MICROBIOLOGICAL QUALITY OF RAW EDIBLE VEGETABLES SAMPLED FROM CAFETERIAS AT UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN, KELANTAN

By

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-	To/Until
%	Percentage
/	Over to
=	Equal to
>	More than
°C	Degree Celsius
CFU	Colony Forming Units
E.coli	*Escherichia coli*
S.aureus	*Staphylococcus aureus*
B.cereus	*Bacillus cereus*
mL	milliliter
L	Liter
g	gram
XLD	Xylose Lysine Deoxycholate
EMB	Eosin Methylene Blue
RV	Rappaport Vassiliadis
MSC	Mannitol Selenite Cystine
BSA	Bismuth Sulphite agar
BPA	Baird Parker agar
BCA	Bacillus cereus agar
MKKM	Makmal Keselamatan dan Kualiti Makanan
WHO	World Health Organization
USFDA	United States Food and Drug Administration
USM	Universiti Sains Malaysia

HUSM	Hospital Universiti Sains Malaysia
CDCP	Centre for Disease Control and Prevention

ABSTRAK

KUALITI MIKROBIAL SAYURAN MENTAH YANG DISAMPEL DARI KAFETERIA UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN, KELANTAN

Kes keracunan makanan yang berpunca daripada pengambilan buah-buahan dan sayuran mentah telah meningkat kebelakangan ini. Sayuran mentah yang tercemar dengan bacteria merbahaya boleh menjejaskan kesihatan kerana sehingga kini masih tiada lagi rawatan berkesan yang dapat digunakan untuk proses membasmi kuman yang terdapat pada sayuran mentah. Satu kajian telah dijalankan untuk mengesan bacteria merbahaya pada sayuran mentah yang dijual di kafeteria-kafeteria di Kampus Kesihatan Universiti Sains Malaysia. Sebanyak 30 sampel daripada enam jenis sayuran mentah seperti timun, pegaga, selom, daun salad, ulam raja dan kacang botol telah dianalisa. Persampelan tersebut mengambil masa dua bulan dan 50 gram telah diambil bagi setiap sampel untuk dianalisa. Pengesanan kehadiran dan identifikasi bakteria merbahaya seperti *E.coli, Bacillus cereus, Staphylococcus aureus, Salmonella* dan "total coliforms" adalah berdasarkan "Australian Microbiological Examination of Food", (1993) dan kualiti mikrobial sayuran mentah dianalisa mengikut "Microbiology Guidelines for Ready to Eat Food " MOH Malaysia (1985). Keputusan kajian menunjukkan "total coliforms" telah dikesan dalam semua sampel yang dianalisa . Sebanyak enam (20%) sampel (1 timun, 1 pegaga, 3 selom dan 1 ulam raja) telah dikesan dengan kehadiran bakteria seperti *E.coli* and *Salmonella* manakala sampel daun salad dan kacang botol tidak dikesan dengan bakteria tersebut. *E.coli* telah dikesan (3-100 CFU/g, sederhana) dalam 25% sampel pegaga, 22.2% sampel selom ($3x10^3$ -$5x10^3$ CFU/g, tidak memuaskan), dan

50% sampel ulam raja ($1x10^3$ CFU/g, tidak memuaskan). Manakala, *Salmonella* telah dikesan dalam 12.5% sampel timun dan 11.1% sampel selom. *Bacillus cereus* dan *Staphylococcus aureus* tidak dikesan dalam semua sampel sayuran mentah yang dianalisa. Keputusan kajian menunjukkan "total coliforms" telah dikesan dalam semua sampel sayuran mentah dan terdapat juga sampel-sampel yang dikesan dengan *E.coli* dan *Salmonella,* melebihi tahap keselamatan yang ditetapkan oleh "Microbiology Guidelines for Ready to Eat Food " MOH Malaysia (1985). Maka sayuran mentah yang tercemar mungkin boleh membahayakan kesihatan. Ujian lanjut telah membawa kepada penemuan enteric serovar weltevreden yang telah dikaitkan dengan beberapa kes keracunan makanan (Bangtrakulnonth *et al.*, 2004). Namun, *E.coli* merbahaya tidak dikesan dalam kajian ini. Oleh itu, kajian lanjut harus dijalankan untuk mengenal pasti punca pencemaran agar langkah-langkah penjagaan dapat diambil untuk meningkatkan kualiti mikrobial sayuran mentah yang dijual di Kampus Kesihatan, USM.

ABSTRACT

MICROBIOLOGICAL QUALITY OF RAW EDIBLE VEGETABLES SAMPLED FROM CAFETERIAS AT UNIVERSITI SAINS MALAYSIA, KUBANG KERIAN, KELANTAN

Raw fruits and vegetables have been known to serve as vehicles of human diseases and food borne outbreaks have increased in recent years. Raw vegetables contaminated with pathogenic bacteria may lead to health hazard as there is no treatment that can be relied upon for decontamination of the vegetables. A study was conducted to screen and identify the bacterial pathogens present in selected raw vegetables sold in the cafeterias in USM Health Campus. A total of 30 samples from six commonly consumed raw vegetables viz., cucumber, winged bean, lettuce, wild cosmos, pennywort leaves and water dropwort were analyzed. Samples were randomly collected over a period of two months. Fifty grams (50g) from each sample were taken for microbiological analysis. The presence and identification of pathogenic bacteria viz., *E.coli, Bacillus cereus, Staphylococcus aureus, Salmonella* and total coliforms was determined based on the "Australian Microbiological Examination of Food", (1993) and the analysis was conducted in triplicates. Compliance to the microbiological quality (CFU/g) of raw vegetables is based on the "Microbiology Guidelines for Ready to Eat Food" MOH Malaysia (1985). The results showed that the total coliforms were presence in all vegetables samples (≥1,000 CFU/g, unsatisfactory). A total of 6 (20%) samples (1 cucumber, 1 pennywort, 3 water dropwort and 1 wild cosmos) showed presence of pathogenic bacteria namely *E.coli* and *Salmonella*. However, lettuce and winged bean did not

show presence of any of the pathogenic bacteria. *E.coli* was detected (3-100 CFU/g , marginal) in 25% of the pennywort, 22.2% water dropwort ($3x10^3$ -$5x10^3$ CFU/g, unsatisfactory), and 50% wild cosmos samples ($1x10^3$ CFU/g, unsatisfactory). Meanwhile, *Salmonella* was detected in 12.5% of the cucumber and 11.1% water dropwort samples. However, the presence of *Bacillus cereus* and *Staphylococcus aureus* were not detected in any of the raw vegetables sampled. Our findings revealed that all vegetables samples showed presence of total coliforms, and some with *E.coli* and *Salmonella*, exceeding the safe level described by "Microbiology Guidelines for Ready to Eat Food" MOH Malaysia (1985), and this can be construed that the contaminated vegetables may pose considerable hazards on human health. Furthermore, further identification has led to the detection of an enteric serovar weltevreden which has been implicated with several cases of food borne illnesses (Bangtrakulnonth, 2004). However, no pathogenic *E.coli* was isolated in this study. Thus, further study should be carried out to determine the source of contamination so that measures can be taken to improve the microbiological quality of raw vegetables sold in Health Campus, USM.

CHAPTER 1 INTRODUCTION

1.1 Background of Study

Food borne illness refers to any type of illness resulting from consumption of contaminated food or beverage. According to the World Health Organization (WHO), contaminated food contributes to 1.5 billion cases of diarrhea in children each year, resulting in more than three million premature deaths.

Food poisoning is a major health risk for those consuming raw foods, and thus increased intake of raw vegetables is associated with greater incidence of food borne illness. According to Sivapalasinga *et al* (2004), food poisoning attributed to contaminated raw produce has risen tenfold since the 1970s.

According to the Department of Statistics, Malaysia, food poisoning incidences certainly are not rare in the country, especially in Kelantan, Terengganu and Kuala Lumpur, with the incidence rate of 85.1, 83.4 and 75.1 per 100,000 of the populations, respectively (The Star Online, 2012).

Food that is contaminated with pathogenic bacteria does not exhibit any particular appearance indicating contamination. Therefore, it is very difficult to differentiate contaminated food from uncontaminated food, unless microbiological analysis is done.

Vegetables play a vital role in our diets, as they support the normal functioning of the body systems by providing vitamins, minerals and fibres. Examples of vegetables that are eaten raw are cucumber, lettuce, winged bean, wild cosmos, tomatoes, long beans and etc.

Raw vegetables are a rich source of antioxidants which include vitamins C and E, folic acid, lycopene, alpha-carotene and beta-carotene. Antioxidants are the chemical compounds that help prevent or reverse cellular damage caused by free radicals.

According to the University of Michigan Integrative Medicine program, in addition to aiding in the prevention of heart disease and cancer, antioxidants also play a role in reducing the risk of Alzheimer's disease and arthritis and in slowing the aging process. Some of the antioxidants are not as readily absorbed by the body from cooked vegetables as they are from raw vegetables (http://www.livestrong.com/article/258893-what-are-the-benefits-of-eating-raw-vegetables/).

Besides that, raw vegetables contain vitamins A, C, E, B-complex vitamins, potassium and calcium. Vitamin A is essential to our vision and skin health while vitamin C is involved in collagen production and iron absorption. Vitamin E is a powerful antioxidant. B-complex vitamins aid in red blood cell production. Potassium helps to regulate blood pressure while calcium is essential for healthy bones.

In addition, raw vegetables contains abundant of dietary fibres. Diets rich in fibres lower cholesterol level, help prevent cardiovascular disease and also can prevent constipation.

The practice of consuming edible raw vegetables had long existed. Nowadays with the increase in health consciousness among the people and along with the scientific

evidence showing raw vegetables is good for our health, many people had chosen to increase raw edible vegetables intake in their diet. Although consuming raw vegetables provides nutritional benefits, issues of public health concern have arisen. Fruits and vegetables carry natural non-pathogenic microorganisms. During the process of growth, harvest, transportation and further processing and handling, these fruits and vegetables can be contaminated with pathogens. Reports have indicated that these raw vegetables can be contaminated with various bacterial pathogens, including *Salmonella, Shigella, E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter* (Beuchat, 1996).

Therefore, it is imperative to screen raw edible vegetables for the presence of several selected pathogens commonly associated with food borne illnesses. This is to ensure the raw edible vegetables are free from potential hazardous microorganisms prior to consumption.

1.2 Rationale of Study

Raw vegetables if contaminated with pathogenic bacteria, may lead to health hazard as there is no effective treatment that can be relied upon for decontamination. Investigations had been done on outbreaks and have identified issues such as the use of manure as fertilizers, agricultural water quality, the presence of animals in fields or packing areas, and the health and hygiene of workers handling the fresh produce during production, packing, processing, transportation, distribution, or preparation of produce are associated with the outbreak of illness.

In addition, increased risk of illness outbreaks associated with the consumption of raw produce is due to the globalized distribution of fresh fruit and vegetables which involves extended food storage (Sivapalasingam *et al* 2004). Human exposure to a wider variety of food-borne pathogens is potentially increased due to the distribution of fruit and vegetables over larger geographical areas (USFDA 2000).

Furthermore, as Universiti Sains Malaysia (Kubang Kerian) is known as the health campus, the standards of hygiene should be better compared to other places. If our cafeterias do not have satisfactory hygiene standards, this will lead to an increase in incidence of food borne illness which will involve the students and staff of USM and HUSM and eventually causing an increase in the unnecessary treatment of which can be avoided if our hygiene is up to the standard.

Therefore, it is important to carry out this study on the presence of potential hazardous microorganisms in raw edible vegetables sold in the cafeterias to find out whether the raw vegetables served are safe for consumption.

1.3 Research Objectives

The research objectives are:

1. To determine the microbial quality of raw edible vegetables sold in the cafeterias of Health Campus, USM, Kubang Kerian.
2. To screen and identify the presence of microorganisms namely *Salmonella, Bacillus cereus, Staphylococcus aureus* , *E.coli* and total coliforms present in the raw edible vegetables sampled from the cafeterias at Health Campus, USM Kubang Kerian.

1.4 Research Conceptual Framework

The research conceptual framework is as shown in Figure 1.1. This laboratory study include the analysis of common raw vegetables such as cucumber, pennywort, winged bean, lettuce, wild cosmos and water dropwort and involve the enumeration and speciation of microorganisms present on the raw vegetables sampled. These samples have been analyzed for the presence of *E.coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* and total coliforms which are commonly isolated from the fresh produce.

Microbiological methods used were based on the method recommended by Makmal Kualiti dan Keselamatan Makanan (MKKM) which was adopted from Standards Australia (1993). Thirty samples (n = 30) were collected randomly over a period of two months from cafeterias in USM Health Campus, Kubang Kerian. The samples were transferred to sterile plastic bags and were immediately transported on ice in an icebox to the laboratory for analysis. At least 50 g of sample were taken for processing.

The presence and identification of pathogenic bacteria viz., *E. coli, Bacillus cereus, Staphylococcus aureus, Salmonella* and total coliforms were determined based on the "Australian Microbiological Examination of Food", (1993) and the analysis was conducted in triplicates. Compliance to the microbiological quality (CFU/g) of raw vegetables is based on the "Microbiology Guidelines for Ready to Eat Food" MOH Malaysia (1985).

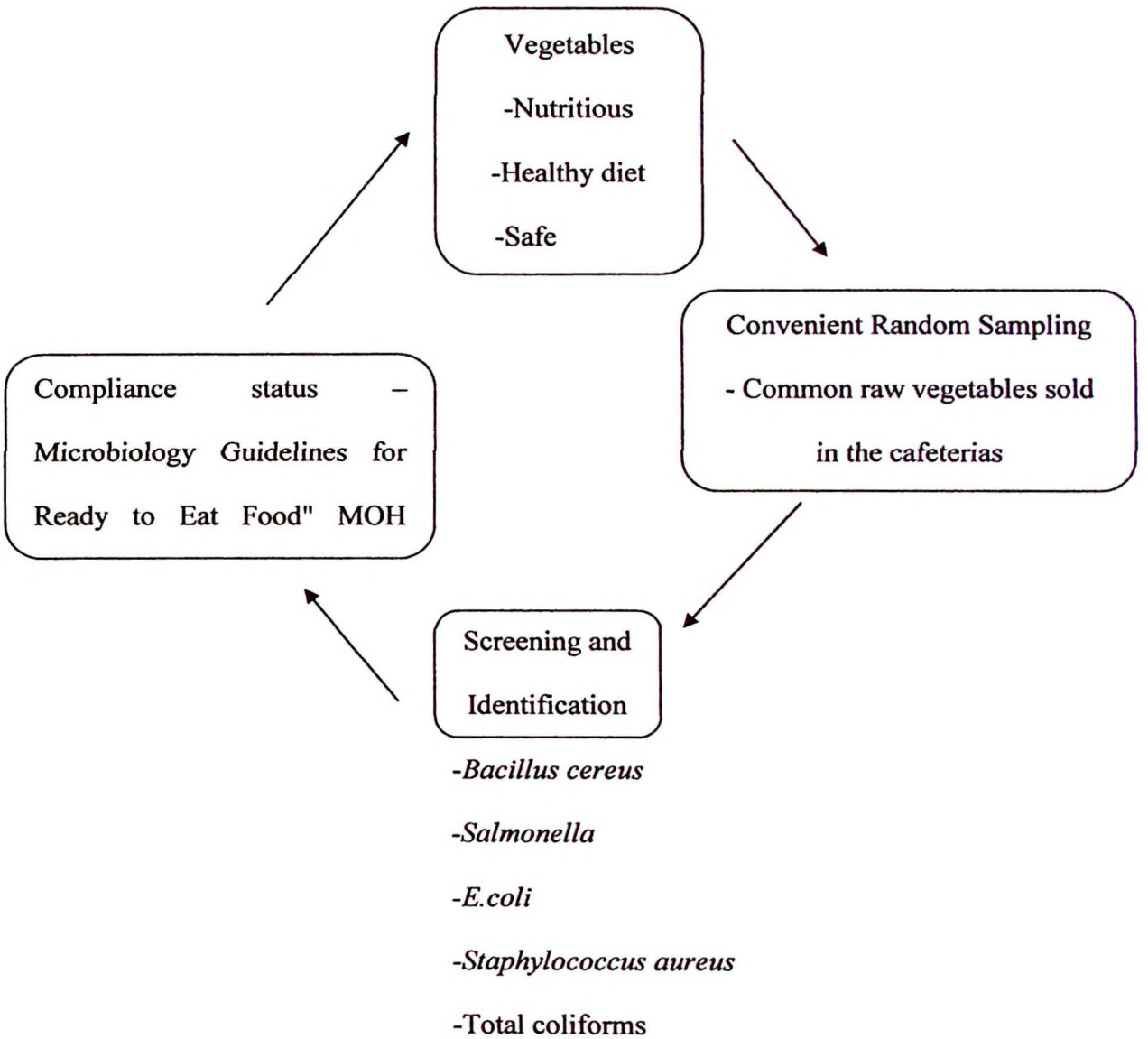

Figure 1.1 Research Conceptual Framework

CHAPTER 2 LITERATURE REVIEW

Raw fruits and vegetables have been known to serve as vehicles of human disease for at least a century. The food borne outbreaks caused by contaminated fresh fruit and vegetables has increased in recent years (Mukherjee *et al.*, 2006). There are a number of reports indicating that raw vegetables may harbour potential food borne pathogens (Nyuyen-the and Carlin, 1994; Beuchat, 1996). Through various studies, *Listeria monocytogenes* (Schlech *et al.*, 1983), *Salmonella* (Doyle, 1990) and *Escherechia coli* (Nyuyen-the and Carlin, 1994) have been isolated from raw vegetables.

According to a review on surface decontamination of fruits and vegetables eaten raw done by Larry R. Beuchat, there were many cases of the past during eighteen and nineteen century that had linked consuming raw vegetables with illness. Morse (1899) linked typhoid infection to the consumption of celery while Warry (1903) on the other hand, attributed an outbreak of typhoid fever to eating watercress grown in soil fertilized with sewage. Ten years later, Pixley (1913) recorded two cases of typhoid from eating uncooked rhubarb which was grown in soil known to have been fertilized with typhoid excreta. In 1912, Creel demonstrated that lettuce and radishes grown in soil containing *Bacillus typhosa* (now *Salmonella typhi*) harboured the organism on their surfaces for up to 31 days. Melick (1917) recovered typhoid bacilli from mature lettuce and radish harvested from soil that had been inoculated at the time seeds were planted.

Salmonella have been isolated from many types of raw fruits and vegetables (Beuchat, 1996b; Wells and Butterfield, 1997). Salmonellosis is characterised by fever, diarrhoea, abdominal cramps and vomiting usually lasting 4-7 days (Anon., 2001). Outbreaks of salmonellosis have been linked to various fruits and vegetables,

including tomatoes (CDCP, 1993; Hedberg *et al.*, 1993; Wood *et al.*, 1991), bean sprouts (Mahon *et al.*, 1996), melons (Blostein, 1991), unpasteurized orange juice (Cook *et al.*, 1990) and apple juice (CDCP, 1975).

Escherichia coli is commonly found in the normal microflora of the intestinal tracts of humans and other warm-blooded animals. Strains that cause diarrhoeal illness are categorized into groups such as enterotoxigenic, enterohaemorrhagic, enteropathogenic, enteroinvasive, diffuse-adhering and enteroaggregative (Doyle *et al.*, 1997) on the basis of virulence properties, mechanisms of pathogenicity, clinical syndromes and antigenic characteristics. Fruits and vegetables can become contaminated with one or more of these groups while in the field or during post-harvest handling.

Enterohaemorrhagic *E. coli* O157:H7 which has recently been recognized as a food borne pathogen was found growing on cantaloupe and watermelon cubes (del Rosario and Beuchat, 1995), shredded lettuce (Diaz and Hotchkiss, 1996) and sliced cucumbers (Abdul-Raouf *et al.*, 1993), and in apple cider (Zhao *et al.*, 1993).

The world's largest reported vegetable borne outbreak to date occurred in Japan in 1996 and of the over 11,000 people affected, about 6,000 were culture confirmed. The outbreak involved the death of three school children and was caused by E. coli O157:H7 (Ministry of Health and Welfare of Japan, 1997).

The microbial ecology of raw fruits and vegetables, particularly cut products suggest that pathogens such as *Staphylococcus aureus*, *Aeromonas* and *Yersinia enterocolitica* have the potential to cause outbreaks (Anon., 2000a). *Staphylococcus*

aureus is known to be carried in the nasal passages of healthy food handlers and has been detected on raw produce (Abdelnoor *et al.*, 1983) and in ready-to-eat vegetable salads (Houang *et al.*, 1991).

Vegetables can become contaminated with such pathogenic organisms while growing, during harvest, from post-harvest handling, or during distribution. Bacteria such as *Clostridium botulinum, Bacillus cereus* and *Listeria monocytogenes*, which are capable of causing illness, are normal inhabitants of many soils, whereas *Salmonella, Shigella, Escherichia coli* and *Campylobacter* are found in the intestinal tracts of animals, including humans, and are more likely to contaminate raw fruits and vegetables through contact with faeces, sewage, untreated irrigation water or surface water. Contamination may also occur during post-harvest handling, including at points of preparation by street vendors, in food-service establishments and in the home (Beuchat LR *et al*, 1998).

Several reasons for the increase in produce-related human infections have been proposed. These include changes in dietary habits, including a higher per capita consumption of fresh or minimally processed fruits and vegetables, and the increased use of salad bars and meals eaten outside the home (Altekruse *et al*, 1996).

During harvest and transport raw vegetables may be bruised resulting in the release of plant nutrients, providing nutrients for microorganisms present on the surface of the vegetable to grow.

Possible sources are soil, faeces (manure, both of human and animal origin), water (irrigation, cleaning), ice, animals (including insects and birds), handling of the

products, harvesting and processing equipment, and transportation (Beuchat, 2002; Johannessen *et al.*, 2002).

The mechanism in which raw fruits and vegetables may become contaminated with pathogenic microorganisms is shown in Figure 2.1. The bacterial pathogens which are commonly isolated from raw vegetables are summarized in Table 2.1.

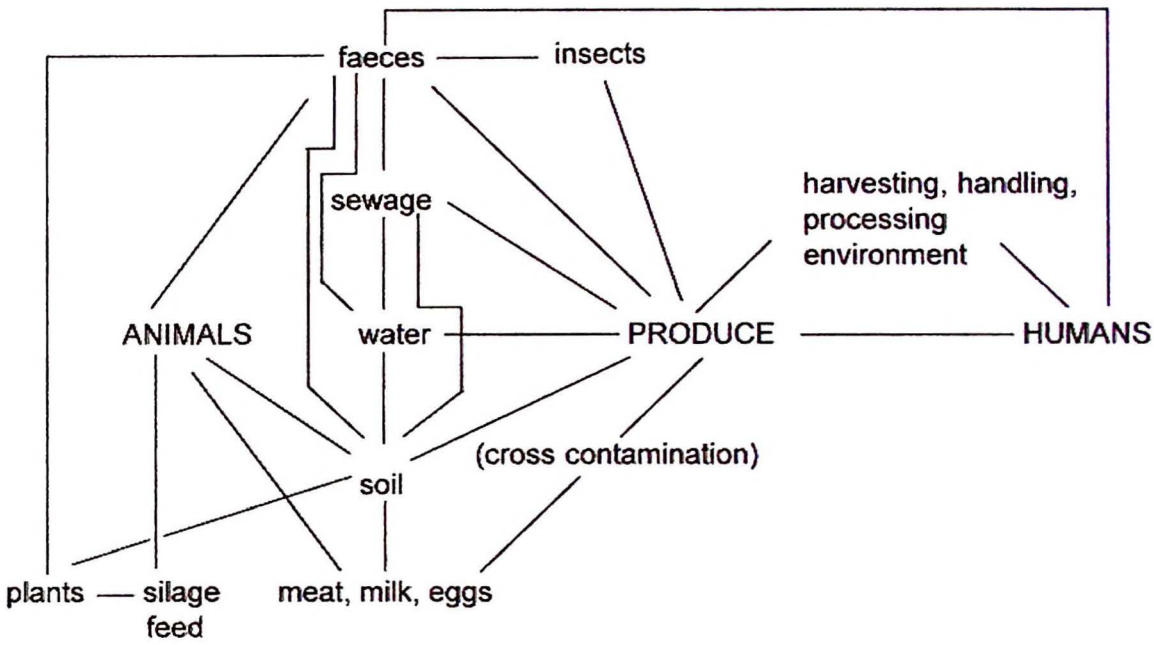

Figure 2.1 Mechanism of contamination of raw fruits and vegetables with pathogenic microorganisms (adapted from Beuchat, 1996b)

Table 2.1 Bacterial pathogens isolated from raw vegetables

Vegetable	Country	Pathogen	Prevalence	Reference
Lettuce	Italy	*Salmonella*	82/120 (68%)	Ercolani (1976)
	Canada	*Campylobacter*	2/67 (3.1%)	Park and Sanders (1992)
	Canada	*L. monocytogenes*	3/15 (20%)	Odumeru *et al.* (1997)
	Lebanon	*Staphylococcus*	(14.3%)	Abdelnoor *et al.* (1983)
	Netherlands	*Salmonella*	2/28 (7.1%)	Tamminga *et al.* (1978)
	Saudi Arabia	*L. monocytogenes*		Salamah (1993)
	Saudi Arabia	*Y. enterocolitica*		Salamah (1993)
	Spain	*Salmonella*		Garcia-Villanova Ruizetal.(1987b)
	United States	*Aeromonas*		Callister and Agger (1989)
Cucumber	Malaysia	*L. monocytogenes*	4/5 (80%)	Arumugaswamy *et al.* (1994)
	Pakistan	*L. monocytogenes*	1/15 (6.7%)	Vahidy (1992)
	Saudi Arabia	*L. monocytogenes*		Salamah (1993)
	Saudi Arabia	*Y. enterocolitica*		Salamah (1993)
	United States	*L. monocytogenes*		Heisick *et al.* (1989b)
Leafy vegetables	Malaysia	*Salmonella*	1/24 (4%)	Arumugaswamy *et al.* (1995)
	Malaysia	*L. monocytogenes*	5/22 (22.7%)	Arumugaswamy *et al.* (1994)
Bean sprouts	Malaysia	*L. monocytogenes*	6/7 (85%)	Arumugaswamy *et al.* (1994)
	Malaysia	*Salmonella*	2/10 (20%)	Arumugaswamy *et al.* (1994)
	Sweden	*Salmonella*		Andersson and Jong (1989)
	Thailand	*Salmonella*	30/344 (8.7%)	Jerngklinchan and Saitanu (1993)
Tomato	Pakistan	*L. monocytogenes*	2/15 (13.3%)	Vahidy (1992)
Carrot	Lebanon	*Staphylococcus*	(14.3%)	Abdelnoor *et al.* (1983)

	Saudi Arabia	*L. monocytogenes*		Salamah (1993)
	Saudi Arabia	*Y. enterocolitica*		Salamah (1993)
Soybean sprouts	United States	*B. cereus*		Portnoy *et al.* (1976)
Mungbean sprouts	United States	*Salmonella*		O'Mahony *et al.* (1990)
Mushrooms	United States	*C. jejuni*	3/200 (1.5%)	Doyle and Schoeni (1986)
Prepacked salads	Northern Ireland	*L. monocytogenes*	3/21 (14.3%)	Harvey and Gilmour (1993)
	United Kingdom	*L. monocytogenes*	4/60 (13.3%)	Sizmur and Walker (1988)
	United Kingdom	*L. monocytogenes*		Velani and Roberts (1991)
Cabbage	Canada	*L. monocytogenes*	2/92 (2.2%)	Schlech *et al.* (1983)
	Canada	*L. monocytogenes*	1/15 (6.7%)	Odumeru *et al.* (1997)
	Mexico	*E. coli O157:H7*	1/4 (25.0%)	Zepeda-Lopez et al. (1995)
	Peru	*V. chlolerae*		Swerdlow *et al.* (1992)
	Saudi Arabia	*L. monocytogenes*		Salamah (1993)
	Saudi Arabia	*Y. enterocolitica*		Salamah (1993)
	Spain	*Salmonella*	7/41 (17.1%)	Garcia-VillanovaRuiz et al.(1987b)
	United States	*C. botulinum*	1/337 (0.3%)	Lilly *et al.* (1996)
	United States	*L. monocytogenes*	1/92 (1.1%)	Heisick *et al.* (1989b)
Cauliflower	Netherlands	*Salmonella*	1/13 (7.7%)	Tamminga *et al.* (1978)
	Spain	*Salmonella*	1/23 (4.5%)	Garcia-Villonova Ruizetal. (1987b)
	United States	*Aeromonas*		Berrang *et al.* (1989)
Egg plant	Netherlands	*Salmonella*	2/13 (1.5%)	Tamminga *et al.* (1978)
Spinach	Canada	*Campylobacter*		Park and Sanders (1992)
	Spain	*Salmonella*	2/60 (3.3%)	Garcia-Villanova Ruizetal.(1987b)
	United States	*Aeromonas*	2/38(5.2%)	Callister and

				Agger (1989)
Salad vegetables	Canada	*L. monocytogenes*	6/15 (40%)	Odumeru *et al.* (1997)
	Egypt	*Shigella*	3/250 (1.2%)	Satchell *et al.* (1990)
	Egypt	*Saureus*	3/36 (8.3%)	Satchell *et al.* (1990)
	Spain	*Aeromonas*	2/33 (6.1%)	Garcia-Gimeno *et al.* (1996a)
	Spain	*L. monocytogenes*	21/70 (30%)	Garcia-Gimeno et al. (1996b)
	United States	*Staphylococcus*		Harris *et al.* (1975)
	Germany	*L. monocytogenes*	6/263 (2.3%)	Breer and Baumgartner (1992)
	Northern Ireland	*L. monocytogenes*	4/16 (25%)	Harvey and Gilmour (1993)
	United States	*C. botulinum*	2/82 (2.4%)	Lilly *et al.* (1996)
	United Kingdom	*Y. enterocolitica*		Brockelhurst *et al.* (1987)
Celery	Mexico	*E. coli O157:H7*	6/34 (17.6%)	Zepeda-Lopez *et al.* (1995)
	Spain	*Salmonella*	2/26 (7.7%)	Garcia-Villanova Ruizetal.(1987b)
Chili	Surinam	*Salmonella*	5/16 (31.3%)	Tamminga et al. (1978)

(adapted from Beuchat ,1996b)

CHAPTER 3 METHODOLOGY

3.1 Materials and Instruments

This study requires the use of several laboratory apparatus and equipments. The materials and equipments are listed below:

- Bunsen burner
- Interscience Stomacher 400ml Bagmixer-400P
- Interscience Stomacher Bag
- Incubators with various temperatures
- Wire loop
- Petri dish
- Hockey stick
- Pipettes
- Pipette tips
- Biohazard bags
- Universal bottles
- Duran bottles
- 70% alcohol
- Culture media

  -3M ™ Petrifilm E.coli/Coliform Count Plate

  -Peptone water (Oxoid)

  -Buffered Peptone water (Oxoid)

  -Tryptone water (Oxoid)

  -Rappaport Vassiliadis broth (Oxoid)

  -Mannitol Selenite Cystine broth (Oxoid)

  -Xylose Lysine Deoxycholate medium (Oxoid)

-Bismuth Sulphite (Merck)

-Eosin Methylene Blue (Oxoid)

-Baird Parker (Merck)

-Bacillus cereus agar base (Oxoid)

3.2 Study Design

Prior to sampling, a quick survey was done to determine the types of commonly sold raw vegetables in the cafeterias. Thirty raw vegetables were sampled by convenience sampling at cafeterias in Universiti Sains Malaysia, Kubang Kerian, Kelantan. Six types of commonly eaten raw vegetables viz., cucumber, pennywort, winged beans, wild cosmos, water dropwort and lettuce were used in this study. The vegetables sampled were subjected to microbial analysis. The summary of the methodology was shown in Figure 3.1.

3.3 Sample Collection

A total of 30 raw vegetables samples were collected during November 2012 through January 2013. The types of raw vegetables collected consist of nine water dropwort samples (30.0%), eight cucumber samples (26.67%), four pennywort samples (13.33%), four winged bean samples (13.33%), three lettuce samples (10%) and two wild cosmos samples (6.67%). The samples were transferred into sterile plastic bags and were transported on ice (4°C) immediately to the laboratory for analysis. At least 50 g of each sample was taken for processing. The samples were analyzed for the

presence of *Escherichia coli*, total coliforms, *Salmonella* species, *Staphylococcus aureus* and *Bacillus cereus*.

3.4 Isolation and Identification of Microorganisms

3.4.1 Isolation of total coliform and fecal coliform

Samples were prepared through standard weighing and serial dilution, with stomaching and pH adjustment if necessary. The petrifilms were placed on a flat surface. Next, to inoculate, the top layer was lifted to expose the plating surface, and with a pipette, 1 mL of the diluted sample was added. For one sample, several dilutions were used. The top film was then slowly rolled down and the "spreader" was used for even distribution.

The petrifilms were left undisturbed for one minute for the solidification of the gel. The petrifilms were then incubated at 37±1°C for up to 48 hours. Confirmed coliforms are red and blue colonies with associated gas bubbles. Confirmed *E.coli* coliforms are blue colonies with associated gas bubbles.

For confirmation of presence of *E.coli*, the blue colony was streaked on eosin methylene blue agar and incubated at 37±1°C for 18-24 hours, where confirmation is made by the presence of green metallic sheen colonies. Three typical green metallic sheen colonies was chosen and sub-cultured into tryptone water at 44.0-44.5°C for 24 hours before carrying out the indole test. Positive indole test is confirmative of *E.coli*. The positive control and negative control used were *E.coli* and *E.aerogenes* respectively.

3.4.2 Isolation of *Salmonella* species

A 25 g of sample was pummeled in a stomacher with 225 mL of Buffered Peptone water and the homogenate was pre-enriched at 37±1°C for 16-20 hours. 0.1 mL of sample was subcultured into 10 mL Rappaport Vassiliadis broth and enriched at 42±1°C for 18-24 hours.

Another 1.0 mL of sample was sub-cultured into 10 mL of Mannitol Selenite Cystine broth and enriched at 37±1°C for 18-24 hours. Using wire loop, both enrichment broth were streaked on XLD and BS agar and incubated for 18-24 hours for XLD agar and 48 hours for BS agar at 37±1°C.

If present, three typical colourless colonies with or without black dot in the centre per XLD plate were taken, sub-cultured into peptone water and identified by biochemical and serological tests. The positive control and negative control used were Salmonella sp. and *Citrobacter freundii* respectively.

3.4.3 Isolation of *Staphylococcus aureus*

1 mL of sample is added to 9 mL of 0.1% peptone water and serial dilutions were made. With appropriate dilutions, enriched samples were streaked on Baird Parker Agar (BPA) and the plate was incubated at 37±1°C for 48 hours. Appearances of jet black colonies surrounded by white halo were considered to be presumptive *S. aureus*. Positive coagulase test is confirmative of presence of *S.aureus*. The positive control and negative control used were *S.aureus* and *S. epidermidis* respectively.

3.4.4 Isolation of *Baccilus cereus*

1 mL of sample is added to 9 mL of 0.1% peptone water and serial dilutions were made. With appropriate dilutions, enriched samples were streaked on Bacillus Cereus Agar (BCA) and the plate was incubated at 37±1°C for 24 hours. The numbers of typical colonies were counted. The plate was then re-incubated at room temperature for 24 hours. Again, the numbers of the typical colonies formed were counted.

If present, 10 colonies were taken randomly for smear and stain which is confirmative of *Bacillus cereus. B. cereus* will appear as large Gram-positive bacilli in short-to-long chains; spores are ellipsoidal, central to sub-terminal, and do not swell. The positive control used was *Bacillus cereus.*

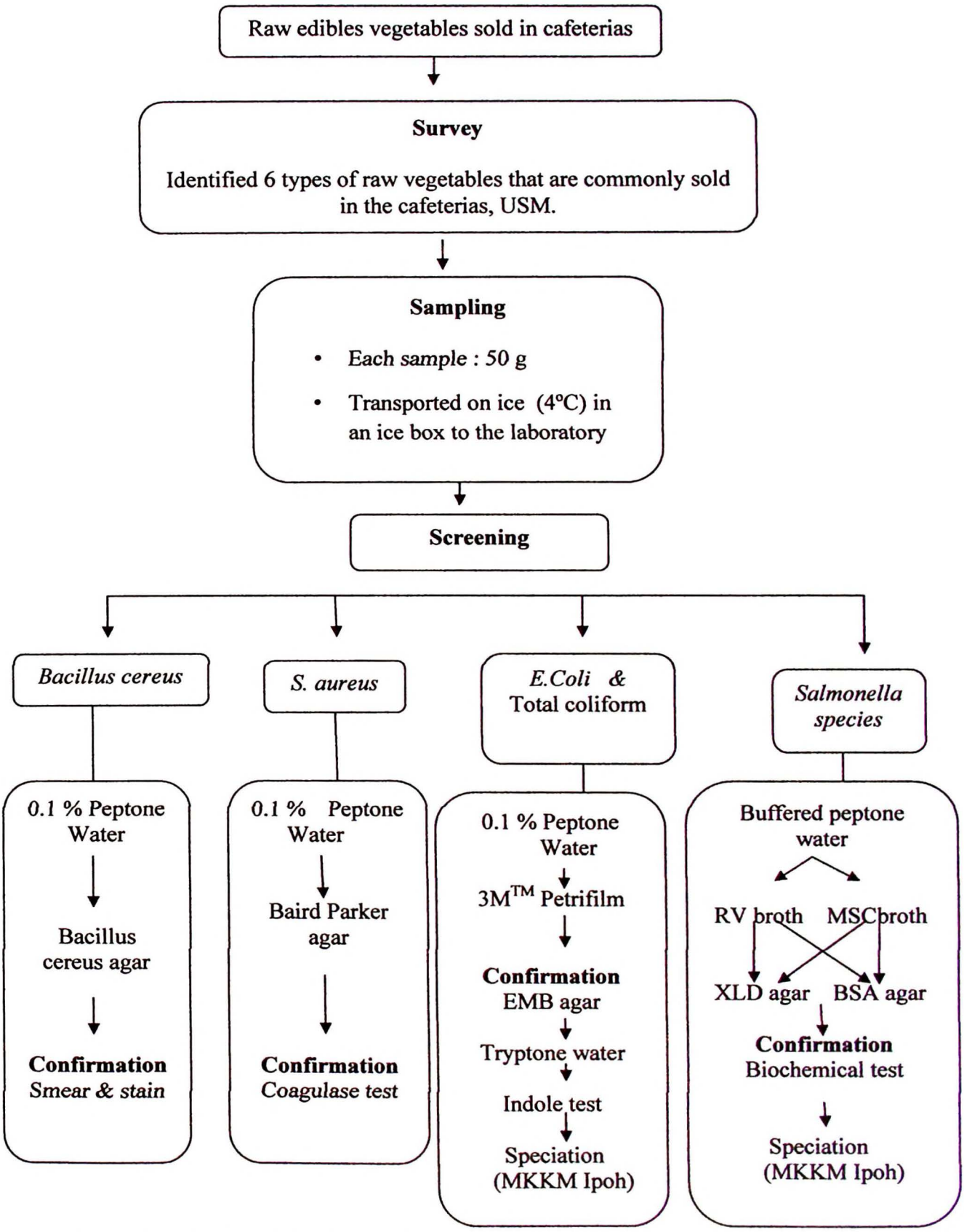

Figure 3.1 Flow chart of Methodology

CHAPTER 4 RESULTS AND DISCUSSION

Raw fruits and vegetables have been known to serve as vehicles of human diseases and food borne outbreaks has increased in recent years. Raw vegetables that are contaminated with pathogenic bacteria may lead to health hazard as there is no treatment that can be relied upon for decontamination of the vegetables. Despite the nutritional benefits of consuming raw vegetables, an increased intake of such vegetables is associated with greater incidence of food borne illness. Therefore, it is imperative to screen raw vegetables for the presence of pathogenic bacteria to ensure the safety and quality of the vegetables.

A study was conducted to screen and identify the bacterial pathogens present in selected raw vegetables sold in the cafeterias in USM Health Campus. A total of 30 samples from six commonly consumed raw vegetables viz., cucumber, winged bean, lettuce, wild cosmos, pennywort leaves and water dropwort were analyzed. Samples were randomly collected over a period of two months. Fifty grams (50 g) from each sample were taken for microbiological analysis.

Guideline levels for determining the microbiological quality of ready-to-eat food

In this study, microbiological criteria are used to distinguish between an acceptable and an unacceptable level of microorganisms in raw vegetables or between acceptable and unacceptable food processing practices. The numbers and types of microorganisms associated with raw vegetables were used to judge its microbiological safety and quality.

The presence and identification of pathogenic bacteria viz., *E.coli, Bacillus cereus, Staphylococcus aureus, Salmonella* and total coliforms was determined based on the

"Australian Microbiological Examination of Food", (1993) and the analysis was conducted in triplicates. Compliance to the microbiological quality (CFU/g) of raw vegetables is based on the "Microbiology Guidelines for Ready to Eat Food" MOH Malaysia (1985).

Four categories of microbiological quality which are satisfactory, marginal, unsatisfactory and potentially hazardous have been assigned based on the standard plate counts, number or presence of pathogens and the levels of indicator organisms.

Satisfactory results indicate good microbiological quality and no action required. Marginal results indicate that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the preparation of the food. Unsatisfactory results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices. For samples that are in potentially hazardous level, it may cause food borne illness and immediate remedial action should be initiated.

Selected guidelines of microbiological quality of ready-to-eat food (1985)

Test	Microbiological Quality (cfu/g)			
	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Escherechia coli	< 3	3 - 100	≥ 100	Presence of pathogenic strains of *E.coli*
Staphylococcus aureus	$< 10^2$	10^2 - 10^3	10^3 - 10^4	$\geq 10^4$
Bacillus cereus	$< 10^2$	10^2 - 10^3	10^3 - 10^4	$\geq 10^4$
Salmonella spp.	Not detected in 25 g	-	-	Detected in 25 g
Total coliform	< 100	< 1,000	≥1,000	-

4.1 Presence of microorganisms in raw vegetables samples

A total of 6 (20%) samples (1 cucumber, 1 pennywort, 3 water dropwort and 1 wild cosmos) showed presence of pathogenic bacteria namely *E.coli* and *Salmonella*. However, lettuce and winged bean did not show presence of any of the pathogenic bacteria. *Staphylococcus aureus* and *Bacillus cereus* were not detected in any of the raw vegetables sampled. Total coliforms was detected in all of the thirty raw vegetables samples. The presence of microorganisms in edible raw vegetables samples were shown in Table 4.1.

3M ™ Petrifilms were used for the isolation of total colifoms and fecal coliforms. It consists of ready to use plates containing a specific growth media for coliforms and E.coli. These petrifilms contain lactose and Violet Red Bile (VRB).

Coliforms use lactose in metabolic reaction, producing gas and acid. The acid produce will cause a change in the colour of the pH indicator, producing red colonies with bubbles.

On the other hand, *E.coli* contain β-glucoronidase enzyme and will react with the glucoronidase indicator, VRB. In addition, *E.coli* also ferments lactose, therefore producing blue colonies with gas bubbles.

From Table 4.1, it is observed that water dropwort, pennywort and wild cosmos with abundant, small leaves are detected with *E.coli*. This shared property of theirs provide more surface area and "hiding place" for the microorganism which can cause removal of microorganism by washing difficult.