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The Antimicrobial Properties of 'Sambung Nyawa" Leaves (Gynuraprocumbens) Towards Bacterial Growth

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Abstract

'Sambung nyawa' leaves (*Gynura procumbens*) is a local medicinal herbs that is highly useful in treating various illnesses. The leaves itself lack in commercialisation and consumption among the consumer, the leaves contains amount of flavonoid and saponin that acts as an antimicrobial agents. The purpose of this study is to determine the antimicrobial effect and inhibition zone of 'Sambung nyawa' leaves (*Gynura procumbent)* towards the growth of bacteria. Amount of 1.50g 'Sambung nyawa' (*Gynura procumbens*) leaves was able to inhibit the growth of *Salmonela typhimurium* (17.7 ± 2.51 mm), *Staphylococcus aureus* (12.7 ± 1.15 mm) and *Escherichia coli* (12 ± 0.00 mm) but failed to inhibit the growth of *Bacillus cereus*. This shows that "sambung nyawa" leaves has the ability to act as antimicrobial properties towards food borne illness bacteria

Keywords: '*Sambung nyawa' leaves (Gynura procumbens), Antimicrobia effect, inhibition zone*

Abstrak

Daun 'sambung nyawa' (*Gynura procumbens*) adalah herba tempatan yang sangat berguna dalam merawat pelbagai penyakit, daun ini kurang dari segi pengunaan dan pengkomersilannya kepada penguna, daunnya mengandungi sejumlah flavonoid dan saponin yang bertindak sebagai agen antimikrob. Kajian ini bertujuan untuk menentukan kesan antimikrob dan zon perencatan bagi daun 'sambung nyawa' (*Gynura procumbens*). Sejumlah 1.50 g daun 'sambung nyawa' (*Gynura procumbens*) digunakan dan ia menunjukkan zon perencatan pertumbuhan *Salmonela typhimurium* (17.7 ± 2.51 mm), *Staphylococcus aureus* (12.7 ± 1.15 mm) dan *Escherichia coli* (12 ± 0.00 mm) tetapi gagal untuk menghalang pertumbuhan *Bacillus cereus*.

KATAKUNCI: *Daun ' sambung nyawa',(Gynura procumbens), kesan antimikrob, zon perencatan*

1. INTRODUCTION

Herbs have been used for thousands of years as natural medicines, natural does not always mean safe. Medicinal plants constitute a source of raw materials for both traditional systems of medicine (e.g. Ayurvedic, Chinese, Unani, Homeopathy, and Siddha) and modern medicine. All medicinal agents have potentially unexpected effects including toxicity and interactions, and herbs are no different. Nowadays, plant materials are employed throughout the industrialized and developing world as home remedies, over-the-counter drugs and ingredients for the pharmaceutical industry (Chirag *et al*, 2012).

Herbal medicine has been widely used in Asian country as a supplementary medicine after modern medicine. Most of the herbal medicine contains medicinal value in treating illness in human.

One of the traditional plants is 'Sambung nyawa' leaves (*Gynura procumbens*) that can be found in Thailand and Southeast Asia (Iskander *et al.*, 2002). Sambung nyawa can be identified as evergreen shrub with a green or purple tint and a fleshy stem (Kaewseejan *et al.,* 2012). This plant has great advantage in treating various illness. Based on various literature reviews, this plant is widely known to cure hypertension (Jawaid *et al*, 2011), antidiabetic (Lee *et al*, 2011), anti- inflammatory (Iskander *et al.*,2002) herpes (Nawawi *et al*.,1999), antibacterial property (Kaewseejan *et al.,* 2012) and wound healing (*Zahra et al*., 2011). 'Sambung nyawa' leaves normally consumed as raw leaves (Rosidah *et al*., 2009).

Consumer has no trace on the amount of chemical that they consumed,So this may lead to intoxication. The safe amount of 'Sambung nyawa' to be consumed is between 1000-5000 mg/kg for a normal person (Rosidah *et al*., 2009). There is an evidence that show 'Sambung nyawa' consists of flavanoid , saponins, tannins, terpenoids and sterol glycosides (Puanpronpitag *et al*., 2010). This active ingredient is being used as antimicrobial constituent.

2. LITERATURE REVIEW

2.1 'Sambung nyawa' leaves

'Sambung nyawa' leaves or scientifically known as *Gynura procumbens* is one of the herbal medicines that can be found in various parts of Asia, Thailand and Southeast Asia (Kaewseejan *et al*., 2012). It also can be found with other name such as *Daun Dewa, Leaves of the Gods, Googoolipid, Mollucan Spinach, Sam Akar, Akar Sebiak, Kelemai Merah and Bai Bing Cao*. It is normally consumed as raw leaves by the consumer (Rosidah *et al*., 2009). Previous study by Nazmul *et al*., (2010) has shown that 'Sambung nyawa' leaves have the antifungal properties. It prevented the growth of *Aspergillus flavus*, *candida albicans*, *Microsporum canis*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*.

The active compound found inside Sambung nyawa'' leaves include flavonoids, saponins, tannins, terpenoids and sterol glycosides (Puangpronpitag *et al*., 2010). Saponins are known to have the antimicrobial properties as well as inhibit mould growth (Francis and Doyle, 2006). Saponin also reported increased the immune system, it function by induced the production of cytokines such as interleukins and interferons that mediate immune stimulant effects (Francis and Doyle, 2006). Increasingly, flavonoids have been reported to possess many useful properties, including anti-inflammatory activity, estrogenic activity, enzyme inhibition, antimicrobial activity (Tim and Lamb, 2005). The extract of 'Sambung nyawa' leaves also has shown growth inhibition of pathogenic microbes such as *Candida albicans, Escherichia coli, Pseudomonas sp*. and *Bacillus subtilis*, this could be a promising source for antimicrobial agent (Rumella *et al,*. 2007).

2.2 Antimicrobial agents of 'Sambung nyawa' leaves

Antimicrobial activities, mode of action and potential uses of plant volatile oils have been applied in food. This is true with regards to plant volatile oils and their antimicrobial evaluation (Dorman and Deans, 2000). According to Food and Agriculture organization of United States (2011), "Antimicrobial agent can be define as any substance of natural, semi-synthetic, or synthetic origin that *in vivo* concentrations kills or inhibits the growth of microorganisms by interacting with a specific target".

The antimicrobial agents work in different kind of action mechanism and they can be classified according to their action mechanism. There are four major modes of action: (1) Interference with cell wall synthesis (e.g β- lactams and glycopeptides agents), (2) inhibition of protein synthesis (macrolides and tetracyclines), (3) interference with nucleic acid synthesis (fluoroquinolones and rifampin), and (4) inhibition of a metabolic pathway (trimethoprim-sulfamethoxazole) (Tenover , 2006).

As for interference of bacterial cell wall synthesis, this process leads to bacterial lysis (bursting) and death of the bacteria. Since animal cells do not have a cell wall, they are unaffected by such agents. The microbial agent may inhibit the protein synthesis. This happens because essential enzymes required for the cell's survival can no longer being produced. The antimicrobial agent may also inhibit the nucleic acid synthesis. The agents prevent cell division and /or the synthesis of essential enzymes. For inhibition of metabolic pathway, Antimicrobial agents act by inhibiting the cell metabolism that is called antimetabolites.

These compounds inhibit the metabolism of a microorganism, but not the metabolism of the host. Antimetabolites inhibit an enzyme- catalysed reaction which is present in the bacterial cell, but not in animal cells (Patrick, 1995).

The active compound found inside 'Sambung nyawa' leaves include flavonoids, saponins,tannins, terpenoids and sterol glycosides (Puangpronpitag *et al*., 2010). Saponins are known to have the antimicrobial properties as well as inhibit mould growth. Increasingly, flavonoids have been reported to possess many useful properties, including anti-inflammatory activity, estrogenic activity, enzyme inhibition, antimicrobial activity (Tim and Lamb, 2005). Saponins are glycosylated phytoanticipins that are found in a wide range of plant species and can be divided into three major groups, triterpenoid, steroid or steroidal glycoalkaloid, depending on the structure of their aglycones.

Because they have high antimicrobial activities it is stated that the natural role of these molecules in plants is to give protection against potential pathogens (Lamothe *et al*., 2009). Flavonoids are classified under phenolic groups in plants which have been known to possess antimicrobial activity. The mechanisms of flavonoids that are antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Hendra *et al.*, 2011).

2.3 Active chemical of 'Sambung nyawa' leaves as antimicrobial agent

2.3.1 *Flavonoids*

Flavonoid compounds, which are a large group of secondary metabolites in higher plants, are known to have antibacterial activity (Liu *et al.*, 2010). Flavonoids are normally found in photosynthesising cells and are commonly found in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey. Increasingly, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity (Tim and Lamb,2005). Flavonoids are classified under phenolic groups in plants which have been known to possess antimicrobial activity. The mechanisms of flavonoids that are antimicrobial can be classified as the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Hendra *et al.*, 2011). Flavonoids are secondary metabolites characterised by flavan nucleus and C6-C8-C6 carbon-skeleton. These are group of structurally related compounds with a chromane-type skelton having phenyl substituent in C2-C3 position. The basic structural feature of flavonoid is 2-phenyl-benzo-γ-pyrane nucleus consisting of two benzene rings linked through a heterocyclic pyran ring (Kumar *et al*.,2011) :

2.3.2 Saponins

Saponins are glycosylated phytoanticipins that are found in a wide range of plant species and can be divided into three major groups, which are triterpenoid, steroid or steroidal glycoalkaloid, depending on the structure of their aglycones. Because they have great antimicrobial activities it is stated that the natural role of these molecules in plants is to give protection against potential pathogens (Lamothe *et al*., 2009).

Saponins are synthesised by a common metabolic pathway starting from acetyl coenzyme A. Mevalonic acid and then squalene is the intermediary products for both triterpenoidal and steroidal saponins. In general, synthesis of cholesterol, other steroids, and saponins proceed through a common synthetic pathway. Saponins are glycoside compounds whose chemical structures are composed of a fat-soluble nucleus called the aglycone that is either triterpenoid (C-30) or neutral or alkaloid steroids (C-27) (Hassan, 2008).

3.0 METHODOLOGY

3.1 Preparation of 'Sambung nyawa' leaves

Fresh young leaves were collected and washed under running tap water, dried in oven at a temperature of 45°C for three days and homogenised to coarse powder and stored in airtight bottles. (Lee *et al*., 2012).

3.1.1 Aqueous extraction

The leaves of 'Sambung nyawa' were extracted using aqueous extraction method, based on method by Lee et al., (2011). Fresh leaves of 'Sambung nyawa' were washed, weighed and dried in oven at temperature of 45 °C for period of three days (3 days). Upon drying, dried leaves would be blended into powder form, then 20 g of dried leaves powder would be mixed with 400 mL of distilled water (ratio of 1:20) and was heated in water bath (50°C) for three hour (3 hour) with stirring of the extract at every 20 minutes interval. The extract was cooled and centrifuged at 1500 rpm for 10 minutes. Supernatant obtained was separated and the pellet was subjected to another round of centrifugation at 3000 rpm for 10 minutes. Both of the supernatants would be combined and freeze-dried to yield brown powder of 'Sambung nyawa' leaves crude aqueous extract and stored in labelled bottles in chiller. Table 3.1.1 show the amount of 'Sambung nyawa' leaves extract used in each formulation.

Table 3.1.1 Formulation 'Sambung nyawa' leaves extract

Formulation	Weight of Sambung nyawa leaves extract (g)
Control	Nil
Formulation 1	1.50
Formulation 2	1.25
Formulation 3	0.50

3.2 Nutrient Agar Preparation

An amount of 23 g of nutrient agar powder was weighed, and dissolved in 1.0 L of distilled water. It was sterilised by using autoclaved at 121 °C for 15 minutes. The agar was poured into ½ of Petri dish and left to solidify at 27 °C in laminar flow and exposed for 15 minutes under UV condition to maintain free of contaminant. The prepared nutrient agar plates were stored inside the chiller prior to usage

3.3 Nutrient broth preparation

Eight grams of the medium was suspended in one liter of distilled water and was well mixed, it dissolved by heating with frequent agitation. It was boiled for one minute until complete dissolution. The nutrient broth was dispensed into Scott bottles and sterilised in autoclave at 121 °C for 15 minutes. The prepared medium was stored at 2-8 °C. The colour of the nutrient broth would turn into amber and slightly opalescent. The dehydrated medium should be homogeneous, free-flowing and beige in colour. If there were any physical changes, discard the medium (Marshall, 1993)

3.4 Susceptibility/sensitivity test

Determination of bacterial susceptibility was determined by using disc diffusion method. The bacterial broth from 24 hours test culture ($1x10^5$ organisms per mL) was dipped with sterile cotton bud and swabbed on the surface of the nutrient agar. The nutrient agar plates were incubated at 37 °C for about 30 minutes until bacterial layer dried on the surface of the agar. Four filter papers discs (6 mm diameter Whatman) were placed on the surface of one nutrient agar plate 40 µL of test sample (10 mg/ml for sample and 0.1 mg/ml for penicillin) was dispersed in distilled water and then sterilized by filtration through 0.2 µM membrane filter and gently injected on the disc. Finally, the nutrient agar plate was incubated at the 37 °C for 24 hours. Zone of inhibitions were measured by measuring diameter in millimeters including the discs (Cavalieri, 2005). The aqueous extraction of 'Sambung nyawa' leaves was used as a replacement for penicillin that was proposed by Cavalieri (2005)

3.5 Statistical analysis

Each of the analysis repeated triplicate. All the results obtained from the study were analysed using Statistical Packaged for Social Science (SPSS) software version 15 and to determine whether there was significance different on each "sambung nyawa" leaves extract formulation

4.0 RESULTS

4.1 Extraction of 'Sambung nyawa'

The extraction was done using aqueous extraction method and methanol extraction method. The ratio of mixture used was 1 part of dried 'Sambung nyawa' leaves to 20 part of water/methanol. The amount of 'Sambung nyawa' extracted by using aqueous extraction was 15 g equal to 37.5% yield by dry basis. This amount can be referred in Table 4.1. The number of leaves used for this extraction was 40 g. This was supported by Puangpronpitag *et al*., (2010) stated that, aqueous extraction produces the highest yield of 'Sambung nyawa' leaves.

Table 4.1 Amount of 'Sambung nyawa' extracted with aqueous

Types of extraction	Weight of 'sambung nyawa' extract (g)	Percentage yield (%)
Aqueous extraction	15g	37.5

4.2 Susceptibility/ sensitivity test

The aqueous extraction of 'Sambung nyawa' leaves showed antimicrobial activity which could be summarised in Figure 4.2 the tested bacteria were *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*.

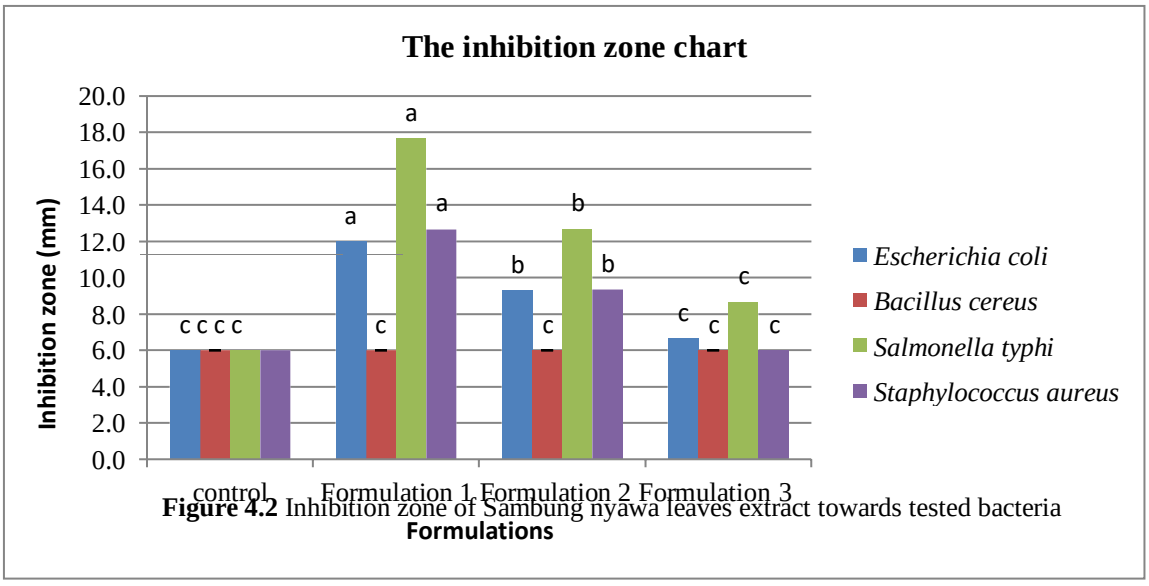

Figure 4.2 Inhibition zone of Sambung nyawa leaves extract towards tested bacteria

The highest antimicrobial activities was shown by Formulation 1 of 'Sambung nyawa' leaves extraction, which inhibits the growth of *Salmonella typhi* with inhibition zone of 17.7 mm, followed by Formulation 2 with inhibition of 12.7 mm and Formulation 3 with 8.7 mm (refer to Figure 4.3). According to Habsah *et al*., (2009) asserted that inhibitory zone diameter ≥ 15 mm was classified as strong, while 10-14.5 mm was classified as moderate and < 10 mm was classified as weak. With reference to this, aqueous extraction of 'Sambung nyawa' has strong antimicrobial effect towards *Salmonella typhi*. For the control, all the bacterial strains did not show any inhibition zone towards the control. The control sample consist only disk immersed with sterilised distilled water.

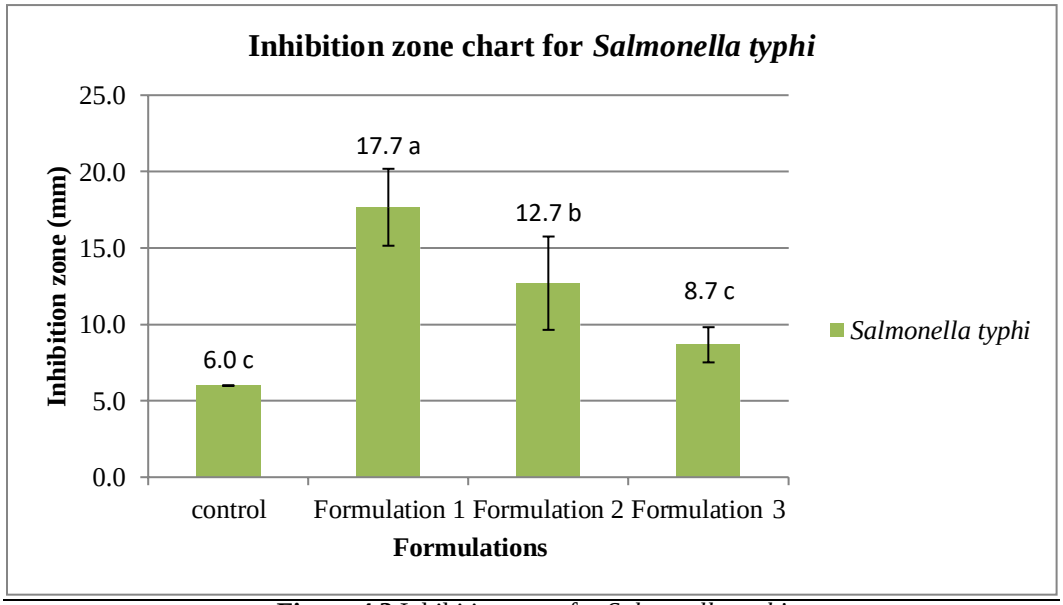

Figure 4.3 Inhibition zone for *Salmonella typhi*

Staphylococcus aureus have the second largest inhibition zone after *Salmonella typhi*, by using the aqueous extraction of 'Sambung nyawa' leaves. For control sample, no inhibition zone can be seen on the plates. For formulation 1, the inhibition zone shows clear zone around the disks with diameter of 12.7 mm, followed by Formulation 2 with 9.3 mm and Formulation 3 with 6.0 mm. Formulation 3 failed to inhibit the growth of *Staphylococcus aureus*. The inhibition zone for *Staphylococcus aureus* can be seen in the Figure 4.4.

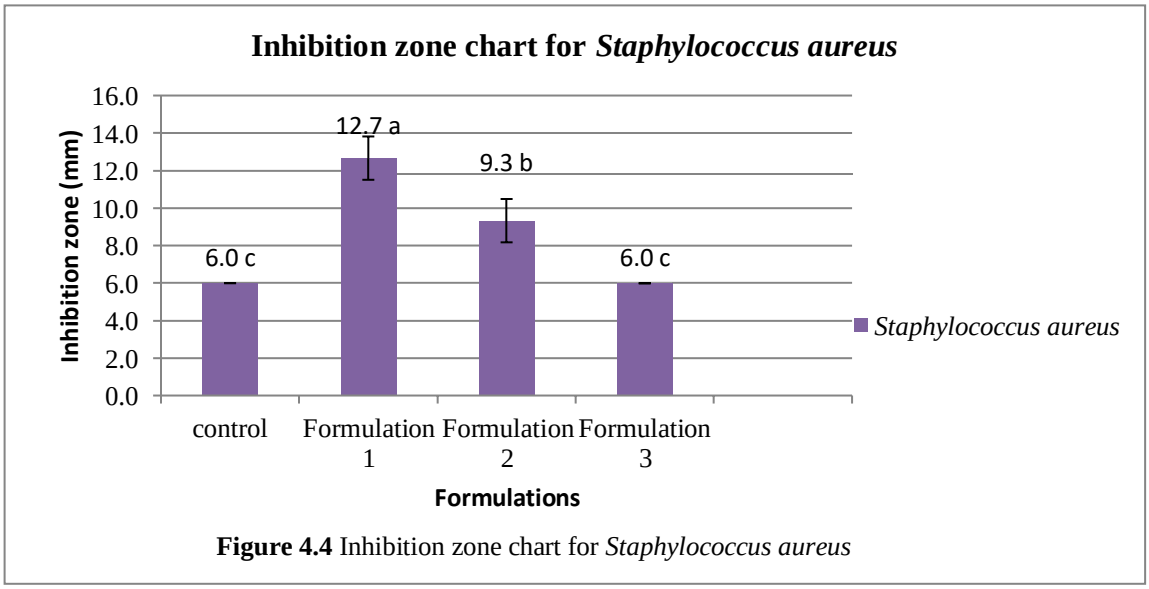

Figure 4.4 Inhibition zone chart for *Staphylococcus aureus*

However, there were antimicrobial activities for Formulation of 1, 2 and 3 of 'Sambung nyawa' leaves. The inhibition can be seen towards all the bacteria; *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*, except for *Bacillus cereus*. There was no inhibition zone observed for *Bacillus cereus* for Formulation of 1, 2 and 3.

The present of inhibition zone towards *Escherichia* coli, *Staphylococcus aureus* and *Salmonella typhi* shows the present of flavonoid which presence in the Sambung nyawa leaves itself; a chemical that can inhibit the growth of bacteria. According to Goyal (2010), "the efficacy of flavonoids was found to be significant against Gram-positive bacteria in comparison to Gram-negative bacteria. These results can be caused by the cell wall structure of both types of bacterial cells. In Gram-negative bacteria, presence of lipopolysaccharide layer outside the peptidoglycan layer makes cell wall more complex and makes it to almost impenetrable for foreign molecules to enter. However, Gram positive bacteria do have only peptidoglycans in their cell wall. This will help in an easy penetration of the molecules inside the cell; thus, shows greater sensitivity".

The inhibition of aqueous extraction of 'Sambung nyawa' towards *Escherichia coli* was in medium antimicrobial strength of aqueous extraction 'Sambung nyawa'. Formulation 1 shows inhibition zone of 12.0 mm, Formulation 2 with 9.3 mm and Formulation 3 with 6.7 mm (refer to Figure 4.5).

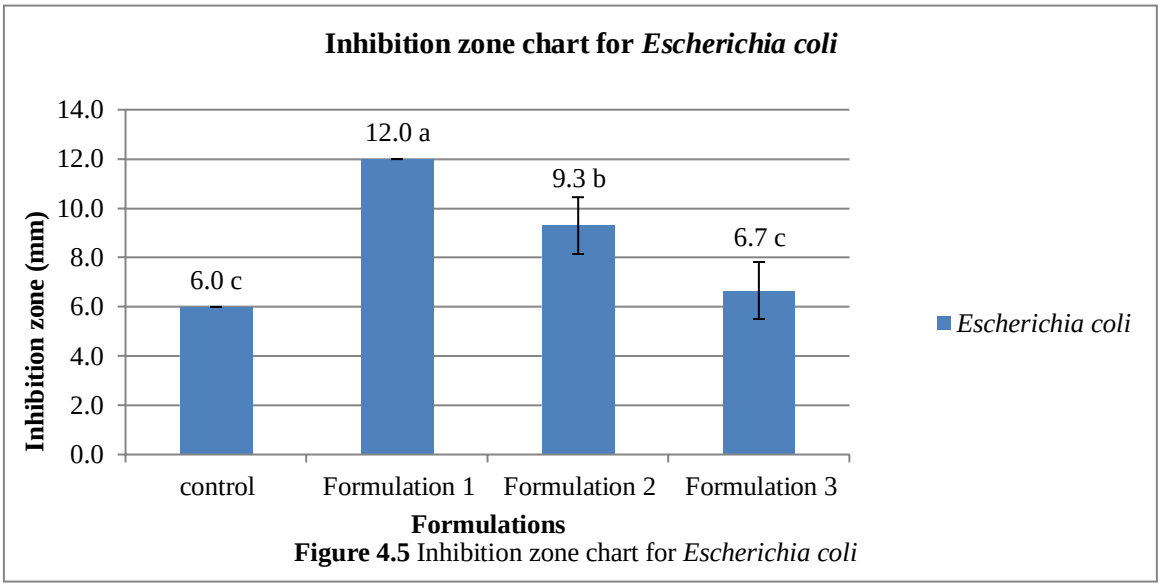

Figure 4.5 Inhibition zone chart for *Escherichia coli*

For inhibition of *Bacillus cereus*, all of the formulations failed to inhibit the growth of *Bacillus cereus*, no clear zone can be observed around the diffusion disks By referring to Figure 4.6, the diameter of the inhibition were only 6 mm for Formulation 1, Formulation 2 and Formulation 3, this show that aqueous extraction of 'Sambung nyawa' was not strong enough to inhibit the growth of *Bacillus cereus*

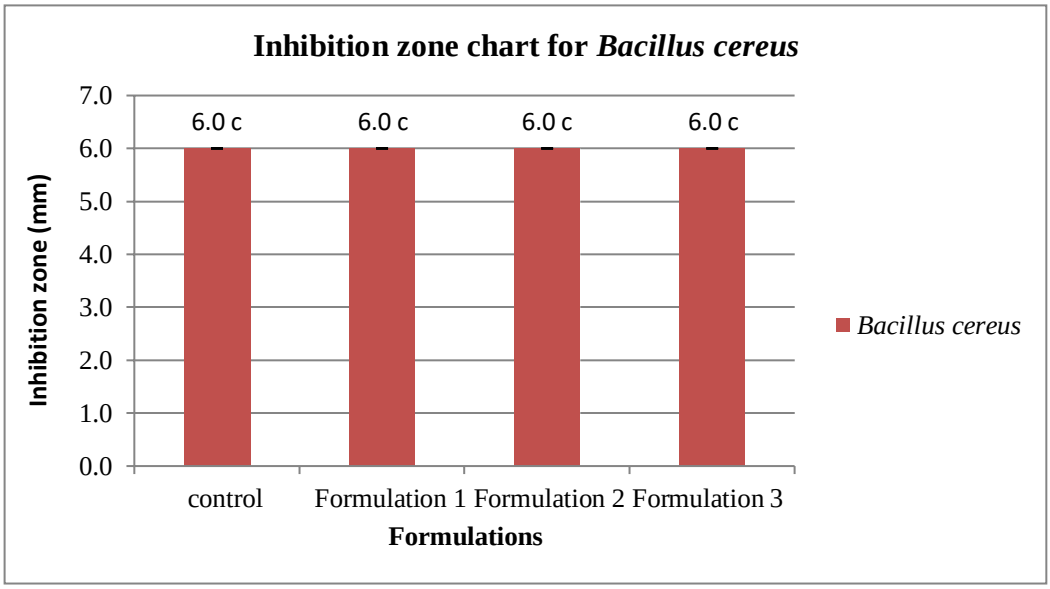

Figure 4.6 Inhibition zone chart for *Bacillus cereus*

Although *Bacillus cereus* is a gram-positive bacterium, it cannot be inhibited by flavonoid that present in 'Sambung nyawa' leaves. This was supported by Kaewseejan *et al*., (2012), saying that aqueous extract and ethanolic failed to inhibit the growth of *Bacillus cereus*. The cell wall thickness differ between Gram positive and Gram negative bacteria. Gram positive bacteria such as *Bacillus cereus* contain a thick layer of peptidoglycan; 20µm to 80µm, that surrounding the cell compare to gram negative bacteria that contains only a thin layer of peptidoglycan between cytoplasmic membrane and the outer membrane. The cell wall essential for the bacterial shape, prevent the cell from bursting due to osmotic pressure and protect the bacterial strain in the environment. According to Bottone (2010), *Bacillus cereus* has a saprophytic life cycle in which spores germinate in soil, with the production of vegetative *Bacillus*, which could then sporulate, maintaining the life cycle. The thick wall of *Bacillus cereus* allows them to do better in dry conditions such as on soils, this thick cell wall also caused it to be insensitive towards flavonoid.

5.0 Conclusion

Overall, in sensitivity test, Formulation 1 (1.50 g dried 'Sambung nyawa' leaves) able to inhibits 17.7 mm of *Salmonella typhimurium*, *Staphylococcus aureus* inhibition zone of 12.7 mm and 12.0 mm inhibition zone for *Escherichia coli*. The recommendations for this study are to variant the extraction methods of 'Sambung nyawa' leaves for better extracts yield. In addition, the 'Sambung nyawa' leaves itself can be incorporated into other product such as candy for it to have more commercialise value.

References

Bottone, E. J., (2010). *Bacillus cereus*, a volatile human pathogen. *Journal of clinical microbiology*. 23(2). 382-398.
Cavalieri, S.J., (2005). Manual of antimicrobial Susceptibility testing. American society for microbiology. 1-481
Chirag, M.M, Shailesh, K.M., and Patel, H.B., (2012). Herbal remedies and its effect: An ultimate review. The southeast Asia Journal of case report and review. 3, 73- 88
Dorman, H.J.D and Deans, S.G., (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88, 308-316.
Food and Agriculture organization of United Nations (FAO) (2011). Guidelines for risk analysis of foodborne antimicrobial resistance. 1-29
Francis, F.B., and Doyle, M.P., (2006). Antimicrobial resistance: Implications for the food system. An expert report, funded by the IFT Foundation. 1-185
Goyal, P., Aggarwal, B.P., Garg. S., (2010). A study on combinatorial effects of various flavonoids for their

antibacterial potential against clinically significant bacterial species (2010). Hacettepe Journal biotechnology and Chemistry. 38 (4), 255-258
Hassan, S.M,. (2008). Antimicrobial activities of Saponin rich guar meal extract. Ph.D for University of Texas A & M, 1-172
Habsah, M.,Zilawati, M.R., Khozirah, S., Jalifah, L., Md. Nordin, H.J.and Abd.Manaf,A.,(2009). Antibacterial and DPPH free scavenging activities of methanolic extracts of *Aapus sp*. (Marine sponges).Pertanika J. Trop. Agri. Sci., 32(1), 43-50
Hendra, R., Ahmad, S., Sukari, A., Shukor, M.Y., and Oskoueian, O., (2011). Flavonoid analyses and antimicrobial activity of various part of Phaleria macrocarpa (Scheff) Boerl Fruit. *International Journal of Molecular Sciences*, 3422-3431
Iskander, M.N., Song, Y., Coupar, I.M., and Jiratchariyakul, W., (2002). Anti-inflammatory screening of the medicinal plant *Gynura procumbens*. Plant Food for human Nutrition, 57, 233-244 .
Jawaid, T., Priyanka, M., and Akanksha, A., (2011). Hypertension and Herbal Plants. *International Research Journal of Pharmacy*, 2, 26-30.
Kaewseejan, N., Puangpronpitag, D., and Nakornriab, M., (2012). Evaluation of Phytochemical Composition and Antibacterial Property of *Gynura Procumbens* extract. *Asian journal of Plant Sciences*, 1-6
Kumar, B., Sandhar, H.K., Prasher, S., Tiwari, P., Salhan, M., and Sharma,. P (2011). A review of phytochemistry and pharmacology of Flavonoids. *International of Pharmaceutical Science*, 1, 1, 25-41
Lamothe, R.B, Mitchell.G, Gattuso. M, Maussa S.D, Malouin.F, anda Boubrab K (2009). Plant antimicrobial agents and their effects on plant and human pathogens. International Journal of molecular Science, Aug : 10(8) : 3400 – 3419.
Lee, H.W., Hakim, P., Rabu, A., and Sani, H.A,. (2011). Antidiabetic effect of *Gynuran Procumbens* leaves extracts involvemodulation of hepatic carbohydrate metabolism in streptozotocin-induced diabetic rats. *Journal of Medicinal Plants Research*,796-812
Marshall, R.T., (1993). Standard methods for the microbiological examination of dairy products. American Public Health Association, Washington, DC. 1-2
Nawawi, A., Nakamura, N., Hattori, M., Kurokawa, M., and Shiraki, K., (1999). Inhibitory Effects of Indonesian Medicinal Plants on the Infection of Herpes Simplex Virus Type 1. Phytotheraphy Research, 13, 37-41
Nazmul, M.H.M., Salmah, I., Syahid, A., and mahmood, A.A., (2010). In vitro screening of antifungal activity of plants in Malaysia. Biomedical research, 22, 28-30.
Puangpronpitag, D., Chaichanadee, S., Naowaratwattana, W., Sittiwet, C., Thammasarn, K.,Luerang,A., and Kaewseejan, N., (2010). Evaluation of nutritional value and antioxidative properties of the medicinal plant *Gynura procumbens* extract. *Asian Journal of Plant Sciences* ,9, 146-151
Patrick, G.L., (1995). An introduction to medicinal chemistry, Oxford University press, 154-204
Rumella, S., Lekatompessy, S., and Sukiman, H., (2007). Isolasi Mikroba Endofitik dari Tanaman Obat Sambung Nyawa (*Gynura Procumbens*) dan Analisis Potensinya sebagai Antimikroba. Pusat Penelitian Bioteknologi,Lembaga Ilmu Pengetahuan, LIPI, Cibinong-Bogor,13,85-90
Rosidah, Yam, M.F., Sadikun, A., Ahmad, M., Akowuah, G.A., and Asmawi, M.A., (2009). Toxicology evaluation of standardized methanol extract of *Gynura Procumbens. Journal of Ethnopharmacology*, 123, 244-249
Tim, C.T.P., and Lamb, A.J., (2005). Antimicrobial activity of flavonoids. *International Journal of Flavonoids*. 343-356
Zahra, A.A., Kadir, F.A., Mahmood, A.A., Hadi, A.A.A., Suzy, S.M., Sabri, S.Z., Latif, I.I.,and Ketuly, K.A., (2011). Acute Toxicity study and wound healing potential of *Gynura procumbens* leaf extract in rats. *Journal of medicinal Plants Research*, 5 (12), 2251-2558