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# Potential Inhibitor Tripsin Enzyme in the Treatment of Colorectal Cancer: A Review

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***Abstract:***
***Background****: Trypsin inhibitor (TI) is an enzyme that can be used to treat colorectal cancer because it inhibits the activity of trypsin enzymes that generate NF-KB signaling and MMPs, which promote colorectal cancer cell proliferation, invasion, and metastasis.*
***Materials and Methods****: The data was gathered using Google Scholar, Science Direct, MDPI, and PubMed search engines, as well as data from various published studies on trypsin inhibitors and colorectal cancer.*
***Results****: Trypsin inhibitor enzymes that could be used to treat colorectal cancer have been isolated from organisms (Glycine max, (Linn) Merrill), (Vigna radiata (L.) R. Wilczek), (Pisum sativum L), (Lens culinaris), and (Momordica charantia) using the purification methods of gel filtration chromatography and ion chromatography, according to several studies. The IC50 value indicates cytotoxicity activity.In HT29 cell growth, the IC50 for IBB1 and IBBD2 was (39.9 2.3 and 48.3 3.5 m). GBP-TI inhibits the proliferation of colorectal cancer cells in female rats with an IC50 of 20 m, Pisum sativum L with an IC50 of 31 m, lentils with an IC50 of 32 m, and bitter melon with an IC50 of 217 g/ml.*
***Conclusion:*** *The five organism trypsin inhibitor enzymes could be used as drug active ingredients in colorectal cancer treatment. And the IC50 value of TI or bowman-birk inhibitor demonstrated the best action, inducing apoptosis and reducing the proliferation of colorectal cancer HT29 cells.*
***Key Word****: Bowman-birk Inhibitor, Colorectal Cancer, Trypsin Enzyme Inhibitor*

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## I. Introduction

Colorectal cancer (CRC) is an abnormal condition that occurs in the large intestine (colon) or rectum. This disease knows no age, so it can happen to anyone. Incidence and death rates increase with age. The World Health Organization (WHO) in 2018 cases of cancer that occurred around 18.1 million and deaths caused by cancer 9.6 million [1]. With 8,751,000 cancer cases in 2018, Asia is a big contributor to the many cancer cases that occur throughout the world [2]. Colorectal cancer in Indonesia in 2018 was reported to have 30,017 new cases [3].

The actual cause of colorectal cancer has remained unknown until now. Colorectal cancer can be caused by a variety of risk factor, including hereditary diseases and environmental influences [4]. Surgical surgery, radiation, and adjuvant chemotherapy are some of the options for colorectal cancer treatment. Surgical therapy, radiation, adjuvant therapy [5], and targeted therapy to block or eliminate the proliferation of cancer cells are all options for colorectal cancer treatment. Nausea, vomiting, diarrhea, stomatitis, leukopenia, elevated bilirubin, peripheral nervous system, gastrointestinal, insomnia, allergies, edema, and fever are all side effects of adjuvant chemotherapy. adverse effects that occur as a result of the prescribed treatment [5]. Enzyme therapy is a different type of treatment that can be used.

Trypsin enzyme levels are reported to be higher than normal in colorectal cancer patients. The trypsin enzyme is known to play a role in colorectal cancer corsinogesis, cell proliferation, invasion, and metastasis [6],[7]. Antimicrobial, anticancer, and biomarker properties have all been discovered for trypsin inhibitor enzymes. One of its anticancer properties is against colorectal cancer, as demonstrated by a study that used the xenography method on mice to conduct an in vivo test and found that the trypsin inhibitor enzyme inhibits the growth of colorectal cancer cells [8]. Colorectal cancer necessitates the use of a trypsin inhibitor to prevent the trypsin enzyme from degrading proteins and thereby decreasing the molecular activity of trypsin signals and matrix metalloproteinases (MMPs). The process of colorectal cancer cell proliferation, invasion, and metastasis is decreased when MMP activity is suppressed [6],[9]. In nature, trypsin inhibitor enzymes are found in plants, animals, and microorganisms. Trypsin inhibitors are primarily found in plants. The following plants generate trypsin inhibitor enzymes, which have been shown to have anticancer properties: mung bean [10]; Lentil [11]; pea [12]; black soybeans [12]; soybean [13]; potato [14]; Yunnan bean (*Gymnoclodus Chinensis)* [15]; pinto bean [16]. *Geoffroeadecorticansseeds* have anticoagulant properties [17]. *Solanum tuberosum* has antibacterial

properties [14]. TI generated by microorganisms, such as *Oceanimonas sp.* BPMS22 [18] and *Streptomyces trypsin*[19], is recognized to have cancer-causing properties. The purpose of this scientific article is to examine various studies of trypsin inhibitors (TIs) which have good activity in targeted therapy for colorectal cancer patients. This review will discuss the producer of TI enzymes, their activities and methods of isolation and purification of enzymes.

## II. Material And Methods

This article review was conducted based on data obtained from research articles that have been published and indexed in Google Scholar, Scopus, Web of Science, Copernicus, EBSCO, DOAJ, PubMed, and Science Direct. The literature search was conducted using the keywords "Enzyme Trypsin inhibitor", "Colorectal cancer". References to research articles that have been used a maximum of the last 10 years.

## III. Result

The trypsin inhibitors, either kunitz inhibitors or bowman-brik inhibitors, extracted from several species, the purification method utilized, and the action of colorectal cancer are all described in this scientific paper.

**Table no 1 :Trypsin inhibitor enzymes that have colorectal cancer activity**

| **Organism** | Protein Name | Purification Method | Enzyme Molecular Weight (kDa) | Specific Enzyme Activities (U/mg) | IC50 µm (µg/ml) | Referensi |
|---|---|---|---|---|---|---|
| *Glycine max* L. Seeds | IBB1 , IBBD2 | Gel filtration chromatography | 8-10 | 3828/2917 | 39.9 µm | ([13], [20]) |
| *Vigna radiata* (L.) R. Wilczek | GBP-TI | Gel filtration chromatography | 14 | 5727 | 20 µm | ([10],[8]) |
| *Pisum sativum* L | rTI1B , rTI2B | Gel filtration chromatography | 7-8 | 2476/2956 | 31 µm | [21] |
| *Lens culinaris* | BBI | Gel filtration chromatography | 7 | - | 32 µm | [11] |
| *Momordica charantia* | Trypsin inhibitor | Ion exchange chromatography | 12 | 96 | 217 µg/ml | [22], [23] |

## IV. Discussion

According to the findings of diverse scientific literature from scientific organisms [24], plants are one of the organisms most reported to have trypsin inhibitor enzymes. Trypsin-inhibiting enzymes have been found in various species. Species considered are species known to inhibit cancer cells.

### 1. Organisms that produce enzymes

In nature, trypsin inhibitor enzymes, also known as kunitz trypsin inhibitors or bowman-birk inhibitors, have been discovered in a wide range of organism, including plants, animals, and microbes. Trypsin inhibitor enzymes can be found in a variety of species, including: *Glycine max* L. Seeds [13], *Macrolepiota procera, Armillaria* [25], *Gymnocladus Chinensis* (Yunnan Bean) Seeds [15], mung bean (*Vigna radiata* (L.) R. Wilczek) [10], Arabidopsis plants [26], Solanum tuberosum [14], Geoffroeadecorticans seeds [17], Pisum sativum L [21], Lentil [11], Pinto beans [16], *Glycine soja* (L) merit [12], *Cassia obtusifolia* [27], Catanduva (Piptadenia moniliformis) seeds [28], *Inga laurina* (SW.) Willd [29], *Vigna unguiculata* seeds [29], Faba Bean (*Vicia faba* cv. Giza 843) [30], *Momordica charanti* [23], *Araneus ventricosus* [31]. *Streptomyces trypsin* [19], *Oceanimonas sp*. BPMS22 [32], *Lactobacillus plantarum* FNCC 0270 [33], *Streptomyces griseu* [34], *Gloeobacter violaceus* [35], *Streptomyces misionensis* UMS1 [29] are among the microorganisms known to generate trypsin inhibitor enzymes.. Only five species with activity as colorectal cancer were chosen for the review of this scientific paper from a large number of organisms that generate trypsin inhibitor enzymes, as indicated in table no1. (*Glycine max*, (Linn) Merrill ), (*Vigna radiata* (L.) R. Wilczek ), (*Pisum sativum* L), (*Lens culinaris* ), (*Momordica charantia*).

Soybeans (*Glycine max*, (Linn) Merrill ) are the best source of protein, fat, vitamins, minerals and fiber as well as food ingredients that have high nutritional value. Soybeans have many health benefits, one of which is because they contain trypsin inhibitors. Bowman-birk inhibitor from soybean was isolated and purified with a molecular weight of 8-10 kDa[20]. Soybean trypsin inhibitor enzymes have been identified in a number of research. Two polypeptides found in soybeans are kunitz trypsin inhibitor (KTI) and bowman-birk inhibitor (BBI) [36]. KTI and BBI make about 6% of the seed protein in soybeans. Using two-dimensional liquid chromatography, the Kunitz trypsin inhibitor with a molecular weight of 20 kDa was successfully extracted from soybeans [37]

In green beans (*Vigna radiata* (L.) R. Wilczek) there is a source of vegetable protein, essential amino acids and vitamin B9. In 100 g of green beans contains 159 micrograms of folic acid. has many health benefits. Mung bean is known to contain a high trypsin inhibitor enzyme, so some researchers extracted TI from green beans which were successfully purified with a molecular weight of 14 kDa and stable at 90$^{o}$C [10].

Peas (*Pisum sativum* L) are a type of nut in the Fabaceae family that can be found in a variety of traditional Indonesian marketplaces. Peas, despite their small size and excellent nutritional value, provided four grams of protein per 170 grams. Alkaloids, glycosides, flavonoids, isoflavones, phytic acid, saponins, and tannins are among the bioactive components found in peas [38]. There is also a BBI which is a naturally occurring plant protease that is known to have the potential to improve digestive tract health. BBI has been isolated with a molecular weight of 7-8 kDa [21].

Lentils (*Lens culinaris*) are beans with a lens shape that has four colors. Lentils are high in protein but low in fat and fiber. Lentils are a source of protein, vitamins, minerals, dietary fiber and folic acid. In addition, lentils also contain biologically active substances such as enzyme inhibitors, lectins, phytates, oligosaccharides and phenolic compounds [39]. The polyphenolic compounds contained in it have potential as antioxidants and protect against various diseases such as diabetes, obesity, and cancer [40]. One of the enzyme inhibitors contained in lentils is BBI. BBI was isolated with a molecular weight of 7 kDa[11].

Bitter melon known as bitter melon (*Momordica charantia*) is a plant from the cucurbitaceae family, growing in tropical and sub-tropical regions [41]. Contains triterpene compounds, proteins, steroids, alkaloids, lipids, and phenolic compounds [42]. Pare is known to have benefits as antidiabetic, antioxidant, antiviral and antineoplastic. The trypsin inhibitor that has been isolated from bitter melon has a molecular weight of 12 kDa [22] . The results of the isolation of the TI enzyme from mung beans succeeded in isolating TI with a higher amount than the amount of TI from soybeans, peas, lentils, and bitter melon.

## 2. MetodePurifikasi

Based on the results of the literature review, trypsin inhibitor enzymes were purified by gel filtration chromatography and ion chromatography methods. Purification of the trypsin inhibitor enzyme is a process to increase the purity of the trypsin inhibitor enzyme which is expressed as the specific activity of the enzyme. Gel filtration chromatography is a technique for separating molecules based on size and charge. SDS-PAGE is a detergent that can denature secondary, tertiary and nondisulfide-linked structures and then coated with a negative charge that correlates with length. Gel filtration chromatography is composed of low polyacrylamide which allows the protein to move quickly and accumulate into tight bands before entering into a high percentage of polyacrylamide. Proteins with small sizes will move faster [43].

Ion exchange chromatography has been used for more than 50 years for the separation and purification of proteins [44]. Ion chromatography is used to carry different charges, which are stored in binding to the matrix/column then collected in pure form and stored [45]. The pH of the binding and elution buffer is an important factor for determining the protein charge, it can be done by calculating the isoelectric point of the protein. Protein isoelectric point is pH, protein with acidic pH shows negatively charged protein, the chromatography chosen is ion exchange and protein with alkaline pH shows positively charged protein, so the selected chromatography is cation exchange [46].

The specific activity of an enzyme is the number of enzyme units per milligram of protein. Based on the results of enzyme purification using the gel filtration chromatography method, TI from green beans had high specific enzyme activity ranging from 5727 U/mg, compared to the specific activity of TI enzymes from soybeans which ranged from 3828/2917 U/mg, from peas 2476/2956 U /mg from bitter melon is 96 U/mg. This shows that the TI from green beans has good purity. Because the purer the enzyme obtained, the higher the activity will be [47]. the specific activity of an enzyme as well as a measure of the purity of the enzyme. activity will increase according to the purity of the enzyme.

## 3. Efficacy of Trypsin Inhibitors in the Treatment of Colorectal Cancer

Trypsin inhibitor enzymes have been shown to be useful in the treatment of cancer. Several matrix metalloproteinases (MMPs) involved in the breakdown of collagen and the basal membrane during the early stages of malignancies are activated by serine proteases. MMP will stimulate cancer cells and maintain tumor growth in the next stage. [48]. In colorectal cancer trypsin activates NF-kB signaling and actively expresses matrix metalloproteinases (MMPs), which promote colorectal cancer invasion and metastasis [7]. Several studies on trypsin inhibitor enzymes, which are known to have a variety of biological activities, including action in inhibiting the trypsin enzyme and cancer cells, are now underway. *Oceanimonassp*.BPMS22 TI demonstrated only minor anti-CML action and was non-toxic to normal cells. TI from *Oceanimonassp*.BPMS also has a synergistic impact in the treatment of cancer-related thrombosis. Inhibition of serine proteases from plasminogen activation in K562 cells revealed anti-cancer effect. [18]. Based on information from research literature, the TI enzyme found in pinto beans exhibits antiproliferative action [16]. TI extracted from

*Gymnocladuschinesis* seeds demonstrated antiproliferative action against L1210 leukemia cells with an IC50 of 4.7 µM and antiproliferative activity against MBL2 lymph cells with an IC50 of 9.4 µM.

One of the anticancer activities of the trypsin inhibitor enzyme is to inhibit the growth of colorectal cancer cells. The IC50 value is one of the parameters used for anticancer effectiveness. The standard IC50 values based on their cytotoxic activity are classified into several categories. The IC50 value < 10 µg/mL is categorized as very active in the sense that the cytotoxicity activity is very strong, IC50 10-100 µg/mL has strong cytotoxicity activity, IC50 100-500 µg/mL has moderate cytotoxicity [49]. If the IC50 value is more than 500 µg/mL, the chemical or compound has no cytotoxic effect [50]. Soybean IBB1 and IBBD2 are Bowman-Birkisoinhibitors (BBIs) with antiproliferative effects and a high temperature tolerance. IBBD2's antiproliferative activities were decreased after it was given the glycation effect in the presence of glucose. IBB1 is unaffected, therefore it can keep its antiproliferative capabilities against cancer cells colon HT29 [13]. The IC50 inhibitory concentrations for IBB1 and IBBD2 in HT29 cell growth were not significantly different (39.9 ± 2.3 and 48.3 ± 3.5 µm). The resulting BBI treatment effect at a given dose, cells were blocked in the G0-GI phase [20]. IBB1 and IBBD2 have strong cytotoxic activity because the IC50 values are in the range of 10-100 µg/mL.

Regarding the possible potential of trypsin inhibitors in mung bean in cancer therapy, it was necessary to develop an TI reconstruction study to target the GRP78 cell surface [8]. A glucose-regulated protein of molecular mass 78 (GRP78) is present on the membranes of cancer cells. GRP78 can induce colorectal cancer apoptosis. GRP78 is also involved in tumor cell proliferation, resistance, metastasis and angiogenesis [51]. GBP-TI With an IC50 value of 20 µm, mung bean can inhibit the development of colorectal cancer cells. Both in vitro and in vivo studies have been conducted on GBP-TI. In colorectal cancer cells, GBP-TI has been found to limit cell proliferation and promote apoptosis. The development of G1 phase arrest and the activation of multiple apoptotic pathways are two of its anticancer effects.Green bean GBP-TI can decrease the development of colorectal cancer cells with an IC50 value of IC50 20 µm [8].

Pea (*Pisum sativum*L ) trypsin inhibitor enzyme action on the proliferation of colorectal cancer cells with an IC50 concentration of roughly 31µm. Results According to published studies, rTI1B and rTI2B can inhibit the development of HT29 cells in vitro better than soybean BBI [21]. The inhibitory effect of BBI on adult lentils on the growth of human colon adenocarcinoma HT29 cells was determined using a cytotoxic NR with a BBI concentration (19–76 µM).The results showed that BBI Lentils may suppress the proliferation of HT29 cells from colon cancer cells at a concentration of more than 19 µM. The inhibitory impact was at the given dose. 32 µm IC50 (concentration inhibition value)[11].Trypsin inhibitor from bitter melon has an inhibitory effect with IC50 values of 217 µg/mL and ED50 134 showing its ability to cause cytotoxicity against colon cancer HCT-166 and HT-29, the resulting cytotoxic expresses apoptosis[24]. The mechanism of action of trypsin inhibitors in inhibiting the growth of colorectal cancer cells is by electrostatic interactions between the Arg63 side chain of TI and the Asp189 side chain in the trypsin enzyme, including three catalytic active sites (His57, Asp102, and ser195) and one Asp189 binding site has succeeded in blocking the center active trypsin and effectively prevents substrate binding to trypsin [52], As a result, the trypsin enzyme inhibits NF-KB signaling and MMP expression. The better the cytotoxicity activity produced, the lower the IC50 value. As a result, GBP-TI has the potential to be used as a candidate for herbal medicine in the treatment of colorectal cancer.

## V. Conclusion

Based on the results of a review of scientific articles, some of them are Glycine max L. Seeds, *Vigna radiata* (L.) R. Wilczek, *Pisum sativum* L, *Lens culinaris*, *Momordica charantia* which can produce trypsin inhibitor enzymes that have been proven either in vitro or in vitro. in vitro vivo has colorectal cancer cell growth activity. The enzyme purification method used is gel filtration chromatography and ion exchange chromatography. The most potential trypsin inhibitor enzyme to be investigated as a candidate for herbal medicine in colorectal cancer therapy is GBP-TI from the organism *Vigna radiata* (L.) R. Wilczek GBP_TI with strong cytotoxicity activity allows it to be used as an active ingredient for colorectal cancer treatment therapy.

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