ANTIMICROBIAL ACTIVITY AND PREBIOTIC EFFECTS OF *Senna alata* LEAF EXTRACTS

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by

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

±	Plus–minus sign
α	Alpha
β	Beta
%	Percentage
>	More than
<	Less than
≤	Less than or equal
ºC	Degree Celsius
g	Gram
µL	Microliter
µg	Microgram
µm	Micrometre
CFU	Colony-forming unit
cm	Centimeter
mm	Millimeter
et al.	*et alii* – ‘and others’
s	Second
L	Litre
h	Hour
mg	Milligram
min	Minute
mL	Millilitre

kg	Kilogram
IC_{50}	Half maximal inhibitory concentration
OD	Optical density
pH	Potential of hydrogen
AST	Aspartate transferase
ALT	Alanine transaminase
ALP	Alkaline phosphatase
ATCC	American Type Culture Collection.
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MRS	Man, Rogosa and Sharpe media
SEM	Standard error of the mean
GIT	Gastrointestinal tract
CO_2	Carbon dioxide
SPSS	Statistical Package for Social Sciences
IBS	Irritable bowel syndrome
CAT	Catalase
SOD	Superoxide dismutase
ANOVA	Analysis of variance
USA	United States of America
UK	United Kingdom
UKM	The National University of Malaysia
IIUM	International Islamic University Malaysia

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AKTIVITI ANTIMIKROBIAL DAN KESAN PREBIOTIK EKSTRAK DAUN *Senna alata*

ABSTRAK

Senna alata atau *Cassia alata* adalah tumbuhan perubatan yang banyak dijumpai di kawasan tropika dan subtropika. Daun tumbuhan ini telah digunakan dalam rawatan jangkitan kulit dan masalah berkaitan pencernaan. Tujuan kajian ini adalah untuk menilai aktiviti antimikrob ekstrak daun *S. alata* terhadap beberapa patogen usus dan kesan prebiotik mereka terhadap beberapa strain probiotik, serta mengesan fitokonstituen dalam ekstrak daun *S. alata*. Ujian MIC dan MBC ekstrak daun akueus dan etanol berkepekatan dari 0.39 mg/mL hingga 200 mg/mL telah dilakukan pada patogen usus dalam steril 96-lubang mikrotiter plat. Kesan prebiotik ekstrak daun akueus dan etanol berkepekatan dari 1.25 mg/mL hingga 10.00 mg/mL dinilai berdasarkan kadar pertumbuhan probiotik dalam 24 jam. Fitokonstituen ekstrak daun akueus dan etanol telah dikesan dengan kaedah kualitatif piawai. Kedua-dua ekstrak daun menunjukkan kesan bakterisida terhadap *S. aureus*, manakala hanya ekstrak daun akueus menunjukkan kesan bakteriostatik terhadap *S.* Typhi. Probiotik *L. helveticus* dan *B. longum* menunjukkan kadar pertumbuhan min positif selepas didedahkan dengan kedua-dua ekstrak daun selama 24 jam. Walau bagaimanapun, kadar pertumbuhan kedua-dua bakteria berkurangan apabila kepekatan kedua-dua ekstrak daun meningkat. Kedua-dua ekstrak daun menunjukkan kehadiran tanin, saponin, alkaloid, karbohidrat, dan flavonoid. Kesimpulannya, kedua-dua ekstrak daun *S. alata* menunjukkan aktiviti antimikrob yang ketara terhadap *S. aureus* dan *S.* Typhi pada kepekatan ekstrak yang diuji. Selain itu, kedua-dua ekstrak daun *S. alata* adalah prebiotik yang lemah kerana ia hanya merangsang pertumbuhan probiotik yang minimum.

ANTIMICROBIAL ACTIVITY AND PREBIOTIC EFFECTS OF *Senna alata* LEAF EXTRACTS

ABSTRACT

Senna alata or *Cassia alata* is a medicinal plant found mostly in the tropics and subtropics. The leaves of the plant have been employed in the treatment of skin infection and digestion-related problems. The purpose of this study was to assess the antimicrobial activity of *S. alata* leaf extracts against several intestinal pathogens and their prebiotic effects against a few probiotic strains, as well as to screen the phytoconstituents in *S. alata* leaf extracts. MIC and MBC assays of aqueous and ethanolic leaf extract in a concentration ranging from 0.39 mg/mL to 200 mg/mL were performed on the intestinal pathogens in a sterile 96-well microtiter plate. The prebiotic effects of aqueous and ethanolic leaf extracts in concentrations from 1.25 mg/mL to 10.00 mg/mL were evaluated based on the growth rate of the probiotic within 24 hours. The phytoconstituents of aqueous and ethanolic leaf extracts were screened by standard qualitative methods. Both leaf extracts showed a bactericidal effect against *S. aureus,* while only aqueous leaf extract showed a bacteriostatic effect against *S.* Typhi. Probiotics of *L. helveticus* and *B. longum* showed a positive mean growth rate after being treated with both leaf extracts for 24 hours. However, the growth rate of both bacteria decreases as the concentration of both leaf extracts increases. Both leaf extracts showed the presence of tannins, saponins, alkaloids, carbohydrates, and flavonoids. In conclusion, both *S. alata* leaf extracts showed significant antimicrobial activity against *S. aureus* and *S.* Typhi at the tested extract concentrations. Besides, both *S. alata* leaf extracts are weak prebiotics because they only stimulate a minimal growth of probiotics.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Herbal medicine has been practised since the ancient period. Today, the use of plant-based herbal medicines is increasing globally and is rapidly gaining broad acceptability. *Senna alata* is a plant in the Leguminosae family that is used for medicinal purposes (Eziuche *et al.,* 2016). The shrub grows to a height of 3-4 metres and has leaves that can reach a length of 50-80 centimetres (Donkor, Mosobil and Suurbaar, 2016). The plant flower resembles a golden candle. According to Hennebelle *et al*. (2009), the plant grows best in sunny, damp environments and emits an unpleasant odour.

The plant can be found in Malaysia, Thailand, Nigeria, Australia, tropical America, and many other places across the world (Abdulwaliyu *et al*., 2018). The plant, also known as the Ringworm plant, Craw-Craw plant, and Winged Senna, has a long history of use in traditional medicine in many developing countries (Kazeem *et al*., 2015). In Malaysia, the plant known as "Gelenggang" and its leaf was used traditionally to treat skin diseases (Fatmawati *et al*., 2020).

The plant contains antibacterial activity, particularly against fungal dermatophytes, and has traditionally been used to treat skin problems in humans (Ehiowemwenguan, Inetianbor, and Yakubu, 2014). This plant's antimicrobial effect can be linked to the presence of metabolites such as phenols, tannins, saponins, alkaloids, steroids, flavonoids, and carbohydrates (Abdulwaliyu *et al*., 2018). According to ethnopharmacological studies, the leaves of the plant also have been employed in the treatment of digestion-related problems such as constipation, stomach discomfort, and liver diseases (Hennebelle *et al*., 2009).

Nowadays, there is a growing demand for prebiotic products due to the increasing health consciousness of consumers. Prebiotics are non-digestible oligosaccharides-based food that can boost gut health by promoting normal gastrointestinal flora and limiting pathogen growth in the intestine (Ahuja and Deb, 2017). Prebiotics are not digestible by α-amylase or other hydrolases in the upper part of the intestine, and they can be considered "food" for probiotics (Azmi *et al*., 2012). Probiotics are non-pathogenic, living microorganisms that can improve human intestinal microbial balance (Manzoor *et al*., 2022).

Lactobacilli and *Bifidobacteria*, the most common species of gut microbiota, use prebiotics in the intestinal tract because they contain enzymes like β-fructosidase and glycotransferase that break down polymers (the prebiotic) into smaller units for fermentation (Lee *et al*., 2002; Azmi *et al*., 2012). Lactic acid bacteria will make organic acids during fermentation, creating an acidic environment in the colon that indirectly inhibits the growth of pathogenic bacteria (Azmi *et al*., 2012). With this mechanism, prebiotics can change the composition of colonic microbiota in the human gut, improving immune function, digestion, faeces removal, and preventing irritable bowel syndrome (Douglas and Sanders, 2008).

A study by Abdulwaliyu *et al*. (2013) found that *S. alata* leaf contains a lot of carbohydrates and fibre. Dietary fibre refers to complex carbohydrates that cannot be broken down by human enzymes, and it absorbs a large amount of water and toxin produced by gut bacteria (Abdulwaliyu *et al*., 2013). As the leaf of *S. alata* contains carbohydrates and fibre, the plant could be a potential source of prebiotics. Hence, this study aimed to assess the antimicrobial activity of *S. alata* leaf extracts against several intestinal pathogens and their prebiotic effects against a few probiotic strains, as well as to screen the phytoconstituents in *S. alata* leaf extracts.

1.2 Problem statements

Changing lifestyles and eating habits are the cause of an increase in digestive and gastrointestinal problems in the present day (Ahuja and Deb, 2017). According to the U.S. Department of Health and Human Services (2019), the most prevalent issues affecting the gastrointestinal tract include chronic constipation, irritable bowel syndrome (IBS), gastrointestinal infection and Crohn's disease. The prevalence of gastrointestinal (GI) illnesses has significant economic and societal repercussions. In the United States, it is estimated that 11% of the population suffers from a chronic digestive ailment, with a prevalence rate as high as 35% among those aged 65 and older (Avramidou *et al.*, 2018). Various bacteria, viruses, and parasites can cause gastrointestinal (GI) infections. Approximately 1.7 billion cases of diarrhoea occur annually among children under the age of five worldwide (Centers for Disease Control and Prevention, 2020). This problem is becoming more serious as new threats emerge, such as antibiotic-resistant infections.

In today's world, there is a growing pattern of consumer awareness towards the trend of increasing demand for functional foods, which are foods that are promoted as having the ability to improve the health of the consumer. In addition to several other components of foods, prebiotics are among those that have garnered a lot of interest in recent times. Prebiotics are defined as substrates selectively utilized by the host's microorganisms resulting in benefits for metabolic health, gastrointestinal system, bone health and mental health (Alves-Santos *et al.*, 2020). According to Manzoor *et al.* (2022), most of the prebiotic compounds are carbohydrates with different molecular structures that help good bacteria grow in the gut. As a result of carbohydrate fermentation, *Bifidobacterium* and *Lactobacillus* make certain metabolites that lower the pH of the intestines and inhibit the growth of pathogens in the gastrointestinal tract.

1.3 Significance of the study

Medicinal plants are a rich source of antibacterial and prebiotic properties, which can be used to treat a variety of human diseases. *S. alata* leaf has many health benefits and has been used traditionally to treat stomach pain, convulsions, sexual diseases, fever, heart failure, oedema, asthma, snake bites, and as a laxative. (Abdulwaliyu *et al*., 2018). The plant also contains many different compounds such as alkaloids, lectins, glycosides, isoflavones, phytoestrogens, chrysophanic acid, kampferin, and sennosides A and B (Boy *et al*., 2018).

Many studies have found that the plant leaves exhibit anti-allergic, anti-inflammatory, antioxidant, thrombolytic, antitumor, anti-diabetic, choleretic, analgesic, anti-microbial, anti-viral, anti-ulcer, hepatoprotective, anti-depressant, anti-malarial, anti-helminthic, cardiovascular, and anaesthetic (Fatmawati *et al*., 2020). Despite many findings on *S. alata* health benefits, little is known about its antimicrobial activity against intestinal pathogens. Besides, there are no research studies conducted on the prebiotic effects of *S. alata* against probiotics such as *Lactobacillus* and *Bifidobacterium* species.

In consideration of this, conducting a study on the antimicrobial activity and prebiotic effects of *S. alata* leaf extracts would be an excellent opportunity, providing useful information to both the pharmaceutical and food industries. Besides, the findings of the research might have the possibility of leading to the development of a brand-new prebiotic and antibiotic. Furthermore, this study can provide the community with useful information about the effect of *S. alata* leaf extracts on intestinal bacteria, which may aid in the resolution of digestive-related issues.

1.4 Objectives of the study

The objectives of the study are:

1.4.1 General objective

To study the antimicrobial activity of *S. alata* leaf extracts against pathogenic intestinal bacteria and their prebiotic effects on probiotics.

1.4.2 Specific objectives

1. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S. alata* aqueous and ethanolic leaf extracts on pathogenic intestinal bacteria.

2. To evaluate the prebiotic effects of *S. alata* aqueous and ethanolic leaf extracts on beneficial intestinal bacteria (probiotics).

3. To screen the phytoconstituents of *S. alata* aqueous and ethanolic leaf extracts.

1.5 Overview of the study

As illustrated in Figure 1.1, the overall flowchart for this study summarises the methods involved.

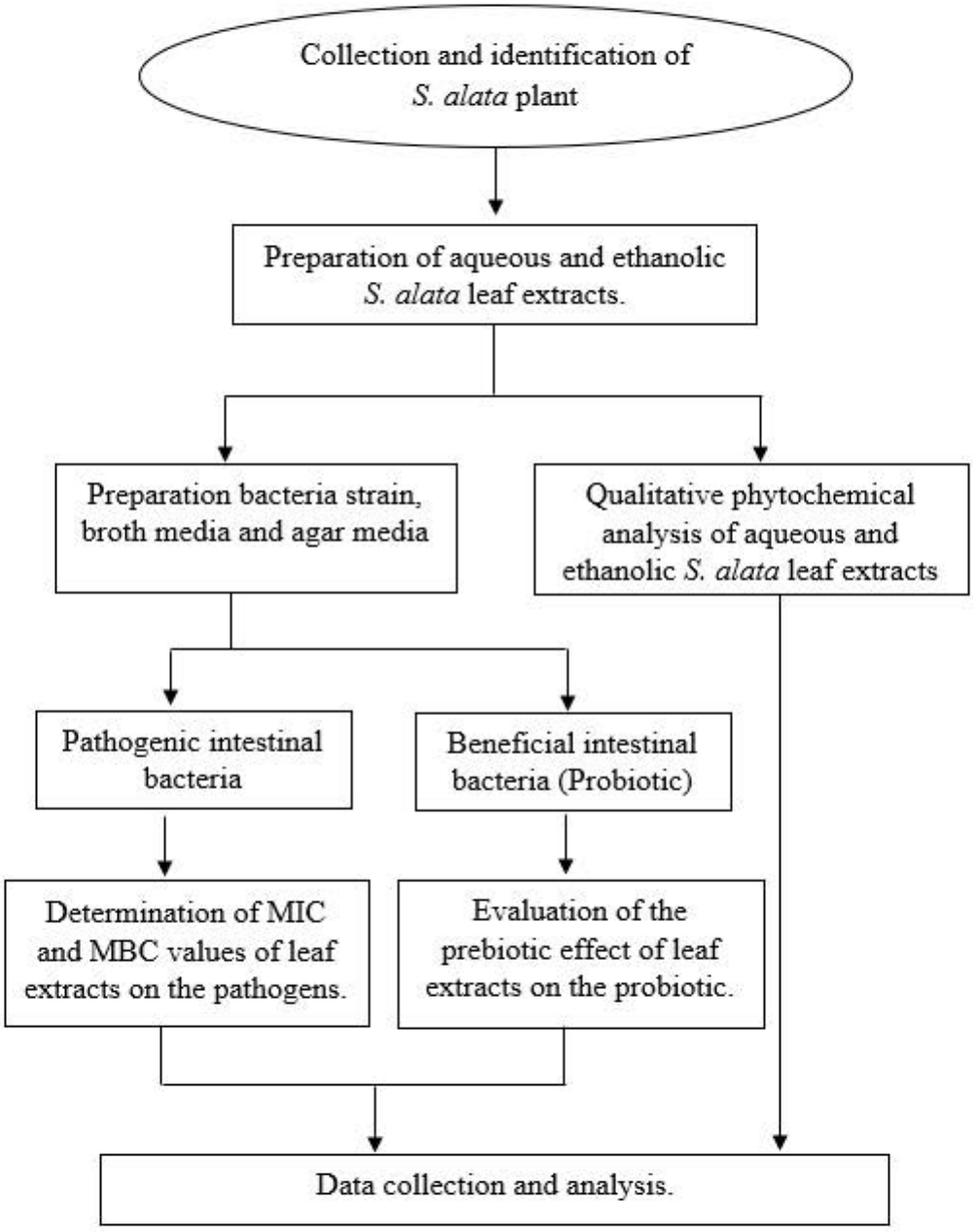

Figure 1.1 Flowchart of methods involved in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of *S. alata.*

Senna alata or *Cassia alata*, sometimes known as 'Ringworm Bush or Senna,' is an erect medicinal plant found mostly in the tropics and subtropics (Boy *et al*., 2018). The plant belongs to the Fabaceae or Leguminosae family, which can grow freely in the tropics (Fatmawati *et al*., 2020). According to Hennebelle *et al*. (2009), the plant is indigenous to Central America and mostly found in the Caribbean region but has been introduced to many tropical nations and islands worldwide. This plant has a foul odour, tall stems, skin of thin stems that is not spiked, yellowish green leaves that are a slightly wider, bright yellow race of flowers and the fruit is firm that resembles a brown pod with brown seeds when ripe (Figure 2.1).

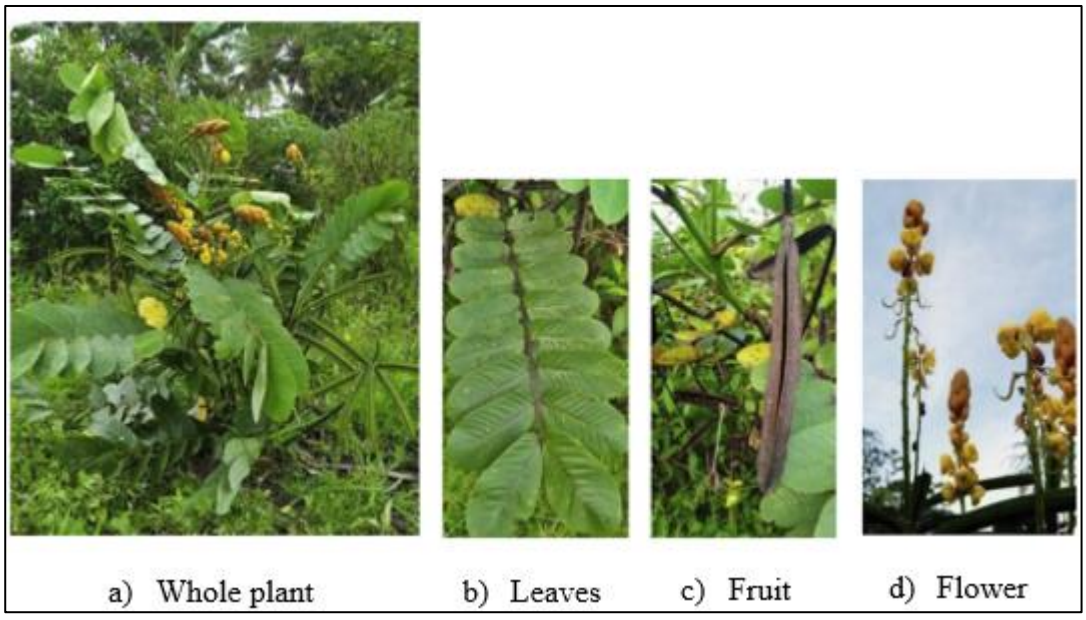

Figure 2.1 The photo of *S. alata* plant (Adapted from Fatmawati *et al*., 2020).

2.1.1 Ethnopharmacological uses of *S. alata*

S. alata has been mentioned in a number of ethnopharmacological investigations. According to Hennebelle *et al*. (2009), the only available ethnopharmacological study in the French West Indies, conducted on 100 traditional medicine users in Martinique, revealed a high level of knowledge and extensive use of this species' leaves for medicinal purposes with two main indications such as skin rashes and constipation.

In the Afro-Caribbean neighbourhood of Livingston, Guatemala, where 403 individuals were interviewed, it was revealed that only the leaves of *S. alata* were used for medical purposes (Hennebelle *et al*., 2009). People there believed that a decoction made from the leaves could not only cure constipation, but also diabetes and malaria. In addition, the infusion of the leaves was used to alleviate stomach aches, while the macerate was applied to the skin to treat skin problems. In Africa, *S. alata* appears to be more well-known for its dermatological benefits than for its purgative properties. A study by Ajibesin *et al.* (2008) reported that the leaves are applied topically and orally to treat skin problems in the Nigerian state of Akwa Ibom.

In Southwestern Nigeria, a macerate of *S. alata* leaves was the second most frequently reported treatment for diabetes (Abo, Fred-Jaiyesimi and Jaiyesimi, 2008). Besides, an occasional usage for pre-hepatic jaundice was discovered in Southern Uganda (Ssegawa and Kasenene, 2007). In Thailand, *S. alata* leaves are used to treat constipation, while in Indonesia they are used to eradicate fungus on the skin that can cause hives and other symptoms by rubbing directly on the affected area (Fatmawati *et al*., 2020). According to Table 2.1, which classifies this research according to its ethnopharmacological applications, *S. alata* is usually regarded as useful for treating digestive disorders (mostly constipation) and dermatological symptoms, regardless of their infectious origin.

Table 2.1 Uses of *S. alata* as reported by ethnopharmacological surveys. (Adapted from Hennebelle *et al*., 2009)

Type of use	Health problem	Geographic zone or population (country)	Part used, method, mode of consumption
Digestive	Constipation	Livingston (Guatemala)	Leaves, decoction, oral
		Trinidad	n.r.
		Martinique (France)	Leaves, decoction, oral
	Stomach pains	Livingston (Guatemala)	Leaves, infusion, oral
	Liver disease	Martinique (France)	Leaves, decoction, oral
	Pre-hepatic jaundice	Sango Bay area (Uganda)	Leaves, infusion, oral
Dermatologic	Skin diseases in general	Livingston (Guatemala)	Leaves, maceration, bath
		Akwa Ibom state (Nigeria)	Leaves, powder, external
			Leaves, decoction, oral
	Dermatitis	Sukajadi, West Java (Indonesia)	Leaves, pounded, topical
	Skin rash	Martinique (France)	Leaves, crushed, bath
	Athlete's foot	Martinique (France)	Leaves, crushed, external
	Herpes zoster	Akwapim-North district (Ghana)*	Leaves, juice/infusion, topical
	Eczema		
	Mycosis		
Anti-infectious	Malaria	Livingston (Guatemala)	Leaves, decoction, oral
	Flu	Quilombola de Olho D'água dos Pires, Esperantina, state of Piauí (Brazil)	Flowers, “tea”, oral
		Guinea*	
	“Infectious diseases”		Fruit, decoction, oral
Anti-diabetic	Diabetes	Livingston (Guatemala)	Leaves, decoction, oral
		Lagos, Ogun, Oyo, Osun states (Nigeria)*	Leaves, maceration, oral
Miscellaneous	Inflammation	Martinique (France)	Leaves, decoction, oral
	Thoracic pain	Martinique (France)	Flower buds, decoction, oral

n.r.: Not recorded.
*Exclusively traditional healers.

2.1.2 Chemical constituents of *S. alata* leaves

The leaves of *S. alata* have been shown to contain a variety of bioactive chemicals. According to Boy *et al.* (2018), many diverse chemicals are found in the plant, including alkaloids, lectins, glycosides, isoflavones, phytoestrogens, chrysophanic acid, kampferin, and sennosides A and B. A study by Agnaniet *et al*. (2005) reported that linalool (23%), borneol (8.6%), pentadecanal (9.3%), and α-terpineol (5.9%) are the primary constituents in the essential oil of *S. alata* leaves.

A study by Doss, Sugumar and Prasad (2016) on qualitative phytochemical analysis of ethanolic *S. alata* leaf extract revealed the presence of carbohydrates, tannins, saponins, flavonoids, quinones, cardiac glycosides, terpenoids, triterpenoids, phenols, coumarins, steroids, and phlobatannins. The study also reported the presence of carbohydrates, tannins, saponins, flavonoids, terpenoids, triterpenoids, phenols, and phlobatannins in water extract of *S. alata* leaves. The presence of important phytoconstituents was shown to be higher in the ethanol extract.

Chemical constituents of *S. alata* leaves have been documented in Thailand's Pattalung Province (Panichayupakaranant and Kaewsuwan, 2004). The methanol extract was fractionated using a liquid vacuum gel chromatography technique and eluted with chloroform: methanol to get six fractions. The fractions were separated using LH-20 sephadex column chromatography and eluted with methanol solvent to yield 8 fractions. Fraction VII (yellow) demonstrated high antioxidant activity. The structure was identified using IR, ^{1}H NMR, and ^{13}C NMR. The identification findings suggested that it was a flavonol, namely kaempferol. Another study using HPLC to analyse *S. alata* leaves extract identified kaempferol-3-O-β-D-glucopyranoside (Saito *et al*., 2012).

In the BCSIR region at Rajshahi Campus, flavone compounds have been identified from *S. alata* leaf ethanol extracts. It was a flavone compound that was known by the names 3,5,7,40-tetrahydroxy flavone and 2,5,7,40-tetrahydroxy isoflavones (Rahman, Ali and Ali, 2009). Other flavonoid compounds like anthraquinone and kaempferol 3-O-gentiobioside were also reported in other studies. (Adiana and Mazura, 2011).

The GC/MS analysis of *S. alata* leaves conducted by Igwe and Onwu (2015) revealed the presence of 7 compounds such as ((6Z)-7,11-dimethyl-3-methyl-idenedodeca-1,6,10-triene), (4a,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octa-hydronaphthalene), (4,4.7a-trimethyl-5,6,7,7a-tetrahydro-1-benzofur-an-2(4H)-one), (3,7-dimethylocta-1,6-diene), hexadecanoic acid methyl ester, hexadecanoic acid and octadecanoic acid methyl ester. Fatmawati *et al*. (2020) reviewed that compounds of the alkaloids have also been discovered from *S. alata* leaves which include adenine, chrysoeriol, quercetin, (5,7,40-trihydroflavanone), (kaempferol-3-O-beta-D-glucopyranosyl-(16)-beta-D-glucopyranoside), n-dotriacontanol, n-triacontanol, stearic acid, palmitic acid, diomestin, luteolin, and (1,3,5-trihydroxy-7-methyl-anthracene-9,10-dione).

2.1.3 Biological activity of *S. alata*

Fatmawati *et al*. (2020) provided a summary of all the research that reported various biological activities of *S. alata* leaf, such as anti-allergic, anti-inflammatory, antioxidant, anti-diabetic, choleretic, analgesic, antimicrobial, antiviral, antiulcer, hepatoprotective, antidepressant, anti-malarial, anti-helminthic, cardiovascular, anaesthetic, and anticancer. Table 2.2 summarises the biological activities of the *S. alata* leaf.

Table 2.2 Biological activity of *S. alata* (Adapted from Fatmawati *et al*., 2020)

Biological activity	Method	Result	Source
Anti-allergic	In vivo (mast cell stabilization).	At 200 mg/kg (75 % inhibition), whereas rhein and kaempferol at 5 mg/kg (76 % inhibition).	(Singh *et al*., 2012)
Anti-inflammatory	Oral gavage to CFA arthritic rats (500 mg/kg, n = 6).	The extract significantly (P=0.0032) decreased knee circumference (swelling) in CFA arthritic rats.	(Lewis and Levy, 2012)
Antioxidant	DPPH radical scavenging	Methanol extract had a lower IC_{50} concentration (54 ± 2.20) than BHT standard (72 ± 2.20)	(Chatterjee, 2012)
Anticancer and antitumor	Bearing carcinomatous cells on Nude mice.	MDA levels decreased significantly (3.44 ± 0.76 to 1.97 ± 0.48) at 100 and 200 mg/kg body weight, whereas glutathione, as well as CAT and SOD activity, rose significantly.	(Pieme *et al*., 2008)
Anti-diabetic	Blood glucose levels used albino Swiss Webster mice.	*S. alata* ethyl acetate extract demonstrated efficacy as an antidiabetic agent, with a 56.7 % drop in blood glucose level compared to CMC control (38.0 %).	(Villasenor *et al.*, 2002)
Choleretics	Bile secretion of rats.	The activity of choleretic extract of *S. alata* at 15 mg/kg was better than Hebucol ND. However, plants suppress bile secretion at high concentrations.	(Assane *et al*., 1993)
Analgesic	In vivo employing an albino rat with tail clamping, tail wagging, tail immersion, and writhing reflexes induced by acetic acid.	The analgesic effect of *S. alata* was substantially greater at 400 mg/kg compared to 200 and 100 mg/kg. In 120 minutes, kaempferol 3-O-sophoroside produced its highest analgesic effect. Assay writhing of acetic acid at 400 mg/kg S. alata demonstrated a significant increase in analgesic effect (56.4%) compared to dosages of 200 and 100 mg/kg (46.4% and 35.9%), whereas the percentage of inhibitory stretching produced by kaempferol 3-O-sophoroside was close to 100 mg/kg *S. alata* extract (36.9%).	(Palanichamy and Nagarajan, 1990)

Table 2.2 (continued)

Biological activity	Method	Result	Source
Antimicrobial	Disk diffusion.	*Salmonella* Typhi, *Proteus mirabilis*, *Bacillus coagulans*, and *Micrococcus luteus* were all inhibited by methanol extract. The growth of *B. coagulans* was inhibited by a petroleum ether extract. *Lactobacillus casei*, *Staphylococcus epidermidis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* were all inhibited by dichloromethane extract. *B. coagulans* and *T. vaginalis* growth was inhibited by ethyl acetate extracts.	(Khan, Kihara and Omoloso, 2001)
Antiviral	In vitro MMT.	Several extracts of *S. alata* leaves (methanol, chloroform, ethyl acetate, n-butanol, and aqueous) were found to be effective against rotavirus (RV) infection.	(Shaheen and Mostafa, 2015)
Antiulcer	Pylorus ligation and ethanol-induced ulcer models in experimental rats.	Ethanolic extract of *S. alata* leaves at doses of 150 and 300 mg/kg significantly inhibited the gastric lesions caused by pylorus ligation-induced ulcers and ethanol-induced gastric ulcers. In comparison to the control, the extract (150 mg/kg and 300 mg/kg) reduced gastric volume, free acidity, and ulcer index significantly ($p < 0.05$).	(Babu *et al.*, 2012)
Hepatoprotective	Hepatic injury in albino rats.	The hepatoprotective effect of an alcoholic extract of *S. alata* leaves has been reported. The methanol extract was found to be effective against Paracetamol-induced hepatic injury in albino rats. Furthermore, pre-treatment with the extract decreased biochemical markers of hepatic injury such as serum glutamate pyruvate transaminase (SGPT), serum oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, and gamma glutamate transpeptidase from paracetamol-induced liver damage.	(Anandan, Jayakar and Manavalan, 2009)
Antimalarial	WHO microtest assay (Mark III)	The results demonstrated that *S. alata* leaves have an action against the 3D7 strain of the *Plasmodium falciparum* parasite, with an IC_{50} of 17.270 µg/mL. The IC_{50} for artesunate-amodiaquine was 0.313 µg/mL.	(Yaw *et al.*, 2015)

Table 2.2 (continued)

Biological activity	Method	Result	Source
Antidepressant	Forced Swim Test (FST) and Tail Suspension Test (TST)	*S. alata* significantly reduced the time of immobility in both the standard medication (22.0 ± 0.26 s) and the test extract (21.25 ± 0.12 s) as compared to the control group (62.5 ± 0.54 s). *S. alata* leaf extracts (200 mg/kg) outperformed the conventional medication fluoxetine (10 mg/kg). As a result, there was a substantial decrease in the immobility of mice in the test extract (59.6 ± 2.23 s) and a standard medicine (57 ± 3.5 s) compared to the control group (153.4 ± 1.97 s) after administration of the standard and test extract. These findings demonstrated that aqueous leaf extracts of *S. alata* had substantial antidepressant efficacy comparable to the standard of care (Imipramine).	(Pamulaparthi *et al*., 2016)
Anti-helminthic	Scanning electron microscope studies (SEM)	Paralysis occurred at 1.68 ± 0.06 h for the ethanol extract at 40 mg/mL, which is equivalent to praziquantel (1.18 ± 0.04 h) at 0.001 mg/mL. The post-paralytic period was shorter in all concentrations of *S. alata*, but longer in all concentrations of praziquantel. The control parasite, on the other hand, survived for up to 69.22 ± 0.23 h.	(Kundu, Roy and Lyndem, 2012)
Cardiovascular	DPPH and lipid peroxidation.	The aorta and heart of hyperglycaemic rats showed a considerable increase in lipid peroxidation, a decrease in total antioxidant activity (DPPH), and a decrease in antioxidant catalase activity. In hyperglycaemic rats, administration of *S. alata* leaf aqueous extract reduced lipid peroxidation (MDA levels), enhanced total antioxidant activity and antioxidant catalase activity, and decreased blood glucose levels.	(Ishak *et al*., 2015)
Anaesthetic	ALT (Alanine Transaminase), AST (Aspartate Transaminase) and ALP (Alkaline Phosphatase)	The results revealed that AST, ALT, and ALP were lowered in groups 3 to 7 compared to group 2 and that ALP and ALT were significantly reduced ($P < 0.05$) when treated with 4000 mg/kg body weight.	(Onyegeme-Okerenta, Nwosu and Wegwu, 2017)

2.2 Prebiotic effects of plant-based food

A review by Manzoor *et al.* (2022) revealed that prebiotics are indigestible compounds that improve a person's health and can change the flora in their gut. In addition, most of the prebiotic compounds are carbohydrates with a variety of molecular structures, and they help promote the growth of bacteria in the gastrointestinal tract. Numerous food crops, including wax ground, shallots, onions, garlic, and artichokes of the gourd family, contain prebiotics in the form of carbohydrates (Althubiani *et al.*, 2019). Some mushroom species, such *Agaricus bisporus*, have been found to offer possible prebiotic effects (Althubiani *et al.*, 2019). The fruiting body extract of *Pleurotous* species (pleuran) contains β-glucan and has been utilised in a variety of dietary supplements to impart immunosuppression and gut probiotic growth for the benefit of human health (Synytsya *et al*., 2009).

Other prebiotic sources include soybeans, inulin compounds, unprocessed wheat, uncooked oats, and nondigestible oligosaccharides (Manzoor *et al.*, 2022). Inulin is mostly found in fruits like yacon and chicory (Althubiani *et al.*, 2019). Prebiotics like inulin and pectin contains antioxidant characteristics that help prevent cancer, shorten the duration of diarrhoea, reduce inflammation, and lessen the risk of intestinal tract issues (Naseer *et al*., 2020). Besides, they enhance the bioavailability and absorption of minerals, hence lowering the risk of cardiovascular disease and preventing obesity through weight loss. (Pokusaeva, Fitzgerald and Van Sinderen, 2011).

Prebiotics are characterised by a number of characteristics, such as the fact that they are not completely fermented by a bacterial community in the mouth and are not absorbed by the small intestine (Manzoor *et al.*, 2022). Furthermore, they are fermented

well by beneficial intestinal bacteria and inadequately fermented by pathogens in the intestine. Dietary fibres and prebiotics are interchangeable words for dietary components that the gastrointestinal tract (GIT) cannot digest. Prebiotics are fermented by a few specialised organisms, whereas dietary fibres can be fermented by most colonic bacteria (Patterson and Burkholder, 2003). Dietary fibres also include a range of prebiotics but not all dietary fibres can be prebiotics (Manzoor *et al.*, 2022).

The primary purpose of prebiotics is to stimulate the growth of beneficial microbes in the GIT, hence enhancing health (Manzoor *et al.*, 2022). Short-chain fatty acids (SCFAs) including propionic, acetic, and butyric acids appear to be key components of carbohydrate metabolism (Manzoor *et al.*, 2022). These SCFAs are used as energy sources by the host (Grajek, Olejnik and Sip, 2005). *Bifidobacterium* and *Lactobacillus* produce specific metabolites as a result of carbohydrate fermentation, which lower intestinal pH and inhibit pathogens growth in the GIT (Manzoor *et al.*, 2022).

Moreover, *Bifidobacterium* has a higher tolerance for SCFAs and a lower pH, making it an appealing candidate for improving human health. Studies have demonstrated that prebiotics improves the body's ability to absorb minerals including magnesium and calcium (Demigné *et al.*, 2008). Prebiotics can be used to replace probiotics or as a supplement to assist probiotics in improving human health (Manzoor *et al.*, 2022). Prebiotics may affect the gut microbiota at the species and strain levels while inhibiting the growth of other microbes (Markowiak and Śliżewska, 2017). The beneficial effects of prebiotics and probiotics on human health are shown in Figure 2.2.

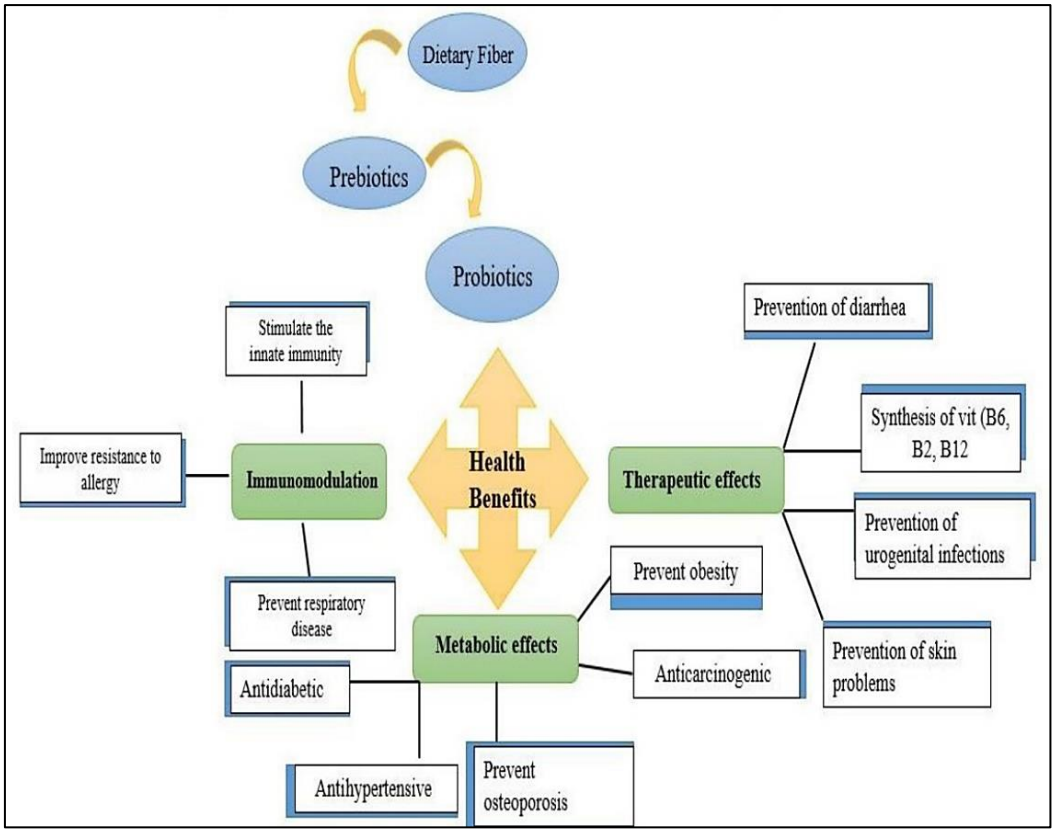

Figure 2.2 The health benefits of prebiotics and probiotics on humans (Adapted from Manzoor *et al.*, 2022).

2.3 Mode of action probiotics

According to a review by Manzoor *et al*. (2022), probiotics are beneficial bacteria that live naturally in the gastrointestinal and can improve human health when consumed in appropriate quantities. Furthermore, they contribute to the maintenance of a healthy balance of gut flora and are resistant to acids as well as bile salts. The most well-known species include the *Lactobacillus* family (*acidophilus, sporogenes, lactis*), *Bifidobacterium* (*bifidum, longum, infantis*), *Streptococcus* group (*thermophillus, lactis, fecalis*), and non-pathogenic yeast (*S. boulardii*). Manzoor *et al.* (2022) further stated that most probiotics are comprised of *Lactobacilli* and *Bifidobacteria*, which are naturally present in the human digestive tract and are capable of fermenting lactic acid.

Some probiotics can make antimicrobial substances like bacteriocins, which can help a person's overall health and well-being by making bowel movements more regular.

Food and Agriculture Organization of the United Nations (2001) stated that probiotics are "live microorganisms that, when administered in suitable quantities, have a favourable impact on the host when taken orally." The key advantage of probiotics is that they dwell in an organism, therefore maintaining a balance between pathogens and essential bacteria for optimal human body function (Oelschlaeger, 2010).

Probiotics can effectively prevent food poisoning by inhibiting the growth of pathogenic bacteria such as *Escherichia coli*, *Clostridium perfringens*, *Shigella sonnei*, *Salmonella enteritidis*, *Campylobacter jejuni*, *Staphylococcus*, and *Yersinia* (Manzoor *et al.*, 2022). Based on molecular and genetic studies of probiotics, researchers have identified four mechanisms by which they exert their beneficial effects. These include antagonism through the production of several antimicrobial chemicals, competition between pathogens for adhesion to epithelial cells and nutrients, modulation of the host immune response, and inhibition of bacterial toxin production (Manzoor *et al.*, 2022).

Probiotics limit the spread of infection by binding to epithelial cells. In addition, when the probiotic strain encounters epithelial cells, a signalling cascade is initiated, therefore modifying the immune response (Manzoor *et al.*, 2022). The release of certain soluble components may directly or indirectly activate innate immune cells, which might assist in the treatment and prevention of many communicable illnesses and cure severe gastrointestinal inflammation (Oelschlaeger, 2010).

2.4 Pathogenic intestinal bacteria

The human intestinal mucosa is a highly dynamic environment in which the host interacts continually with trillions of commensal bacteria, known as the microbiota, and intermittently with different pathogens (Perez-Lopez *et al*., 2016). Nearly 1.6 million individuals died worldwide in 2017 from diarrheal illnesses, and many of those deaths were caused by enteric pathogens (Dadonaite, Ritchie and Roser, 2019). Many microbes can cause food-borne infections, with varying illness severity, clinical signs, and persistence. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhi, *Vibrio cholerae*, and *Klebsiella pneumoniae* are among the microbes that can cause diarrheal diseases in humans.

According to a review by Desmarchelier and Fegan (2016), *Escherichia coli* is a member of the Enterobacteriaceae family, which contains gram-negative, facultatively anaerobic rod-shaped bacteria that lack the enzyme oxidase. Besides, *E. coli* cells are generally 1.1-1.5 µm broad, 2-6 µm long, and appear as single straight rods. They can be motile or nonmotile, and when motile, they generate lateral rather than polar flagella. In addition to flagella, several strains develop additional appendages like fimbriae or pili, which are proteinaceous structures that extend outward from the bacterial surface and aid in adhesion to surfaces such as other cells or host tissues. *E. coli* causes a variety of diarrheal illnesses, including traveller's diarrhoea and dysentery (Mueller and Tainter, 2020).

Staphylococcus aureus is a Gram-positive bacterium with cocci-shaped cells that are grouped in "grape-like" clusters (Taylor and Unakal, 2018). Besides, these organisms may thrive in a medium containing up to 10% salt, and colonies are often golden or yellow (aureus means golden or yellow). These organisms can thrive aerobically or anaerobically (facultatively) and at temperatures ranging from 18 to 40

degrees Celsius (Taylor and Unakal, 2018). *S. aureus* is found on the skin and mucous membranes, and humans are the bacteria' primary reservoir (Boucher and Corey, 2008). *S. aureus* toxins are linked to food poisoning, and the alpha toxin of *S. aureus* may disrupt the barrier function of intestinal cells in vitro by affecting their junctional integrity (Kwak *et al*., 2012). In addition, *S. aureus* may induce antibiotic-associated diarrhoea in hospitalised patients (Lane *et al*., 2018). *S. aureus* is linked to inflammatory bowel disease (IBD) because the antigens produced from the gut may promote inflammatory responses (Lu *et al*., 2003).

Salmonella Typhi is a gram-negative, rod-shaped, flagellated bacterium with solely a human reservoir (Crump *et al.,* 2015). *S.* Typhi is a bacterium that causes typhoid fever and has been a burden on impoverished nations for decades (Ashurst and Woodbury, 2019). *S.* Typhi is often transmitted by the consumption of infected food or drink, and the organism must survive the gastric pH barrier in the stomach before reaching and adhering to the small intestine (Parry *et al*., 2002). *S.* Typhi reach the small bowel submucosal area by either direct epithelial tissue penetration mediated by the cystic fibrosis transmembrane conductance regulator (CFTR) or via the M-cell, a specialised lymphoid epithelial cell (Ashurst and Woodbury, 2019). The bacteria produce hypertrophy of the Peyer's patches after it enters the submucosa.

Vibrio cholerae strains produce toxins that cause the disease. *V. cholerae* is a comma-shaped, gram-negative, extremely motile bacterium with a single polar flagellum (Ojeda Rodriguez and Kahwaji, 2020). There are hundreds of serogroups, including both pathogenic and non-pathogenic strains. Recently, the disease was caused by just two of these serotypes, Inaba and Ogawa, and two biotypes of toxigenic serogroup O1, classical and El Tor (Ojeda Rodriguez and Kahwaji, 2020). In 1992, serogroup O139, sometimes known as Bengal, appeared as another *V. cholerae*

pandemic variety (Somboonwit *et al*., 2017). Cholera is a well-known disease caused by the toxin-producing bacterium *V. cholerae* in the digestive tract. This potentially deadly diarrheal condition is characterised by huge quantities of watery faeces, resulting in rapid dehydration that can lead to hypovolemic shock and metabolic acidosis (Ojeda Rodriguez and Kahwaji, 2020).

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Raw materials

S. alata leaves were used as raw materials in this study. Fresh *S. alata* leaves were purchased and obtained from local people in Machang, Kelantan, Malaysia.

3.1.2 Bacterial strains

In this study, six bacteria strains were used such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* Typhi, *Vibrio cholerae*, *Lactobacillus helveticus* and *Bifidobacterium longum* (Table 3.1). Bacteria strains of *E. coli*, *S. aureus*, *S.* Typhi and *V. cholerae* were provided by the Microbiology Laboratory, School of Health Sciences, Universiti Sains Malaysia, Kelantan. For *L. helveticus* and *B. longum*, these strains were provided by I Medikel Pharmacy, Kelantan.

Table 3.1 List of bacteria strains used in this study

Bacteria strains	Source
E. coli	ATCC 25922 (Manassas, USA)
S. aureus	ATCC 25923 (Manassas, USA)
S. Typhi	Clinical (Kelantan, Malaysia)
V. cholerae	Clinical (Kelantan, Malaysia)
L. helveticus	Rosell-52 (Rosell Institute, France)
B. longum	Rosell-175 (Rosell Institute, France)

ATCC: American Type Culture Collection

3.1.3 Chemicals and reagents

All the chemicals used in this study are listed in Table 3.2.

Table 3.2 List of chemicals and reagents used in this study

Chemicals and reagents	Brands
Absolute ethanol	ACS Chemicals, India
Ammonia solution	Sigma-Aldrich, USA
Molisch's reagent	Bendosen, Malaysia
Sulphuric acid concentrate	Supelco, USA
Sodium hydroxide	Sigma-Aldrich, USA
Ferric chloride	Sigma-Aldrich, USA
Iodine	Sigma-Aldrich, USA
Potassium iodide	Sigma-Aldrich, USA
Mueller-Hinton broth (MHB)	Oxoid, UK
Mueller-Hinton agar (MHA)	Oxoid, UK
De Man, Rogosa and Sharpe (MRS) broth	Sigma-Aldrich, USA
De Man, Rogosa and Sharpe (MRS) agar	Sigma-Aldrich, USA
Tween 80	Sigma-Aldrich, USA
0.5 Polymer McFarland Standard	Thermo Fisher Scientific, USA

3.1.4 Apparatus and consumables

All apparatus and consumables used in this study are listed in Table 3.3.

Table 3.3 List of apparatus and consumables used in this study

Apparatus and consumables	Brands
Conical flask (100 mL and 1 L)	Labmart GQ, Malaysia
Beaker 500 mL	Labmart GQ, Malaysia
Measuring cylinder 25 mL	Labmart GQ, Malaysia
Sterile plastic petri dish	Labmart GQ, Malaysia
Disposable glass culture test tubes	Kimble Chase, USA
Specimen containers	Starplex, Canada
Glass dropper	Fisher Scientific, USA
96-well microtiter plate	Greiner Bio-One 96, USA
Micropipette tips (200 µl)	Axygen, USA
Aluminium foil	Diamond, Bangladesh
Filter paper	Whatman, UK
CO_2 anaerobic pack	Oxoid, UK
Scott bottles (500 mL)	Pyrex, USA
Glass filter funnel	Labmart GQ, Malaysia
Sterile cotton bud	Jiaxin Medical, China
Gloves	Xcel, Malaysia
Parafilm	Bemis, USA
Face mask	Medicos, Malaysia

3.1.5 Laboratory equipment

All the laboratory equipment used in this study are listed in Table 3.4.

Table 3.4 List of laboratory equipment used in this study

Laboratory equipment	Supplier
Hot plate	Stuart Scientific, UK
Weighing balance	Sartorius AG, Germany
Rotary evaporator	Buchi, Switzerland
Autoclave machine	MaxLab Technology, Malaysia
Microplate reader (SUNRISE)	Tecan, Switzerland
Blender	Conair, USA
Laminar flow hood (ESC II)	ERLA, Malaysia
Freeze dryer	LabsNova, China
Refrigerator (-4°C)	Philips, Netherlands
Carbon dioxide (CO_2) incubator	Binder, Germany
Fume hood	ERLA, Malaysia
Micropipette gun (100 µL-1000 µL)	Eppendorf, Germany
Class II Biohazard Safety Cabinet	Esco, Singapore

3.1.6 Computer application program and software

All computer application and software used in this study are listed in Table 3.5

Table 3.5 List of computer application and software used in this study

Computer application program and software	Brands
Microsoft Excel 2021	Microsoft Corporation, USA
Microsoft Word 2021	Microsoft Corporation, USA
Statistical Package of Social Sciences (SPSS) software, version 26	IBM, New York, USA