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JUDUL: SCREENING FOR PHYTOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY OF BERMUDA GRASS (Cynodon dactylon L.) EXTRACTS

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SCREENING FOR PHYTOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY OF BERMUDA GRASS(*Cynodon dactylon* L.) EXTRACTS

NURUL ATIKA AFIFA BINTI MOHD DZULKEFLY

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

CROP PRODUCTION PROGRAMME
SCHOOL OF SUSTAINABLE AGRICULTURE
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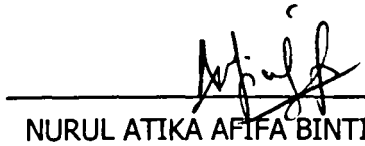

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Screening For Phytochemical Properties and Antimicrobial Activity of Bermuda Grass (*Cynodondactylon*L.) Extracts

The uses of weedy plant or herb such as *Cynodon dactylon* is not fully explored in Malaysia. The bioactive compounds present in the plants are very useful in medicinal purposes. Chemical compounds of interest were extracted and examined for their phytochemical activity and antimicrobial properties. For the purposes of this study, screening of *Cynodon dactylon* for their phytochemical properties and antimicrobial activity were conducted on three ecotypes of *C. datylon* that growing wild around University Malaysia Sabah Campus Sandakan. Chemical compounds that will be tested are saponin, flavonoids, tannins and glycoside, while the microorganisms used in this study were *Escherichia coli, Staphylococcus aureus, Salmonella enteritis* and *Citrobacter freundii.* There were five replications for each treatment. This study was conducted in School of Sustainable Agriculture Laboratory, University Malaysia Sabah,Campus Sandakan. Based on the result, I found that all ecotypes showed positive result for phytochemical screening as well as had the abilities to inhibit all bacteria for antimicrobial screening. Ecotype 1 showed the best ecotypes where it presence the highest concentration of tannins and glycosides. It also gave the best inhibition effect especially towards *Salmonella enteritis* bacteria. As a conclusion, *Cynodon dactylon* has a high potential to be commercialized as a medicinal purposes.

Keywords: *Cynodon dactylon*, phytochemical, antimicrobial, *Escherichia coli and Staphylococcus aureus.*

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UJIAN SARINGAN TERHADAP FITOKIMIA AND AKTIVITI ANTIMIKROB DALAM Cynodondactylon

ABSTRAK

Penggunaan rumpai atau herba seperti Cynodon dactylon tidak diterokai sepenuhnya di Malaysia. Sebatian bioaktif yang terdapatdalamtumbuh tumbuhan adalah sangat berguna dalam tujuan perubatan. Sebatian kimia yang diingini telah diekstrak dan diperiksa untuk aktiviti fitokimia dan antimikrob. Bagi tujuan kajiani ni, ujian saringan Cynodon dactylon terhadap fitokimia dan aktiviti antimikrob telah dijalankan keatas tiga 'ecotype' C.dactylon yang tumbuh liar di sekitar di Universiti Malaysia Sabah, Kampus Sandakan. Sebatian kimia yang diuji adalah saponin, flavonoid, tannin, dan glikosid, manakala mikroorganisma yang digunakan dalam kajian ini adalah Escherichia coli, Staphylococcus aureus, Salmonella enteritis dan Citrobacter freundii.Terdapat lima replikasi untuk setiap rawatan. Kajian ini telah dijalankan di Makmal Sekolah Pertanian Lestari, Universiti Malaysia Sabah, Kampus Sandakan.Berdasarkan keputusan, saya mendapati bahawa semua ecotypes menunjukkan hasil yang positif untuk pemeriksaan fitokimia serta mempunyai kebolehan untuk menghalang pertumbuhan semua bacteria untuk pemeriksaan antimikrobial. 'Ecotype' 1 menunjukkan ecotypes' terbaik di mana ia mempunyai kehadiran kepekatan tertinggi iaitu tannin dan glikosida. Ia juga member kesan perencatan yang terbaik terutamanya kearah bakteria Salmonella enteritis. Kesimpulan, Cynodon dactylon mempunyai potensi tinggi untuk dikomersilkan sebagai tujuan perubatan.

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Kata kunci *:Cynodon dactylon, fitokimia, antimikrob, Escherichia coli dan Staphylococcus aureus.*

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

cm	Centimeter
^0C	Degree Celsius
^0F	Fahrenheit
β	Beta
CNS	Central Nervous System
µm	micrometer
%	Percentage
ml	millimeter
mg	milligram
g	gram
HCl	Hydrochloric acid
h	Hour
±	plus-minus

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Malaysia is among the most biologically diverse countries in the world. There are approximately 12,500 species of flowering plants, including species of valuable and marketable timber and fruit (Khairunnisa, 2010). Therefore, one of it is *Cynodon dactylon*. This plant is also known as Bermuda grass, Devil grass, Bahama grass, vine grass, wiregrass and scutchgrass (Guertin, 2003) and it is come from family Poaceae. It is usually found in north and east of Africa, Asia and Australia as well as southern Europe. According to Nagori and Solanki (2011), this grass is a rapid growing perennial grass and has creeping runner or stolons. This grass can grow to a height of one foot. The leaves are grayish green colour and are four to 15 cm long. The flower heads have about 2-6 spikes which formed like a wheel spokes. *Cynodon dactylon* have no stalks, and are arranged in two rows along one side of a more or less flattened triangular ranchis. It also has a ligule of short, white hairs and fringe of hairs along the keel of the lemma (Guertin, 2003). It is a short C4 grass, which is rhizomatous, stoloniferous and water-stress tolerant (Burton *et al.*, 1988; Naidu and Harwood, 1997) and also recommended for soil revegetation (Osvaldo *et al.*, 2005).

Besides, it is also tolerant to extreme heat and alkaline soils and is extremely drought resistance. University of California (1998) notes that *Cynodon dactylon* seed can germinate and regrowth can occur when the soil temperatures are above 55- 60^{o}F (13-16^{o}C). This plant is used as a major turf for the fields, lawns, golf courses and other landscaping purposes. Aggressive root systems minimize soil

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erosion, stabilize ditch banks, and cover roadsides and airfield, and beautify landscapes, along with providing a smooth, resilient playing surface for sports fields and playground (Duble, 2004). Several bermudagrass cultivars have been selected specifically for putting green due to their tolerance for short sowing, fine texture, higher shoot density, deeper root and less thatch development and they include 'Tifgreen', 'Tifdwarf', 'Floradwart' and 'TifEagle' (Fresenburg, 2010).

Traditionally, *Cynodon dactylon* L. juice is used for freshness and to threat several common diseases (Kaleeswaran *et al.*, 2010). It also said to have many medicinal properties including antimicrobial and antiviral properties. The plant is a rich source of metabolites such as proteins, carbohydrates, mineral constituents, β-sitosterol, flavanoids, alkaloids, glycosides and triterpenoides (Nagori and Solanki, 2011). This plant can cure many diseases such as cancer, convulsions, cough, cramps, diarrhea, dropsy, dysentery, epilepsy, headache, hypertension, hysteria, sore, wounds and others. Besides that, the juice from the stem can be used as a diuretic and astringent to stop bleeding.

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1.1 Justification

The uses of weedy plant such as *Cynodon dactylon* is not fully explored in Malaysia. This is due in part to the complexity of the molecules and also to the lack of pathway intermediates for biochemicals studies (Mert-Türk, 2006). The bioactive compounds that are present in the plants are potentially used in medicinal purposes. They can act as antimicrobial, antidepressant, antibiotic, sedative and others. They also can cure diseases such as cough, skin problem, asthma, headache and others. Therefore, screening of weedy or herb plants for medicinal purposes should be fully explored, so that the uses can be known by Malaysian citizens. Besides, all weedy or herb plant can be found anyway, even around our house because of it is aggressively growth habits.

Successful detection of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily waters as the solvent but researchers have found that plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water. These observations can be rationalized in terms of the polarity of the compounds a being extracted by each solvent and, in addition to their

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intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay (Parekh *et al.*, 2005).

1.2 Objectives

i. To determine the phytochemical constituents present in the three ecotypes of *Cynodon dactylon.*

ii. To determine the phytochemical activity present in the three ecotypes of *Cynodon dactylon..*

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CHAPTER 2

LITERATURE REVIEW

2.1 Plant Materials

2.1.1 *Cynodon dactylon*

Cynodon dactylon (L) Pers. is a perennial grass that has a variety of medicinal properties (Singh *et al.*, 2009) and the whole herb and its root stalk are used for medicinal use (Kritikar and Basu, 1980). Cynodon dactylon belongs to the family poaceae. It is also known as Durva grass, Bermuda grass, Dog's Tooth grass, Bahama grass, Devil's grass, Couch grass, Indian Doab, Scutch grass, Dhub, Doob and Durba in different parts of the world (Oudhia, 2003). Durva is a Sanskrit word that means, which is cut or eaten by the animals. It is the most sacred plant of India nest to tulsi (Nagori and Solanki, 2011).

Cynodon dactylon is a rapid growing perennial grass. The plant has creeping runner or stolons. It roots at nodes forming a dense tuft on the surface of the soil. The runners are sometimes 20 m long and have leaves that are variable in size, from 2.5-20 cm long, 0.5-1.0 cm broad, flat or sometimes folded or convolute, tapering towards the apex (Nagori and Solanki, 2011). The flowers are green or brinjal in color and the fruit grains are tiny and grayish in color (Bhandari, 1990).

This plant can be found as a weed along the roadsides, in lawns and can readily take possession of any uncultivated area. Growth is promoted by full sun and retarded by full shade (Nagori and Solanki, 2011).

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Doob is used as a folk remedy for anasarca, calculus, cancer, carbuncles, convulsion, cough, cramp, cystitis, diarrhea, dropsy, dysentery, epilepsy, hemmorhoids and others (Nagori and Solanki, 2011). The pharmacological activities of *C. dactylon* are antidiabetic, antioxidant, antidiarrheal, immunomodulatory, antiulcer, antiarrhythmic, CNS depressant, hepatoprotective and cardioprotective.

2.1 Chemical Compounds

2.1.1 Saponin

Saponins are natural glycosides of steroid or triterpene which exhibited many biological and pharmacological activities (Hong-Xiang Sun *et al.*, 2009). Saponins have detergent or surfactant properties because they contain both water-soluble and fat-soluble components consist of a fat-soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water-soluble carbohydrate (Cheeke, 2000). The saponin can be found throughout the plant, the roots, stems or leaves. It was most abundant in the basal parts of the plant, where the fibro vascular bundles were numerous and not especially fibrous and the trachea or tracheids formed an essential part of the bundles (Carlo *et al.*, 1922).

Besides medicinal purposes, saponins also can be used as pest control. The data showed that extracts of these two natural saponin plant sources can be used for an effective bioactive preparation in *Aedes aegypti* and *Culex pipiens* mosquito as control (Zeew and Bishnu, 2003).

2.1.2 Tannins

Tannins are complex, polyphenol, nitrogen-free, amorphous and non-toxic compound, soluble in water and the application of tannins in medicine is based on their astringent as well as anti bacterial and fungicidal action (Rakić *et al.*, 2004). They are classified into two major categories, the hydrolysable and the condensed tannins (Koukoura and Nastis, n.d) (Figure 2.1). Besides that, tannins usually found in the bark but often in fruits and leaves; complex chemicals which act as an astringent, drawing the tissues together and contracting them which is useful for treating surfaces such as the

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inflamed mucous membranes characteristic of coughs and colds and bathing wounds (Australian National Botanic Gardens Education Services, 2000).

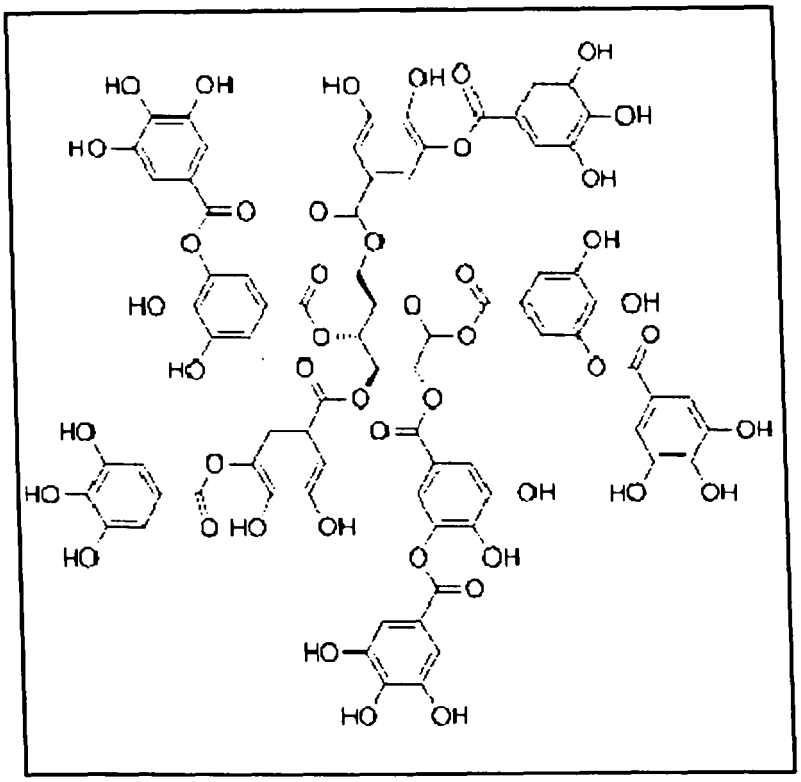

Figure 2.1 Structural of Tannins

(Source: Koukoura and Nastis, n.d)

2.1.3 Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitously in plants that present in practically all dietary plants, like fruits and vegetables (Ren *et al.*, 2003) (Figure 2.2). A great number of plant medicinal contain flavonoids, which have been reported by many authors as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, vasodilatory actions (Alan and Miller, 1996), radical scavenger and antileukemic (Sharma, 2006). According to Nataraj (2009), in the present study, the total phenol content & total flavonoidal content were determined and this in terms helps in gauging the antioxidant potential of the tuberous plant and not only helping for establishing the phytochemical standardization but also in authentication of this drug.

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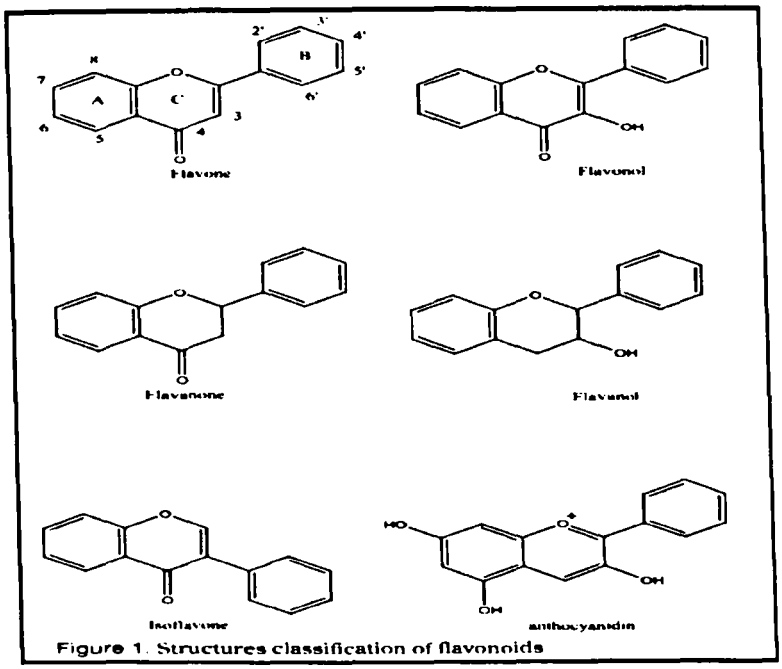

Figure 2.2 Structural of flavonoids
(Source: Takashi Kinoshita *et al.*, 2005)

2.1.4 Glycoside

In chemistry, a glycoside is a molecule in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. The glycosidic residue can be crucial for their activity or can only improve pharmacokinetic parameters. Owing to recent developments in molecular glycobiology, the relations of aglycone vs. glycoside activity are now clearer and -based on these findings - it is possible to develop new, more active or more effective glycodrugs (Kren and Martinkova, 2001). Cyanogenic glycosides are widely distributed among 100 families of flowering plants. They are also found in some species of ferns, fungi and bacteria (Harborne, 1972, 1993). Many biologically active compounds are glycosides. Glycosides comprise several important classes of compounds such as hormones, sweeteners, alkaloids, flavonoids, antibiotics, etc (Kren and Martinkova, 2001).

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C. dactylon the presence of reveals the presence of flavanoids, sterols and cardiac glycosides. Cyanogenic glycosides are defined as O-β-glycosides of α-hydroxynitriles (cyanohydrins), biosynthetically derived from amino acids. They generally co-occur with β-glycosidases that can cleave the glycoside and release cyanide and carbonyl compounds upon damage to the plant (Hay-Roe *et. al.*, 2011). Both cyanide and carbonyl compounds are capable of conferring protection from herbivory and/or pathogens (Harborne, 1982). Many cynodon grasses are cyanogenic (Mahmoodzadeh, 2010). The cardiac glycosides have been also proved for its cardioprotective & cardiotonic activity, thus the significant antihypertensive activity of aq. extact of C. dactylon may be due to the presence of cardiac glycosides (Bharti *et. al.*, 2012). According to Hay-Roe, 2011, they concluded that the evidence is presented that plant cyanogenic glycosides might play a significant role in defining the host range of the two fall armyworm strains. Differences in cyanide levels were observed in the host plants, and the two strains differed in their sensitivity to cyanogenic compounds and in their capacity to metabolize and/or eliminate these compounds.

2.2 Streptomycin

Antibiotics are the antimicrobial agents, which are produced by some micro-organisms to inhibit or to kill many other micro-organisms including different bacteria, viruses and eukaryotic cells. It can be purified from microbial fermentation and modified chemically or enzymatically (Abbas, S. *et. al.*, 2010). Antibiotics, as secondary metabolites, are generally produced by multistep biosynthetic pathways starting from intermediates of primary metabolism to specific moieties (Abbas, S. *et. al.*, 2010).

Streptomycin is an antibiotic drug, the first of a class of drugs called aminoglycosides to be considered, and it was the first antibiotic remedy for tuberculosis. It is derived from the actinobacterium *Streptomyces griseus*. Streptomycin is a bactericidal antibiotic. Streptomycin is a protein synthesis inhibitor. It cannot be given orally, but must be administered by regular intramuscular injections. Adverse effects of this medicine are ototoxicity, nephrotoxicity, fetal auditory toxicity, and neuromuscular paralysis. At low concentrations, however, Streptomycin only inhibits growth of the bacteria by inducing prokaryotic ribosomes to misread mRNA. Streptomycin is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria, and is therefore a useful broad-spectrum antibiotic.

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Streptomycin is a human antibiotic drug which also is used as a pesticide, to control bacteria, fungi and algae. Streptomycin controls bacterial and fungal diseases of certain fruit, vegetables, seed, and ornamental crops, and controls algae in ornamental ponds and aquaria. The use of streptomycin to control fireblight on apples and pears accounts for 58% of its total use. Other significant uses are on nursery stock and in landscape maintenance (17% of use), and on tobacco (7% of use) (R.E.D. FACTS, 1992). Streptomycetes are best known for their synthesis of a vast array of antibiotics, some of which are useful in medicine and biological research. Examples include amphotericin B, chloramphenicol, erythromycin, neomycin, nystatin, streptomycin, tetracycline and so forth. Although most streptomyces are non-pathogenic saprophytes, a few are associated with plant and animal diseases (Prescot *et al.* 1999).

The results also confirmed that the gram-positive bacterial strains were more susceptible to the plants extracts as compared to gram negative bacteria (Shahwar and Raza, 2009). This is in agreement with the fact that gram positive bacteria have only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). Therefore, antibacterial activity of local medicinal plants should be studied to provide alternative antibacterial regimens (Kowti *et. al.*, 2010)

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2.3 Antimicrobial Activity

2.3.1 *Staphylococcus aureus*

Staphylococcus aureus is a gram positive bacterium that has been a pathogen (capable of causing human infection) for as long as we have had medical literature. *Staphylococcus aureus* secrets exotoxins called superantigens, which stimulate a large proportion of T cell. On the other hand, staphylococcal antigens may induce allergic reactions caused by the release of inflammatory mediators such as leukotrienes and histamine (Mustafa *et al.*, 1996). In fact, when this microorganism enters the blood, it represents one of the most lethal human pathogens also because it is often characterized by multidrug resistance (Boyce, 1997). Photoactivated disinfection appears as an effective methos of *Staphylococcus aureus* inactivation (Tzvetelina *et al.*, 2010).

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2.3.2 *Escherichia coli*

Escherichia coli are a member of the family of *Enterobacteriaceae*, is related to the genus Shigella and is closely related to the genera *Citrobacter*, *Salmonella* and Klebsiella. The *Escherichia coli* bacterium is a gram-negative rod of about 1.1-1.5 µm x 2.0-6.0 µm in size. It grows under aerobic and anaerobic conditions (facultatively anaerobic) (Schulze *et al.*, 2006). *Escherichia coli* are normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins (Oie, 2009). According to Health People, 2010, *Escherichia coli* showed progress toward its target of 50 percent reduction from baseline issues. The number of outbreaks from food infections caused by *Escherichia coli* has actually increased. In October 1995, the Food Safety and Inspection Service (FSIC) declared raw ground beef can contain traces of *Escherichia coli* and they began a sampling program to test for the *Escherichia coli* in retail stores (Food Safety and Inspection Service).

2.3.3 *Citrobacter freundii*

The Citrobacter species, including *Citrobacter freundii*, are aerobic gram-negative bacilli. *Citrobacter freundii* are long rod-shaped bacteria typically 1-5 µm in length (Wang *et al.*, 2000). Most *C. freundii* cells are surrounded by many flagella used to move about, but a few are non-motile. Its habitat includes the environment (soil, water, and sewage), food, and the intestinal tracts of animals and humans. It belongs to the family of Enterobacteriaceae.

It is known to be the cause of a variety of nosocomial infections of the respiratory tract, urinary tract, blood and several other normally sterile sites in patients (Whalen J.G. *et al.*, 2007). Therefore, one of the chief reasons many different strains and plasmids of the *C. freundii* genome are being sequenced is in order to find antibiotics that can fight these opportunistic infections.

Unlike gram-positive bacteria, *C. freundii* cells do not contain a thick cell wall made up of peptidoglycan. *Citrobacter freundii* are commonly found in the environment, mainly in soil, water, and sewages. They are an indicator of potential contamination of water. They are also found on different organs of diseased animals, including mammals, birds, reptiles, and amphibians (Wang *et al.*, 2000). They are not known to interact with other organism.

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2.3.4 *Salmonella enteritis*

Salmonella is a genus of rod-shaped, Gram negative, non-spore-forming predominantly motile enterobacteria. They are obtaining their energy from oxidation and reduction reactions using organics sources, and are facultative anaerobes. Salmonella is closely related to the *Escherichia* genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and foodborne illness. Salmonella infections are zoonotic and can be transferred between humans and nonhuman animals. Many infections are due to ingestion of contaminated food. It can be a source of salmonella diarrhoea epidemics, especially when it occurs in public food handlers or nurseries (Sirinavin and Garner, 2009). Salmonella diarrhoea can cause: chronic diarrhoea, fluid & electrolyte disturbance, malnutrition, necrotising enterocolitis in young infants, and systemic and localised infections (Gomez HF, 1998). All of these conditions are serious and life threatening and can occur without known diarrhoeal episodes although the gastrointestinal tract is the likely route of acquiring the organism. These complications are more likely to occur in young infants, old age and immunocompromised patients, and fatality rates are higher in these groups.

Salmonellosis is a disease caused by raw or undercooked food. Infection usually occurs when a person ingests foods that contain a high concentration of the bacteria, similar to a culture medium. Enteritis Salmonella, for example Salmonella enteric subsp. enteric serovar enteritidis, can cause diarrhea, which usually does not require antibiotic treatment. However, in people at risk such as infants, small children, the elderly, Salmonella infections can become very serious, leading to complications. If these are not treated, HIV patients and those with suppressed immunity can become seriously ill. Children with sickle cell anaemia who are infected with Salmonella may develop osteomyelitis. Salmonella is one important cause of opportunistic infections in HIV/AIDS patients. It is a common cause of severe morbidity in these patients. Problems with eradication of salmonella intestinal colonization and prevention of extra-intestinal invasion need to be addressed (Sirinavin and Garner, 2009).

Most people with salmonellosis develop diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours after infection. In most cases, the illness lasts four to

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seven days, and most people recover without treatment. In some cases, though, the diarrhea may be so severe, the patient becomes dangerously dehydrated and must be taken to a hospital. At the hospital, the patient may receive intravenous fluids to treat the dehydration, and may be given medications to provide symptomatic relief, such as fever reduction. In severe cases, the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites, and can cause death, unless the person is treated promptly with antibiotics. The elderly, infants, and those with impaired immune systems are more likely to develop severe illness.

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