs showing that sitosterol (30 mg/mL) triggered apoptosis in Caco-2 cell lines were obtained. The cytotoxicity of γ-sitosterol from *S. crispus* against Caco-2 was highlighted by these results, which showed IC_{50} values of 8.3, 21.8, and 28.8 mg/mL, respectively. The substance reduced the articulation of the c-*myc* gene in the treated cells and caused apoptosis in the Caco-2 cell line(15).

The MTT cell proliferation assay was employed in a different study by N.S. et al. (14) to evaluate the cytotoxic activities of *S. crispus*. On colon cancer cell lines (HCT 116), extracts were added in different quantities. Nevertheless, the data revealed that the extracts' cytotoxicity was only noticeable at high doses when it came to HCT 116 colon cancer cell lines. The extracts' IC_{50} values for colon cancer cell lines HCT 116 were higher than 200 µg/mL(14).

Endrini et al. (20) conducted a study to analyze the cytotoxic effects of leaf extracts from two plants, *Lawsonia inermis* and *S. crispus*. Utilizing the MTT assay, the cytotoxicity of the chloroform extracts from these plants was investigated on a range of cancer cell lines, including Chang liver cell lines, HepG2, MCF-7, Caco-2, and MDA-MB-231. The outcomes showed that the *S. crispus* extract was cytotoxic, with IC_{50} values on Caco-2 cell lines of 25.1 µg/ml, respectively. By evaluating the result of supplementing the extracts on c-*myc* gene expression utilizing RT-PCR and sequencing techniques, the study also looked into the cytotoxic mechanism. The researchers came to the conclusion that the extract's cytotoxic effects are potentially related to the reduction of c-*myc* articulation(20). Table I summarizes the findings of anti-proliferative activities of *S. crispus* against colon cancer cell lines from numerous studies.

Table I: Anti-proliferative activity of *Strobilanthes crispus* against colon cancer cells.

References	Type of extracts	Cytotoxicity assay	Cytotoxicity value	Effect
(18)	β-sitosterol and stigmasterol	MTT assay	IC_{50} value of 8.3 µg/mL	Activation of apoptosis
(16)	Ethanol	MTT and BrdU assay	IC_{50} value of 52 ± 6.3 µg/mL	Inducing cancer cell death
(11)	Ethyl acetate and methanolic	MTS assay	IC_{50} values of 70.2 ± 1.4 and 59.0 ± 0.8 µg/mL	Exhibited anticancer properties
(11)	Dichloromethane and ethyl acetate	MTS assay	IC_{50} values of 90.3 ± 1.1 and 42.0 ± 1.8 µg/mL	Anti-proliferative effect
(15)	γ-sitosterol	MTT assay	IC_{50} values of 8.3, 21.8, and 28.8 mg/mL	Induced apoptosis in the Caco-2 cell line and suppressed c-*myc* gene expression in the treated cells

CONTINUE

Table I: Anti-proliferative activity of *Strobilanthes crispus* against colon cancer cells. (CONT.)

References	Type of extracts	Cytotoxicity assay	Cytotoxicity value	Effect
(20)	Chloroform	MTT assay	IC_{50} values of 25.1 µg/ml	Down-regulation of c-*myc* expression

DISCUSSION

The *Acanthaceae* family particularly *Strobilanthes crispus*, is indigenous to subtropical nations including Malaysia, Indonesia, and Madagascar. In Malaysia, it is frequently referred to as "pecah beling," "pokok pecah," "pecah kaca," or "jin batu." It is a spreading shrub with woody features that has glossy, opposite, elliptical, dark-green leaves. *Strobilanthes crispus* is a plant that has been applied for medical purposes in Malaysia and Indonesia for a long time. It is believed to have diuretic, antilithic, and antidiabetic properties. It is also used to treat constipation by acting as a laxative. It is recognized locally as Hei Mian Jiang Jun (Black-faced General) in the Chinese population. The leaves are usually boiled and then drunk as tea or blended with other herbs(21). In *S. crispus*, a number of active substances with pharmacological and biological properties have been discovered. Particularly, the plant's leaves have been used to separate and determine seven phenolic acids, including p-hydroxy benzoic, p-coumaric, caffeic, vanilic, gentinic, ferulic, and syryngic acid. Verbascoside is a glycosidic ester of caffeic acid(22). The leaves also include a high concentration of flavonoid constituents such as tannin, catechins, and caffeine, as well as minerals, vitamins C, B1, and B2, and antioxidants(23).

It has been stated that plants from the identical botanical family typically shield cells from oxidative damage and hinder the development of reactive oxygen species (ROS) in cell lines, ultimately preventing cell demise(9). Different methods of providing this safeguard have been proposed, including the enhancement of intracellular superoxide dismutase (SOD) activity. SOD facilitates the conversion of superoxide into oxygen and hydrogen peroxide through dismutation, thereby shielding the cell from the toxic effects of superoxide(24). The significant antioxidant potency of SOD has been documented as a crucial element in addressing colon inflammation associated with colitis and preventing the activation of endothelial cells(25). Furthermore, the reduction in malondialdehyde (MDA) levels results in a decrease in ROS levels, redirecting the metabolic pathway in alignment with the existence of hydroxylated C3, unsaturated C ring, and the hydrophobic nature of the extract(26).

Phytochemicals Content in *Strobilanthes Crispus*

Numerous researches have revealed that *S. crispus* has a wide range of phytochemicals as shown in Table II, and these substances have been analytically shown to have a number of beneficial medical effects, including wound healing(26), anti-oxidant(14), anti-microbial(27), anti-diabetic(28), and anti-ulcerogenic(13) qualities. Most notably, in this review, several investigations that were conducted revealed that this herb also possessed anti-cancer properties.

Table II: Phytochemicals content in *Strobilanthes crispus*.

Phytochemicals	Reference
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-:26.21% Squalene:26.11% Stigmasterol:10.93% Vitamin E: 9.75% γ-sitosterol: 6.70% Campesterol: 3.57%	(29)
Methanol extract; Hexadecanoic acid, methylester: 12.11% 3-octadedecyne: 9.25% Stigmasterol: 7.89% α-sitosterol: 7.08% Phytol: 3.78% Lupeol: 3.60% 3,7,11,15-tetramethyl-2-hexadecen-1-ol: 3.48%	(14)
Aqueous extract; Nitrous Oxide: 21.44% Cyclobutanol: 13.56% Monoethanolamine: 5.53% n-propylacetate: 5.38% hydrazine carboxamide: 3.32% 3,5-dithiahexanol 5,5-dioxide: 3.09%	
Flavonoid Content (%): 3.98	
Contents of Flavonoid (mg/g); Kampferol: 19.45 Luteolin: 12.52 Rutin: 8.47 (+)-Catechin: 4.83 (–)-Epicatechin: 4.55 Myricetin: 4.10 Apigenin: 3.75 Naringenin: 3.63	(30)
Contents of Phenolic Acids; Caffeic acid Ferulic acid Gentisic acid p-caumeric acid p-hydroxybenzoic acid Syringic acid Vanilic acid	(18)
Ester glycoside; Verbascoside	
Proximate Analysis (%); Crude Fibre: 13.9 ± 0.6 Protein Content: 13.3 ± 0.9 Total Carbohydrate: 4.3 ± 0.7	
Minerals (mg/100 g sample); Potassium: 10,900 ± 498 Calcium: 5185 ± 359 Sodium: 2953 ± 60 Iron: 255 ± 163 Phosphorus: 201 ± 22	(23)
Vitamins (%); Ascorbic Acid (C): 9.8 ± 1.2 Thiamin (B1): 0.14 ± 0.001 Riboflavin (B2): 0.11 ± 0.04 Other components (%); Alkaloid: 3.2 ± 0.60 Catechin: 1.18 ± 0.08 Tannin: 1.0 ± 0.30	

Protective Mechanisms of *Strobilanthes Crispus* Against Colon Cancer Cell

SOD activity significantly increased in rats treated with *S. crispus*, in contrast to untreated controls in a study by Al-Henhena et al. (19), demonstrating the plant's antioxidant qualities. The study delved into the defense measures of *S. crispus* ethanolic extracts on the intestinal crypt morphology, mitigating carcinogenic alterations incited by AOM. The ethanolic extracts from *S. crispus* effectively preserved the structure of epithelial cells, evident in minimal nuclear and cytoplasmic vacuole condensation, reduced early apoptosis, maintained luminal space, and prevented significant alterations in size and shape. Moreover, the extracts countered the accumulation of ROS particles, resulting in the suppression of *Bax* gene overexpression and an elevation in lactate dehydrogenase (LDH) levels in animal serum. Additionally, the ethanolic extracts of *S. crispus* dose-dependently reduced the number of ACF over time. The study employed real-time PCR to genetically examine the defensive function of the plant extract and its active components against cancer development. The findings indicated that the downregulation of mRNA, induced by *Apc* gene mutation, led to progression in colon cancer formation, similar to previous reports. Another plausible explanation is that *S. crispus* ethanolic extract may safeguard protein structure from detrimental changes initiated by free radicals at the genetic degree(19).

To find out how well the plant extract and its active ingredients prevented the development of cancer, real-time PCR was used for genetic analysis. Consistent with previous publications, the study's findings showed that the *Apc* gene mutation-induced reduction in mRNA was the primary factor contributing to the advancement of colon cancer(31). In the commencement of genetic reconfiguration cascades, the decreased expression of *Apc* was associated with an upsurge in mRNA levels and an elevation in *Bcl-2*, which prevented cells from going through apoptosis and subsequently promoted the progression of cancer(32). Thus, the findings pointed to a possible instance of dysregulation in the apoptotic process(33).

In order to clarify potential preventive mechanisms against malignant progression, Al-Henhena et al. (19) examined the outcomes of subjecting two cell lines to the plant extract and its components. The subjection of HT29 and CCD-841 to the plant extract and components resulted in a dose-dependent reduction in cell survival, as established by the MTT assay. The induction of apoptosis in HT29 and CCD-841 was evidenced by the articulation of apoptotic and antiapoptotic indicators, namely the *Bax* and *Bcl-2* genes. *S. crispus* fragments STF2 and STF3 demonstrated a notable impact on HT29 cell death at 27.43% and 9.09%, respectively. These two fragments also exhibited effectiveness on CCD-841 colon cells, with viabilities of 59.66% and 55.53%, respectively. Conversely, the remaining four *S. crispus* fragments (STF1, STF4, STF5, and STF6) were substandard on both cell lines. The primary constituents characterized were i*cariin* and *epigallocatechin*, both associated to the flavonoid family of configurations(19).

Flavonoids have been suggested to be pivotal in inhibiting carcinogenesis. Previous research has highlighted the antioxidant potential of flavonoids and their chemo preventive activity against various types of cancer. Many of these studies have utilized intrinsic sources of flavonoids to assess their tumor-suppressing effects on animal models. Additionally, some investigations have revealed the anticancer effects through *in vitro* experiments. In a research conducted by Al-Henhena et al. (19), varied components of *S. crispus* exhibited varying outcomes on cells. The collective flavonoid constituents of *S. crispus*, including a concoction of flavonoids, caffeic acid, ferulic acid, and urosolic acid, provided protection to colorectal cells from cellular damage and apoptosis. The synergistic effect of flavonoids and other active compounds was evident in the *in vivo* preliminary setup. Overall, *S. crispus* and its polyphenolic compounds exhibited a notable reduction in MDA levels and LDH activity, a significant reduction in entire colonic ACF structuring, an amplification in SOD function, a notable downregulation of both *Defa24* and *Bcl-2*, activation of *Apc* and *Bax*, and activation of Slc24a3. In conclusion, both *in vitro* and *in vivo* analysis of *S. crispus* and its polyphenolic chemical composition demonstrated chemopreventive qualities against colon cancer(19).

A number of human cancers are initiated by the c-*myc* oncogene, and increased knowledge about its expression and activity has led to novel therapeutic options for the treatment of cancer(34). It has been shown that a number of plant extracts inhibit cell growth by downregulating the expression of c-*myc*(35). In a study conducted by Endrini, Rahmat, Ismail, et al. (15), *S. crispus* ɣ-sitosterol was discovered to inhibit c-*myc* expression. mRNA was taken out of the treated cells to determine how well ɣ-sitosterol inhibited oncogenes. mRNA was transformed to cDNA preceding to the PCR procedure because of RNA instability and for PCR purposes. The best method for amplifying oncogene amounts was determined to be PCR, which allowed for the unambiguous visibility of oncogene suppression via gel electrophoresis analysis. Furthermore, in order to produce the best PCR products, various oncogenes have varied temperature requirements for denaturation, annealing, and elongation in a particular number of cycles. The findings indicated a dose-dependent effect, and this effect appeared to be interrelated with the IC_{50} value of each treatment(15).

The techniques employed for evaluating breaks in DNA strands rely on tagging or staining the cellular DNA. After labelling or staining, the DNA is later examined using fluorescence microscopy or confocal laser scanning microscopy to enhance the precision of

the results. Significant degradation of DNA is a typical occurrence in the commencement of apoptosis. The DNA fragmentation can produce low molecular weight DNA residues, such as mono- and oligonucleosomes, along with single-strand pieces (nicks) in high molecular weight DNA. These pieces in DNA strands are able to be identified by enzymatically labelling the unbound 3-OH ends with reconfigured nucleotides. *Terminal deoxynucleotidyl transferase* (TdT), a suitable labelling enzyme, is capable of end labelling and can label blunt ends of double-stranded DNA pieces individually of a template. This investigation illustrated that γ-sitosterol extracted from *S. crispus* may induce apoptosis in Caco-2 colon carcinoma cell lines. Confocal laser scanning microscopy revealed morphological alterations in chromatin condensation, DNA fragmentation, and the vicinity of several apoptotic bodies in the administered groups, while no apoptotic events were monitored in the untreated group(15).

While the findings from various research by several researchers showed positive and promising results, existing research has not yet provided current, evidence-based conclusions on this topic, necessitating further study. Notably, the existing findings as of today lack clinical trials and result from larger sample size. The limitations of current studies highlight the potential for future research into the medicinal properties of this traditional herb. Some of the potentials could be studying the anticancer effects of this herb when combined with other compounds such as nanoparticles and testing this herb in clinical trials settings.

CONCLUSION

This review delineates the tumor-suppressing outcomes of *Strobilanthes crispus* on colon cancer cell lines, drawing upon *in vitro* and *in vivo* analyses undertaken by various researchers. Evidently, the current literature lacks up-to-date, evidence-based findings on this subject, prompting ongoing investigations. Researchers are actively exploring *Strobilanthes crispus's* potential as a safer alternative treatment for colon cancer, free from concerns about side effects akin to chemotherapy. The quantitative assessment of intervention effectiveness and the qualification of each study through systematic reviews and meta-analyses are now imperative. Additionally, the absence of recent findings creates an opportunity for future research on the medicinal properties of this traditional herb, not only for colon cancer but also for other severe ailments.

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