CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Department of Crop Sciences and Agroforestry

In vitro growth-inhibitory effect of Philippine medicinal plants traditionally used to cure diarrhoea against intestinal bacterial pathogens

MASTER'S THESIS

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DIPLOMA THESIS ASSIGNMENT

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Thesis title

In vitro growth-inhibitory effect of Philippine medicinal plants traditionally used to cure diarrhoea against intestinal bacterial pathogens

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Objectives of thesis

The main maim of the thesis is evaluation of in vitro growth inhibitory effect of extracts from medicinal plants used in traditional Philippian medicine for treatment of infectious diarrheal diseases against diarrhoea-causing pathogens such as Listeria monocytogenes, Escherichia coli, and Shigella flexneri.

Methodology

Susceptibility of diarrhoea causing bacteria to ethanol extracts obtained by maceration from species of medicinal plants identified based on literature data on their ethnomedicinal use in Philippian medicine for treatment of diarrhoea will be evaluated using standard broth microdilution method.

The proposed extent of the thesis

60-70

Keywords

Diarrhoea, Philippines, medicinal plants, antibacterial, extracts

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Recommended information sources

Clinical and Laboratory Standards Institute (CLSI), 2009, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard, 3rd edn. CLSI document M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.

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Edwino SF, Marciano MR, Galang AP, Angara GM, Berrova LG, Sarmiento IP, 2018, Philippine Native Trees 303 : Up Close and Personal. Green Convergence: Quezon City, 484 p.

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Declaration

I declare that I elaborated this Diploma thesis *"In vitro growth-inhibitory effect of Philippine medicinal plants traditionally used to cure diarrhoea against main representatives of intestinal bacterial pathogens"* alone, and that I have used only literature, web resources and other resources mentioned in references at the end of the thesis.

I agree with placing this work in the library of CULS Prague and make it accessible for study purposes.

Prague …….

26th of April 2019 Barbora Fišerová, Bc.

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Abstract

Infectious diarrhoea is serious public health problem worldwide, mainly in developing countries, including Philippines. Diarrhoeal disease is the third leading cause of death worldwide with 2.2 million deaths. Plants used in traditional medicine for treatment of diarrhoea are tested for antimicrobial activity, because of increasing resistance of bacteria to antibiotics, and in countries such as Philippines antibiotics can be hard to reach or expensive for people from rural areas, where 80 % of them is still dependent on indigenous medicine.

In this study, 19 species were collected, air-dried and tested for their antimicrobial effects by broth microdilution method. Six of them showed positive results against up to 5 out of 8 tested representatives of pathogenic bacteria. *Artocarpus camansi* species showed the highest antibacterial effect against *Clostridium perfringens* (MIC = 128 μg/mL) and *Clostridium difficile* (MIC = 128 μg/mL). Significant anti-bacterial effects exhibited also *Artocarpus blancoi* against *Enterococcus faecalis* (MIC = 128 μg/mL), *Acalypha grandis* against *Vibrio parahaemolyticus* (MIC = 256 μg/mL), and *Diplazium esculentum* and *Picrasma javanica* produced growth-inhibitory action against *Escherichia coli* with MICs 256 and 512 μg/mL, respectively. *Carmona retusa* possessed moderate effect against *Vibrio parahaemolyticus* (512 μg/mL).

These results suggest above-mentioned species as perspective plant materials for development of pharmaceutical and food applications effective for treatment of diarrhoea. However, further research focused on identification of their antimicrobial principles and evaluation of their safety will be necessary.

Keywords: antibacterial, diarrhoea, extracts, medicinal plants, Philippines

Abstrakt

Infekční průjem je závažným zdravotním a celosvětovým problémem, a to především v rozvojových zemích, do kterých se řadí i Filipíny. Průjmová onemocnění jsou třetí nejčastější příčinou úmrtí na celém světě, které dohromady sčítá 2,2 milionu objetí ročně. Rostliny využívané v tradiční medicíně k léčbě průjmu byly testovány na jejich antimikrobiální účinky, kvůli rostoucí rezistenci bakterií na antibiotika, která v zemích jako jsou Filipíny mohou navíc být buď drahá či těžko dostupná, pro obyvatele venkova, kde je stále 80 % z nich odkázaná na tradiční medicínu.

V této studii bylo sesbíráno 19 rostlinných druhů, které byly usušeny na vzduchu a poté testovány na antimikrobiální účinky pomocí bujonové mikrodiluční metody. Šest z nich prokázalo pozitivní výsledky proti až 5 z 8 testovaných zástupců patogenních bakterií. *Artocarpus camansi* vykazoval nejvyšší antibakteriální účinek proti bakteriím *Clostridium perfringens* (MIC = 128 μg/ml) a *Clostridium difficile* (MIC = 128 μg/ml). Významné antibakteriální účinky byly zjištěny také u druhu *Artocarpus blancoi* proti *Enterococcus faecalis* (MIC = 128 μg/ml), *Acalypha grandis* proti *Vibrio parahaemolyticus* (MIC = 256 μg/ml) a *Diplazium esculentum* a *Picrasma javanica* prokázaly inhibiční účinky růstu *Escherichia coli* s MIC 256 a 512 ug/ml. *Carmona retusa* měla mírný účinek proti bakterii *Vibrio parahaemolyticus* (512 μg/ml).

Zmíněné výsledky, testování výše uvedených druhů rostlin, mohou sloužit jako perspektivní materiál pro vývoj farmaceutických a potravinářských produktů účinných při léčbě průjmů. Bude však nutný další výzkum zaměřený na vysvětlení principů jejich antimikrobiálních účinků a vyhodnocení bezpečnosti jejich užití.

Klíčová slova: antibakteriální, extrakty, Filipíny, léčivé rostliny, průjem

Contents

List of Abbreviations

ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
DSMZ	German Resource Centre for Biological Material
IPNI	International Plant Name Index
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
VSU	Visayas State University
WCB	Wilkins-Chalgren broth

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1 Introduction

Diarrhoea is a disease of gastrointestinal tract, which can be caused by many different agents such as chemicals, heavy metals, parasites, fungi, and viruses, but mainly by pathogenic bacteria (Wagner 2008), such as *Clostridium difficile*, *Clostridium perfringens*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella enterica*, *Shigella flexneri*, and *Yersinia enterocolitica*. Infectious form of this malady is a worldwide problem responsible for 8 million deaths each year, particularly among children due to the poor sanitation and more limited access to health care (Seupaul 2012). Worldwide, more than one billion people suffer with one or more episodes of acute diarrhoea each year, whereas acute infectious form of this disease remains one of the most common causes of mortality in developing countries (Sur & Bhattacharya 2006). Antibiotics treatment is possible way of the cure for such ailments, however, the growing resistance of bacterial pathogens leads to a need to look for alternatives, which are able to inhibit growth of bacteria in the same ways as antibiotics (World Health Organization 2014).

One of the potential sources of alternative medicine to cure such disease are medicinal plants, thanks to their active substances such as alkaloids, phenolics, steroids, and terpenoids (Pöll & Álvarez 2015) which has a potential to inhibit growth of bacteria. Examples of such plants can be *Arctostaphylos uva-ursi* which cure infections of urinary tracts, *Melissa officinalis* and *Allium sativum* which by their content of essential oils are able to treat respiratory problems (Etkin 2006). *Melaleuca alternifolia* is known for its ability of therapeutic effects of skin diseases (Cortés et al. 2003). Berberine and bismuth subsalicylate are examples of plant compounds and their derivatives that are used in praxis for treatment of diarrhoea (Kokoska et al. 2018).

Philippines represents region with uniqueness of flora, which includes many endemic plants that can be found only in Philippines. Traditional herbal medicine has a long tradition here and is still being practice until today. Despite the rich traditional use of Philippian medicinal plants in treatment of infectious diseases including diarrhoea (World Health Organization 1997) the number of studies dealing with this topic is limited.

2 Diarrhoeal diseases

Diarrhoea is a common malady, sometimes mentioned as a symptom (Guandalini 2011), which increase stool frequency, fecal thinning, or both at the same time. This disease can be divided into different classes according to the agents responsible for them. Acute diarrhoea is the first and most common type initiated by different reasons. It is caused mainly by infections and manifested by inflammation of mucosa of the stomach and intestines (gastroenteritis). Large amount of fluid is transferred to the intestine. Likewise, this type is caused by some laxatives. Further agents are toxins, which are poisonous substances that secretes bacteria. Next is ingestion of many non-digestible substances. Further mental causes, such as anxiety, can be based on acute form intestines shift their content very quickly, so only a small amount of fluid is absorbed back into the blood. Side effects of drugs (e.g. antibiotics, and medicines containing magnesium) cause acute diarrhoea as well as ingestion of large amounts of fluid when the intestine cannot absorb it, same with large quantities of alcohol. Second type is chronic, where one of the most common causes of long-term diarrhoea is the so-called irritable bowel.

Infectious diarrhoea, which is caused by microorganisms, can be classified as follows: acute watery, acute dysenteric, shigellosis, cholera, and pseudomembranous enterocolitis. Manifestations of infectious form is a sudden onset, sometimes with fever, unwillingness, vomiting and later abdominal pain, usually diffuse with a chuckle. The stool may be mushy at first, later it becomes watery, sometimes with mucus and blood especially in case of dysentery. *Shigella* genus can be mentioned as an example of bacteria causing this kind of disease.

The human gastrointestinal tract serves as a home to complex bacterial ecosystem, hosting about 400 different species counting about 2.3 to 6.8 kg of living bacteria. In healthy individuals, the most common bacteria are *Bacillus*, *Bifidobacterium*, *Clostridium*, *E. coli*, *Eubacterium Peptococcus*, *Fusobacterium*, *Klebsiella*, *Lactobacillus*, *Pepto-streptoccus Staphylococcus*, and *Streptococcus*. Without beneficial microbiota we cannot live due to various reasons, such gut microflora plays a dynamic role in human health and execute important metabolic functions and its sustenance for the digestive system which is essential for us.

Research reveals that the gut coating is primarily nurtured by nutrients produced from auspicious bacteria. Our complete dependence upon the activities of these microorganisms is obvious, especially to produce key vitamins, assimilation and delivery of nutrients, and for

building defense against pathogenic and putrefactive bacteria, where their fermentation is a vital component of our digestive process, producing essential nutrients and short chain fatty acids. Without prosperous bacteria which produce these nutrients, cell damage can happen, with further causing of losing of guts coating. Thus, maintenance of the proper balance of germs in the intestine is necessary for our good health (English & Dean 2013).

2.1 Epidemiology of diarrhoeal diseases

Every year, more than one billion people worldwide suffer with one or more episodes of acute infectious diarrhoea, when 8 million of them die (Seupaul 2012). Acute infectious form of this disease remains one of the most common causes of mortality in developing countries. The bacteria responsible for causing diarrhoea are usually transmitted by the faecal-oral route, which includes the ingestion of faecally contaminated water or food, person-to-person transmission, and direct contact with infected faeces. All these ways of spreading mostly occur because of poor sanitation and problematic access to health care, particularly among children. Diarrhoea is a leading cause of illness and death among children in developing countries, where an estimated 1.3 thousand million episodes and 0.5 million deaths occur each year in under-fives (World Health Organization 2017), and it is the leading causes of morbidity and mortality globally and account for more deaths in early childhood after the neonatal period than any other (Mokomane et al. 2018). These children experience an average of 3.3 episodes each year, but in some areas the average exceeds nine episodes per year. Where episodes are frequent, young children may spend more than 15% of their days with diarrhoea. About 80% of deaths due to diarrhoea occur in the first two years of life. The main cause of death from acute diarrhoea is dehydration, which results from the loss of fluid and electrolytes in diarrhoeal stools (Rehydration Project 2016). The mostly affected countries are Sub-Saharan Africa, Nigeria, Niger, Pakistan, Malaysia, India and Philippines (Mokomane et al. 2018). It can be cured by antibiotics, but bacteria responsible for diarrhoea are becoming resistant and it is necessary to look for alternatives which are able to inhibit growth of bacteria in the same ways as antibiotics do.

2.2 Causes

Acute infectious diarrhoea can be caused by various reasons. Bacteria are responsible for bacterial type of this malady with invasive or toxigenic mechanisms of the disease. This kind can be found especially in hot regions. Travelers are the group who suffers the most. Another is viral type. Mainly children suffer, but adults are also affected. Last kind is parasitic

diarrhoea. It occurs generally in subtropical areas and is less frequent. The major concerns involve the risk of complications is in case in vulnerable patients as young children, the elderly, and patients with immunosuppression. These complications are mainly dehydration and malnutrition. For these patients, rehydration is urgent. Diagnosis of this disease requires history and clinical assessment as underlying illnesses, severity of symptoms, presence and extent of dehydration and other clinical symptoms, travel history, known outbreaks, and pathogenic mechanism, where is necessary to know if it is invasive or toxigenic (Schertzer & Garmel 2008). This thesis is focused on the type of illness which are caused by various bacteria and their description follows in subchapter 2.3.

2.3 Diarrhoea-causing bacteria

More than 90 % of food poisoning is each year caused by 9 representatives of bacteria which can be commonly found on raw food. Mishandling of raw or cooked foods and warmer temperatures allows bacteria to grow faster. Representatives of taxa that are the most responsible for food poisoning are *Bacillus cereus*, *Campylobacter*, *Clostridium*, *E. coli*, *L. monocytogenes*, *Salmonella*, *Staphylococcus*, and *V. parahaemolyticus*. Most of them produce heat-sensitive toxin, so after cooking the toxin is eliminated. Nevertheless, some of them can produce toxins or spores which are heat-resistible and therefore it can be a serious problem. Some of these bacteria species require special conditions, different from those which all the bacteria have in common. *Clostridium* needs to have low-oxygen atmosphere and *Vibrio* needs salt for its growth. Certain bacteria responsible for intestinal infections such as *Salmonella* may result in additional symptoms. Some of them can survive in adverse conditions for very long time as a *Listeria.* Most of them are found on protein-rich foods as a meat, seafood, tofu, poultry, fish, egg, milk or starchy food (Wagner 2008).

Mechanism of infectious diarrhoea can be described as a noninflammatory, inflammatory or invasive, according to pathogen which is causing certain disease. The noninflammatory diarrhoeas are caused by bacteria such as *Vibrio cholerae,* which is enterotoxin producing or another is enterotoxigenic *E. coli*. They adhere to the mucosa and disrupt the absorptive or secretory processes of the enterocyte without causing acute inflammation or mucosal destruction. Second type, the diarrhoea with inflammatory effects on human gut is caused by two groups of organisms. First of them produce cytotoxins and bacteria itself is noninvasive. Bacteria causing this kind of diarrhoea can be *E. coli* and *C. difficile*, and by invasive organisms such as *Campylobacter* spp., *Entamoeba histolytica*, *Salmonella* spp., and *Shigella* spp. Cytotoxin-producing organisms adhere to the mucosa, activate cytokines and stimulate

the intestinal mucosa to release mediators, which are responsible for further inflammation. Invasive organisms producing cytotoxins attack the intestinal mucosa and induce an acute inflammatory reaction, involving activation of cytokines and inflammatory mediators. Regardless the basic mechanism they use, these different types of pathogens have all been developed successfully to avoid and modulate the host defense systems (Scotland & Wortley 2003).

Enterococcus faecalis

This bacterium belongs to Gram-positive cocci, usually occurs in pairs or chains and it naturally occupies human intestine in all healthy individuals, where it is usually beneficial for our organism, but it can cause gastrointestinal problems such as diarrhoea or can be even life-threatening (Murray 1990). In general, this bacterium belongs to the main representatives of bacterial causes of diarrhoea. It is tolerant to 8.5 pH and salinity. Usually most of known bacteria need acid surrounding, which means 7 pH and lower. In nature, it can grow in range of temperatures from 10 to 45°C and survive up to temperatures of 60°C for 30 minutes (Stuart et al. 2006). For laboratory testing, it requires special MHB with 1% glucose. *E. faecalis* is highly antibiotic resistant to vancomycin, penicillin and aminoglycosides. Tigecycline and rifampicin shown to have antienteroccocal activity. It is responsible for infections of urinary and gastrointestinal tract. Urinary tract is usually treated with nitrofurantoin (Zhanel et al. 2001), more sensitive strains can be treated by penicillin, ampicillin and vancomycin (Pelletier 1996).

Listeria monocytogenes

L. monocytogenes is a type of facultative anaerobic bacteria and Gram-positive. It is capable of surviving long time periods under adverse conditions as to grow at refrigeration temperatures. *L. monocytogenes* is pathogenic species which can causes infection of listeriosis and its vector of transport is usually food. For pregnant women, newborn, very young or the elderly which are immunocompromised, there is higher risk of subsection to virulent *Listeria*. Death is rare in healthy adult individuals. It is believed that up to 10 % of human gastrointestinal tracts may be colonized by this bacterium (Ramaswamy et al. 2007). It grows in pH at level from 5.0 to 9.5 pH when the growth medium is good. It is also salt tolerant till concentrations as high as 30.5 percent it can survive for about one hundred days at 4°C. When the temperature is over 38°C the vitality of bacteria is getting shorter and then is able to survive 5 days (Wagner 2008). Disease caused by this bacterium are cured with antibiotics. It is responsible for listeriosis and meningitis and it is the most common food

pathogen. Known successful treatment of *Lysteria* is done by penicillin or ampicilin (Temple & Nahata 2000).

Escherichia coli

It is one of the most commonly known and occurring Gram-negative, facultative anaerobic, non-spore forming and rod-shaped bacterium. Most of its strains are harmless and commonly found in human lower intestine (Tenaillon et al. 2010), but some variations can cause serious food poisoning to their hosts followed by recalls due to food contamination (Vogt & Dippold 2005). Enteropathogenic (tending to produce disease in the intestinal tract) *E. coli* is a significant cause of diarrhoea in developing countries and localities with low level of sanitation. The major source of the germs in the surroundings is probably the inoculated feces of human and animal individuals which are infected. To avoid contaminated food and further illnesses is done mainly by prevention. Prevention should include sufficient cooking and avoidance of recontamination of cooked meat by contaminated equipment, water and infected food handlers. Virulent strains cause gastroenteritis, infections of urinary tract, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Treatment is done by ampicillin and penicillin, when some strains are resistant (Wagner 2008).

Shigella flexneri

Shigella is Gram-negative and facultative anaerobe (it can grow in environment full of oxygen gas or in oxygen-free atmosphere). They are the least chemically active of all Enterobacteriaceae family. It is recorded 165 million cases of shigellosis each year, when most of the cases occurs among children of developing countries, when 1.1 million of them die (Jennison & Verma 2004). People after 40 years of age suffer less with this illness (Von Seidlein et al. 2006). It causes diarrhoea and shigellosis which is dysentery and it is specific for humankind only. Many of virulence factors contribute to this disease as the potent Shiga toxin of Infections caused by this bacterium, can be cured with antibiotics anyway there exist some resistance already. Vaccination is possible but with just little success (Mumy 2014).

Salmonella enterica

Salmonella belongs to category of Gram-negative bacteria. About its epidemiology, the source is exclusively human, symptomatic and bacilli, contaminated water, soil, waste, food and countries with low hygienic standards. The infection is possibly transmitted from an infected person (the patient excretes the bacteria by urine and feces) to alimentary tract (contaminated water). Common sources of *S. enterica* is gastrointestinal tracts of animals and man and high protein foods such as meat, eggs, poultry and fish are most frequently

associated. Any food that becomes contaminated by this bacterium, and is held after at improper temperatures and storage, can cause salmonellosis. If *Salmonella* is present on raw and cooked foods, its growth can be controlled by refrigeration below 4°C and furthermore is destroyed by cooking above 65°C. Another contamination of cooked meals occurs from contact with utensils and surfaces that were not properly washed and disinfected after use with raw products. These bacterium are also responsible for diarrhoea and to treat that antibiotics such as chloramphenicol (Wagner 2008).

Clostridium perfringens

C. perfringens is nonmotile, anaerobic bacterium, which is found in soil, dust and the gastrointestinal tracts of animals and man. The vegetative cell has the shape of a rod with depth exceeds 1 μm and the length is from 2 to 10 μm. Spores are oval in shape, thermosensitive, paracentral. Sporulation occurs in the intestine, never in the affected tissues. *C. perfringens* produce many biologically active substances including toxins and enterotoxin. Depending on the type of toxin produced, it is divided into five groups. *C. perfringens* are part of the normal intestinal microflora of humans and animals. When food with a large amount of this bacteria is consumed, a toxin in the intestinal tract is produced and causes illness. When the environment where *C. perfringens* occurs is inhospitable, it can exist as a heat-resistant spore, so it may survive cooking. After that it grow to large numbers if the cooked food is held between 4°C and 60°C for a long time period. High protein meals are the foods most frequently involved. Hot foods should be served immediately after heating. If this condition is not respected, the contingency of getting ill with diarrhoea is getting higher. For colic and watery diarrhoea, the incubation time is 8-14 hours after consuming contaminated food, symptoms as a diarrhoea, convulsions and abdominal pain, persist for about 24 hours. *C. perfringens* is quite sensitive bacterium and can be treated with regular antibiotics such as ciprofloxacin (Wagner 2008).

Clostridium difficile

C. difficile is a species of Gram-positive, motile and spore-forming bacterium (Moreno et al. 2013). As well as *C. perfringens* and all known genus *Clostridium*, this bacterium is anaerobic. It needs for its successful growth and vitality oxygen-free environment. In nature is ubiquitous, mainly in soil. When conditions are not optimal for active bacteria, it produces spores which can survive in extreme conditions. From 2 to 5 % of adult humans has this bacteria present in their colon (Ryan & Ray 2004). This pathogenic strain produce various toxins which are responsible for production of diarrhoea and inflammation of patients (Di

Bella et al. 2016). The diarrhoea caused by *C. difficile* may range from few days with light symptoms to life-threatening pseudomembranous colitis which is intense inflammation of the colon (Ryan & Ray 2004). *C. difficile* is responsible for 10 % of cases of antibiotics-associated diarrhoea and almost all cases of antibiotic-associated pseudomembranous colitis, which can be life-threatening. This type occurs due to the alteration of gut microflora by antibiotics Treatment by antibiotics could be difficult due to its resistance and producing of spore, anyway metronidazole, vancomycin, fidaxomicin are commonly used (Bartlett 2015)..

Vibrio parahaemolyticus

V. parahaemolyticus is a curved, non-controversial, facultative anaerobic, gram-negative stick 1.4-6.6 μm long. Bacteria are mobile with one polar flagellum, are oxidase positive, catalase positive and ferment glucose. *V. parahaemolyticus* is a human pathogen that is widely distributed in marine environments. This organism is frequently isolated from a variety of raw seafood (Guthrie 1976). *V. parahaemolyticus* is found on seafoods, requires the salt environment of sea water for growth (Wagner 2008). The consumption of raw and undercooked seafood contaminated with *V. parahaemolyticus* can lead to the development of an acute gastroenteritis characterized by diarrhoea, headache, vomiting, nausea and abdominal cramps. It has also been isolated from wound infection and septicemia (Guthrie 1976). *V. parahaemolyticus* is very sensitive to cold temperatures and heat and also antibiotics. Proper storage of seafoods, which is perishable, below 4°C, and subsequent cooking and holding above 60°C, destroy all the contained bacterium. Food poisoning caused by this bacteria is a result of insufficient cooking followed by contamination of the cooked product by a raw product and by improper storage temperature (Wagner 2008). Diseases are cured with quinolone, tetracycline, cephalosporins and penicillin (Wong et al. 2015). For growth requires more than 2% NaCl, in laboratory conditions is cultivated in MHB with 3% NaCl.

2.4 Management and conventional treatment

Since food-poisoning bacteria are often contained in many foods, the characteristics of such bacteria is essential for knowing the management of treatment. The program of curing depends on seriousness of certain disease. Besides food-borne bacteria, other way of spreading of these microorganisms in human gastro-intestinal tract, is through fecal-oral way with contaminated water connected with poor sanitation and low personal hygiene.

2.4.1 Prevention

The first and most important step before any others in preventing food poisoning is to assume that all foods may cause food-borne illness (Wagner 2008) and to have an access to drinking water (World Health Organization 2017). Further we can comply mostly simple precaution to avoid from diseases such as gastro-intestinal disorders connected with pathogenic bacteria like washing hands which is the most powerful step. Washing surfaces used for food preparation and utensils thoroughly before and after handling raw foods is also essential. This step is done to avoid recontamination of cooked foods. Highly recommended is to keep foods refrigerated below 4°C, serving foods hot and immediately after warming it up or keep them heated above 60°C. Dividing large volumes of food into small portions for rapid cooling might help as well. If exists any doubt about harmlessness of our food, it is always better to throw it away then eating it (Wagner 2008). Another option is to observe kind of food which is expected to be responsible for our problems in a long period of time and start avoiding that food by following a specific diet. One example can be that we are lactose-intolerant (Ratini 2017), which is not kind of problem connected with bacteria.

The other option of diarrhoeal disease is caused by spreading of bacteria through fecally-oral way. The main option how to avoid getting ill with such disease is respect our personal hygiene and in case of drinking water the main key is sufficient treatment of water before drinking, to avoid any possible occurrence of potentially dangerous bacteria.

Probiotics and Functional Food make important part of prevention, when probiotics have been extensively studied over the past several years as a tool of prevention of treatment of diarrhoeal diseases. Besides probiotics we can also use so called functional food which is defined as a specialized food product that can promote human health. The most widely used and known functional food can be for example yogurt. Yogurt is a product made by fermenting milk with benneficial bacteria. Intestinal microflora could be improved by the ingestion of yogurt. Fermented dairy products has a potential to decrease risk of having various intestinal disorders – especially diarrhoea. Unfortunately, probiotics can be destroyed

or depleted by stress, aging, antibiotics, changes in one's diet, or ingestion of foreign bacteria which is often connected with traveling in foreign countries. Probiotics are also food products that contain health-promoting bacteria, primarily lactic-acid bacteria that have a positive influence on the metabolic activity of our microflora in our intestine (English & Dean 2013). The evidence of their efficacy is only modest for the prevention of diarrhoea according to Guandalini (Guandalini 2011). Most commonly used probiotics are *Bifidobacterium*, *Lactobacillus*, and *Streptococcus*. The published trials have shown a known and identified probiotic strains, mostly *Lactobacillus G.G.*, strain ATCC 53103 and *Saccharomyces boulardii*, where statistically significant benefit and moderate clinical benefit were shown, in the treatment of acute watery diarrhoea. The interval of reduction of its duration is approximately only one day. The effect is strain-dependent and also depends on the dosage of the probiotics (Guandalini 2011). Use of probiotics may shorten the duration of this illness (Goenka & Kapoor 2012). If our gut microflora is healthy, the risk of falling ill with diarrhoea is decreasing so we should take care of our microorganisms living in our intestine. The beneficial bacteria naturally occur in our body and digest our food in intestinal tract. Without these microorganisms we are not able to digest properly. When we suffer from acute diarrhoea, pathogenic bacteria can overgrow our beneficial bacteria in meaning of quantity and we cannot digest.

2.4.2 Treatment

Management of diarrhoea depends on the stage of the disease, timing and where appropriate, other diseases and possible complications (allergy to medicaments) must be considered before treatment itself. At the first place, rehydration of patient should be managed and as a second step antimicrobial therapy by certain medicament, mostly antibiotics is used.

Oral rehydration therapy

If prevention fails and not even probiotics are not helping to cure diarrhoea, the last method how to cure out diarrhoea is taking medicaments with specific medicinal substances. Initial therapy should always include oral or parenteral rehydration as fast as possible, especially among children. Antimotility agents are usually not signposted (Schertzer & Garmel 2008). Oral rehydration therapy is the mainstay of treatment for mild dehydration caused by diarrhoea and is as effective as intravenous rehydration and serves as a prevention before need of hospitalization. For moderate dehydration oral rehydration solutions are recommended as well as insurance of prevention before vomiting. Improving tolerance of oral rehydration

solutions can be also required. To assure intravenous fluids is recommended for children for whom the previous oral rehydration therapy was not successful (Webb & Starr 2005).

Antimicrobial therapy

If all previously mentioned methods such as a prevention and usage of probiotics, fail, it is necessary to treat diarrhoea by antibiotics. Although the application of these agents is effective, it is the last option for curing of diarrhoea because of their numerous side effects and inhibitory action on beneficial gut microflora. Antibiotics are type of antimicrobial substance active against bacteria. Is the most important type of antibacterial agent for fighting against bacterial infections. They are used to avoid or treat bacterial infections by their ability of prevent them from its reproducing and spreading by inhibition or elimination of their growth. If they are needed to be taken, their doses can be provided in two ways, such as oral application and by injecting of them. Oral antibiotics are most likely tablets, capsules or a liquid to be drink. This method is used to treat most types of mild to moderate bacterial infections. Second way is injections, that is usually earmarked for more serious infections. Injection can be applied to patient as a direct injection or infusion. Infusion is done through a drip directly into the blood or muscle (Department of Health & Social Care 2016). In case of invasive and dysenteric diarrhoea specific antibiotic treatment is systematically indicated in immunosuppressed patients (Schertzer & Garmel 2008). Side effects of taking medicaments such as antibiotics can lead to feeling and being sick, bloating and indigestion and further causing of diarrhoea. If some individuals are sensitive, they can suffer from allergic reaction or even serious allergic reaction such as anaphylaxis (Department of Health & Social Care 2016).

Some of hundreds known antibiotics are classified as broad-spectrum and they inhibit wide range of microorganisms. The opposite are specific, inhibiting growth of particular bacterium. Six groups of antibiotics can be mentioned: penicillins (such as penicillin and amoxicillin), cephalosporins (such as cephalexin), aminoglycosides (such as gentamicin and tobramycin), macrolides (such as erythromycin and clarithromycin), fluoroquinolones (such as ciprofloxacin and levofloxacin), and tetracyclines (such as tetracycline and doxycycline). Last two groups, fluoroquinolines and tetracyclines can be used to treat a wide range of infections caused by bacteria in human gut. Broad-spectrum antibiotics that can be used to treat a wide range of infections (Department of Health & Social Care 2016). Further, four antibiotics most commonly prescribed for initial of empirical therapy of acute diarrhoeal diseases are discussed: ciprofloxacin, co-trimoxazol, clarithromycin, rifaximin (Sur & Bhattacharya 2006).

For this research were used three main antibiotics, ciprofloxacin, metronidazole, and, tetracycline.

Tetracycline is used to cure numerous infections. Tetracycline belongs to family of tetracyclines. By blocking the ability of bacteria to make proteins works. It has a broad range of antibiotic action. Originally, they possessed some level of bacteriostatic activity against almost all medically pertinent aerobic and anaerobic bacterial genera, both Gram-positive and Gram-negative, with some exceptions (World Health Organization 2014).

Another antibiotic which was used is metronidazole. Besides it is antibiotics, also serves as an antiprotozoal medicament. It is the most effective and safe medicines in a health system and available worldwide and is applied by mouth, topically as a cream and intravenously. Is used to inhibit mainly *C. difficile.* Common side effects after application can be nausea, a metallic taste, loss of appetite and headaches (World Health Organization 2014)

Third and last kind of antibiotic used to cure bacterial infections is ciprofloxacin, which was also used mostly during testing. Is used to cure many diseases including infectious diarrhoea caused by bacteria. Is used in treatment of serious infections, such as those which are caused by Gram-negative bacteria. Furthermore, ciprofloxacin in combination with metronidazole is one of several first-line antibiotic recommended for the treatment of abdominal infections for adult persons (Zhou & Li 2010).

3 Medicinal plants

Medicinal plants have been discovered already in prehistoric ages and have been used in traditional medicine worldwide. More than 50,000 of them (Schippmann et al. 2005) out of 422,000 flowering plants on our planet (Scotland & Wortley 2003) are used somehow for their medicinal purposes. Active substances and their properties for curing various maladies are generally compounds such as phenolics, terpenoids, steroids and alkaloids (Pöll & Álvarez 2015). Some of these substances can serve as a source of inhibition of bacterial and viral growth. Due to these properties they are able to cure various diseases by synthesizing chemical compounds which usually works as defense against insects, fungi or herbivorous animals or human (Ahn 2017). Plants are able to do so by their phytochemicals properties with potential biological activity and to produce primary and secondary metabolites during their life. Secondary metabolites are organic substances formed in the body of organisms that are not directly involved in the process of growth, development or reproduction of the organism. If missing, in the long run, it reduces the immunity of the individual. Secondary metabolites primarily serve as a defense but can serve also as an attraction for pollinators etc. By known taxonomy of medicinal plants we can estimate chemotaxonomy, and it can be assumed that representative individuals of the same family will have similar chemical and healing properties. To give examples of herbal medicaments used in Ayurveda practices to cure diarrhoea can be *Cymbopogon citratus*, *Glycyrrhiza glabra*, *Elettaria cardamomum*, and *Tinospora cordifolia*.

3.1 Phytochemistry and pharmacology

Phytochemistry and phytopharmacology deals with structure and functions of this compounds because they are known as biological active and beneficial for human health (Kurmukov 2013). Phytochemistry is a science which study phytochemicals. These substances are plant derivates and product of secondary metabolism. Phytochemicals are the active biological component including substances such as tannins and quinones, which determine pigments, alkaloids, and terpenoids, responsible for their odor, glycosides, polyphenols and flavonoids. These substances possess antimicrobial activity. Phytochemistry can be considered as a part of botany and chemistry. They are and always have been an important source of antimicrobial compounds. It has a long tradition and traditional healers are still used to use plants to prevent or cure infectious diseases. Today up to 50 % of current pharmaceuticals are direct plant derivates (Cowan 1999). Due to increasing resistance of antibiotics it is necessary to search

for new agents which can serve as an inhibitors of bacterial growth and further investigation is required (Kenny et al. 2015).

3.2 Drug discovery

Phytochemistry by its applications can be used in pharmacognosy, for studies of plant physiology and for discovery of new drugs. Drugs are substances which are biologically active and can affect our body. Searching for new drugs is a process of finding mediators which can specifically interact with biological materials. This process is measured in molecules, most of the time these substances are proteins and nucleic acids. After finding and isolation of these substances, they can be synthetized in artificial ways of preparation by tools of organic chemistry. In modern era of pharmacology pure chemicals are used, instead of crude extracts of medicinal plants which were used as treatment or still are used in folklore medicine.

Science oriented in this topic is called ethnobotany, with further division to ethnopharmacology. Ethnobotany is a study of relationships between plants and humans. This science deals with usage of plants in various and ancient human cultures. The main interest is how plants have been or still are being used, processed and perceived in human societies. It includes both wild-crafted and domesticated plants, used as food, medicine, poison, for ritual prophecy, and many other purposes. New drugs are discovered with many different approaches, including wide range of previous knowledge. Firstly, observation as tool and knowledge of science such as botany, mycology, taxonomy, anthropology, ethnography, archaeology, comparative folklore, religious studies, medicine, chemistry, pharmacology are essential for discovery of new drugs in modern science. Ethnopharmacology study ways of using, modes of actions and effects of occurring drug compounds that can be found in nature. This science has an ability to explain the effectiveness of herbal medicine, stimulants, analgesics, inebriants or psychoactive species (Botanical Dimensions 2013).

3.3 Plant-derived antidiarrhoeal products

Traditional use of plants with potential of curing diarrhoea is based on various mechanisms of their action such as antispasmodic effects, delay intestinal transit, suppression of gut motility, stimulation of water adsorption, and reduction of electrolyte secretion (Palombo 2006). Examples of such compound is berberine, an isoquinoline alkaloid having positive anti-diarrhoeal effect which can be found in *Berberis* ssp. It is sold especially in Asia in pure form as a pharmaceutical drug for the treatment of intestinal infectious diseases such as diarrhoea.

Usually it is available in tablets, in three forms: berberine tannate, berberine sulphate and berberine hydrochloride (Kokoska et al. 2018). It is believed that the anti-diarrhoeal activity of berberine works thanks to a complex of different mechanisms such as a decrease of bacterial adherence to mucosal and epithelial surfaces (Sun et al. 1988). Mastic is another example. This compound is a resin of yellow-to-white pastel color, extracted from *Pistacia lentiscus*. It is obtained from trunk and used in various dietary supplements and traditional medicines in the form of capsules or oil extracts, and tablets for relief of gastric and intestinal inflammation and as a natural treatment for infections. GutGard is *Glycyrrhiza glabra* extract and contains flavonoids as a glabridin and others, saponins, such as glycyrrhizin and phenylpropanoids. This substance is another example of herbal supplement with purpose to inhibit growth of bacteria, such as *Helicobacter pylori*. Bismuth subsalicylate is another compound which is plant-derived and used as a medicament. Many bismuth-containing preparations are available worldwide. The combination drug Pepto-Bismol with the active substance bismuth subsalicylate is available in the USA. It contains two potentially active substances - bismuth and salicylate. It is not clear how effective bismuth compounds are, but their results have been confirmed in the treatment of so-called diarrhoea and gastric infection. It affects diarrhoea causing bacteria by hydrolysis of salicin (glycoside of salicyl alcohol) present in bark of *Salix alba* which is responsible for formation of salicylic acid and this compound has anti-inflammatory effects with ability of reduction of water secretion and inflammation. It is sold in form of liquid or tablets and is used to treat temporary discomforts of the stomach and gastrointestinal tract, such as diarrhoea, indigestion, heartburn and nausea. (Kokoska et al. 2018).

4 Philippines and local traditional medicine

Republic of Philippines is an island country of Southeast Asia situated in the western Pacific Ocean and it consists of 7,641 islands (CNN Philippines 2016). Philippines has tropical maritime hot humid climate and are prone to typhoons, earthquakes and experiences with frequent seismic and volcanic activity due to its location within the Pacific Ring of Fire with combination of close distance to the equator. Most islands are covered by tropical rainforest and they have mountainous and volcanic origin and they are spread out more than 300,000 sq km (The World Bank 2017) with the fifth longest coastline in the world by its length of almost 36,300 sq km (Philippine Statistics Authority 2011). Whole Philippines can be divided into 3 main parts: Luzon, Visayas and Mindanao. These are further divided into 17 regions, 81 provinces, 145 cities, 1,489 municipalities, and 42,036 villages (Philippine Statistics Authority 2011). Its population exceeded number of 100 million (Bersales 2016). They were Spanish colony over 300 years which resulted in fact that Catholicism become to be a dominant religion and naming of Philippines after King Philip II. In these days president Duterte let himself be heard he would like to rename his country to name Maharlika, which in the local language refers to the historical social class of noblemen-warriors (The national 2019). With one exception of autonomous region in Muslim Mindanao, which is situated in the south of the country, it is governed as a unitary state. Christians and Muslims are leading religions. Philippines has many ethnic groups according to the 2000 census, 28.1 % of locals are Tagalog, 13.1 % Cebuano, 9 % Ilocano, 7.6 % Visayans, 7.5 % Hiligaynon, 6 % Bikol, 3.4 % Waray, and 25.3 % of others (CIA Staff 2015) with 182 living languages (Simons & Fenning 2017).

Philippines biodiversity is one of the most diverse due the highest rates of discovery in the world, since 100 mammals and 170 bird species exist only there (Rowthorn et al. 2018). Rate of endemism for the Philippines is very high and will continue to rise (Heaney 2001). In local rainforest it can be found extraordinary flora as some species of orchids of *Rafflesia pilippinensis* which is endemic to Philippines and grows only on one specific mountain in Luzon island (Barcelona et al. 2009). Philippine's flora is unique to because Philippines never was a part of continental mainland. In The Philippines about 13,500 plant species was estimated (Rowthorn et al. 2018).

Traditional medicine in Philippines has a long history. It is believed that some practices are brought from China. Anyway, in these days in rural and remote areas of Philippines the traditional medicine is still very popular and, in many cases, indispensable. There are either a

handful practicing herbalists or licenses modern medicine doctors and approximately 250,000 unregulated traditional herbalists (World Health Organization 1997).

4.1 History of traditional medicine

Since ancient times plants have played an important role in traditional medicine in the Philippines. It is believed that Chinese traders introduced some medicinal plant there. By this fact were surprised also Spanish colonizers in year 1521 when they arrived. During Spanish regime was written many books about traditional medicine of Philippines. Later, during years of occupation by Americans, further research on medicinal plants was conducted. During the Second World War, indigenous people of Philippines were depended entirely on plants as sources of medicine due to lack of medicaments.

Local herb doctor is a respected member in his community and medicinal plants are part of the cultural heritage. Knowledge is of traditional practice is pass from one generation to another. They use plants for the treatment of diseases, for pain relieve and for physical suffering. Traditional practice was coupled with native beliefs and superstition connected with religion. For example, it is believed that disease is caused presence of evil spirits in the human body. That can be removed by using some bitter-tasting substances, usually derived from plants. The practice of traditional practitioners is based on nature principles.

When modern Western drugs became available, many locals, especially those in urban centers lost contact with their herbal heritage. This leads to loss of attraction about knowledge and difficult work of herbalists and researchers. Especially the widespread adoption of medicinal plants became to be not easily accessible.

Many Western trained doctors are now prescribing herbal remedies and plant-based medicine for their patients and to continue providing Philippine health care, especially in remote areas and islands where drug shortages are critically felt.

As in most developing countries, more than 80% of the population still use herbal remedies. Dosage forms such as tablets, capsules, syrups, ointments, lubricants, tinctures, lozenges, lotions and herbal teas are available and other products as a herbal soaps, shampoos and other body care products are popular (Padua et al. 2001). To give examples, traditionally used plants are *Vitex negundo* as anti-asthma and anticough medicament, *Mentha cordifolia* used as analgesic, *Blumea balsamifera* is diuretic and anti-urolithiasis, *Carmona retusa* against gastro-intestinal hypomotility, *Ouisqualis indica* is anti-parasitic agent, *Psidium guajava* as a wound antiseptic, *Cassia alata* for skin anti-fungal and anti-scabies properties, *Peperomia pellucida* can lower blood uric acid, *Allium sativum* is able to lower blood cholesterol, and

Momordica charanti can serve as hypoglycemic agent in Philippines (World Health Organization 1997).

4.2 Current practices of traditional medicine

In the communities through the Philippines exist 250,000 practitioners of the traditional medicine. They instruct people suffering with various diseases and coming for help, to prepare decoctions, poultices and other ways of traditionally prepared herbs. The prescriptions are connected with prayers, incantations and changes in everyday life. People of Philippines have special names for the practitioners according to their way of use of the herbal medicaments. Name "hilot" or "manghihilot" is designation for midwifes or massage therapist who promote health and healing. "Tawas" (or "mangtatawas") is practitioner which uses alum, candles, smoke, paper, eggs and other mediums for diagnosis of cause of specific illness. The healing can be also connected with prayers. Name "albularyo" means person, who uses a combination of both healing methods. That may include prayers, incantations, mysticism and herbalism. These healers could be named also shamans. Similar name used in Philippines is "medico", which comes from Spanish language and this person use similar ways of healing of his patients as the "albularyo", but integrates western medicine to promote healing. They are more as a faith healer, a practitioner who claims divine power bestowed by the Holy Spirit or God so that his patient is required to have faith and believe in the powers (World Health Organization 1997).

4.3 Antidiarrhoeal herbal preparations

Preparation of medicinal plants is process of adjusting the plant so in the end the healing substances are quickly and easily accessible. Historically, these types of treatments are used to cure or relieve symptoms such as diarrhoea. Many ways of plants preparation exist, where the most common are eating or chewing raw of plant part containing curing substance. The part eaten are whole raw plant, raw fruit, and raw seeds. Usually are eaten young leaves or shoots. Second way of use require some preparations such as juice, decoction, tea, suppositories, and powder. Decoction is the most commonly used method. The product of decoction, which includes mashing of material and boiling the extract oils and organic compounds is drunken when it is ready. For decoction usually parts as a leaves, flowers, seeds and sometimes even roots of the plants are used (Padua et al. 2003). Some plants (e.g. *Cyanthillium cinereum*) in Philippines are used in the way of suppository to cure diarrhoea (Abe & Ohtani 2013). They have a positive impact on health and alleviate various problems. Tea is prepared by pouring boiling water to dried or fresh herb and let to leach. Preparation of plant as a paste consistency

is achieved by milling the herb and after it is mixed with water and the whole mixture is heated. Powder preparation means drying and milling herbs into powder. It can be mixed with a little bit of water followed by heating and eaten (Górnicka 2014). Example of plant used in Philippines to cure diarrhoea by drinking powdered plant dissolved in water could be *Manihot esculenta.* Not prepared and eaten raw are different parts of plants. To get juice from plant is necessary to squeeze out the liquid content. The processed juice could be diluted by water (e.g. *Pueraria phaseoloides).* Recorded are also information about topical usage of medicinal plants to cure diarrhoea, as for example application of heated leaves on a stomach of the patience (Abe & Ohtani 2013).

4.4 Specific Philippine plants with potential of diarrhoea treatment

Although there are several reports showing *in vitro* antimicrobial effects of certain plants used in traditional Philippian medicine for treatment of diarrhoea, e.g. *Alstonia scholaris, A. macrophylla* (Khyade et al. 2014), *Sargassum oligocystum* (Baleta et al. 2011), *Ipomoea muricata* (Ysrael 2003), *Coffea arabica, Psidium guajava, Mangifera indica*, *Vitex negundo, Tithonia divesrifolia,* and *Cassia occidentalis* (Balangcod et al. 2012).

Acalypha grandis

It can be found under different synonyms according to Plant List (Theplantlist.org 2013) such as *Acalypha amentacea, A. consimilis, A. exaltata, A. finitima, Ricinocarpus consimilis, R. exaltatus*, or *R. grandis* and belongs to Euphorbiaceae family. **Botany:** *A. grandis* is a spreading shrub or a small tree growing up to 10 meters tall. Spreading shrub or small tree up to 10 m tall, young parts are fulvous in color. Leaves are broadly ovate, 25 cm x 20 cm, base is variably cordate, apex can be shortly caudate and acuminate. Margin of leaves is variably crenate-serrate, petiole is up to 17 cm long, stipules are lanceolate and up to 1 cm long. Male inflorescence is up to 20 cm long and female inflorescence up to 17 cm long, lax-flowered, bract is very variable, up to 10 cm in diameter, toothed and accrescent. **Geographical distribution:** It occurs in Province Albay of Philippines, island Luzon: Ilocos Sur, Banaue, Isabela Province Northern Sierra Madre Natural Park, Palanan municipality, Barangay Alomanay, Dipudyen River, island Panay: Antique province Mun. Libertad, Bulanao river. Island Mindoro: Oriental Mindoro prov. Mountain ridge behind Puerto Galera. San Teodoro, island: Cebu: Cebu Prov. Mun. Argao, Brgy. Cansuje. This plant can be found in primary and secondary forests, stream banks, and in regrowth along lava flows, at elevations up to 150 meters. It can be found growing around houses. The plant is sometimes harvested from the

wild for local medicinal use. **Traditional use:** Finely crushed leaves and flowers are added to food as an antidiarrhoeal substance. Sap of the pounded inner bark is employed as a mouthwash against thrush. The sap of heated leaves in combination with *Citrus* sap is given as a remedy for thrush in children. A poultice of the leaves is an effective remedy for boils and other skin affections. In Milne Bay, Papua New Guinea, leaf sap is drunk with water, to treat diarrhoea and dysentery (Ken Fem 2014). **Chemistry and biological activities:** *A. grandis* has positive effects on various maladies (Padua et al. 2001) including ability of decreasing level of breast cancer and antiplasmodial activities were observed with obvious cytotoxic potential (Bradacs et al. 2010). However, other information on chemistry and biological activity of this species are missing.

Acanthus ebracteatus

Philippine name for this plant is Lagiwliw or Ragoyroy, english name is Sea holly and it belongs to Acanthaceae family. Synonyms of these plants can be also found and follows: *A. ilicifolius* and *Dilivaria ebracteata*. **Botany:** It grows as an erect, spreading or scrambling shrubby herb, up to 1.5 meters tall, usually with a great many stems. Its leaves are green to dark green, stiff, with sharp spines at the end of each deep lobe, very much like those of holly (*Ilex*). The spines are very typical for this plant. Flowers are blue, purple or white, and occur in spikes terminal on the branches. The fruit is a square-shaped capsule, which explodes when ripe, projecting the seeds up to two meters from the plant. Seeds are flat and off-white in color. **Geographical distribution:** *A. ebracteatus* species occurs in the undergrowth of mangrove plants or trees, thus in highly wet and humid marine areas, usually along the seacoast, were mangrove grows, thought the South-East Asia. **Traditional Use:** This species is used traditionally in South-east Asia, for wound healing and against diarrhoea. **Chemistry and biological activities**: In Thailand *A. ebracteatus* is used for wound healing, due to its ability of increasing wound closer and healing skin infections (Sittiwet et al. 2009), it has a angiogenic (Somchaichana et al. 2012) and antitumor properties and it is able to treat neurodegenerative diseases associated with oxidative stress (Prasansuklab & Tencomnao 2018). Stem of this species contains bioactive polysaccharides (Hokputsa et al. 2004). Fibers have performed antioxidant activity (Vongsetskul et al. 2016).

Aleurites moluccana

This plant is also known under many different synonyms as *A. moluccanus, A. ambinux, A. angustifolius, A. commutatus, A. cordifolius, A. integrifolius, A. javanicus, A. lanceolatus, A. lobatus, A. pentaphyllus, A. remyi, A. trilobus, Camerium moluccanum, Camirium cordifolium, C. oleosum, Dryandra oleifera, Jatropha moluccana, Juglans camirium, Mallotus moluccanus, Manihot moluccana, Ricinus dicoccus, Rottlera moluccana* or *Telopea perspicua* according to Plant List (Theplantlist.org 2013). This plant belongs to family Euphorbiaceae. Philippine names for this plant are various according to Philippine local language. Local names are Lumbang and Biau in Bagio language, Rumbang in Bisaya, Biau in Cebu Bisaya, Kami in Sulu or Kalumban and Kapili, Lumbang and Lumbang-Bato in Tagalog language, which is spread all around the Philippines. English name for *A. moluccana* is Bankul nut tree, Candleberry, Candle nut oil tree, Indian walnut, Lumbang tree or Varnish tree. **Botany:** Large tree reaching a diameter of 80 to 150 centimeters, younger parts and the inflorescences are hairy. Leaves have long petioles. Blades are ovate to lanceolate, 10 to 20 centimeters long, entire or lobed. Flowers are white, numerous, 6 to 8 millimeters long, borne on panicles 10 to 15 centimeters long. Fruit is fleshy, ovoid, smooth, 5 to 6 centimeters long, containing 1 or 2 hard-shelled, oily seeds. Shell of the seed is very hard, rough, ridged, about 2.5 millimeters thick. Within the seed is a white, oily, fleshy kernel consisting of a very thin embryo surrounded by a large endosperm. Candle nuts are toxic when are eaten raw. **Geographical distribution:** Occurrence of this plant is throughout the Philippines. It can be found at low and medium altitudes, in second-growth forests or thickets. In island Cebu: Cebu Prov. Alcoy, Brgy. Nug-as; island Negros: Negros Occidental Prov. Mt. Kanlaon Natural Park; island Luzon: Metro-Manila Quezon City, Diliman, Ninoy Aquino Parks and Wildlife Center. **Traditional Use:** Traditionally used parts of this plant are bark against diarrhoea or dysentery, to treat pain, fever, asthma, hepatitis, gastric ulcer and inflammatory process in general, and the nut oil had been topically applied to treat arthritis and other joint pain, however the seeds are classified as toxic for oral use (Quintão et al. 2019). In Philippines, the seeds are used as a mild purgative. For example: in Java the bark is used for blood diarrhoea. **Chemistry and biological activities**: *A. moluccana* extracts have shown inhibitory activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Ken Fem 2014). Its main active constituent, flavonoid 2 "-O-rhamnosylswertisin is able to treat rheumatoid arthritis and it has anti-hypersensitivity and anti-inflammatory properties (Quintão et al. 2019).

Artocarpus blancoi

It is also known under name *Artocarpus communis* var. *blancoi.* and is from family Moraceae. Local name in Philippines is Antipolo or Tipolo. It is an endemic species, which means it grows only in one place and not anywhere else. **Botany:** *A. blancoi* is a medium-sized, evergreen, dioecious tree, up to 15 meters tall but it can grow even up to double size of 30 meters. In diameter it proximately has 100 cm, exuding white latex when wounded. Leaves arranged spirally and leave blade is ovate-elliptical, 40 cm × 30 cm, base cuneate to rounded, margin entire or pinnatifid with 1-4 pairs of lateral lobes, apex acute, almost glabrous above, pubescent throughout below, with about 12 pairs of secondary veins, stipules amplexicaul. Inflorescence is capitate, solitary, axillary with numerous flowers densely packed together, embedded in the receptacle, the perianth enclosing a single stamen or ovary, mixed with abundant stalked interfloral bracts; male head are cylindrical, 15 cm × 1.5 cm, on a 17-37 mm long peduncle; female head are with bifid styles. Fruit is an ellipsoid syncarp, up to 10 cm × 6.5 cm big, yellow-brown in color, covered with flexuous, tapering with spines 8-15 mm long, with rough, inflated hairs, with scattered interfloral bracts and with numerous non-fleshy fruiting perianths with free proximal region. Seeds are ellipsoid in shape, 12 mm × 9 mm big (Ken Fem 2014). **Geographical distribution:** It originates on Philippines and can be found throughout the whole country in thickets and forests, mainly at lowlands but also at medium altitudes. **Traditional Use:** We can find in literature that various parts are used as a leaves, fruit or bark. Part used which is used in traditional medicine for curing diarrhoea is fruit but if not found, bark arises as a good alternative (Tan 1980). **Chemistry and biological activities**: Glycosides, sterols and trace amounts of triterpenes, flavonoids, saponins and tannins are present. *A. blancoi* is a potential anti-larvicidal and anti-ovicidal agent against *Aedes aegypti* (Pineda-Cortel et al. 2019). Isolated 5,7,4'-trihydroxyflavanone-3-O-alpha-L-rhamnopyranoside from its bark exhibited xanthine oxidase inhibitory activity (Fabian & Chichioco-Hernandez 2018).

Artocarpus camansi

Family of this species is the same as for the rest of *Artocarpus* species - Moraceae. In Philippines the name for this plant is Kamansi, for English speaking world it can be found name Breadnut. No synonyms are recorded for this species according to the plantlist.org. **Botany:** *A. camansi* is a large tree, 10 to 15 meters high. Leaves are large, leathery, ovate to oblong-ovate, 40 to 60 centimeters long, 25 to 45 centimeters wide, with deeply incised

margins, 4- to 5-lobed. Male spikes are cylindric, greenish-yellow, 15 to 25 centimeters long, and 3 to 4 centimeters in diameter. Fruit is green, ovoid or somewhat rounded, 10 to 15 centimeters long, 7 to 12 centimeters in diameter, with a spiny surface, with little pulp. Seeds are numerous, light brown, ovoid or somewhat rounded, about 2.5 centimeters in diameter. **Geographical distribution:** Its occurrence can be described as it is plant of low to moderate elevations in the moist tropics, where it is found at elevations from sea level to 1,550 meters - alluvial forests, the first species to appear on the tops of frequently flooded banks of rivers. It is now found only in cultivation in the Philippines, where it is typically grown as a backyard tree - cultivated in Manila. **Traditional Use:** Fruit and bark seem to be also valuable. Diluted latex used for diarrhoea, stomach aches and dysentery. In the Visayas, also bark decoction is used for dysentery (Ken Fem 2014). Decoction of leaves is used for diabetes and baths of people with rheumatism (Salonga et al. 2014). **Chemistry and biological activities**: Cytotoxic activity is recorded (Tantengco & Jacinto 2015) and it is known for its exceptional medicine value, possessing phytochemicals and also nutritional value (Hari et al. 2014). Its leaves has an antioxidant and antimicrobial properties (Vianney et al. 2018) and fruit contains of both water soluble fibers, such as pectin and gum, and water insoluble fibers (Suryanti et al. 2016). *A. camansi* leaves containing polysaccharide and/or glycoprotein might be an effective natural product to treat allergic contact dermatitis (Salonga et al. 2014). Its seeds are high in phosphorus, potassium and sodium, respectively (Adeleke & Abiodun 2010) and high in protein (Adeleke & Abiodun 2010; Nelson-Quartey et al. 2007).

Artocarpus elasticus

Another species from genus *Artocarpus* and also from Moraceae family is *A. elasticus.* English name of this plant is Terap which is similar to local Philippine name. We can find synonyms for this plant as an *A. blumei* or *A. kunstleri.* **Botany:** *A.* is an evergreen tree growing up to 45 meters in height, with reports that it can reach 65 meters. The straight bole can be free of branches for up to 30 meters, it is up to 125 cm in diameter with prominent buttresses up to 300 cm high. The plant has extraordinarily large leaves on juvenile trees they can be over 1 meter long. It can be deciduous when growing in areas with a short dry season. The wood is a source of ‚terap' timber and is usually traded on market (Ken Fem 2014). **Geographical distribution:** It is spread all over East Asia in countries such as Myanmar, Thailand, Malaysia or Indonesia. This plant we can find within evergreen forests, usually at elevations up to 300 meters, but occasionally even up to 1,350 meters. **Traditional Use:** Traditionally used parts are leaves and bark or latex against dysentery according to Lim 2016.

Roots are used as an aperient decoction. **Chemistry and biological activities**: Compounds cycloartelastoxanthone, artelastoheterol, cycloartobiloxanthone and artonol A were isolated from *A. elasticus*, where all showed inhibition of oxidative DNA damage and they are also promising antioxidant agents (Lin et al. 2009). Leaves contains prenylated dihydrochalcones, namely elastichalcones B, C, D, E, F and artocarpanone, where elastichalcone C exhibited good antibacterial activity and cytotoxicity (Daus et al. 2017). Bark contains artonin E, dihydrobenzoxanthone derivative named elastixanthone, cycloartobiloxanthone and artobiloxanthone and compounds 1,1-diphenylpicrylhydrazyl, which displayed broad spectrum antimicrobial activities against thirteen different bacterial strains. Cytotoxic screening revealed that artonin E constantly exhibited strong cytotoxic activity against human estrogen receptor, positive breast cancer, and human estrogen receptor negative cells (Ramli et al. 2016). Stem bark extracts can play an important role in the bioreduction and stabilization of silver ions to Ag-NPs (Abdullah et al. 2015). Leaves contains diprenylated dihyrochalcones elastichalcone A and B (Ramli et al. 2013). Wood contains prenylated flavones: artocarpin, cycloartocarpin, cudraflavones A and C, and oxepinoflavone artoindonesianin E1, which has cytotoxic activity against P-388 cells (Musthapa et al. 2009). Artonol A exhibited cytotoxic activity against the A549 human cancer cell (Ko et al. 2005). It contains also prenylated flavonoids such as artelastofuran (Kijjoa et al. 1998) artelastocarpin, carpelastofuranartelastin, artelastochromene, artelasticin, artocarpesin, and cyclocommunin, where all of them besides artelastofuran showed cytotoxic effects (Cidade et al. 2001).

Artocarpus odoratissimus

Philippine name of this plant is Marang in Sulu language, Madang in Lanao and Loloi in Tagalog. Used English name is Terap and family is Moraceae as for the rest of the *Artocarpus* genus. Synonyms are *A. mutabilis* and *A. terap*. **Botany:** *A. odoratissimus* is a medium-sized tree growing to a height of 18 up to 25 meters in height, sometimes with low buttresses. Twigs are 4 to 10 millimeters thick, with long, yellow to red, spreading hair and stipule-scar rings. Leaves are alternate, ovate and 7/9-lobed. The lobes are lanceolate, glossy, dark green above, green below, stiff and petioled. Inflorescences occur in the leaf axils. Male flowers are minute in stiff spikes. Female flowers are in conical heads. Fruit is subglobose, up to 20 centimeters in diameter, green to yellow in color, densely covered with stiff, hairy processes about 1 centimeter long, borne occurs at the end of long flexible branches, with a mass of seeds embedded in pulp. Fruit flesh is white, edible, juicy, and fragrant but strong-smelling edible pulp. **Geographical distribution:** This plant can be found in Mindanao island and is

cultivated there for its edible fruit. It is also occurring in Mindoro, Basilan, and the Sulu Archipelago. Usually is wild-crafted. Further it can be found in San Teodoro; island Mindanao: Lanao del Norte prov. Iligan City, Brgy. Ditucalan, Tinago Falls; island Palawan: Palawan prov. Puerto Princesa; Mindoro, Oriental Mindoro, Calapan, Mindoro, Calapan. **Traditional Use:** Traditionally used parts are roots used against diarrhoea (Lim 2016). **Chemistry and biological activities**: From sugars in the highest amount fructose is present and from vitamins it is potassium (Tang et al. 2013). This species is a source of antioxidants due to presence of phenolic compounds such as flavonoids (Abu Bakar et al. 2009). in smaller amount, it contains also carotenoids (Bakar et al. 2015). Its fruits contain prenylated pyranoflavone derivatives artosimmin and traxateryl acetate. The first compound exhibited significant cytotoxicity against cancer cell lines (HL-60 & MCF-7) and also possessed antioxidant properties toward 1,1-diphenyl-2-picrylhydrazyl radical (Ee et al. 2010).

Breynia cernua

Synonyms for this plant besides the original name are *B. rubra, B. rumpens, Melanthesa cernua, M. rubra, Phyllanthus blumei, P. cernuus* or *P. ruber*. In Philippines local names that are normally used are Matang-ulang, Bagbagotot or Tintug. English version can be Gagilamo or Gambiran. Plant is from family Phyllantaceae. **Botany:** It grows as a shrub or treelet, up to 2 till 7 m tall, glabrous, leaves are ovate, 1.7-6.5 cm x 1.2-3.7 cm big, base attenuate, apex obtuse to bluntly acute, margin is entire flat and papery, light green above, tinged glaucous underneath, petiole is 1.5-4 mm long, stipules are 1.5-2.5 mm long. Flowers are staminate with 1-1.5 mm in diameter, pedicel 2.5-4 mm long, calyx about 1-1.5mm long, very thick, green, lobes rim-like, androphore 0.7-1 mm high, anthers about 0.7 mm long; pistillate flowers about 3.5 mm in diameter, pedicel about 1.7 mm long, calyx about 2.8 mm long, flat, accrescent in fruit up to 11 mm in diameter, red, lobes about 1.7 mm wide, ovary with stigmas very short, undivided; fruit about 3.5-4 mm x 6-7 mm, red turning black when mature. Seeds are 2.5 mm x 3.3 mm x 2 mm big. *B. cernua* is a very variable species, the most typical characters being the accrescent pistillate calyx and ovate leaves. **Geographical distribution:** It is usually encountered in very disturbed and anthropogenic habitats and can be found also in coastal vegetation. Often grow on limestone, up to 450 m altitude. In Philippines voucher specimens were collected in Luzon island: Quezon prov. Dolores, Brgy. Kinabuhayan, Mt Banahaw. **Traditional Use:** Traditionally used parts are probably in New Guinea, infusion of the bark is used to cure dysentery (Lim 2015a). **Chemistry and biological activities**: Bark of

root exhibited a good level of antifungal activity (Khan & Omoloso 2008). However, other information on chemistry and biological activity of this species are missing.

Carmona retusa

Tsaang-gubat is a local Philippine name for this species. English name is Philippine tea tree and it belongs to Boraginaceae family. **Botany:** *C. retusa* is an erect, much branched shrub growing up to 1 to 4 meters high. Leaves are in clusters on short branches, obovate to oblong-obovate, 3 to 6 centimeters long, entire or somewhat toothed or lobed near the apex and pointed at the base, short stalked and rough on the upper surface. Flowers are white, small, axillary, solitary, 2 or 4 on a common stalk, borne in inflorescences shorter than the leaves. Calyx lobes are green, somewhat hairy, and linear, about 5 to 6 millimeters long. Corolla is white, 5 millimeters long, and divided into oblong lobes. Fruit is a drupe, rounded, yellow when ripe, 4 to 5 millimeters in diameter, fleshy, with a 4-seeded stone, fleshy on the outer part, and stony inside. **Geographical distribution:** This plant is easily found from the Batan Islands and northern Luzon to Palawan and Mindanao, in most or all islands and provinces, in thickets and secondary forests at low and medium altitudes. Countries where it can be also find are China, Japan, Malaysia, Indonesia, Vietnam, New Guinea, Australia and the Solomon Islands. **Traditional Use:** Leaves are used for curing diarrhoea in a way of tea preparation or infusion is used for diarrhoea or as tea in the Philippines. A decoction is usually prepared from the dried leaves (Villaseñor et al. 2004). **Chemistry and biological activities**: Leaves of this species were obtained to have many properties such as antibacterial (Chandrappa et al. 2012), anticancer (Chandrappa et al. 2014), antioxidant (Reglos-Zara et al. 2010), anti-larvicidal (Rajkumar et al. 2018) or antimutagenic (Villaseñor & Edu 1993). It contains triterpenes (Villaseñor et al. 2004), phenolics, flavonoids, and tannins (Reglos-Zara et al. 2010).

Commelina communis

This species we can find under english name Day Flower or Asiatic day flower and it becomes to family Commelinaceae. Synonyms for this plant besides name *C. communis* are *C. coreana, C. polygama, C. vulgaris, C. willdenowii* or *Disecocarpus polygamus.* **Botany:** *C. communis* is an erect or decumbent, annual plant producing stems 25 – 70 cm long from a fibrous rootstock. Roots at proximal nodes. Stems diffusely branched. Leave blade are narrowly lanceolate to ovate-elliptic, 1 to 4 cm long, apex acute to acuminate. Inflorescences

is type of distal cyme usually vestigial, included, sometimes one-flowered, green, whitish basally with contrasting, dark green veins, pedunculate, usually not falcate, 1.5 to 4 centimeters long, margins distinct, scabrous, not ciliate, apex is acute to acuminate and glabrous to puberulent. Peduncles are 0.8-5 cm of length, flowers are bisexual, proximal petal paler or white, very reduced where petals are blue to bluish purple. Antherodes are usually yellow sometimes with central brown spot, cruciform. Capsules are two-locular, two-valved, 4.5-8 mm long. Seeds are 4, brown, 2 to 4 mm big and rugose is pitted-reticulate (Ken Fem 2014). **Geographical distribution:** It is commonly cultivated as a vegetable in China. It can be found Eastern Europe, through temperate Asia to China, Japan, Korea, Myanmar, Thailand, Cambodia, Laos, Vietnam. Occurrence of this plant is in partly shaded grassy places, cultivated fields and roadsides usually in moist places. It has many different habitats, often in humid localities, along ditches and roadsides. In Philippines it can be found in Luzon island – San Fernando. **Traditional Use:** The plant is harvested from the wild for local use as a food, medicine and source of a dye. Traditionally used parts are theaerial parts of the plant against diarrhoea. A decoction of the dried plant is used to treat bleeding diarrhoea, fever, diabetes. **Chemistry and biological activities**: Extracts of the plant showed antibacterial, antioxidant activity (Shibano et al. 2008) and anti-hyperglycemic properties in studies searching for treatment of diabetes (Youn et al. 2004). It contains alkaloids having antiviral activity (Bing et al. 2009) against viruses in the lungs (Zhang et al. 2010), C-glucosides: 3,4-epoxy-5-hydroxymethyl benzoate 2-C-beta-glucoside and alkaloid: 1H-indole-3-carbaldehyde (Yang & Ye 2009). A blue complex pigment is present, and it is called commelinin which is a tetranuclear (4 Mg^{2+}) metal complex, in which two Mg2+ ions chelate to six anthocyanin molecules, while the other two Mg2+ ions bind to six flavone molecules, stabilizing the commelinin complex (Shiono et al. 2008). Flavocommelin, 7-O-methylapigenin 6-C-,4 '-O-bis- beta-D-glucoside is component of the blue supramolecular pigment from *C. communis* (Misawa et al. 2013).

Cyathula prostrata

Philippine name of this plant is dayang, tuhod-manok and bakbaka. English name for this species is Pastureweed and it belongs to Amaranthaceae family. Synonyms are *Achyranthes alternifolia, A. prostrata* or *Pupalia prostrata* according to Plant list (Theplantlist.org 2013). **Botany:** *C. prostrata* is an annual, branched herb, reaching a length of 1 meter or more, with the stems prostrate and creeping below. Leaves are rhomboid-oblong, 2 to 8 centimeters long, and gradually tapering to an acute base. Spikes are usually terminal and axillary, slender,

peduncles, and 5 to 20 centimeters long. Flowers are numerous, green in color, ovoid, and about 3 millimeters long. Sepals has small hair at the surface. Fruit is an ellipsoid utricle, 1.5 to 2 millimeters long, thin-walled, hairless, one-seeded, surrounded by a stiff perianth. Seed is ovoid, 1.5 to 1.5 millimeters long, shiny and brown. **Geographical distribution:** This species can be found in waste places, thickets, etc., at low and medium altitudes, throughout the Philippines as Luzon island: Quezon prov. Mun. Dolores, Brgy. Kinabuhayan, Mt Banahaw, Los Banos, Mt. Makiling; Cebu island: Cebu Prov. Mun. Argao, Brgy. Cansuje. In other places such as many parts of Africa, through Asia to New Guinea, northern Australia and in western Pacific it can be found. **Traditional Use:** Traditionally used parts are roots for dysentery by decoction. In Indonesia, the leaves are mashed with water are a remedy for cholera, and an infusion of the whole plant is taken for fever and dysentery (Ken Fem 2014). **Chemistry and biological activities**: Anti-inflammatory, analgesic and antioxidant activities have been reported about this species (Ibrahim et al. 2012) as well as anticancer (Mayakrishnan et al. 2014). Antibacterial effects are also included for example against *E. coli* (Unni et al. 2009). However, no further information about biological activities of this species are recorded.

Cyperus brevifolius

Synonym for this species are *C. cruciformis, Kyllinga brevifolia, K. aurata, K. cruciate, K. cruciformis, K. elongate, K. fuscata, K. gracilis, K. hohenackeri, K. honolulu, K. intermedia, K. intricate, K. laxa, K.longiculmis, K. monocephala, K. monocephala, K. nivea, K. odorata, K. oligostachya, K. pumilio, K. sojauxii, K. sororia, K. tenuis, K. tenuissima, K. tricephala, Mariscus kyllingioides,* and *Schoenus capitatus* (Theplantlist.org 2013). Name which is known in Philippines is Pugo-Pugo, Boto-botonisan, Kadkadot, or Pugo-pugo according to the location. Known English names are Mullumbimby couch, Short leaved Kyllinga, Shortleaf spikesedge, Green K., Green water sedge or K. weed for this plant and it belongs to Cyperaceae family. **Botany:** *C. brevifolius* is an annual herb with slender stems, 10 to 50 centimeters high, usually scattered, rising from slender creeping rootstocks. Leaves are narrow, 3 to 10 centimeters long and less than 3 millimeters wide. Color is shining green. Spikes are mostly solitary, ovoid, green to white, up to 8 millimeters long. Spikelets are very numerous, lanceolate, about 3 millimeters long, with the keel of the flowering glume not winged. Whole inflorescence is subtended by 3 long leafy bracts. Fruit is a nut, about 1 millimeters long and compressed. **Geographical distribution:** It is spread all over Hawaii and it occur throughout the whole Philippines, at low to medium altitudes, especially in open

grasslands, waste places, along dikes, rice paddies and other moist places, at low and medium altitudes. Pantropic and wild-crafted. Occurs in open grasslands, meadows, along roads, in forest clearings, on riverbanks, waste places, along dikes, rice paddies and other moist places in the Philippines. Grows at elevations up to 1,500 meters. It can be find within Philippines in island Luzon: Metro-Manila Quezon City, Diliman, Ninoy Aquino Parks and Wildlife Center, Luzon central, Rio and Benguet. **Traditional Use:** Traditionally used parts against diarrhoea are leaves (Ken Fem 2014). **Chemistry and biological activities**: It contain allelopathic essential oils and it is also rich in terpenes, including α-cyperone, β-selinene, and α-humulene (Komai & Tang 1989). However, other information on chemistry and biological activity of this species are missing.

Diplazium esculentum

This plant species can be found under about 20 synonyms due to plantlist.org, but the most common name is *D. esculentum*. We can find it in family Woodsiaceae or Anthyriaceae. It is vegetable fern, which is edible found throughout Asia and Oceania. It is probably the most commonly consumed fern (The World Vegetable Center 2012). It is known as pakô in the Philippines (Copeland 2006). Common English name is Vegetable fern. **Botany:** Its botanical features can be described as a rhizomes are erect; trunk line has a stipes stout and is blackish at the base, fronds are 60 × 90 cm big and triangular, young fronds are 1-pinnate, older are 2-pinnate. Veins are netted and kidney-shaped on alongside of veinlets. It grows in habitats with enough of sun light and it needs a hot climate. It prefers slightly acid soils and it's quite tolerant to high humidity, heat and shade but is highly intolerant to frosts. **Geographical distribution:** This species can be found in whole tropics, especially in Asia or Oceania. Production Methods System of this fern is mainly gathering in the wild on streambanks or companion cropping with trees or under shade. Mono-cropping can be also used. For planting material offshoots are used and for planting method, young shoots are transplanted, and irrigation is required. If fertilizer is used, then is organic matter. Crop management: light and shading. From planting to harvest needs 90 days. Harvesting is done as a repeated cutting of unextended young fronds 15 cm in length. Yield is about 20 t/ha/year. **Traditional Use:** It is consumed both fresh and boiled. Leaves have antioxidative activities (The World Vegetable Center 2012). It is used in Asia as herbal edible medicine against various diseases (Paul et al. 2015; Chai et al. 2015; Tag et al. 2012). **Chemistry and biological activities**: About its nutrition value, beta-carotene, riboflavin, ascorbic acid, calcium, and iron are present but in low concentrations, on the opposite is relatively high in vitamin E. It is rich in protein (Rana

et al. 2015) in concentration of 3.2 %. Antifungal and antioxidant properties were recorded (Khoo et al. 2014) as well as antidiabetic and hepatoprotective activity (Junejo et al. 2018). *D. esculentum* leaf extract serves as a coagulant aid in leachate treatment (Zainol et al. 2017). Content of esculentic acid, which is kind of triterpene acid (Tandon et al. 1980) and flavonone glycosides is also recorded (Srivastava et al. 1981).

Emilia sonchifolia

This species belongs to Asteraceae family, Tagunilaw is name used in Philippines and English name for it is Cupid's Shaving Brush. **Botany:** This plant is an erect or ascending, variable, smooth or sparingly hairy, more or less branched plant with 10 and up to 40 centimeters of high. Leaves are sessile, somewhat fleshy and clasping where the lower ones being lyrate-lobed or sinuate toothed and 5 to 10 centimeters long. On the opposite the upper ones can be much smaller and usually entire. Undersurface is usually tinged with violet hue. Flowering heads are 12 to 24 millimeters in length and long-peduncled; the branches are usually dichotomous. Involucral-bracts are green, cylindric, somewhat inflated below, and about as long as the purple flowers. Flowers all perfect and tubular, the limb long, 5-toothed. No ray flowers. Fruits are achenes, narrowly oblong, about 2.4 millimeters long and ribbed. The pappus is white, soft and copious. **Geographical distribution:** We can find it in open places, wastelands, cultivated lands or even in gardens. In and about towns and settlements at low and medium altitudes throughout the Philippines as well. It is native to Asia but is distributed thought whole tropics, including America, Australia and Africa. **Traditional Use:** Whole plant or roots are used in traditional medicine. It is used for enteritis, dysentery, and infections of the respiratory system (Ken Fem 2014). **Chemistry and biological activities**: Hepatotoxic pyrrolizidine alkaloids are recorded to be part of this species (Hsieh et al. 2015) and its extracts have antinociceptive (Couto et al. 2011), anti-inflammatory and analgesic effects (Md. Atiar Rahman 2012). Its seeds contains flavonoids (Wang et al. 2013) and flavones such as luteolin, quercetin and isorhamnetin (Yadava & Raj 2011) and 3,7,3',4'-tetrahydroxy-flavone-3-O-beta-D-xylopyranosyl-(1 -> 3)-O-beta-D-gal-actopyranosyl-(1 -> 4)-O-alpha-L-rhamnopyranoside, when the last has antiviral properties (Yadava & Raj 2012). Pyrrolidine alkaloid, emiline was recorded to be present with its skeleton derived from a bicyclo-[2.2.2]-oct-5-one ring and a pyrrolidine unit (Shen et al. 2013b) as well as cyclohexylacetic acid derivative, named 2-{4-hydroxy-7-oxabicyclo [2.2.1] heptanyl}-acetic acid together with 2-(1,4-dihydroxy cyclohexanyl)-acetic acid (Shen et al. 2013a). Volatile constituents such as γ-

muurolene, β-caryophyllene, (E)-β-ocimene, α-muurolene, δ-cadinene, epi-α-cadinol, and sesquiterpene hydrocarbon are present in its aerial parts (Joshi 2018).

Hyptis capitata

This plant has synonyms such as *Clinopodium capitatum, H. celebica, H. decurrens, H. macrochila, H. mariannarum, H. rhomboidei, Mesosphaerum capitatum, M. rhombodeum, Pycnanthemum decurrens* and *Thymus virginicus* (Theplantlist.org 2013). Philippine term is Botonesan, Palapasagi or Tultulisan according to Philippine local language. English designation are names as a bachelor's button, Buttonweed, False ironwort, Knobweed or. This plant is from Lamiaceae family. **Botany:** *H. capitata* is a stout, erect, nonaromatic, hairy, annual herb, about 0.5 to 1.5 meters high, with green or purplish 4-angled stems. Leaves are lanceolate, 8 to 14 centimeters long, with toothed margins. Flowers are numerous, crowded in long-peduncles, growing up to 10 centimeters in length and the heads 1to 2 centimeters in diameter with basal involucres of hairy bracts. Calyx is green, 4 millimeters long, accrescent, 8 millimeters long in fruit. Corolla is white, 6 millimeters long. **Geographical distribution:** It is native to Florida and can be found in Mexico, Central America, the West Indies, and South America but naturalized in Australia, Southeast Asia, and islands. It grows in Philippines in places from northern Luzon (Cagayan) to Mindanao. In all or most islands and provinces, as a weed in settled areas, occurring in open, waste places, fallow rice paddies, etc. In island Luzon: Metro Manila Quezon City, Diliman, Ninoy Aquino Parks and Wildlife Center; Cagayan prov. Sierra Madre Mtn Range, Penablanca Protected Landscape & Seascape, Barangay Minanga, Sitio Lowak; Isabela prov. Northern Sierra Madre Natural Park, Palanan municipality; Luzon Irosin, Mt. Bulusan, Sorsogon Prov; Luzon Camarines Norte, Bicol National Park; Mindoro Pola voucher speciement were found. **Traditional Use:** The whole plant is traditionally used to cure gastrointestinal disorders (Ken Fem 2014). **Chemistry and biological activities**: Antimicrobial and antioxidants properties have been recorded for this species (Kusuma et al. 2015). It contains triterpene hyptic acid (Yamagishi et al. 1998) which has a anti-tumor properties (Yamagishi 1987). Aerial parts caontains: 2,3-di(3',4'-methylenedioxybenzyl)-2-buten-4-olide, a lignan with a gamma-butenolide structure and 10-epiolguine, a 5,6-dihydro-alpha-pyrone. Also isolated were stigmasterol, 5-hydroxy-4',7-dimethoxyflavone (apigenin-4',7-dimethyl ether), oleanolic, ursolic and rosmarinic acids. The crude extracts prepared with petrol, dichloromethane and methanol showed no pronounced fungicidal or insecticidal effects. The plant contains no alkaloids (Almtorp et al. 1991).

Parkia javanica

Belongs to Fabaceae family, local name is Kupang and the English one is Tree bean. Synonyms for this species are *P. roxburghii* and *P. timoriana.* **Botany:** *Pa. javanica* is a very large tree growing to a height of 25 to 40 meters. Leaves are evenly bipinnate, 30 to 80 centimeters long. Pinnae are 40 to 60, 8 to 20 centimeters long. Leaflets are 60 to 140, linear-oblong, 6-12 millimeters long, close-set, shining above, and pointed at the tip. Heads are dense, obovoid or pyriform, axillary, long-peduncled, up to 6 centimeters long. Flowers are white, about 1 centimeter long. The pods are 25 to 30 centimeters long, about 3.5 centimeters wide, rather thick, pendulous, black and shining when mature, containing 15 to 20 seeds (Ken Fem 2014). **Geographical distribution:** We can find this plant commonly in forests at low and medium altitudes of Philippines in La Union to Laguna Provinces in Luzon, and in Palawan. **Traditional Use:** Part used are seeds and bark seem to be also valuable. Seeds contain glycosides, resin and tannin and are antidiarrhoeal. Seeds are used, in lieu of peppermint, for abdominal colic. Pods are used for bleeding hemorrhoids. In India bark extract used for diarrhoea and dysentery (Tan 1980). Indonesia seeds are used for medicinal industry. In addition, the bark, leaves and the root are also used. It is most sought after for its anti-bacterial properties and is applied in traditional medicine for infections and stomach disorders besides the gastro-intestinal. **Chemistry and biological activities**: It contains phenolics (Reihani & Azhar 2012) due to it has a antimicrobial and antioxidant properties (Das et al. 2017). Bioactive compounds present in *Pa. javanica* seeds are reported to have anticancer effect (Khangembam et al. 2019). Phenyl propanoid, parkinol was isolated from leaves (Dinda et al. 2010) and iridoid glucosides, javanicosides 8-O-p-hydroxybenzoyl-6'-O-p-coumaroyl-mussaenosidic acid and 7-O-E-3,4-dimethoxycinnamoyl-6'-O-d glucopyranosylloganic acid were isolated from the leaf and stem bark (Dinda et al. 2009). Its beans are rich in protein and purified lectin showed to have two forms of proteins, where both of them appeared to be single polypeptide chains (Utarabhand & Akkayanont 1995).

Picrasma javanica

It is from Simaroubaceae family. No synonyms are found according to The Plant list (Theplantlist.org 2013). **Botany:** *Pi. javanica* grows up to 24 meters of height tall. Its trunk in diameter has up to 25 centimeters. The bark is smooth and dark in color. The blooms are white, yellow or green. Its fruits are red, blue or green mostly. Ovoid to roundish in shape and measure up to 1.2 cm in diameter. It can be used as an antidote, when roots of *Pi. javanica*,

which is also called Manunggal in Philippines, are chopped off and the juice is spit on the snake bite. **Geographical distribution:** It grow in rainforest from level of the sea up to 1,500 meters of altitude (Mabberley et al. 2006) from northern India to Indochina and Malesia. **Traditional Use:** Against diarrhoea leaves and bark are used (Prigge 2005). In case of food poisoning *Pi. javanica* vomiting can be induced by swallowing the juice of this plant species. It cures stomach ache or liver disorders (Khan et al. 2001). **Chemistry and biological activities**: Substances such as alkaloids and quassinoids (Koike et al. 1995) were found in this species (Arbain et al. 1990) and also antibacterial activity is recorded (Khan et al. 2001). Found quassinoids are picrajavanicins, which exhibited potent and selective anti proliferative activities against human pancreatic cancer (Win et al. 2016), des-4-methylpicrasane, (Koike et al. 1991), picrasan isolated from bark (Koike et al. 1995) and javanicinoside isolated from stem (Koike & Ohmoto 1992). From alkaloids it contains javacarboline, which is type of beta-carboline alkaloid (Koike et al. 1994).

Pseudelephantopus spicatus

This plant belongs to Asteraceae family. Known synonyms are *Ageratum dubium, A. quadriflorum, Distreptus spicatus, Elephantopus dubius* and *Matamoria spicata* (Theplantlist.org 2013). Common English names used are dog's tongue or false elephant's foot (Natural Resources Conservation Service 2018). Philippine name used is Kokumbanog (Prigge 2005). **Botany:** This is a perennial herb spreading by underground rhizomes. Leaf blades are mostly 3–15 (up to 20 cm and more) cm × 10–30 (up to 45 mm and more) mm big, including petioles. Both faces are sparsely pilose or hirsute and often glabrescent and abaxial resin-gland-dotted. Bracts are 30 × 3.5 mm big. Inner phyllaries is 9–12 mm long, sparsely is hispidulous (covered with stiff, short hairs) where hairs if 0.1–0.3 mm are often glabrescent (lacking hair or similar growth or tending to become hairless). Flowers are white to light pink color or can be even purplish it is flowering in January. **Geographical distribution:** This plant is native to tropical hot areas and grows in open places and prefers to grow on sandy soils in 0–10 m of dept. It can be found in Mexico, West Indies, Central America, South America, Asia and Pacific Islands where was introduced to all of these places (Gray 2002). **Chemistry and biological activities**: Biological activity towards *Leishmania amazonensis* by sesquiterpenes was discovered (Odonne et al. 2011). However, other information on chemistry and biological activity of this species are missing.

Tabernaemontana pandacaqui

Name used in Philippines is Pandakaking-puti and common name of this plant is Banana bush. It belongs to Apocynaceae family. **Botany:** This plant is known for its erect appearance and branched and smooth look. It can be characterized as a shrub, 1 to 4 meters high. Leaves are elliptic-lanceolate to oblong-elliptic, 5 to 12 centimeters long, narrowed at both ends, shining and short-stalked. Inflorescences are axillary and terminal, peduncled, and have rather few flowers. Calyx is green, ovoid, and short. Corolla is white, tinged with green, slender-tubed, 1.7 centimeters long and slightly enlarged upward; limb is 2 to 2.5 centimeters in diameter, composed of five, spreading, falcate, lanceolate lobes. Follicles are red or yellowish-red, oblong, 2 to 4 centimeters long, and longitudinally ridged or keeled. **Geographical distribution:** It is common in thickets at low altitudes at low altitudes from the Babuyan Islands and Luzon to Mindanao, in most Islands and provinces of Philippines. **Traditional Use:** Roots and bark are traditionally used against diarrhoea (Ken Fem 2014). **Chemistry and biological activities**: It has a many biologically active properties including effects on cardiovascular system (Taesotikul et al. 1989), cytotoxic, antiprotozoal and antimicrobial properties (Bradacs et al. 2010) and it showed anti-inflammatory, antipyretic and antinociceptive activities (Taesotikul et al. 2003). It contains alkaloids, such as indol (Abe et al. 1993) and triterpenes (Lopes Cardoso et al. 1997).

Triumfetta bartramia

Kulutkulutan, Bulagun and Kollo-kolot are names for this plant used is Philippines. English names are Diamond bulbark, Chinese burr or Burweed. It is from Malvaceae family. **Botany:** It is erect, more or less hairy, branched annual, often half-woody shrub, growing to a height of 0.5 to 1.5 meters. Leaves are variable, usually orbicular to rhomboid-ovate, 2 to 6 centimeters in length, entire or 3-lobed, the upper ones oblong to ovate-lanceolate, smaller and not lobed. Flowers are yellow, numerous, about 6 millimeters long, borne on dense axillary fascicles. Fruit is small, rounded, hairy smooth spines covered with hooked (Ken Fem 2014). **Geographical distribution:** This species is very common in open waste places in all islands and provinces of Philippines, at low and medium altitudes. Can be found in Caramoan; Aurora Memorial National Park. Also, it can be found in rest of Asia and Western Australia. **Traditional Use:** Traditionally used parts are bark, root and fresh leaves used in diarrhoea and dysentery curing (Padua et al. 2001) and is used mainly in Taiwan. **Chemistry and biological activities**: This species contains β-sitosterol, friedelin, friedelinol, quercetin, 2,6-

dimethoxy-1,4-benzoquinone, and rosmarinic acid (Ho et al. 1995). However, other information on chemistry and biological activity of this species are missing.

5 Aims of the Thesis

The aim of this thesis is to evaluate *in vitro* growth-inhibitory effects of extracts derived from medicinal plants, which are traditionally used in Philippine medicine for treatment of infectious diarrhoeal diseases against representatives of diarrhoea-causing bacterial pathogens.

The specific objectives of the thesis are as follows:

a) The collection, identification and processing of medicinal plants used in Philippian traditional medicine to treat diarrhoea.
b) Determination of minimum inhibitory concentrations (MICs) of ethanol extracts against diarrhoea-causing bacterial pathogens.

6 Materials and methods

6.1 Plant materials

The plant species were selected based on literature data and internet sources on their use in traditional medicine for treatment of diarrhoea (Lim 2016; Lim 2015b; Padua et al. 2003; Pardo de Tavera 1901; Padua et al. 2001; Godofredo & Stuart. 2018). All plant samples were collected during May 2017 and 2018 in Philippines, mainly on Leyte island close to Baybay city and within campus of Visayas State University. Subsequently, plants were also collected through following islands: Bohol, Cebu and Negros (Figure 1). For all plants, voucher specimens were collected

Reference specimen sheets with botanical descriptions, natural habitat, and illustrations were elaborated. The plants were authenticated by Bc. Barbora Fišerová and Ing. Tomáš Kudera and confirmed by local expert from Visayas State University Institute of Tropical Ecology and Environmental Management (Dr. Bande) according to their morphology and habitat. Voucher specimens have been deposited in the herbarium of the Department of Botany and Plant Physiology of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague. List of plant species collected is shown in Table 1.

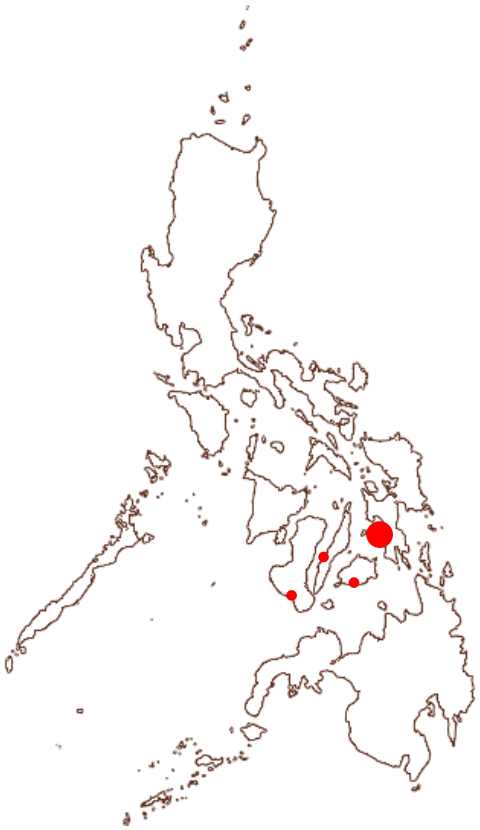

Figure 1: Map of Philippines with marked places of collection of samples

6.2 Plant extract preparation

Before the transportation, samples were air dried in the area of collection. Samples were homogenized by Grindomix mill (Retsch, Haan, DE) and 15 g of dry matter was extracted for 24 h in 450 ml 80% ethanol (Sigma-Aldrich, Prague, CZ) at room temperature prior the testing. Laboratory shaker (GFL, Burgwedel, DE) was used for the extraction of samples into the ethanol. The extracts were then filtered and concentrated using rotary evaporator (Büchi Labortechnik, Flawil, CH) in vacuum at 40°C. Dried residues were subsequently diluted in 100% dimethyl sulfoxide (DMSO), which serves as a preservative (Penta, Prague, CZ) to obtain stock solution of the final concentration 51.2 mg/ml and stored in Eppendorf Tubes® 2.0 ml at -20°C until their use. The yield of dry residues was calculated (Table 1).

6.3 Used microorganisms and growth medium

The antibacterial activity was determined against 8 representatives of both Gram-positive/-negative and aerobic/anerobic diarrhoea-causing bacteria. Standard American Type Culture Collection (ATCC) strains were obtained from Oxoid [Basingstoke (UK)] and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DMSZ) from German Resource Centre for Biological Material (Braunschweig, DE). *C. difficile* infant KK4 was isolated from faecal samples of healthy infants aged from 1 to 6 months. The pathogenic bacteria follow: *S. flexneri* ATCC 12022, *L. monocytogenes* ATCC 7644, *S. enterica* ATCC 13076, *E. coli* ATCC 25922, *E. faecalis* ATCC 51299, *C. perfringens* DSMZ 11778, *C. difficil*e DSMZ 12056, *V. parahaemolyticus* ATTC 17802.

Mueller-Hinton broth (MHB), (Oxoid, Basingstoke, UK) was used as growth medium for aerobic group of bacteria and Wilkins-Chalgren broth (WCB), (Oxoid, Basingstoke, UK) was used for anaerobic bacteria. Buffered and standard forms of broth were used for testing, where buffered versions of both WCB and MHB were enriched by KCl, NaCl and Trizma base. Into one liter of distilled water is added 0.2g KCl, 6.1g of Tris Base and 8.0g of NaCl and after adding of all substances the mixture needs to be stirred properly. After mixing all substances together in the distilled water, the pH is around 10.0 and by adding 35% HCl the pH equilibrated to pH 7.6. Further 21 g of MHB is added to the solution and mixed. After this step, autoclave is used for sterilization of the broth and after cooling down we store broth in fridge in 4°C till using. For not buffered version of broth the process is the same but without additives of salts and Trizma base. For *V. parahaemolyticus* NaCl (Sigma-Aldrich, Prague,

CZ) has to be added to have a final concentration 1%. For *E. faecalis*, the substance that is necessary to add to broth is glucose to have final concentration of 3 % (Sigma-Aldrich, Prague, CZ).

6.4 Determination of the minimal inhibitory concentration (MIC)

Broth microdilution method was used for analysis, using 96-well microtiter plates according to Clinical and Laboratory Standards Institute (CLSI) guidelines, modified by Cos *et al.* (Cos et al. 2006), where MIC [μg/mL] have been assessed. The MIC was defined as the lowest concentration of antimicrobial compound that inhibits visible growth of the microorganism after overnight incubation and it make it important indicator for the resistance of micro-organisms to antimicrobial agents (Andrews 2002). All 20 plant extracts were dissolved in DMSO and 2-fold diluted in appropriate growth media (100 μL) in a ranges of 512, 256, 128 and 64 μg/mL using automated pipetting platform Freedom EVO 100 (Tecan, Männedorf, CH) or by manual pipetting using multichannel pipette (Eppendorf, Wesseling-Berzdorf, DE). 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 aerobs were prepared in aerobic flow box workstation and incubated in Biological Thermostat BT 120, whereas the plates with anaerobes, specifically *Clostridium* strings, were handled under anaerobic conditions in Whitley A35 Anaerobic Workstation (Don Whitley Scientific, West Yorkshire, UK). Plates with bacteria which require oxygen-free environment were inoculated and incubated in the anaerobic box. Dimethyl sulfoxide was used as a negative control and ciprofloxacin, tetracycline and metronidazole as a positive control. Bacterial growth was determined by measuring the optical density by Cytation 3 Imaging Reader (BioTek, Vermont, USA) at 405 nm. MICs were calculated as the lowest concentration that showed ≥80% reduction of microbial growth compared to compound-free growth control after 24 h of growth. The solvents used (DMSO, ethanol and *d* H_2O) did not inhibit bacterial growth at the concentrations tested (1%). All tests were performed as at least three independent experiments each carried out in triplicate, and the results are presented as median/modal values. The average of particular MIC against pathogenic bacteria were calculated and the comparison of values was expressed by selectivity index.

Table 1: Tested Philippine medicinal plants used to cure diarrhoea

Botany name (Family)	Local name	Place of Collection in Philippines	Year of collection	Voucher specimen number	Part of plant used	Yield [%]
Acalypha grandis (Euphorbiaceae)	-	Leyte – Marcos	2018	02537KBFR8	leaves	23.6
Acanthus ebracteatus (Acanthaceae)	Lagiwliw	Leyte – Santo Rosario	2017	02505KBFR3	whole plant	17.9
Artocarpus blancoi (Moraceae)	Antipolo	Leyte – Guadalupe (Utod)	2018	02538KBFR9	fruit	25.7
Artocarpus camansi (Moraceae)	Kamansi	Leyte – Baybay City	2017	02512KBFR1	bark	13.3
Artocarpus elasticus (Moraceae)	Terap	Leyte – Guadalupe (Utod)	2018	02539KBFRA	bark	11.8
Artocarpus odoratissimus (Moraceae)	Marang	Leyte – Guadalupe (Utod)	2018	02540KBFR2	fruit	22.9
Breynia cernua (Phyllantaceae)	Mutang-ulang	Bohol – Pilar	2018	02541KBFR3	bark	10.6
Carmona retusa (Boraginaceae)	Tsaang-gubat	Leyte – Guadalupe (Utod)	2017	02489KBFRE	leaves	15.3
Commelina communis (Commelinaceae)	Alibangon	Leyte – Monterico	2018	02542KBFR4	whole plant	13.3
Cyathula prostrata (Amaranthaceae)	Dayang	Leyte – Guadalupe (Utod)	2018	02543KBFR5	whole plant	12.8
Cyperus brevifolius (Cyperaceae)	Pugo-pugo	Leyte – Ormoc (Lake Danao)	2018	02544KBFR6	whole plant	11.4
Diplazium esculentum (Woodsiaceae)	Paco	Leyte – Pangasungan	2018	02545KBFR7	root	5.4
Emilia sonchifolia (Asteraceae)	Tagunilaw	Leyte - Guadalupe (Utod)	2017	02520KBFR0	whole plant	20.9

Table 1: Continued

Botany name (Family)	Local name	Place of Collection in Philippines	Year of collection	Voucher specimen number	Part of plant used	Yield [%]
Hyptis capitata (Lamiaceae)	Botonesan	Leyte - Pangasungan	2018	02546KBFR8	whole plant	10.1
Parkia javanica (Fabaceae)	Kupang	Leyte - Baybay City (Patag)	2018	02547KBFR9	bark	25.7
Picrasma javanica (Simaroubaceae)	Manunggal	Leyte - Baybay City (Patag)	2018	02548KBFRA	bark	6.3
Pseudelephantopus spicatus (Asteraceae)	Kokunbanog	Bohol - Pilar	2018	02553KBFR6	whole plant	12.5
Tabernaemontana pandacaqui (Apocynaceae)	Pandakaking-puti	Luzon - Los Baños (Dampalit Creek)	2017	02503KBFR1	bark	10.1
Triumfetta bartramia (Malvaceae)	Kulutkulutan	Leyte - Baybay City (Patag)	2018	02554KBFR7	root	14.9

Footnotes: - : no found record of local name

7 Results

In this study, the *in vitro* antimicrobial effect of plant ethanol extracts was determined by broth microdilution method and compared with representatives of major classes of conventional antibiotics used in curing of diarrhoea. Twenty samples were tested to antimicrobial effects and 6 of them strictly proven to have positive results of this tests by showing antimicrobial activity against 5 out of 8 tested representatives of pathogenic bacteria. Most of the families are included only once, anyway two species are representatives of Euphorbiaceae family and four of species which were collected belong to Moraceae family. MICs were found in cases of 5 bacteria out of 8 and the they were inhibited by 6 tested plants. *A. camansi* species showed positive results against two strains of bacteria. One is *C. perfringens* with MIC = 128 μg/mL and the second is another *Clostridium*, *C. difficile*, where MIC was the same, 128 μg/mL. *A. blancoi* showed positive results in antimicrobial testing against *E. faecalis* (MIC = 128 μg/mL), *A. grandis* exhibited anti-bacterial effects against *V. parahaemolyticus* (MIC = 256 μg/mL). *Pi. javanica* showed positive results against *E. coli* with MIC = 256 μg/mL and the antimicrobial activity against the same bacteria, *E. coli*, were observed for species *D. eculentum* with MIC = 512 μg/mL. The last plant species which showed significantly positive results in broth microdilution method was *C. retusa* against *V. parahaemolyticus* as well as *A. grandis*, but with MIC = 512 μg/mL. All results of testing are presented in Table 2. The rest of tested bacteria (*S. flexneri* ATCC 12022, *L. monocytogenes* ATCC 7644, and *S. enterica* ATCC 13076) were not found to be affected by tested extracts. Tested species *A. ebracteatus*, *A. moluccana*, *A. elasticus*, *A. odoratissimus*, *B. cernua*, *C. communis*, *C. prostrata*, *C. brevifolius*, *E. sonchifolia*, *H. capitata*, *Pa. javanica*, *P. spicatus*, *T. pandacaqui*, and *T. bartramia* did not showed any positive results.

Three antibiotics (ciprofloxacin, metronidazole, and tetracycline) were used as a positive control of testing antimicrobial activity of ethanol extract by broth microdilution method in accordance to CLSI (Clinical Laboratory Standard Institute 2018). Only ciprofloxacin was tested for all the bacteria, the rest of antibiotics were used only in case of 4 microorganisms of 8 tested. Results of tested antibiotics are presented in Table 2. Found MIC are in accordance to CLSI (Clinical Laboratory Standard Institute 2018).

Table 2: Antimicrobial activity of ethanol extracts from Philippian medicinal plants

Plant sample/ATB	Microorganisms/ MIC (mg/mL)							
	Shigella flexneri	*Listeria monocytogenes*	*Salmonella enterica*	*Escherichia coli*	*Enterococcus faecalis*	*Clostridium perfringens*	*Clostridium difficile*	*Vibrio parahaemolyticus*
Acalypha grandis	-	-	-	-	-	-	-	256
Acanthus ebracteatus	-	-	-	-	-	-	-	-
Artocarpus blancoi	-	-	-	-	128	-	-	-
Artocarpus camansi	-	-	-	-	-	128	128	-
Artocarpus elasticus	-	-	-	-	-	-	-	-
Artocarpus odoratissimus	-	-	-	-	-	-	-	-
Breynia cernua	-	-	-	-	-	-	-	-
Carmona retusa	-	-	-	-	-	-	-	512
Commelina communis	-	-	-	-	-	-	-	-
Cyathula prostrata	-	-	-	-	-	-	-	-
Cyperus brevifolius	-	-	-	-	-	-	-	-
Diplazium esculentum	-	-	-	512	-	-	-	-
Emilia sonchifolia	-	-	-	-	-	-	-	-
Hyptis capitata	-	-	-	-	-	-	-	-
Parkia javanica	-	-	-	-	-	-	-	-
Picrasma javanica	-	-	-	256	-	-	-	-
Pseudelephantopus spicatus	-	-	-	-	-	-	-	-
Tabernaemontana	-	-	-	-	-	-	-	-
Triumfetta bartramia	-	-	-	-	-	-	-	-
ciprofloxacin	<0.06	2	16	1	32	2	512	<0.50
tetracycline	1	0.5	NT	1	0.5	NT	NT	NT
metronidazole	>32.00	>32.00	NT	>32.00	>32.00	NT	NT	NT

Footnotes: MIC - Minimum inhibitory concentration in µl/mL. (data are median or modal values of three independent experiments, each performed in triplicate); -: Not active (MIC >512 mg/mL); NT: not tested for certain bacteria; ATB: antibiotics

8 Discussion

In this study, the bark extract of *A. camansi* showed significant growth-inhibitory effect against *C. difficile* and *C. perfringens.* Although ethanol extract from leaves of this species exhibited in previous study respective MICs 25 and 50 mg/mL against *S. aureus* and *E. coli* (Vianney et al. 2018), according to our best knowledge, this is the first report on anti-clostridial effect of this plant.

The extract from fruit of *A. blancoi* showed positive antimicrobial activity against *E. faecalis.* In our best knowledge, this is the first report no antimicrobial effect of this plant. Nevertheless, our results can be supported by studies of Kuete et al. (2011) and Teanpaisan et al. (2014) who reported antibacterial activity of related species *A. lakoocha*, against *E. faecalis, S. aureus* and *E. coli*.

Our results showing inhibitory effect of *Pi. javanica* against *E. coli* can be supported by Khan et al. (2001) who observed the similar effect for extract from leaves when tested by disk diffusion method.

Leaves extract of *A. grandis* were biologically active against *V. parahaemolyticus*, whereas in work Bradacs et al. (2010) leaves showed antiprotozoal but not antimicrobial activity. These activities are believed to be caused by triterpenoids and flavonoids (Schwikkard & van Heerden 2002) which are contained in the plant. In our best knowledge, this is the first report no antimicrobial effect of this plant. Different species of *Acalypha* genera, such as *A. australis* (Vianney et al. 2018) or *A. indica* (Seebaluck et al. 2015) showed antimicrobial activity (P. Saranraj 2010; Setty et al. 2017). *A. indica* was active against *E. coli* and *Vibrio cholerae* (Krishnaraj et al. 2010).

C. retusa was found to be active against *V. parahaemolyticus*, which can be supported by various authors who found biological activity (Penecilla & Magno 2011; Villaseñor et al. 2004) of the methanol extract of the stem and leaves of the *C. retusa* (Chandrappa et al. 2012). In Penecilla & Magno (2011) work this species was tested against four pathogenic representatives, such as *S. aureus, Bacillus subtilis, E. coli,* and *Pseudomonas aeruginosa* by disk diffusion method. Ethanol extract showed significant biological activity against all of tested bacteria, besides *E. coli.*

Root of fern *D. esculentum* has shown antimicrobial activity against *E. coli*. Amit et al. found inhibition of growth of various bacteria such as *Salmonella arizonae, Salmonella typhi,* and *S. aureus* but not in case of *E. coli*. Leaves were also tested but with negative results. The rhizome extracts in combination with antibiotics were found to be more effective than positive control of tetracycline alone (Amit et al. 2011). Author used disk diffusion method which is less sensitive in comparison with broth microdilution method. This species should be recommended for further studies including toxicity. Mackeen et al. (2003) analyzed aerial parts of this species, where its antimicrobial properties were confirmed with MIC = 400 μg/ml against *E. coli*.

Research found MIC for *C. perfringens* inhibition by ciprofloxacin to be 2 μg/mL. In comparison with standardized MICs data (Murray et al. 1999), the results indicate that tested strain of *C. perfringens* is sensitive to ciprofloxacin at concentrations ≤ 1 μg/mL. Other authors (Watt & Brown 1986; Nilius et al. 2003) found the MIC at 1 μg/mL. In other studies the results differ when biological activity of ciprofloxacin was observed by Child et al. (1995) at 0.25 μg/ml or Prabhala et al. (1984) at MIC of 4 μg/ml. Anyway, resistance of this species to ciprofloxacin is recorded as well. Results of this study are in accordance with standard data of Murray et al. (1999). *E. faecalis* is resistant to ciprofloxacin (Lee 2013) and the range of MIC varies from 0.125 to 64 μg/ml according to Genaro (Genaro et al. 2006). For *E. coli* MIC was 1 μg/mL which can by supported by research of Baudry-Simner et al. (2012). For *V. parahaemolyticus* MIC found was <0.5 μg/mL when in case of study of Han et al. (2007) the MIC is ≤0.03-1 μg/mL.

9 Conclusion

All objectives of this thesis were accomplished. In this study, 6 of 19 of Philippine medicinal plants traditionally used to cure diarrhoea exhibited *in vitro* growth-inhibitory effect against 5 of 8 representatives of main intestinal bacterial pathogens. *A. camansi* showed the highest antibacterial effect against clostridia tested. Significant antibacterial effects exhibited also *A. blancoi*, *A. grandis*, and *Pi. javanica*. In addition, *D. esculentum* and *C. retusa* produced moderate antibacterial effects. The most susceptible microorganisms tested were *C. perfringens, C. difficile, E. faecalis, E. coli*, and *V.* parahaemolyticus, respectively. The results of this study suggest above-mentioned species as perspective plant materials for development of pharmaceutical and food applications effective for treatment of diarrhoea. However, further research focused on identification of their antimicrobial principles, evaluation of their safety and effectiveness *in vivo* will be necessary.

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The Appendices: Pictures of the research

List of Appendices

11 The Appendix A: Pictures from the fields

Figure 1: Collecting of samples in the fields

Figure 2: Identification of samples with local expert Dr. Bande

Figure 3: Drying of collected material

Figure 4: Preparing samples for transport

12 The Appendix B: Pictures of plants with positive results

Figure 5: *Picrasma javanica* (source: phytoimages.siu.edu)

Figure 6: *Artocarpus camansi* (source: phytoimages.siu.edu)

Figure 7: *Carmona retusa* (source: Useful Tropical Plants)

Figure 8: *Diplazium esculentum* (source: wikimedia)

Figure 9: *Artocarpus camansi* (source: WorldPress)

Figure 10*: Acalypha grandis* (source: Earth.com)