REVIEW ARTICLE

The Effects of Simvastatin and Soursop (*Annona muricata*) Leaf Extract on Colorectal Cancer: A Systematic Review

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ABSTRACT

Colorectal cancer (CRC) is a heterogeneous disease of the colon and rectum. In Indonesia, CRC has the fourth highest cancer incidence, with about 30,000 new cases and causing around 16,000 deaths per year. Numerous studies have shown the benefits of using simvastatin and *Annona muricata* (*A. muricata*) leaf extract to promote cytotoxic effects in CRC cells. This systematic review aimed to evaluate the effects and the possible molecular mechanisms of actions used by simvastatin and *A. muricata* leaf extract to exert anti-cancer effects in CRC cells. We synthesized published studies on the effect of simvastatin and *A. muricata* leaf extract in *in vitro* and *in vivo* CRC studies, and highlighted the potential application of simvastatin and *A. muricata* leaf extract in combination with other anti-cancer treatments. This systematic review was written in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA). Simvastatin and *A. muricata* leaf extract were shown to suppress CRC cell growth and proliferation and generate a synergistic effect when combined with other anti-cancer treatments. Several possible mechanisms of actions of simvastatin include bone morphogenetic protein (BMP) pathway and mevalonate pathway activation, ERKs inactivation, and regulation of the yes-associated protein 1 (YAP1), while for *A. muricata* leaf extract, possible mechanisms of actions include intrinsic apoptosis pathway activation, cytochrome c-mediated apoptosis, mitochondria-mediated apoptosis, and programmed cell death initiation. Further experimental evidence is needed to determine the specific mechanisms on how simvastatin and *A. muricata* leaf extract induce anti-cancer effects against CRC cells.

Keywords: Colorectal cancer; simvastatin; soursop; anticancer agents; combination drug therapy

INTRODUCTION

Colorectal cancer (CRC) is a heterogeneous disease of the colon and rectum. It can be termed as either colon cancer or rectal cancer based on its origin (Chu, 2007; Rawla, Sunkara, & Barsouk, 2019). CRC most often arises from the glandular epithelial cells of the large intestine, and it usually develops from a dysplastic adenomatous polyp. It commonly progresses through a multistep process consisting of the sequential inactivation of various tumor suppressor genes, along with the activation of proto-oncogenes (Chu, 2007; Marley & Nan, 2016; Rawla, Sunkara, & Barsouk, 2019).

CRC is currently the third leading cause of cancer-related death and the fourth most commonly diagnosed cancer with about two

million new cases and approximately one million deaths each year across the world (International Agency for Research on Cancer, 2018; Rawla, Sunkara, & Barsouk, 2019). In Indonesia, CRC has the fourth-highest incidence rate for cancer, constituting about 8.6% of all cases. It causes about 30,000 new cases and 16,000 deaths every year (International Agency for Research on Cancer, 2018). In addition, most CRC cases in Indonesia are detected at either stage three or four, approximately 76.7% and 13.1% cases, respectively (Widjaja & Yo, 2016). At these late stages, metastasis would have occurred, and patients' survival would be significantly low. Thus, it is clear that CRC is considered one of Indonesia's major public health issues (Abdullah *et al.*, 2012).

Current treatments for metastatic CRC include a number of chemotherapy drugs possessing significant antitumor activity, including 5-fluorouracil (5-FU), irinotecan, oxaliplatin, capecitabine, raltitrexed, trifluridine, and tipiracil (El-Shami *et al.*, 2015). However, chemotherapy and radiotherapy, especially in long-term treatments, have various side effects, such as nausea, vomiting, dehydration, dry skin, rashes, easy bruising or bleeding, acne, loss of appetite, sleep difficulty, urinary incontinence, sexual dysfunction, hair loss, and fatigue. Furthermore, serious adverse effects, such as mucositis, allergic reactions, severe diarrhea, hand and foot syndrome, peripheral neuropathy, gastrointestinal problems, anxiety, and depression, may also be found (Denlinger & Barsevick, 2009; El-Shami *et al.*, 2015).

Despite all the breakthroughs and advancements in current clinical research studies, it remains a significant challenge to construct a comprehensive molecular therapy for CRC treatment. The reason for this problem is due to the presence of mutations and mutagens that could lead to the development of CRC, and also the rapid progression of drug-resistant cancer cells (Colussi *et al.*, 2013; Sideris & Papagrigoriadis, 2014; Rawla, Sunkara, & Barsouk, 2019). In addition to this, treatments for CRC in Indonesia are still very expensive and limited. Hence, more research needs to be aimed at developing more accessible and affordable alternative treatments for CRC.

Due to the significantly high time and cost of production required for drug development, repurposing existing drugs or studying natural products for their anti-cancer properties presents an appealing and convenient opportunity (Beckwitt, Shiraha, & Wells, 2018). Thus, the combination of natural products and drugs with favorable safety profiles could be considered as a visible cancer treatment. (Wahab *et al.*, 2018). Combination therapy for cancer treatment has several advantages. Firstly, more potent effects with lower doses may be achieved due to the multiple pathways targeted. Secondly, combination therapy minimizes the possibility of cells developing drug resistance since it is more difficult for the cancer cells to adapt to simultaneous toxic effects produced by two or more therapeutic agents (Karjalainen & Repasky, 2016; Mokhtari *et al.*, 2017).

Simvastatin is one of the most common lipid-lowering drugs for the treatment of hypercholesterolemia (Moghadasian, 1999; Beckwitt, Shiraha, & Wells, 2018; Wang *et al.*, 2018). Simvastatin works by competitively inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is responsible for the conversion of HMG-CoA into mevalonic acid, therefore inhibiting the hepatic synthesis of very-low-density lipoprotein (VLDL) and decreasing plasma low-density lipoprotein (LDL)

and VLDL levels (Moghadasian, 1999; Talreja & Cassagnol, 2019; Wang *et al.*, 2018). The mechanism used by simvastatin to inhibit HMG-CoA reductase, also inhibits proliferation and induces apoptosis in several cancer cell lines, including CRC (Pisanti *et al.*, 2014). Currently, there are several epidemiological studies suggesting a correlation between simvastatin use and lower CRC risk (Dobrzycka *et al.*, 2018; Hassanabad, 2019). Therefore, simvastatin might be a great anti-cancer agent alternative.

A. muricata, mostly known as soursop, is a tree producing soft, edible, green, heart-shaped, spiny skinned fruits that can be found throughout Southeast Asia, including Indonesia (Cassé, 2018; Rady *et al.*, 2018; Wahab *et al.*, 2018). According to numerous phytochemical studies, anti-cancer compounds, such as antioxidants, alkaloids, sterols, phenolic compounds, acetogenins, and megastigmanes, have been found in *A. muricata* leaf extracts (Leboeuf *et al.*, 1980; Yang *et al.*, 2015; Coria-Téllez *et al.*, 2018; Rady *et al.*, 2018). Previous studies have assessed the cytotoxicity of *A. muricata* leaf extract on several cancer cell lines; the results showed the extracts were highly toxic to cancerous cells but not to normal healthy cells (Cassé, 2018; Gavamukulya *et al.*, 2017; Rady *et al.*, 2018; Wahab *et al.*, 2018). Thus, *A. muricata* leaf extract can be considered as an anti-cancer agent candidate.

Numerous studies have explored the anti-cancer effects of simvastatin and the cytotoxic properties of *A. muricata* leaf extract. However, there has not been any review that evaluated the effects and the possible mechanisms of actions on how simvastatin and *A. muricata* leaf extract induce anti-cancer effects against CRC cells, synthesized published studies on the cytotoxic effects of simvastatin and *A. muricata* leaf extract on CRC cells, and highlighted the combinatorial activity of simvastatin or *A. muricata* leaf extract with other drugs or treatments. Therefore, this systematic review aimed to evaluate the effects and the possible molecular mechanisms of actions used by simvastatin and *A. muricata* leaf extract to exert these anti-cancer effects in CRC cells. We synthesized published studies on the cytotoxic effects of simvastatin and *A. muricata* leaf extract in CRC cell lines, animal models, and patients. In addition, we highlighted the potential of simvastatin and *A. muricata* leaf extract to be used in combination with other anti-cancer treatments.

METHODS

This systematic review was performed following the PRISMA guidelines (Moher *et al.*, 2009). Relevant publications have been identified through a systematic literature search of databases, including PubMed, Cochrane Library, SCOPUS, and Web of Science. The combination of keywords that were used in the search were ["colorectal cancer" OR "colon cancer" OR "CRC"], ["simvastatin" OR "statins"], ["*Annona muricata*" OR "*A. muricata*" OR "soursop" OR "graviola" AND "leaf extract"], and ["combination therapy" OR "combinatorial therapy"]. Mendeley was used to store the research articles and Covidence was used to conduct the screening.

This systematic review identified studies on cell lines (*in vitro*), animal models (*in vivo*), and humans (patients). Inclusion criteria for the review were: (i) articles published between the year 2006 until 2020; (ii) articles published in English; (iii) simvastatin for CRC treatment; (iv) *A. muricata* leaf extract for CRC treatment; and (v) combination therapy studies that used simvastatin or *A. muricata* leaf extract in CRC treatment. The types of study designs that were eligible for the review were cell line studies,

animal studies, randomized control trials (RCT), observational studies, cohort studies, and case-control studies.

The author extracted the following information (if applicable) from studies included in the review: (i) name of the first author; (ii) year of publication; (iii) number of subjects; (iv) study design; (v) number of CRC patients in the treatment and control groups; (vi) definition of simvastatin or *A. muricata* leaf extract exposure; (vii) progression-free survival (PFS); (viii) overall survival (OS); (ix) percentage of patients alive and free from progression; (x) 95% confidence intervals (95% CIs); (xi) objective response rate (ORR); (xii) disease control rate (DCR); (xiii) time to progression (TTP); (xiv) complete response (CR); and (xv) partial response (PR).

Risk of bias assessment of the included studies was done using Cochrane risk-of-bias tool (Higgins, Altman, & Sterne, 2011), version 2 of the Cochrane risk-of-bias tool for randomized trials (Sterne *et al.*, 2019), and Joanna Briggs Institute Critical Appraisal Checklist (Joanna Briggs Institute, 2020).

RESULTS AND DISCUSSION

Search Results

Results and details of the study selection process were summarized and presented in the form of a PRISMA flow diagram (Figure 1). A total of 334 relevant articles were identified from the search results. From all of the articles, 73 were excluded due to duplication, while the remaining 261 were screened based on the titles and abstracts. After the exclusion of 192 unrelated articles, 69 full-text articles were further reviewed according to the inclusion and exclusion criteria and 28 articles were excluded with reasons. Finally, 41 studies that met the criteria were included in this review. Among them, 24 studies evaluated the effects of simvastatin or *A. muricata* on CRC cell lines, eight studies were conducted on animals, and the remaining nine studies were performed on humans.

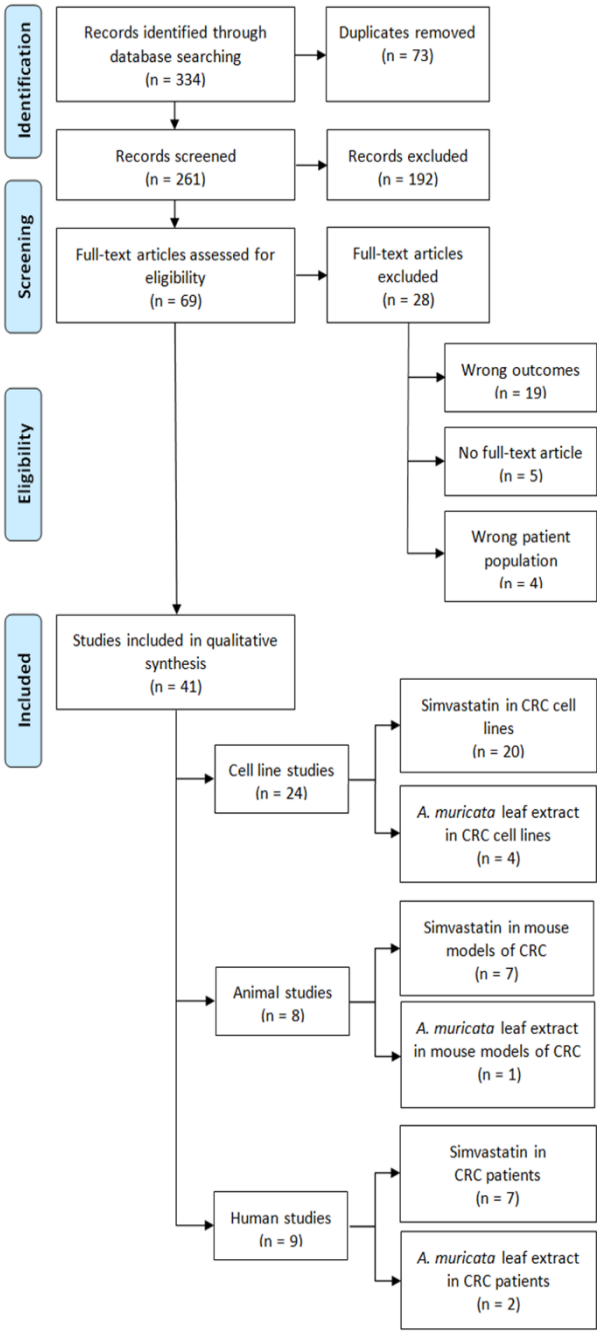

Figure 1. PRISMA flow diagram of the study selection process.

Experimental Evidence of Anti-Cancer Effects of Simvastatin

Over the years, *in vitro* and *in vivo* studies have demonstrated that simvastatin exerts pleiotropic effects independent of its lipid-lowering properties.

Studies have shown that simvastatin exhibits cytotoxic effects, mostly in a dose- and time-dependent manner, against various CRC cell lines, and in tumor xenograft models. Also, it has been demonstrated that simvastatin is capable of suppressing CRC cell growth and proliferation (Chang *et al.*, 2013; Chen *et al.*, 2018; Cho *et al.*, 2008; Gauthaman *et al.*, 2007; Ishikawa *et al.*, 2014; Jang *et al.*, 2016a; Kodach *et al.*, 2007; Luput *et al.*, 2018; Menter *et al.*, 2011; Qi *et al.*, 2010; Zheng *et al.*, 2019), inducing apoptosis (Chang *et al.*, 2013; Cho *et al.*, 2008; Gauthaman *et al.*, 2007; Kodach *et al.*, 2007; Qi *et al.*, 2010), promoting cell cycle arrest at G1 phase (Chen *et al.*, 2018; Gauthaman *et al.*, 2007; Jang *et al.*, 2016a; Lim *et al.*, 2015b), inhibiting epithelial-to-mesenchymal transition (EMT) (Zheng *et al.*, 2019), repressing cell migration and invasion (Zheng *et al.*, 2019), suppressing tumor angiogenesis (Cho *et al.*, 2008; Li *et al.*, 2017; Luput *et al.*, 2018), suppressing inflammation (Luput *et al.*, 2018), and reducing tumor size and volume (Cho *et al.*, 2008; Kodach *et al.*, 2007; Luput *et al.*, 2018).

Several mechanisms of simvastatin-induced apoptosis have been proposed, which includes the activation of the intrinsic pathway for apoptosis (Qi *et al.*, 2010; Jang *et al.*, 2016a) through downregulation of anti-apoptotic B cell lymphoma 2 (BCL2) expression, upregulation of pro-apoptotic BCL2-associated X (BAX) expression (Jang *et al.*, 2016a; Karagkounis *et al.*, 2018; Luput *et al.*, 2018), and activation of the pro-apoptotic ERK pathway (Jang *et al.*, 2016c; Karagkounis *et al.*, 2018). Several possible mechanisms for apoptosis initiation were via activation of the bone morphogenetic protein (BMP) pathway (Kodach *et al.*, 2007; Kodach *et al.*, 2011), and mevalonate pathway (Lu *et al.*, 2019). Meanwhile, a study demonstrated that in most CRC cell lines, simvastatin did not induce mevalonate pathway activation to exert its anti-cancer effect but rather inducing histone deacetylase 5 (HDAC5) upregulation and downregulated the enhancer of zeste homolog 2 (EZH2) (Ishikawa *et al.*, 2014). Another possible mechanism was the inactivation of the ERKs via inhibiting PI3K/Akt pathways (Chen *et al.*, 2018; Jang *et al.*, 2016b; Karagkounis *et al.*, 2018; Zheng *et al.*, 2019), inhibiting ERK1/2 phosphorylation (Karagkounis *et al.*, 2018) downregulation of insulin-like growth factor 1 (IGF-1), activation of IGF-1-induced ERK/Akt (Jang *et al.*, 2016b), activation of p38 mitogen-activated protein kinase MAPK-p53 signaling cascade, and inhibition of Sp1 expression (Chang *et al.*, 2013). Other proposed mechanisms were regulation of the Yes-associated protein 1 (YAP1)-mediated transcription (Liu *et al.*, 2018) via downregulation of survivin and connective tissue growth factor (CTGF) expression (Chang *et al.*, 2013; Gauthaman *et al.*, 2007; Lim *et al.*, 2015b), and inducing oxidative stress and generation of reactive oxygen species (ROS) (Qi *et al.*, 2010).

Chen *et al.* mentioned that simvastatin induced CRC cell cycle arrest at G1 phase via downregulation of cyclin-dependent kinases (CDKs)/cyclins (cyclin D1, cyclin E1, CDK2 and CDK4) genes, and upregulation of glycogen synthase kinase 3 beta (GSK3β) and cyclin-dependent kinase inhibitor 1B (CDKN1B) genes (Chen *et al.*, 2018). Simvastatin suppressed tumor angiogenesis by controlling the HER2/VEGF axis (Li *et al.*, 2017). HRG-b1 has an

important part in promoting angiogenesis via HER2 signaling (Kumar & Yarmand-Bagheri, 2001; Xiong *et al.*, 2001), and simvastatin was capable of repressing HRG-b1/HER2-induced angiogenesis (Li *et al.*, 2017). Simvastatin was also found to inhibit CRC cell migration and invasion by upregulation of the expression of tumor suppressive microRNA, miR-192, and modulating the levels of EMT-associated protein, such as increasing E-cadherin mRNA levels, decreasing β-catenin, Twist, RAB2A, PI3K and ERK levels (Zheng *et al.*, 2019).

In CRC cell lines with p53 mutation (mutp53), simvastatin was found to prevent metastasis and induce anti-proliferative effect through downregulation of Kruppel-like factor 2 (KLF2) and p21WAF1 expressions (Lu *et al.*, 2019). However, several studies found that simvastatin induced the activation of ERK and PI3K/Akt pathways (Karagkounis *et al.*, 2018; Qi *et al.*, 2010), increased NF-*κ*B levels (Riganti *et al.*, 2008), increased multidrug resistance mutation 1 (MDR1) expression (Palko-Łabuz *et al.*, 2019), did not induce cell cycle arrest (Cho *et al.*, 2008), and did not significantly induce apoptosis in primary cancer cell lines (Chen *et al.*, 2018). In addition, *in vitro* and *in vivo* data showed that a low concentration of simvastatin (2 μM) induced CRC cell growth (Kodach *et al.*, 2007).

Simvastatin in Combination Treatments

In vitro and *in vivo* studies demonstrated that simvastatin was able to generate a synergistic effect when combined with other anti-cancer drugs or treatments.

Ishikawa *et al.* found that the combination of simvastatin and MC1568, a selective class IIa HDAC II inhibitor, increased p27Kip1 protein expression and decreased EZH2 protein expression. CRC patients taking simvastatin were found to have higher overall survival (OS) and disease-free survival (DFS) compared to patients without simvastatin intake. This study discovered that simvastatin generates epigenetic alterations, such as EZH2 downregulation, which led to p27KIP1 upregulation which plays a role in simvastatin-induced anti-cancer effects (Ishikawa *et al.*, 2014).

A study combining simvastatin and bevacizumab, a monoclonal antibody that acts as an angiogenesis inhibitor, displayed that simvastatin increased the anti-angiogenic effects of bevacizumab. Simvastatin was found to inhibit angiopoietin 2, binding the immunoglobulin protein (BiP) and heat-shock protein 90 alpha (HSP90α) in CRC cells (Lee *et al.*, 2014). In another study, simvastatin encapsulated in long-circulating liposomes (LCL-SIM) was combined with 5-FU encapsulated in LCL (LCL-5-FU). Combination therapy with LCL-SIM and LCL-5-FU demonstrated anti-angiogenic actions on the tumor microenvironment (TME) *in vivo* and suppressed *in vivo* CRC cell growth (Luput *et al.*, 2020).

Compared to single treatments with simvastatin, cetuximab, or gefitinib alone, the combination of simvastatin and cetuximab or gefitinib was more effective in suppressing cell proliferation and increasing colony inhibition in common CRC cell lines and cetuximab resistant-KRAS mutant CRC cell lines. Combination of simvastatin and EGFR inhibitor (cetuximab or gefitinib) also demonstrated increased apoptosis induction and induced suppression of tumor growth in common CRC cell line xenografts and KRAS mutant xenografts, indicating that simvastatin enhanced cetuximab's cytotoxic effect towards CRC cells (Liu *et al.*, 2018). Similar results were found in studies combining simvastatin with 5-FU chemotherapy drug, class-II HDAC inhibitors,

and radiation therapy (Gauthaman *et al.*, 2007; Ishikawa *et al.*, 2014; Karagkounis *et al.*, 2018; Lim *et al.*, 2015b). Simvastatin caused depletion of GGPP, which led to decreased ERK1/2 phosphorylation; therefore, the EGFR-RAS-ERK1/2 pathway was suggested to be involved in simvastatin's action in enhancing radiation sensitivity (Karagkounis *et al.*, 2018).

Combination of simvastatin and ionizing radiation in p53 wild-type and p53-deficient CRC cells demonstrated that simvastatin can induce cell death by decreasing the protein levels of cell cycle-associated proteins, homologous recombination DNA repair, and mouse double minute 2 homolog (MDM2). In addition, simvastatin was able to become a radiosensitizer in p53-deficient CRC cells by increasing the expression of forkhead box class O 3a (FOXO3a), p21, and E-cadherin in p53-deficient cancer cells and xenograft models (Lee *et al.*, 2018).

Simvastatin elevated the cytotoxic effects of doxorubicin by increasing IκB kinase (IKK) activity, increasing multidrug resistance-associated protein 3 (MRP3) levels, and enhanced doxorubicin-induced tyrosine nitration of MRP3 via Ras homolog gene family, member A (RhoA) inhibition (Riganti *et al.*, 2008). In a similar study, the combination of simvastatin and flavones (6-hydroxyflavone, 7-hydroxyflavone and baicalein) enhanced cytotoxicity of doxorubicin in CRC cells with or without doxorubicin-resistant (Palko-Łabuz *et al.*, 2019). Additionally, simvastatin was found to augment the effectiveness of oxicam derivatives (PR17 or PR18) in inducing apoptosis in doxorubicin-resistant CRC cells (Środa-Pomianek *et al.*, 2019).

In contrast, simvastatin could not inhibit growth and colony formation in BRAF mutant cells, and the cells still showed resistance to cetuximab. Simvastatin decreased BRAF expression and BRAF enzymatic activity in KRAS mutant cells, but did not affect the KRAS levels. Meanwhile, BRAF expression in BRAF mutant cells remains unaffected subsequent to simvastatin treatment. Simvastatin treatment also reduced the levels of phosphorylated MAPK1/2 and MAP2K1 in KRAS and BRAF mutant cells. Results of this study displayed that simvastatin can revert resistance to cetuximab in BRAF wild-type and KRAS mutant CRC cells, but not in CRC cells with BRAF mutation. This study suggested the possibility that simvastatin sensitizes CRC cells to cetuximab by regulating BRAF protein levels and promoting apoptosis induction via the modulation of BCL2-like 11 (BCL2L11) and BCL2-antagonist of cell death (BAD) protein levels (Lee *et al.*, 2011).

Simvastatin in Treatments for CRC Patients

In a prospective observational study involving 25 CRC patients and 25 healthy volunteers, nine CRC patients were treated with 80mg of simvastatin for 14 days, before blood samples were taken. CRC patients treated with simvastatin displayed significant reductions in serum IL-6 levels ($p < 0.05$), while the decrease in IL-8 serum levels was not significant (Malicki *et al.*, 2009)

An open-label, single-arm, phase II study involving 55 patients, evaluated the effect of simvastatin in combination with capecitabine and oxaliplatin (XELOX) and bevacizumab, for the treatment of metastic CRC. Treatment regimens include administration of 130 mg/m^2 of oxaliplatin, 1,000 mg/m^2 of capecitabine (twice a day). After that, bevacizumab (7.5mg/kg) was administered, followed by oxaliplatin. Simvastatin (80 mg) was taken orally once a day during chemotherapy, until the treatment regimens were terminated. After about 15 months of treatments, results

demonstrated that the median PFS was 10.4 months (95% CI, 9.6-11.1), median OS was 19.0 months (95% CI, 11.9-26.0), 88.3% (95% CI, 74-96) DCR, 58.3% ORR (95% CI, 44-77), one CR, and 34 PRs. The most-reported toxic effects were nausea, vomiting, diarrhea, neuropathy, fatigue, rash, stomatitis, and hand-foot syndrome. Results of this study showed that combination treatment of simvastatin (80 mg), XELOX and bevacizumab, was considered safe and demonstrated clinical efficacy in metastatic CRC patients (Kim *et al.*, 2019).

A multicenter phase II study involved 49 CRC patients. The patients received FOLFIRI regimen with irinotecan 180 mg/m^2 diluted in 500 ml 5% dextrose, 200 mg/m^2 of leucovorin, 400 mg/m^2 of 5-FU, and 2,400 mg/m^2 bolus injection. In addition, 40 mg of simvastatin/ day was taken orally during the chemotherapy until the chemotherapy regimen was stopped. After four weeks of treatment, one patient displayed CR while 22 patients showed PRs. Results of this study includes 46.9% ORR (95% CI, 31.0–58.8), 83.7% DCR (95% CI, 73.4–94.0), median OS was 21.8 months (95% CI, 14.4, 29.2), and median TTP was 9.9 months (95% CI, 6.4, 13.3). During the combination treatment, the most common side effects were neutropenia and nausea, and there were no life-threatening adverse effects. This study concluded that the addition of simvastatin in FOLFIRI therapy was safe; however, phase III trial is required to analyze the effect of simvastatin on the OS of metastatic CRC patients (Lee *et al.*, 2009).

In a multi-center, double-blind, placebo-controlled, randomized phase III trial, the combination of capecitabine plus irinotecan (XELIRI) or FOLFIRI chemotherapy with simvastatin was evaluated and compared to the combination with placebo. FOLFIRI chemotherapy regimen include 250 mg/m^2 of irinotecan, 1000 mg/m^2 of capecitabine (twice a day), or 180 mg of irinotecan diluted in 500 ml of 5% dextrose, continued by 200 mg/m^2 of leucovorin, and 400 mg/m^2 and 2400 mg/m^2 of 5-FU. Simvastatin (40 mg), or placebo, was orally administered once a day during the chemotherapy until the regimen was terminated. Out of 269 patients, 134 patients were randomly assigned to receive a combination of XELIRI/ FOLFIRI and simvastatin treatment, while the rest received the combination of XELIRI/ FOLFIRI and placebo. According to the study that was conducted for approximately 39 months, the median PFS in the simvastatin group compared to placebo group was 5.9 months (95% CI, 4.5–7.3 months) VS 7.0 months (95% CI, 5.4–8.6, p = 0.937). The ORR, DCR, CR, and PR in the simvastatin group compared to place group were 11.9% VS 11.8%, 67.2% VS 71.1%, 2 (1.5%) VS 1 (0.7%), and 14 (10.4%) VS 15 (11.1%), respectively. There was no significant difference, in terms of median OS, between CRC patients in the simvastatin and placebo group (15.9 months VS 19.9 months, p = 0.826). The median PFS (simvastatin group = 5.4 months, 95% CI, 1.9–8.9 VS placebo group = 6.0 months, 95% CI, 4.4–7.6, p = 0.859) and OS (simvastatin group = 13.7 months, 95% CI, 11.8–15.6 VS placebo group = 14.5 months, 95% CI, 10.8–18.1, p = 0. 615) were also not significantly different in 83 patients with KRAS mutation (simvastatin group = 49 patients and placebo group = 34 patients). The most common toxic effects during the study were anemia, neutropenia, nausea, alopecia, anorexia, and diarrhea. Simvastatin did not increase any of the adverse effects. At the end of the study, there were 189 progression events and 165 deaths in CRC patients. Overall, results demonstrated that low-dose administration of simvastatin (40 mg) to XELIRI/FOLFIRI treatment did not affect or improve the PFS in metastatic CRC patients (Lim *et al.*, 2015a).

In an RCT, administration of drugs in metastatic CRC patients was randomized between the combination of capecitabine and oxaliplatin (CAPOX) and bevacizumab, or the combination of CAPOX, bevacizumab and cetuximab. A total of 529 patients were involved in this study, with 78 patients receiving statin therapy. Results of the study showed the median PFS in KRAS wild type CRC patients (median PFS of statin users = 10.3 VS non-statin users = 11.4 months, p = 0. 882) and median OS in KRAS wild type CRC patients (median OS of 19 statin users = 22.4 weeks VS 145 non-statin users = 19.8 weeks, p = 0. 650). In summary, results demonstrated that statin did not significantly affect or improve the PFS (median PFS of 16 statin users = 7.6 months VS 83 non-statin users = 6.2 months, p = 0.291) or OS (median OS of 16 statin users = 18.1 weeks VS 83 non-statin users = 14.5 weeks, p = 0. 125) in KRAS mutant CRC patients (Krens *et al.*, 2014).

A single-arm, phase II study investigated the combination of simvastatin and cetuximab in 18 CRC patients with KRAS mutation. Simvastatin (80 mg and 20-35 mg/kg/day) was taken orally, and cetuximab (250 - 400 mg/m^2) was administered a week after simvastatin treatment. At the end of this study, results showed that the median PFS was 9 weeks, median OS was 31.5 weeks, 6% ORR, partial remission in 1 patient, and four patients (22 %) were free from progression (12.5 weeks after the first cetuximab administration). Common side effects that occurred during the study were fatigue, acne, and rashes, and one of the severe adverse effects resulted in the death of a participant. Results of this study showed that the combination treatment of simvastatin and cetuximab was not applicable to treat CRC patients with KRAS mutation (Baas *et al.*, 2015b).

Another single-arm, multicentre phase II study evaluated the safety and efficacy of the combination of simvastatin and panitumumab in 15 CRC patients with KRAS mutation. Simvastatin (80 mg) was taken once a day and after a week, panitumumab (6 mg/kg) was given every two weeks. Results demonstrated that the median TTP was 8.4 weeks, median OS was 19.6 weeks, 0% ORR, no patients had partial remission, and one participant (7%) was free from progression at the end of the study. The most common adverse effects were fatigue, anemia, and hypomagnesemia. In summary, the study in CRC patients with a KRAS mutation demonstrated that administration of simvastatin (80 mg) once a day did not induce sensitivity to panitumumab (Baas *et al.*, 2015a).

Experimental Evidence of Anti-Cancer Effects of *A. muricata* Leaf Extract

In vitro and *in vivo* studies performed on CRC cell lines and animal models, investigated the anti-cancer effects and possible mechanisms of *A. muricata* leaf extract, alone or in combination with other treatments, against CRC cells.

Studies have shown that extract of *A. muricata* leaves exerts cytotoxic effects, mostly in a dose- and time-dependent manner, against various CRC cell lines, and in tumor xenograft models. Studies have demonstrated that extract of *A. muricata* leaves was capable of suppressing CRC cell growth and proliferation (Moghadamtousi *et al.*, 2014; Moghadamtousi *et al.*, 2015), inducing apoptosis (Abdullah *et al.*, 2019; Awad *et al.*, 2020; Moghadamtousi *et al.*, 2014; Moghadamtousi *et al.*, 2015), promoting cell cycle arrest at G1 phase (Moghadamtousi *et al.*, 2014), promoting cell cycle arrest at G2/M phase (Awad *et al.*, 2020), repressing cell migration and invasion (Moghadamtousi *et al.*,

2014), and reducing tumor size and volume (Moghadamtousi *et al.*, 2015).

Possible mechanisms of *A. muricata* leaf extract includes activation of apoptosis intrinsic pathway via upregulation of pro-apoptotic BAX expression and downregulation of anti-apoptotic BCL2 expression (Abdullah *et al.*, 2019; Awad *et al.*, 2020; Moghadamtousi *et al.*, 2014; Moghadamtousi *et al.*, 2015). Other possible mechanisms were cytochrome c-mediated apoptosis through cytochrome c release, initiation of programmed cell death and mitochondria-mediated apoptosis through induction of lactate dehydrogenase (LDH) leakage, depletion of mitochondrial membrane potential (MMP), and induction of DNA fragmentation (Moghadamtousi *et al.*, 2014; Moghadamtousi *et al.*, 2015). Several other possible mechanisms were downregulation of proliferating cell nuclear antigen (PCNA) (Awad *et al.*, 2020; Moghadamtousi *et al.*, 2014), and the downregulation of MDR1 and mitogen-activated protein kinase 1 (MAPK1) expression (Awad *et al.*, 2020). Furthermore, *A. muricata* leaf extract was shown to induce oxidative stress and generate ROS (Moghadamtousi *et al.*, 2014; Moghadamtousi *et al.*, 2015).

A study investigating the *in vivo* effect of ethyl acetate extract of *A. muricata* leaves (EEAML) in azoxymethane (AOM)-induced CRC cell discovered that the inhibitory effect of EEAML in the formation of aberrant crypt foci (ACF), was found to be similar to the effect generated by 5-FU. Compared with 5-FU, EEAML even demonstrated higher antioxidant defense, and displayed a higher decrease in malondialdehyde (MDA) production, indicating that EEAML had stronger protective effects against oxidative stress in CRC cells (Moghadamtousi *et al.*, 2015).

A. muricata Leaf Extract in Combination Treatments

A. muricata leaf extract in combination with cisplatin, a common chemotherapeutic drug for the treatment of various cancers, demonstrated a synergistic effect in inducing cytotoxicity in CRC cells. Interestingly, in this study *A. muricata* leaf extract was found to promote cell cycle arrest at the G2/M checkpoint instead of the G1 phase. However, the combination treatment of *A. muricata* leaf extract and cisplatin was found to induce cell cycle arrest at the S phase. The combination treatment also displayed lower expression of MDR1 and MAPK1 mRNA than the treatment with cisplatin alone (Awad *et al.*, 2020).

A. muricata Leaf Extract in Treatments for CRC Patients

In a double-blind placebo-controlled RCT, 20 patients were randomly assigned into taking a capsule per day consisting of either 300 mg of a water extract of *A. muricata* leaves (ESFAM) (10 ptients) or maltose as the placebo for eight weeks (10 patients). After eight weeks of treatment, serum was taken from the peripheral blood samples of all patients from both ESFAM and placebo groups and was used to treat CRC cells. Based on the results, the caspase-9 activity from ESFAM group was significantly elevated compared to before receiving the treatment (0.59, 95% CI 0.49-0.71 VS 0.68, 95% CI 0.60–0.75, $p = 0.048$). In contrast, serum from placebo group showed a significantly lower activity of caspase-9 compared to before the treatment (0.62, 95% CI 0.52–0.79 VS 0.54, 95% CI 0.47–0.64, $p = 0.014$). However, CRC cells displayed an inconsistent result where the activity of caspase-8 was shown to be decreasing (0.17, 95% CI 0.14–0.20 VS 0.15, 95% CI 0.13–0.19, $p = 0.057$) instead of

increasing after treatment with serum from patients of the ESFAM group. Meanwhile, caspase-8 activity in CRC cells after treatment with serum from patients receiving placebo group showed no changes pre and post treatment (0.19, 95% CI 0.16–0.23 VS 0.19, 95% CI 0.16–0.23, *p = 0.433*). In summary, this study exhibited that ESFAM induced cytotoxic activities in CRC cells through significant elevation of caspase-9 activity (Indrawati *et al.*, 2016).

In a double-blind placebo-controlled RCT, 28 patients were randomly assigned to take a capsule per day consisting of either 300 mg of ESFAM (14 patients), or maltose as the placebo for eight weeks (14 patients). After eight weeks of treatment, there was a significant increase in energy intake in the ESFAM group, at week eight (1787.55±620.2 kcal) compared to week four (1544.31±560.43 kcal, *p = 0.04*) and week six (1596.6±531.11 kcal, *p = 0.02*). In contrast, there was no significant difference in energy intake in the placebo group. ESFAM group displayed a slightly higher BMI (23.3±0.0 VS 21.0±3.2, *p = 0.17*) and haemoglobin levels (12.7±1.6 and 12.6±1.5, *p = 0.88*) compared to the placebo group. Overall, the present study demonstrated that ESFAM inhibited the growth of CRC cells. However, to confirm the findings from this study and to recalculate the sufficient dose for the use of ESFAM, a longer research study with a larger group of CRC patients and a thorough evaluation of the efficacy and safety of ESFAM, is required (Indrawati *et al.*, 2017).

This review was conducted following a protocol designed prior to starting the review process (Pollock & Berge, 2018). A comprehensive search in four databases was performed to avoid evidence selection bias. Screening and selection of potential articles were done by two reviewers, and the draft report of the systematic review was peer-reviewed to avoid reporting bias.

There are possibilities that several biases might occur when selecting the included studies, such as selection bias from poor randomization in randomized blinded studies, detection bias in analyzing study results due to knowledge of treatment allocation in unblinded non-randomized studies, performance bias by the researcher towards the different intervention groups, or changed behaviors in the study volunteers due to knowledge of the allocated treatments, and reporting bias due to selective reporting.

Several limitations in this systematic review include, the exclusion of grey literature, only reviewing articles that are published in English and reviewing the effects of a drug and an extract, and not the individual agents within this drug and extract. Furthermore, the number of studies reporting *A. muricata* leaf extract was considerably lower compared to studies about simvastatin. Therefore, there was less information known about *A. muricata* leaf extract.

CONCLUSION

This review has showed that simvastatin and *A. muricata* leaf extract exhibit anti-cancer effects in CRC cells such as suppressing CRC cell growth and proliferation, inducing apoptosis, promoting cell cycle arrest at G1 phase, repressing cell migration and invasion, and reducing tumor size and volume. There are various possible mechanisms of actions on how simvastatin and *A. muricata* leaf extract induce anti-cancer effects against CRC cells. The possible mechanisms of actions for simvastatin to exert anti-cancer effects were through the activation of intrinsic apoptosis pathway, pro-apoptotic ERK pathway, BMP pathway, and

mevalonate pathway. Other mechanisms include ERKs inactivation, regulation of the Yes-associated protein 1 (YAP1)-mediated transcription, promotes cell cycle arrest at G1 phase, regulating the HER2/VEGF axis, and modulation of the EMT-associated protein levels. The possible mechanisms of actions on how *A. muricata* leaf extract exert anti-cancer effects include promoting cell cycle arrest at G1 or G2/M phase, activation of apoptosis intrinsic pathway, activation of cytochrome c-mediated apoptosis, and initiation of programmed cell death and mitochondria-mediated apoptosis. In combination therapy studies, simvastatin and *A. muricata* leaf extract were mostly found to exhibit a synergistic effect and enhanced the effects of other anti-cancer treatments. However, several studies demonstrated that the addition of simvastatin did not generate a synergistic effect when combined with some treatments. Current findings are still insufficient to determine the specific mechanisms on how simvastatin and *A. muricata* leaf extract induce anti-cancer effects against CRC cells. Therefore, the present evidence is still inadequate to recommend the use of simvastatin or *A. muricata* for CRC treatment. More experimental evidence is required to define the specific molecular mechanisms of actions of simvastatin and *A. muricata* leaf extract to induce anti-cancer effects against CRC cells.

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