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In vitro growth-inhibitory effect of medicinal plants used in Philippine folk medicine for treatment of diarrhoea against intestinal bacteria associated with colorectal cancer

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled *In vitro* growth-inhibitory effect of medicinal plants used in Philippine folk medicine for treatment of diarrhoea against intestinal bacteria associated with colorectal cancer independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague

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Mikuláš Středa

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Abstract

The occurrence of colorectal cancer (CRC) has shown significant increase in the last century and is the third most common cancer and the fourth most the fourth most common cause of cancer-related death. Healthy microbiota environment in intestine is key factor for preventing CRC. In the last years the connection with specific bacteria can be seen in the colorectal tumour microenvironment. Although currently use treatment such as surgery or chemotherapy is quite successful, many patients want to go back to traditional medicine which can have less side effects. It has previously been reported that many plant species are used in traditional Philippine medicine for treatment of health complaints related to CRC such as diarrhoea. Since many of them already showed antibacterial properties, we decided to evaluate the *in vitro* effects of these plants against selected representatives of bacteria associated with CRC. In this thesis 10 representatives of medicinal plants, namely *Acalypha grandis* (inflorescence), *Artocarpus blancoi* (leaves), *Artocarpus camansi* (fruit), *Artocarpus elasticus* (leaves), *Artocarpus lakoocha* (bark, leaves, fruit), *Citrus microcarpa* (leaves, fruit), *Crescentia cujete* (leaves, bark, fruit), *Dysoxylum blancoi* (bark), *Euphorbia thymifolia* (aerial part), *Merremia peltata* (leaves), were tested against 6 CRC associated bacteria *Eischerichia coli, Streptococcus bovis, Clostridium septicum, Fusobacterium necrophorum, Fusobacterium nucleatum*, and *Peptostreptococcus anaerobius* by broth microdilution method. Among all samples tested, *A lakoocha* and *A. elasticus* were only active plant species producing growth-inhibitory effect against *E. coli* (MIC = 256 μg/mL) and *S. bovis* (MIC = 512 μg/mL), respectively. The results of further experiments also showed the time dependent growth-inhibitory effects of three extracts, namely *A. blancoi, A. elasticus* and *D. blancoi,* against *S. bovis*. Other tested bacteria, namely *Clostridium septicum*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum* and *Peptostreptococcus anaerobius*, did not inhibit growth of CRC bacteria. These results represent experimental background for future studies focused on the effects of Philippine medicinal plants on growth of bacteria associated with CRC.

Key words: Plant extract, antimicrobial activity, colorectal cancer

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5-FU – 5-Flouracil

ATCC – American Type Culture Collection

BBR – Berberine

CCM – Czech Collection of Microorganisms

CRC – Colorectal cancer

DMSO – Dimethyl sulfoxide

IBD – Inflammatory bowel disease

MHB – Mueller-Hilton broth

MIC – Minimal inhibitory concentration

TTO – Tea tree oil

WCB – Wilkins-Chalgren broth

1. Introduction

A healthy human body contains at least tenfold more bacteria cells than human cells and the most abundant and diverse microbial community resides in the intestinal tract (Boleij & Tjalsma 2012). The human gut microbial is considered to play a crucial role in gut health (Duncan et al. 2007). Both environmental and generic factors can contribute to the imbalance of intestinal flora, which may promote the occurrence and development of colorectal cancer (CRC) (Liu et al. 2022). New cases are identified annually, and lifetime risk is approaching 1 in 15 for men and 1 in 19 for women. CRC has become increasing problem in developed countries, where it is one of the leading neoplastic diseases (Lewandowska et al. 2022). There are many risk factors which can lead to developing of CRC such as age, lifestyle, and past chronic illness (Mármol et al. 2017). Recently, several studies have identified species of bacteria associated with CRC including examples such as *Bacterioides fragilis, Clostridium septicum, Escherichia coli, Fusobacterium necrophorum, Fusobacterium nucleatum, Helicobacter,* and *Streptococcus bovis* (Sun & Kato 2016). As bacterial resistance to antibiotics continues to increase, there is a growing focus on researching novel medicinal compounds that could offer solutions to this pressing issue. A significant portion of this research is directed towards exploring the potential of plant-based extracts used in traditional medicine. Potential candidates could be plants which are used for treatment of diarrhoea in traditional medicine in Philippines.

2. Colorectal cancer (CRC)

Before the 20th century, CRC was relatively uncommon however the incidence has risen dramatically especially in the last fifty years (Pericleous et al. 2013). Now, CRC is the third most common cancer, the fourth most common cause of cancer-related death and the main cause of death in gastrointestinal cancer (Granados-Romero et al. 2017). Most cases of CRC are detected in Western countries, with its incidence increasing year by year (Mármol et al. 2017). Up to 55% of CRC cases are occurring in more developed countries (Favoriti et al. 2016). It often arises from a combination of genetic predisposition and lifestyle factors such as an unhealthy diet, tobacco use, high BMI, and low physical activity (Baojun et al. 2022). CRC, mostly comprising adenocarcinoma of the colon and rectum, is defined as a transformation of the normal colonic and rectal epithelium to a precancerous lesion (adenomatous intermediate) and possibly to an invasive carcinoma (adenocarcinoma), which may spread to different distant orangs and give rise to metastatic lesions (Alzahrani et al. 2021). The disease typically originates from benign polys or adenomas in the intestinal epithelial layer, progressing to cancer through molecular changes (Chen et al. 2022). CRC often grows slowly, and generally does not produce symptoms until reaching a considerable size of several centimetres, which may block the passage of faeces and lead to cramping, pain, or bleeding that can present as visible bleeding with bowel movements or, rarely dark ‘‘tarry’’ stools (Simon 2016).

2.1. Epidemiology

The epidemiology of the disease varies across regions, age groups, genders, and racial groups. The role-playing factors are exposure, demographics, and genetic mutations as well (Baidoun et al. 2020). CRC is most diagnosed in Australia and New Zealand, followed by Europe and North America. The highest rates of death from CRC are reported in Central Eastern Europe. In contrast, the regions with the lowest rates are Asia and Africa (Pardamean et al. 2023). Of the patients that are diagnosed with colorectal cancer, 90% are older than 50 years, with a median age of 64 years. However, the disease is more aggressive in patients who are diagnosed at younger ages (Granados-Romero et al. 2017). Globally, there were 1,931,590 new cases of CRC and

935,173 deaths in 2020 (Khaleel et al. 2023). Gender differences have been also observed to influence the risk of developing CRC, noting that women have a lower risk and have better overall and cancer-specific survival rates (Yang et al. 2017). During 2020, 10.6% and 9.4% of all worldwide cancer cases were men and women with colorectal cancer, respectively (Sung et al. 2021).

2.2. Causes

Risk factors that place an individual at increased risk of CRC include personal or family history of adenomatous polyps that may lead to colonic neoplasia, hereditary syndromes that predispose to the development of CRC (up to 35% can be explained by hereditary factors), inflammatory bowel disease (IBD), and the genetic contribution to sporadic cancer (Ahmed 2004; Granados-Romero et al. 2017). CRC is caused by mutations that target oncogenes, tumour suppressor genes and genes related to DNA repair mechanisms. Of the cancers that begin in the colorectal region, the vast majority (over 95%) are classified as adenocarcinomas (Alzahrani et al. 2021). Several risk factors have been proposed including the adoption of a Westernized diet (Pericleous et al. 2013), obesity, a diet low in fruits and vegetables, a high intake of red and processed meats, refined grains and high-fat dairy products (Thanikachalam & Khan 2019). These dietary patterns can lead to insulin resistance, which in turn may promote the growth of colon cancer precursor lesions and the development of CRC (Bruce et al. 2000).

2.3. Management

Dietary lifestyle is highly relevant to the health of the colon and rectum. Not only is the balance of what we consume and our overall nutritional state important to the maintenance of bowel health but also to the prevention and treatment of colorectal diseases (Young 2000). It has been estimated that nutrition could account for more than one third of cancer deaths and that dietary factors are responsible for 70% to 90% of all cases. Therefore, diet optimization could potentially help reduce the incidence of this type of malignancy (Pericleous et al. 2013).

In the recent 2 decades, improvements have been achieved in the outcomes of rectal cancer surgery with the advances in surgical techniques as well as adjuvant therapy

(Orsenigo et al. 2007). Surgical treatment of CRC is based on tumour resection and removal of associated lymph glands (Salibasic et al. 2019). Today there are two main types of surgeries performed on CRC. First one is open surgery (open colectomy), where are the malignant tumours of colon and rectum, the segment of bowel containing the tumour and its supplying vascular pedicle excised, which lead to keeping local margins free of malignancy (Brown et al. 2019). The second one is laparoscopic colectomy, which is a minimally invasive surgical technique offering several advantages over the traditional open colectomy. These include reduced postoperative pain, improved cosmetic outcomes, earlier return of bowel function and shorter hospital stay (Chang & Nelson 2005; Wasserberg 2010). This approach is also feasible for patients with previous abdominal surgery, with comparable peri-operative and long-term outcomes to those without previous surgery (Nozaki et al. 2008).

For the treatment, there is also possible palliative chemotherapy, which is used for advanced or metastatic CRC, or as adjuvant chemotherapy after the surgery. 5-Fluorouracil (5-FU) is the most widely used chemotherapeutic agent. It is used to treat different malignant tumours, including breast, pancreatic, skin, stomach, esophageal and neck cancers (Vodenkova et al. 2020). The use of oral 5-FU alone was abandoned early due to its unpredictable gastrointestinal absorption and marked variation in pharmacokinetics. Combination with leucovorin showed better results with fewer side effects (Inoue et al. 2010). The clinical benefits of 5-FU treatment, however, are often short-lived, and the majority of treated patients do not realize complete eradication of tumour cells, resulting in poor outcomes due to recurrence after 5-FU therapy (Cho et al. 2020).

The adjuvant chemotherapy is used after surgical resection of colorectal cancer. The surgery aims to destroy the remaining tumour cells and thus to reduce the risk of recurrence of the disease. The potential CRC recurrence rate after surgery may be as high as 30% (Vail et al. 2024). It is particularly beneficial in stages II and III, however, the efficacy in elderly patients with stage III has shown varying results (Rúa et al. 2022). Also, patients in stage II showed no improvement in overall survival and disease-free survival with AC was not statistically significant. Overall, the effectiveness of adjuvant chemotherapy against CRC varies based on factors like age, disease, stage, and chemotherapy regimen (Breadner et al. 2022). Now AC is established as a standard

treatment worldwide with clearly demonstrated benefits in survival and disease recurrence after curative resection (Demols & Van Laethem 2002).

2.4. Bacteria associated with CRC

The large intestine is a complex microbial ecosystem harbouring more than 500 different bacterial species and around 75% of these remain uncultured (Duncan et al. 2007). The activities of the microbial community that inhabits the large intestine are considered to play an important role in the maintenance of gut health and in the aetiology of gut disease in humans (Gill & Rowland 2002). The large intestinal microbiota is conveniently viewed as being a homogeneous entity, but the reality is quite different, because the bacteria exist in a multiplicity of different microhabitats and metabolic niches that are associated with the mucosa, the mucus layer and particle surfaces in the colonic lumen (Macfarlane et al. 2000). The chance of an individual developing CRC may be impacted by a sequence of consecutive microbial impacts. This idea is reinforced by the unequal bacterial colonization and metabolic results observed throughout the stages of CRC (Dougherty & Jobin 2023). Dysbiosis, characterized by an imbalance between the beneficial and opportunistic gut microbiota, is associated with several gastrointestinal disorders, such as IBD, irritable bowel syndrome or CRC (Quaglio et al. 2022). There are many risk factors that can modify the indigenous microbiota such as obesity or diets. Thus, the microbiota or its metabolites may be the proximate environmental modifiers of risk for CRC (Flemer et al. 2017). Animal studies have shown that bacteria may potentially contribute co CRC development through direct interaction with the host's immune system, production of cancer-associated metabolites, and release of genotoxic virulence factors (Kostic et al. 2013; Eaton et al. 2015; Cipe et al. 2015). The bacteria in the colon's mucus layer, via direct contact or metabolite communication, may affect host cellular balance and inflammation. Gut microbiota disruptions may also link to colorectal cancer development (Carding et al. 2015). Individual bacterial species associated with colorectal cancer are *Bacterioides fragilis, Clostridium septicum, Escherichia coli, Fusobacterium necrophorum, Fusobacterium nucleatum, Helicobacter, Streptococcus bovis* (Sun & Kato 2016). *Escherichia coli* and *Streptococcus bovis* belong to aerobic bacteria, meanwhile

Clostridium septicum, Fusobacterium necrophorum and *Peptostreptococcus anaerobius* are anaerobic bacteria.

E. coli are rod-shaped, gram-negative bacteria that can be commonly found in the human gastrointestinal tract and are frequently identified in stool cultures. Even though they are usually harmless, *E. coli* can become harmful when they acquire genetic material, and it is one of the leading bacterial causes of diarrhoea (Nataro & Kaper 1998). The connection between CRC and *E. coli* started during the observations, where *E. coli* was frequently found to colonize cancer lesions and neighbouring epithelium. Often accumulates there in large numbers, even sometimes it is the only cultivable organism in close contact with the diseased site (Bonnet et al. 2014). Specific strains of intestinal *E. coli* are able to influence the development and initiation of CRC by exploiting virulence factors and inflammatory pathways (Nouri et al. 2021), due to carrying a 54-kb biosynthetic gene cluster (BGC), which can be found primarily in the B2-phylogroup called polyketide synthase that encodes for secondary metabolite named colibactin, which causes DNA damage in mammalian cells (Dougherty & Jobin 2023).

S. bovis belongs to gram-positive, group D *Streptococcus*. Around 5% to 16% of people carry *S. bovis* as a normal part of their gastrointestinal tract flora (Noble 1978). The clinical manifestations happen when the organism invades the bloodstream and causes bacteraemia and infective endocarditis (Hasan et al. 2010). In 1951, *S. bovis* was first linked to CRC (Mathews 1951). Numerous other case reports have since confirmed this correlation. From 25% to 80% of patients with *S. bovis* bacteraemia can develop concomitant colorectal tumours. After some years, colonic neoplasia may arise because of the presentation of bacteraemia or infectious endocarditis of *S. bovis* (Abdulamir et al. 2011). *S. bovis* infection is particularly high in patients with colonic polyps, adenocarcinomas, inflammatory bowel disease, and chronic gastrointestinal tract disease (Al-Jashamy et al. 2010).

C. septicum is an anaerobic, motile, gram-positive, and endospore-forming microorganism that is an uncommon cause of human infections and is generally grown at 37°C (Alves et al. 2021). It can cause bacteraemia and spontaneous myonecrosis, which occurs in the absence of traumatic injury. *C. septicum* bacteraemia is rapidly progressing and can be fatal if not treated within 48 hours (Chipp et al. 2009). Its infections are known to co-exist with malignancy and it is thought that disruption of the

normal mucosal barrier caused by mucosal ulceration of the tumour surface and haematogenous invasion allows a portal of entry for the bacteria (Schaaf et al. 1980). It is held that the gastrointestinal tract is a common point of access for *C. septicum* bacteraemia, which is probably one of the explanations for its close association with gastrointestinal tumours such as colon cancer (Kopliku et al. 2015). In colorectal cancer, the acidic and hypoxic environment provided by anaerobic glycolysis of the tumour may be conducive to spore germination and lead to active infection (Chew & Lubowski 2001).

F. necrophorum is a gram-negative, non-spore-forming, nonmotile, anaerobic, pleomorphic bacterium. It occurs in liver abscesses, oral cavity, gastrointestinal tract and genitourinary tract of humans and animals (Langworth 1977). It is a member of the normal flora that, unlike other commensal anaerobic non-spore-forming bacteria, can become a primary invasive pathogen causing serious and even life-threatening disease (Brazier 2002). However, the incidence of human infections is recently on the increase, and this is attributed largely to alterations in antibiotic usage patterns, malnutrition, and poor oral hygiene (Chukwu et al. 2014). *F. necrophorum* causes the rare, life-threatening Lemierre's syndrome, which is characterised by anaerobic oropharyngeal infection leading to septic thrombophlebitis of the internal jugular vein (Lee et al. 1997). *F. necrophorum* also has connection with CRC, because this bacterium is more frequently present in CRC tumour tissue samples compared to healthy tissue and colorectal adenomas (Villar-Ortega et al. 2022).

P. anaerobius is a gram-positive anaerobic coccus found in the gastrointestinal and vaginal microbiota. The microorganism is mainly found in polymicrobial and scarcely in monobacterial infections such as prosthetic and native endocarditis (Legaria et al. 2021). It is often associated with infections of the abdominal cavity and the female genitourinary tract. The problem with *P. anaerobius* is its increasing resistance to antibiotics (Shenoy et al. 2018). Its association with CRC can be found in faecal samples and in the gut mucosa of patients with CRC, where its amount is significantly increased. *P. anaerobius* mainly colonizes the colon and is often found within foci of intestinal dysplasia (Karpiński et al. 2022).

F. nucleatum is a gram-negative anaerobic bacterium, which is part of the gut, and oral commensal flora, generally found in human dental plaque. Its presence could

be associated with various human diseases, including periodontal angina, and lung and gynecological abscesses (Stokowa-Sołtys et al. 2021). In recent years, several studies have reported that the level of *F. nucleatum* is significantly elevated in human colorectal adenomas and carcinomas compared to that in adjacent normal tissue (Shang & Liu 2018). *F. nucleatum* has been linked through its adhesion proteins (FadA and Fap2), which allow it to specifically attach to colorectal carcinoma cells (Kaiumov et al. 2023). After the bacterium is attached, it indices inflammation and suppresses host immunity, which creates a favourable environment for tumour growth and development (Wu et al. 2019).

3. Antibacterial effect of medicinal plants

Antibiotics have proven to be powerful drugs for the control of infectious diseases and remain one of the most significant discoveries. However, their extensive and unrestricted use has a selective pressure upon bacteria, leading to the development of antimicrobial resistance (Abreu et al. 2012). Research on new antimicrobial substances must therefore be continued and all possible strategies should be explored. Besides small molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agent (Cos et al. 2006). Plants can produce several antibacterial compounds. These plants are often well-known for their medicinal value and are widely used in communities for the treatment of various diseases (Yaseri & Mirzaei 2017). Plants produce over 100,000 small-molecule compounds, where most of them have antimicrobial activity (Lewis & Ausubel 2006). These activities could be described as inhibition of the bacterial growth by interfering with their cellular processes or structure (Ríos & Recio 2005). Some plants can also disrupt bacterial cell membranes thanks to certain bioactive compounds, which can lead to cell lysis and death (Abreu et al. 2012), or inhibit bacterial enzymes in bacterial metabolism, affecting their ability to survive and replicate (Cos et al. 2006). Modulation of bacterial gene expression can be done by plant compounds, which affect influence of the expression of bacterial genes, leading to changes in bacterial behaviour and susceptibility to antimicrobial agents(Gibbons 2008).

Tea tree oil (TTO) is a yellow liquid extracted from Melaleuca alternifolia. TTO has been used in medicine for at least 80 years. It consists of about 100 different compounds, and the major components are terpenes and sesquiterpenes (Li et al. 2016).

In vitro studies have demonstrated a good antimicrobial effect of TTO or individual components towards a broad range of bacteria., yeasts and other fungi. In recent years, TTO has gained additional interest, as it shows growth-inhibiting effects even against resistant microbes (Hammer et al. 1998; Kulik et al. 2000). Terpinen-4-ol is presumed to be the most important antimicrobial agent of TTO. The antimicrobial mechanisms of terpinene-4-ol are due to the lipophilicity of the oil, which can penetrate through cytoplasmic membranes and cell walls (Li et al. 2016).

Curcuma longa, commonly known as turmeric, is widely used as a spice and colouring agent, and is well known for its medicinal properties (Luthra et al. 2001). Curcumin, which can be found in turmeric, belongs among the most researched bioflavonoids today, and many studies have confirmed its antioxidant, antibacterial, gastroprotective and many others health properties (Luthra et al. 2001). The parts used for treatment are rhizomes, which are often short branched. It is used as a disinfectant or antiseptic and for treating burns of various degrees. Externally, the rhizome mixed with alum is also applied as a paste to wounds, bruises, inflamed joints, and sprains (Eigner & Scholz 1999).

3.1. Antidiarrheal medicinal plants

Diarrhoea can be described as a gut disorder characterized by passage of three or more loose or liquid stools per day. There are three clinical types, namely acute watery, acute bloody, and persistent diarrhoea (Casburn-Jones & Farthing 2004). Acute diarrhoea is often treated by antibiotics such as tetracycline, ciprofloxacin, norfloxacin, fleroxacin, etc. However, using antibiotics can lead to depletion of beneficial gut and mucosal microorganism, immune-suppression, and allergic reactions (Tsuji et al. 1990; Dethlefsen & Relman 2011). Also, emergence of antibiotic resistant pathogenic strains can be seen which leads to spread and stabilization of resistant bacteria and resistance genes. This can result to overgrowth of enteropathogens such as *Clostridium difficile*, termed as antibiotic associated diarrhoea (Larcombe et al. 2016). Because of the increasing resistance to currently used therapeutic agents in many common pathogens has revived the interest of scientists in natural product-based drug discovery (Rawat et al. 2017). A range of medicinal plants have been discovered all around the world. The protective of these plants is probably due to their anti-inflammatory, antioxidant, and astringent properties (Mishra

et al. 2016). Secondary metabolites like phenols, tannins, alkaloids, flavonoids, saponins, steroids, and terpenoids can be often found in plant species that are used for treatment of gastrointestinal diseases (diarrhoea included) (Manzo et al. 2017). These phytochemicals present in the plants can help reduce the severity of diarrhoea by enhancing electrolyte reabsorption and colonic water (Bello et al. 2016). Examples such as phytochemicals are curcumin, which is found in turmeric, and quercetin, which is found in fruits. Both have anti-inflammatory properties in the gastrointestinal tract. These compounds have been shown to reduce inflammation and mucosal damage which can lead to aiding electrolyte reabsorption and water balance during diarrhoea (Yao et al. 2021).

Berberine (BBR) is an isoquinoline alkaloid occurring in medicinal plants species of the berberis species including *Berberis aristata* and *Coptis chinensis.* It has been extensively used in China to treat various bacteria-associated diarrhoeas for over 2000 years (Yue et al. 2019). It has shown activity in treating intestinal bacterial infections, including bacterial dysentery and viral infections as well. BBR has significant effect on infectious or non-infectious secretory or exudative diarrhoea (Yu et al. 2020). It is generally found in the roots, rhizomes, and the stem bark (Bandyopadhyay et al. 2013). The extract of stem bark of *B. aristata* is reported to be useful especially useful, evidence indicates that it also has multiple pharmacological effects such as immunomodulatory and anti-inflammatory (Joshi et al. 2011).

3.2. Plant products inhibiting growth of CRC associated bacteria

Due to increasing rate of CRC, less harmful pharmacological treatments are needed now more than ever before. CRC affects a significant portion of the world's population and chemotherapy has its limits and side effects as well. In animal experiments, it has been observed that medicinal herbs possess the ability to inhibit tumours and prevent apoptosis. These anti-tumour and anti-apoptotic properties have shown effectiveness against diverse types of cancers, including CRC (Islam et al. 2022). Recently, some essential oils and plant extracts showed inhibitory effect against bacteria associated with CRC. For example, green tea that contains high contractions of flavanols such as epicatechin, catechin and gallate esters, inhibited the growth of CRC associated bacteria like *F. nucleatum* and *Clostridium septicum* (Lee 2014). In another study, garlic showed inhibitory effect on growth of *F. nucleatum* (Amani 2021).

4. Traditional Philippine medicine

Traditional Philippine medicine is deeply rooted in the country's history and culture, with focus on natural remedies and spiritual healing. Healers, often women, play a significant role in the community, using a variety of methods such as chanting, animal sacrifices, and herbal treatments. The medicine is not fundamentally different from the folk medicine of other cultures (Villegas 1923). The use of medicinal plants has been a consistent feature, with a gradual increase in knowledge over time (Gutierrez 2019). Deep connection between traditional medicine and religion can be also seen. Example can be the successful collaboration between Christian medicinal practitioners and traditional healers in the Philippines (Seale 1993). Traditional medicine was mostly influenced by Spanish during the colonization on the Philippines (DeGracia 1979). The investment in *Materia medica* was done by Spanish authorities and Catholics, which indicates a strong influence on the development of traditional medicine. However, traditional Asian attitudes, beliefs and practices including those related to healing and mental health endured influence of Western culture and traditional Philippine medicine was not completely overshadowed (Lin et al. 1990; Platt 2017). Despite the widespread availability of Western medicines, the utilization of medicinal plants continues to rise due to a variety of reasons, encompassing cultural and socioeconomic factors across diverse communities (Rondilla et al. 2021). However, the World Health Organization and the Department of Health in the Philippines encounter challenges concerning the safety, effectiveness, quality, and appropriate utilization of traditional medicine. Difficulties in accessing Traditional, Complementary, and Alternative Medicine can be attributed to the lack of documentation, as much of the information is orally transmitted and tends to be specific to particular communities, often revolving around health practices at the household level (Lloyd Dapar & Demayo 2017).

4.1. Principles and practices

Folk healing in the present situation of the Philippines was quite similar to what the early Filipinos did. The descriptions of the early Spanish chroniclers on the early

healers are similar to present-day situations (Mercado 1988). Philippine folk healers, known as albularyo, play significant role in the country's traditional healing practices. These healers are considered general practitioners in folk medicine, utilizing herbal remedies and spiritual interventions (Galan 2020). The Department of Health reports that the ration is about 1 folk healer for every 300 Filipinos. Meanwhile the doctor population ratio is as low as 1 to more than 26 000 Filipinos (Lee Mendoza 2009). Polyherbal formulations and other traditional remedies are notably common in rural regions, where their usage remains widespread. Locals often consult their health concerns with folk healers, who commonly prescribe them plants either individually or in polyherbal formulations (Pucot & Demayo 2021). Medicinal plants which are used are often sold in urban markets, supported by laws like the Traditional and Alternative Medicine of 1997. The practice involves utilizing indigenous knowledge of antipyretic plants rich in phytochemical constituents like flavonoids, alkaloids, tannins and saponins for various health issues (Labagnao et al. 2022). Methods and beliefs are specific for regions and can vary. In Zamboanga City, subada (hilot), herbalism and sahuma (mangtatawas) are commonly used. In Laguna, they rely on a variety of medicinal plants, especially those from the *Lamiaceae, Euphorbiaceae* and *Asteraceae* families, to treat a range of ailments. In the Bicon Region, folk healers pay attention on the safety and effectiveness of herbal medicines, with focus on personal responsibility and accountability. In Partido District, Camarines Sur, folk healing practices are used to treat distinct illnesses, with combination of cultural, spiritual, and ritual procedures (Roldán 2017; Galan 2020; Molina & Esperat 2020; T. Cerio 2020).

4.2. Herbal medicines

Herbal medicine (also Herbalism) is the study of pharmacognosy and the use of medicinal plants, with more than 35 000 plant species that can be used for medicinal purposes. People have used plants for medical treatments most of the human history, and such traditional medicine is still widely practiced today (Savithramma & Rao 2012; Matole et al. 2021). About 75 – 80% of the world population still use herbal medicine and 40% directly depends on plant-based medicine for their health care, mainly in the developing countries (Pal & Shukla 2003; Parveen & Sharma 2014a). It is often used to

treat mild to moderate illnesses like urinary tract infection, wound infection, stomach-ache, diarrhoea, cough, diabetes, headache, cold or hypertension (Roldán 2017; Matole et al. 2021). In the Philippines, the herbal medicine has been used for centuries, especially in rural areas. The most common method of preparation for medicinal applications is decoction, done by boiling the material in water to extract water-soluble compounds. Large number of plants are prepared as fresh or dried (Agapin 2019). The most used parts of plants are leaves, followed by stems, whole plant, roots, and fruit. The most used plant families are Convovulaceae, followed by Cucurbitaceae and Moringaceae (Orillaneda 2023). In Philippines 1500 medicinal plants are used by traditional healers and in 1992, the Department of Health circulated a list of 10 scientifically validated Philippine herbal plants, today it is 120 plants which are safe and efficient (Dapar 2020).

4.3. Medicinal plants used for the treatment of diarrhoea

4.3.1. Acalypha grandis

A. grandis is characterized by elliptical leaves with prominent venation, displays a rich, chocolaty hue, contributing to its aesthetic allure. Typically attaining heights of up to 90 cm, this perennial species forms dense clusters, creating an imposing presence within its ecological niche. It is native to New Guinea, Samoa, and Vanuatu, but today can be found in other tropical and subtropical regions of world such as Philippines or Indonesia. *A. grandis* thrives in environments characterized by warm temperatures and moderate humidity levels. It is commonly observed in lush tropical forests, and natural reserves, where it demonstrates adaptability to various soil compositions (Das et al. 2012; Sagun et al. 2006).

In traditional medicine, *Acalypha* is used for treating a range of ailments, including dental issues, stomach pain, diarrhoea, and respiratory problems. The leaf extracts have antimicrobial and antifungal properties, inhibiting the growth of various bacteria and fungi (Emeka et al. 2012; Seebaluck et al. 2015).

4.3.2. Artocarpus blancoi

A. blancoi is a large tree species indigenous to Philippines but can be found in Indonesia and Malaysia as well. It is characterized by its canopy with ovate leaves with deeply serrated margins and a glossy green surface *A. blancoi* typically grows up to 30 m heigh. It thrives in lowland rainforest and moist conditions prevalent in tropical regions. It also serves as a valuable resource for local communities for its fruit (Joel Bicaldo 2022).

Different parts of the tree can be used for various uses. Dried male flowers can be used as mosquito repellent, the fruit contains nutritious seeds which are compared to almonds and macadamia, or you can make flour out of them (Lim 2012b). Leaves are prepared by boiling and then used to relieve hypertension, control diabetes or against diarrhoea (Mohammed & Wickham 2011).

4.3.3. Artocarpus camansi

A. camansi is a large tree species, which exhibits broad ovate leaves with deeply serrated margins, presenting a lush, verdant canopy that characterizes its arboreal stature and grows over 20 m in height. It prefers lowland rainforests and moist conditions. It serves as a food source for numerous fauna species and local people, also known as breadnut, is primarily found in the tropics and is indigenous to New Guinea and was introduced to Philippines and other Pacific islands. *A. camansi* thrives in equatorial lowlands with specific rainfall and soil requirements (Ragone 2006). The fruit's and seed's physical characteristics and chemical composition can vary across different localities indicating its adaptability and potential for diversification in food processing (Marina Silalahi 2022).

The genus *Artocarpus* is rich in bioactive compounds (flavonoids, stilbenoids), which have been used for various medicinal purposes (Jagtap & Bapat 2010). The plant is rich in secondary metabolites such as triterpenoid compounds. It is a tree with a variety of uses. Its fruit extracts have been found to possess significant antioxidant activity and is rich in fibre content, which can help against diarrhoea (Arif et al. 2018).

4.3.4. Artocarpus elasticus

A. elasticus is a large tree species and indigenous to Myanmar, Thailand, Peninsular Malaysia, Sumatra, Java, Lesser Sunda Islands, Borneo, and the Philippines

(Lim 2012b). Tree is characterized by its large, glossy, and leathery leaves, which are often used for making traditional umbrellas and hats. The fruit is edible and has a sweet custard-like flavour. The wood of *A. elasticus* is highly valued for its durability and is used in construction (Jagtap & Bapat 2010).

It has been extensively used in traditional medicine due to its rich bioactive compounds, including phenolic compounds, flavonoids, stilbenoids, and jacalin (Raju et al. 2017). Various parts of the tree are used in Southeast Asia. Leaves are given to consumptive patients and to nursing mother in Malaysia. In Sarawak, young shoots are prescribed as a remedy for vomiting blood in the Iban community. Latex was used for dysentery in Java and roots are used as an aperient decoction (Lim 2012b).

4.3.5. Artocarpus lakoocha

A. lakoocha is a tropical tree species, which can be up to 24 m high, with a spreading canopy. It is used for its fruits and wood (Lim 2012b). The edible fruit is irregular in shape, 5 – 12 centimetres wide, velvety, dull-yellowish and slightly citrus-like in taste. It has served as a novel cosmetic ingredient for anti-tyrosinase and skin whitening activities. It is indigenous to Southeast Asia, particularly prevalent in regions such as India, Thailand, Myanmar, and Philippines (Tengamnuay et al. 2006).

In traditional medicine is used for treating liver ailments, skin conditions, and as an aphrodisiac (Joshee et al. 2002). Extracts from the bark and leaves are consumed orally for treatment of liver, digestive issues, and diarrhoea. They are often prepared as herbal teas or decoctions (Gautam & Patel 2013). Leaves and fruit are rich in flavonoids, phenols, tannins, saponins, and steroids (Bhattacharya et al. 2019).

4.3.6. Citrus microcarpa

C. microcarpa originates in China as a natural hybrid. It is believed to have been introduced in early times to Indonesia and the Philippines where it became the most important citrus juice source. It is evergreen shrub or small tree to 4 m high, densely branched. Leaves are elliptic to broad oval with green to yellowish colour. Fruit is 2 – 4 cm in diameter with green colour turning to yellow or orange. It can be eaten fresh but usually it is made into juice drinks, or dried and used as preserves (Lim 2012a).

Its fruit extract is used in traditional medicine for its antibacterial properties (Roanisca & Mahardika 2020). The juice is traditionally used against diarrhoea, to prevent respiratory diseases, strengthen the bones and act as growth stimulant for children. The leaves can be used in the treatment of skin diseases, relieve headache and act as a mouth wash to treat sore throat. The essential oil from peels is used commercially in perfumes, food, cosmetics, and detergents (Md Othman et al. 2016).

4.3.7. Crescentia cujete

C. cujete is a small or medium sized tree that is native to Central America and introduced to Africa, and Southeast Asia, Philippines included. The plant grows up to 10 m high possessing thick bole and a rounded crown. The leaves are simple, alternate, or fascicles and suspended on a short shoot or stem. The fruit resembles green pumpkins with a diameter of 12 – 14 cm (Balogun & Sabiu 2021).

C. cujete is used in traditional medicine to treat conditions such as hypertension, diarrhoea, respiratory ailments, stomach troubles and snakebites. The plants pharmacological potential is attributed to its various bioactive compounds, including flavonoids, alkaloids, saponins, phenols, or volatile oils (Lima & Feitosa 2021; Gonzales et al. 2023).

4.3.8. Dysoxylum blancoi

D. blancoi is species of tree, native to Southeast Asia, including Philippines. It is characterized by its diffuse-porous wood with simple perforations and small, alternate, or randomly arranged intervascular pits. It has white-villous stems, petioles and abaxial leaf blade, and can grow up to 20 m. The tree thrives in tropical rainforests and areas with ample moisture (Patel 1974; Mabberley 1994).

D. blancoi is used to treat osteomyelitis, abscesses, skin ailments, diarrhoea, or cancer. Decoction from leaves have been used against stomach-aches, diarrhoea, and dysentery (Bhardwaj et al. 2024). The plant contains antibacterial and anti-cancerous properties hence further studies need to be done to validate its efficacy in combating cancer (Arya 2017).

4.3.9. Euphorbia thymifolia

E. thymifolia exhibits slender, lanceolate leaves arranged in whorls along its stems. Typically grows to height of around 30 cm. *E. thymifolia* forms compact clusters and is indigenous to tropical and subtropical regions of America and was introduces to Africa and Asia, including Philippines. It thrives in diverse habitats ranging from open woodlands to disturbed areas with well-draining soils.(Mali & Panchal 2013).

In traditional medicine *E. thymifolia* can be prescribed for cough, skin disease, parasitic infection, or as an ingredient of vegetable soup for diarrhoea. The leaves and seeds are given in worm cases and in certain bowel affections of children. The plant possess diuretic, laxative, anti-diarrheic, anti-malarial, anti-rash and anti-hemorrhoidal activity (Gupta et al. 2007).

4.3.10. Merremia peltata

M. peltata is a climbing vine that can be invasive. It is characterized by its climbing or creeping nature and heart-shaped leaves with prominent veins. Vines can reach lengths exceeding 5 m. It is indigenous to tropical and subtropical regions and in native to Asia, Philippines included. It is commonly found in lowland forests and disturbed habitats (Yudaputra 2022).

Its usage in traditional medicine is for variety of ailments, including arthritis, skin infections, inflammation, fever, diabetes, and diarrhoea. The plant contains saponins, alkaloids and flavonoids, which have been found to have anti-diabetic, anti-inflammatory, wound healing, analgesic and anti-microbial properties (Neyanila et al. 2013; Abdurrahman et al. 2023).

Despite the rich tradition in the medicinal use of above-mentioned species for treatment of diarrhoea in Philippines, information on their effects on CRC causing bacteria are missing in the literature.

5. Aims of the Thesis

The aim of this thesis is to evaluate *in vitro* growth-inhibitory effects of plants used in traditional Philippine medicine for treatment of diarrhoea against representatives of bacteria associated with colorectal cancer (CRC) development.

The specific objectives are as follows:

a) Determination of minimum inhibitory concentrations (MICs) of plant extracts against CRC-causing bacterial pathogens;

b) Monitoring of growth-inhibitory action of antibacterial extracts on growth kinetics and length of lag phase of susceptible bacteria.

6. Materials and Methods

6.1. Plant materials

The plant species selection was made based on literature and internet sources on their use in Philippine traditional medicine for the treatment of diarrhoea. Plant samples were collected during expeditions in 2018 and 2022 in the Philippines, especially on Leyte Island close to Baybay city and in campus of Visayas State University (VSU).

For all plant species, reference specimen sheets with botanical descriptions, natural habitats, and illustrations were elaborated. The plants were authenticated by local expert Dr. Marlito Bande from the Institute of Tropical Ecology and Environmental Management of the VSU. Voucher specimens have been deposited in the herbarium of the Faculty of Tropical AgriSciences of Czech University of Life Sciences Prague. Botanical data on collected plant species are shown in Table 1.

6.2. Plant extract preparation

Before transportation from the Philippines, the samples were air-dried. In the laboratory, 15 g of dry sample was homogenized by Grindomix mill (Retsch, Haan, DE) and then extracted in 450 ml of 80% ethanol (Sigma-Aldrich, Prague, CZ) for 24 hours at room temperature using a laboratory shaker (GFL, Burgwedel, DE). Samples were filtered and concentrated by a rotary evaporator (Büchi Labortechnik, Flawil, CH) at 40°C vacuum. Dried residues were subsequently diluted in 100% dimethyl sulfoxide (DMSO, Penta, Prague, CZ). The final stock solution of the extract at the concentration of 51.2 mg/ml was stored in Eppendorf Tubes 2.0 ml at -20°C until their use. The yields of dry residues are shown in the **Chyba! Nenalezen zdroj odkazů.**.

6.3. Used microorganism and growth medium

The antibacterial activity was determined against 6 representatives of both Gram-positive/-negative and aerobic/anaerobic CRC-associated bacteria. Standard American Type Culture Collection (ATCC), Czech Collection of Microorganisms (CCM) and Czech National Collection of Type Cultures (CNCTC) strains namely *E. coli* ATCC

35218, *S. bovis* ATCC 33317, *C. septicum* ATCC 12364, *F. necrophorum* CCM 5981, *P. anaerobius* CCM 3790, *F.* nucleatum CNCTC 5414 were obtained from Oxoid (Basingstoke, UK), CCM (Brno, CZ) and CNCTC (Prague, CZ).

Mueller-Hinton broth (MHB), (Oxoid, Basingstoke, UK) was used as growth medium for aerobic group of bacteria and Wilkins-Chalgren broth (WBC), (Oxoid, Basingstoke, UK) was used for anaerobic bacteria. of the pH of the broth was equilibrated by buffer (o 0.2 g KCl, 6.1 g of Tris Base and 8.0 g of NaCl in one litre of distilled water) and verified using pH meter (Eutech Instruments pH 510, Chromservis, Prague, CZ). Autoclave (Tuttnauer, 3870ELV) was used for sterilization of the growth media, which were stored in fridge at 4°C until use.

6.4. Determination of the minimal inhibitory concentration (MIC)

Broth microdilution method was used for antimicrobial susceptibility testing folowing Clinical and Laboratory Standards Institute (CLSI) guidelines, modified by Cos et al. (Cos et al. 2006), where MIC [μg/ml] were assessed in 96-well microtiter plates. The MIC was defined as the lowest concentration of an antimicrobial compound that inhibits visible growth of the microorganism after overnight incubation (Andrews 2002). All 15 plant extracts were dissolved in DMSO, and 2-fold diluted in appropriate growth media (100 μL) in concentrations 512, 256, 128, 64, 32, 16, 8, 4, and 1 μg/mL using automated pipetting platform Freedom EVO 100 (Tecan, Männedorf, CH). All bacterial cultures were diluted to contain 1.5×10^8 CFU/mL and subsequently inoculated with the suspension in microtiter plate. Microplates were incubated for 24 h at 37 °C. The plates inoculated with aerobes (*E. coli, S. bovis*) were prepared in flow box workstation (FlowFAST bhg2004) and incubated in Biological Thermostat BT 120, whereas the plates with anaerobes (*C. septicum, F. necrophorum, P. anaerobius, F. nucleatum*) were handled and incubated under anaerobic conditions in Whitley A35 Anaerobic Workstation (Don Whitley Scientific, West Yorkshire, UK). Ciprofloxacin was used as a positive antibiotic control. Bacterial growth was determined by the absorbance measurement by Cytation 3 Imaging Reader (BioTek, Vermont, USA) at 405 nm. The lowest DMSO diluted samples concentration showing at least ≥80% reduction of microbial growth compared to compound-free growth control. The solvents used (DMSO, ethanol and *d* H_2O) did not inhibit bacterial growth at the concentrations tested (1%). All

tests were performed as three independent experiments each carried out in triplicate. The mode and median were used for the final MIC value calculation when the triplicate endpoints were within the two- and three-dilution range, respectively.

6.5. Monitoring of growth kinetics

For monitoring of bacterial growth kinetics, the protocol of broth microdilution method described above was used. The growth of *E. coli* and *S. bovis* was monitored by the assay. Plant extracts were 2-fold diluted in MHB (*E. coli*) and BHI (*S. bovis*) in ranges of 1-512 μg/ml, whereas ciprofloxacin was prepared in ranges of 0.0625-32 μg/ml. During 24 h of incubation, absorbance measurements by Cytation 3 Imaging Reader (BioTek, Vermont, USA) were performed every hour and the regular orbital shaking conditions were selected. The lag time was defined as the time at which the extrapolated slope of the exponential phase intercepts a horizontal line, extrapolated from the starting inoculum concentration. The lag phase values at each concentration of tested agent were calculated by Gen5 Image+ 3.11 software (BioTek, Vermont, USA). The difference between growth control and individual concentrations was calculated based on these values. Growth curves plotting the time dependence of optical density at the wavelength of 405 nm were constructed using Microsoft Excel (Microsoft Corporation, Washington, USA) for plant extracts showing the most significant prolongation and shortening of calculated lag times.

Table 1 Botanical data on medicinal plants used in traditional Philippine medicine for treatment of diarrhoea

Botanical name (Family)	Local name	Place of collection/geographical coordinates	Year of colection	VSN	Part used	Yield [%]
Acalypha grandis. Benth (Euphorbiaceae)	/	Happy valley/10.771746 N, 124.824039 E	2018	02537KBFR8	Inflorescence	19,2
Artocarpus blancoi Elmer (Moraceae)	Antipolo	Molave Hill/10.745301 N, 124.797645 E	2018	02538KBFR9	Leaves	8,9
Artocarpus camansi Blanco (Moraceae)	Kamansi	Duncaan riverbank/10.654201 N, 124.853644 E	2018	02512KBFR1	Fruit	13,7
Artocarpus elasticus Reinw. Ex Blume (Moraceae)	Terap	Molave Hill/10.745044 N, 124.798286 E	2018	02539KBFRA	Leaves	13,3
Arocarpus lakoocha Roxb (Moraceae)	Monkey Jack	Campus VSU/10.70434227 N, 124.802194 E	2022	02636KBFR8	Bark	9,8
					Leaves	30
					Fruit	9,2
Citrus microcarpa Bunge (Rutaceae)	Philippine orange	Campus VSU/10.7446653 N, 124.7895516 E	2022	02635KBFR7	Leaves	8,1
					Fruit	33,3
Crescentia cujete Schum (Bignoniaceae)	Colabash tree	Campus VSU/10.7477332 N, 124.7928907 E	2022	02633KBFR5	Leaves	4,2

					Bark	9
					Fruit	2,2
Dysoxylum blancoi Vidal (Meliaceae)	Ivory mohagony	Campus VSU/10.7478155 N, 124.7909320 E	2022	02637KBFR9	Bark	10
Euphorbia thymifolia Forssk (Euphorbiaceae)	Makikitot	Campus VSU/10.7477332 N, 124.7928907 E	2022	02638KBFRA	Aerial part	8,9
Merremia peltate Merr (Convolvulaceae)	Merremia	Campus VSU/10.7477211 N, 124.7964415 E	2022	02634KBFR6	Leaves	9,8

7. Results and discussion

7.1. Determination of MICs

The inhibitory effect of plant extracts was determined *in vitro* against six representatives of both aerobic and anaerobic bacteria associated with CRC. Among all samples tested, *Artocarpus lakoocha* and *Artocarpus elasticus* were only active plant species producing growth-inhibitory effect against *E. coli* (MIC = 256 μg/mL) and *S. bovis* (MIC = 512 μg/mL), respectively. Our result can be supported by findings of Biswas & Chakraborty (2013), who reported the inhibitory effect of hexane fraction of *A. lakoocha* leaves against *E. coli.* Although Daus et al (2017) have previously observed that extracts from the leaves of *A. elasticus* showed significant activity against *Staphylococcus aureus* (MIC 128mg/mL), according to our best knowledge, there are no studies reporting antibacterial action against *S. bovis.* Since various antibacterial prenylated compounds have been isolated from the leaves of both *Artocarpus* species (Jagtap & Bapat 2010; Daus et al. 2017), it can be suggested that these agents can significantly contribute to the total antibacterial activity of extracts tested. All other plant samples did not produce any antibacterial effect (MIC > 512 μg/mL). Anaerobic bacteria, namely *C. septicum, F. necrophorum, P. anaerobius* and *F. nucleatum,* were resistant to all plants tested. Teanpaisan et al. (2014), who tested aqueous extract of *A. lakoocha* purchased from a traditional drug in Thailand, found out that *F. nucleatum* ATCC 25586 was among the most susceptible strains (MIC = 100 μg/mL). Since the tested part is not indicated in previously mentioned study, the comparison with our results is difficult. Moreover, different bacterial strain was assayed in our study. Ciprofloxacin used as a positive antibiotic control showed MIC ranging from 0.5 to 8 μg/mL.

Table 2 *In vitro* growth-inhibitory effect of extracts from Philippine medicinal plants against CRC-associated bacteria

Plant species/ATB	Microorganisms/ Minimum inhibitory concentration (μg/mL)					
	Escherichia coli	*Streptococcus bovis*	*Clostridium septicum*	*Fusobacterium necrophorum*	*Peptostreptococcus anaerobius*	*Fusobacterium nucleatum*
Artocarpus blancoi	>512	>512	>512	>512	>512	>512
Artocarpus elasticus	>512	512	>512	>512	>512	>512
Artocarpus lakoocha	256	>512	>512	>512	>512	>512
Dysoxylum blancoi	>512	>512	>512	>512	>512	>512
Ciprofloxacin	4	0.5	4	8	8	0.5

Footnotes: MIC -. (data are median or modal values of three independent experiments, each performed in triplicate); >512: Not active; -: Not determined; ATB: antibiotics

7.2. Monitoring of growth kinetics and length of lag phase *E. coli* and *S. bovis*

The bacterial growth kinetics were measured for two CRC-associated aerobic microorganisms susceptible to extracts tested, namely *E. coli* and *S. bovis.* The results showed the time dependent growth-inhibitory effects of three extracts, namely *A. blancoi, A. elasticus* and *D. blancoi,* against *S. bovis*. At the concentration of 512 μg/mL, *A. blancoi* and *D. blancoi* extracts prolonged the exponential growth of S. *bovis* for 309 and 120 minutes, respectively (Figures 1 and 2). Although the initial growth of *S. bovis* in the presence of *A. elasticus* extract at concentration 256 μg/mL started 121 minutes earlier than in case of growth control, full exponential growth appears after 14 hours of cultivation (Figure 3). Tested extracts did not prolong lag phase of *E. coli*.

Table 3 Kinetics growth of *S. bovis*

Plant species	Lag phase (minutes)
Artocarpus blancoi	**309**[a]
Artocarpus elasticus	**-121**[b]
Dysoxylum blancoi	**120**[a]

Footnotes: LP - Lag phase (Extract lagtime - Growth control lagtime); a: 512 μg/mL, b: 256 μg/mL

Although it has previously been demonstrated that the plant extract markedly inhibits the growth of certain pathogens by significant extension of the lag phase (Kudera et al. 2017), according to our best knowledge, there are no literature data describing the bacterial growth kinetics of *E. coli* and *S. bovis* in the presence of plant extracts from *A. blancoi*, *A. elasticus* and *D. blancoi*.

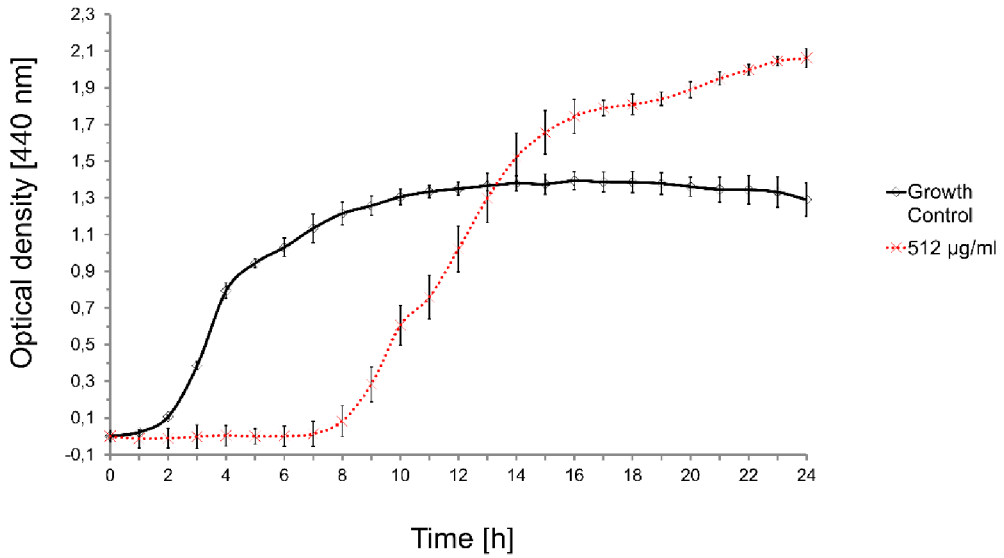

Figure 1. Growth of *S. bovis* in the presence of *A. blancoi* at the concentration of 512 µg/ml

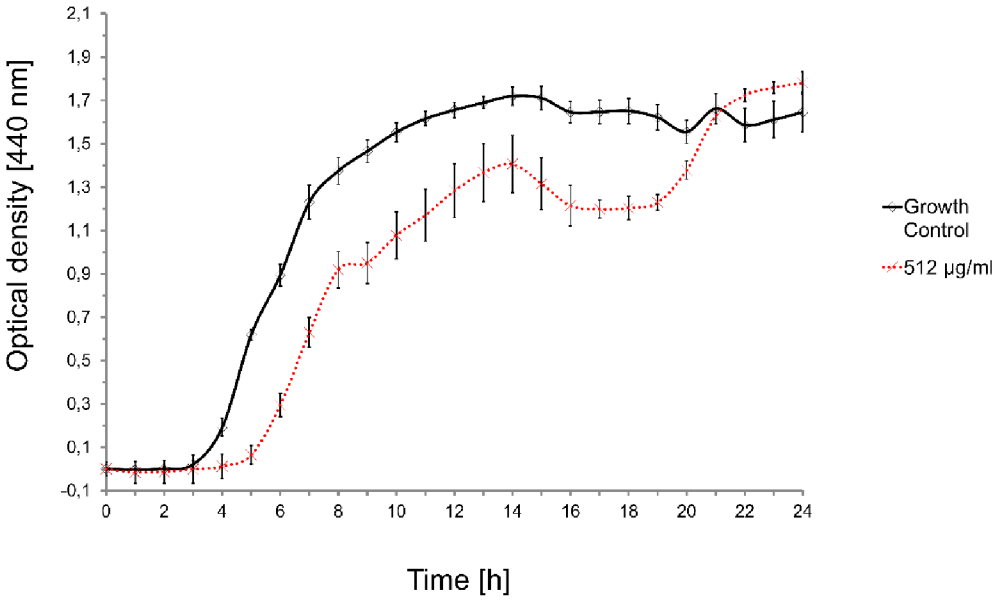

Figure 2. Growth of *S. bovis* in the presence of *D. blancoi* at the concentration of 512 µg/ml

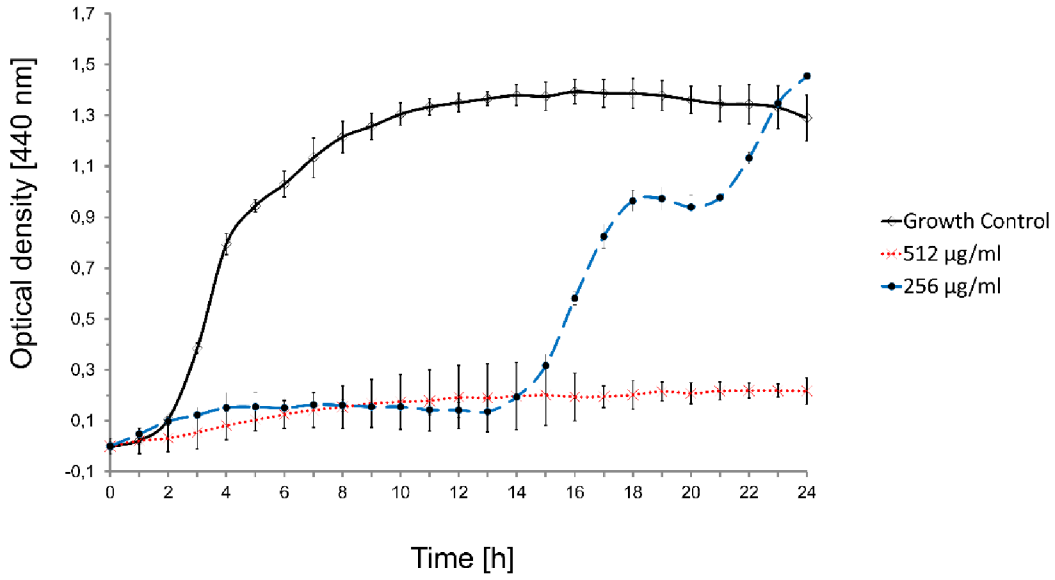

Figure 3 Growth of S. bovis in the presence of A. elasticus at concentrations 256 and 512 µg/ml

8. Conclusion

In summary, 4 of 15 extracts of Philippine medicinal plants traditionally used to cure diarrhoea exhibited *in vitro* growth-inhibitory effects against 2 out of 6 representatives of bacteria associated with CRC. Among all samples tested, the extract of *A. elasticus* showed the highest antibacterial activity against *S. bovis*. The extracts of *A. blancoi, A. lakoocha, D. blancoi* also exhibited noteworthy antibacterial activity. *E. coli* and *S. bovis* were the most susceptible aerobic bacteria. All anaerobic bacteria, namely *C. septicum, F. necrophorum, P. anaerobius* and *F. nucleatum* were resistant to extracts tested. The results suggest possible use of above-mentioned extracts for development of pharmaceutical applications effective against CRC related bacteria. However, further research must be done in focus on identification of their antimicrobial principles, evaluation of their safety and effectiveness *in vivo*.

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