CHAPTER II

LITRATURE REVIEW

2.1 Honey

The relation between bees and man started as early as Stone Age (Crane, 1983). Honey is a thick, sweet liquid produced by bees from the nectar of flowers. The ancient Egyptians observed that honey not only a delicious food but also pointed out its use as a healing substance. Such concept is referred to Surat El-Nahl (verses 98 & 69) in the Holy Quraan. The first written reference about honey mentioned honey's use as a drug and an ointment (Crane, 1975). In most cultures honey has been used for medical and nutritional purposes (Jones, 2001). The use of honey as drug, nutrient and ointment has been carried into our days. Since a long time ago honey was very important source for carbohydrates and used as sweetener until it was replaced by industrial sugar after 1800 (Crane, 1975). Alternative medicine called apitherapy has developed offering treatments based on honey and the other bee products against many diseases (Potschinkova, 1992; Cherbuliez & Domerego, 2003). The use of honey in healing application is seen in the treatment of wounds, burns and infections were reviewed by (Molan, 1999; Bogdanov *et al.*, 2008 & 2012; Shakespeare, 2011).

2.2 Chemical composition of honey

The carbohydrates are the main contents, comprising about 95% of the honey dry weight. Additionally, honey contains numerous compounds such as organic acids, amino acids, proteins, minerals, polyphones, aroma compounds and vitamins (Table 1). It is suggested that the chemical composition of honey is related on the botanical origin (Persano & Piro, 2004).

The monosaccharide fructose and glucose are the main sugars in honey; about 25 different oligosaccharides have been detected (Doner, 1977; Siddiqui, 1970). The main oligosaccharides in blossom honey are the disaccharides sucrose, maltose,

Table 1: Nutrient Values of Honey (Bogdanov *et al*., 2008)

Nutrient	Average amount per 1 Tbsp serving (21g)	Average amount per 100g
Water	3.62g	17.10g
Calories	64	304
Total Carbohydrate	17.46g	82.40g
Fructose	8.16g	38.50g
Glucose	6.57g	31.00g
Maltose	1.53g	7.20g
Sucrose	0.32g	1.50g
Other carbohydrates	0.85g	4.00g
Dietary Fiber	0.04g	0.20g
Total Fat	0	0
Cholesterol	0	0
Total Protein	0.06g	0.30g
Ash	0.04g	g0.20
Vitamins		
Thiamin	0	0
Riboflavin	0.01mg	0.04mg
Niacin	0.03mg	0.12mg
Pantothenic acid	0.01mg	0.07mg
Vitamin B-6	0.01mg	0.02mg
Vitamin B-12		0
Folate	0.42mcg	2.00mcg
Vitamin C	0.11mg	0.50mg
Vitamin A	0	0
Vitamin D	0	0
Vitamin E	0	0
Vitamin K	0	0
Minerals		
Calcium	1.27mg	6.00mg
Phosphorus	0.85mg	4.00mg
Sodium	0.85mg	4.00mg
Potassium	11.02mg	52.00mg
Iron	0.09mg	0.42mg
Zinc	0.05mg	0.22mg
Magnesium	0.42mg	2.00mg
Selenium	0.17mg	0.80mg
Copper	0.01mg	0.04mg
Manganese	0.02mg	0.08mg

turanose and trehalose, as well as some nutrition relevant ones like panose, 1-kestose, palatinose and 6-kestose. Compared to blossom honey, honeydew honey contains higher amounts of the oligosaccharides melezitose and ranffinose. After honey intake the main carbohydrates fructose and glucose are transported quickly into the blood and can be utilized for energy requirements by human body. A daily dose of 20 g honey could cover about 3% of the daily energy (Bogdanov *et al*., 2008).

Honey contains about 0.5% proteins, free amino acids and mainly enzymes. The three main honey enzymes are amylase (decomposing starch into smaller sugar units), α-glucosidase (decomposing sucrose into fructose and glucose), as well as glucose oxidase (producing hydrogen peroxide and gluconic acid from glucose) (Bogdanov *et al*., 2008). It is known that different unifloral honeys contain varying amounts of minerals and trace elements (Bogdanov *et al*., 2008). Nutritionally, honey contains manganese and selenium and the elements sulphur, boron, cobalt, fluoride, iodide, molybdenum and silicon are important in human nutrition (Bogdanov *et al*., 2008). Honey contains 0.06 to 5 mg/kg acetylcholine and 0.3-25 mg/kg choline (Heitkamp, 1984).

The color of honeys is different from one to other, depending on botanical origin (Crane, 1984). Generally, honeys with high content of fructose are sweeter compared to those with high concentration of glucose. The honey aroma depends also on the content and kind of acids and amino acids (Bogdanov *et al*., 2007). Polyphenols are also important compounds for the appearance and functional properties of honey (Al-Mamary *et al*., 2002, Gheldof & Engeseth, 2002). They are majorly flavonoids (kaempferol, luteolin, quercetin, apigenin, galangin, chrysin), phenolic acids and their derivatives (Tomás-Barberán *et al*., 2001) that are known for their antioxidant activity. Flavonoids content can vary between 60 and 460 µg/100 g of honey and this amount increase in dry seasons with high temperatures (Kenjeric *et al*., 2007).

Glycemic index (GI) is the only understood data for honey and is based on the data from Australian different honey samples. GI and fructose have negative interaction and that could be due to the differences of the fructose and glucose ratios in the tested honey types (Arcot & Brand-Miller, 2005). The low GI in some honey samples could have beneficial physiological effects and these types of honey can be used for diabetes patients. Several types of honey were given to healthy people and diabetes patients in amount of 50 g and this led to increase of blood insulin and glucose compared to same amount of glucose or sugar mixture added to honey (Al-

Khalidi *et al.*, 1980; Jawad *et al.*, 1981).

2.3 Physiological properties

2.3.1 Antioxidant effects

Antioxidant activity was reported to be found in honey in significant amount including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins (Bogdanov *et al.*, 2008; Perez *et al.*, 2007). Honey contains cenamic acid, antioxidant agent and some flavonoids which have been approved for antibacterial applications (Rahman *et al.*, 2010). Antioxidants in honey may play a positive role in food safety beyond food preservation (Taormina *et al.*, 2001). The antioxidative activity of honey can be measured *in vitro* by comparing the oxygen radical absorbance capacity (ORAC) with the total phenolics concentration. There is a significant interaction between phenolic content and antioxidant activity of honey and lipoprotein oxidation of human serum inhibition (Gheldof *et al.*, 2003). Antioxidant activity rely on botanical origin of honey and varies base on honey samples from different botanical sources (Al-Mamary *et al.*, 2002; Frankel *et al.*, 1998; Baltrusaityte *et al.*, 2007; Vela *et al.*, 2007).

2.3.2 Antimutagenic and antitumor activity

Mutagenic substances can affect the genetic structure directly or indirectly (Doner, 1977; Siddiqui, 1970). The anti-metastatic effect of honey and its possible mode of anti-tumor action were studied (Orsolic & Basic, 2004). Anti-metastatic effect was proved by oral application of honey and it was highly significant. These reports showed that honey activity effect the immune system and honey ingestion could be advantageous with respect to cancer and metastasis prevention. On the other hand, it is suggested that orally consumption of honey before tumor cell inoculation could reduce the spreading of tumor (Orsolic *et al.*, 2003). Swellam *et al.*, (2003) reported honey has anti-tumor activity *in vivo* and *in vitro* against bladder cancer in mice. This study supports the earlier studies elaborated that honey is an effective agent against several types of tumor especially the bladder cancer.

2.3.3 Anti-inflammatory effects

Honey was evaluated for their anti-inflammatory effects, the ingestion of honey reduced inflammatory of bowel disease in rats by inflammatory experimental model (Al-Waili & Boni, 2003; Bilsel *et al*., 2002). The antibacterial effects of honey are possible to be responsible for the reduction of inflammation or to a direct anti-inflammatory effect. The anti-inflammatory effects of honey were supported by animal study observed in wounds with no bacterial infection (Postmes, 2001).

2.3.4 Athletic performance

Honey in the forms of gels and powder has physiological action as carbohydrates source for athlete was studied under controlled conditions (Kreider *et al*., 2002; Earnest *et al*., 2004). Honey is a natural fructose-glucose carbohydrate mixture with some oligosaccharides, protein, vitamins and minerals. The potential of honey to be added to beverages and gels to be used by athletes are very high (Ustunol, 2000). In previous study the result showed that honey is a perfect mixture of carbohydrates for pre-exercise ingestion and it can produce only modest increases in glucose and insulin without a threat of hypoglycemia (Kreider *et al*., 2000b; Rasmussen, *et al*., 2000). In other study the result were varied for the different carbohydrate and it was suggested that the type of sugar ingested with protein after the exercises could affect the anabolic hormonal profile (Kreider *et al*., 2000a). Heart frequency and the blood glucose were increased significantly by honey during performance of athlete (Molan, 2001c).

2.3.5 Infants benefits

Honey supplied to infants showed better blood formation and higher weight gain compared to other infants who were on diet without honey (Frauenfelder, 1921). Feeding the infants on honey rather than on sucrose had increased the haemoglobin content and better skin color, also no digestion problems were reported (Tropp, 1957; Takuma, 1955). The possible cause of the good effect of honey in infant diet is due to the effects of oligosaccharide on *B. bifidus* (Rivero-Urgell & Santamaria-Orleans, 2001). Mixture of honey and milk given to infants regularly showed that these infants had steady weight gain and had an acidophilic microorganism flora rich in *B. bifidus*

(Hu¨ bner, 1958).

2.3.6 Different health effects

As reported by Australia Ministry for Health and Aged Care (1999) that the value of honey is recognized by medical authorities the world over. In Australia, it has been approved as a "Therapeutic Good" to be used as:

- Antiseptic dressing to promote healing of wounds, burns and skin ulcers.
- Topical antibacterial agent for the treatment of acne and other skin infections.
- Topical antibacterial agent for the treatment and prevention of diaper-rash.
- Topical antibacterial and moisturizing agent for the treatment of atopic eczema.
- Topical antifungal agent for the treatment of tineas.
- Antiseptic salve for conjunctivitis and blepharitis.
- Antibacterial agent and rehydrating agent for the treatment of gastroenteritis.
- Antibacterial agent and healing-promoting agent for the treatment of dyspepsia and peptic ulcers.

A patient of hepatitis A was found to decrease in the alanine aminotranferase activity and a decrease of bilirubin production after rape honey ingestion (Baltuskevicius *et al.*, 2001). A reduction in the incidence of radiation mucositis of patient who has undergone a cancer radiation therapy was reported by Zidan *et al.*, (2006) and that was because of the supportive effect of honey.

2.3.7 Physiological effects

Antibody production can be affected by honey especially against thymus-dependent antigen in sheep red blood cells and thymus-independent antigen (*Escherichia coli*) in mice (Al-Waili & Haq, 2004). The antibody production was enhanced by the honey intake during primary and secondary immune responses against thymus-dependent and thymus-independent antigens (Bogdanov *et al.*, 2008). In animal experiments honey showed an immunosuppressive activity (Dddukuri *et al.*, 1997).

2.3.8 Antimicrobial activity and health benefits

Honey has the ability to inhibit microorganisms such as pathogenic bacteria (Table 2), spoilage fungi and yeast and viruses. The antibacterial effect of honey especially against Gram positive bacteria is well documented (Molan, 1997; Bogdanov, 1997). Both bacteriostatic and bactericidal effects have been reported for many strains especially, pathogenic bacteria (Bogdanov *et al.*, 2008). *Burkholderia cepacia* which causes pulmonary infections and chronic granulomatous disease patients such as urinary tract infections and wound infections in hospitalized patients was sensitive to manuka honey at concentrations less than 6% (v/v). It is suggested that clinical applications for treating *B. cepacia* with honey include honey aerosols as an adjunct to antibiotic therapy and topically for infections and wounds (Cooper, *et al.*, 2000a & b).

Table 2: List of bacteria that were found to be sensitive to honey (Bogdanov *et al.*, 2008)

Actinomyces pyogenes
Bacillus anthracis
Corynebacterium diphtheria
Epidermophyton floccosum
Escherichia coli
Haemophilus influenza
Helicobacter pylori
Klebsiella pneumonia
Microsporum canis, M. gypseum
Mycobacterium tuberculosis
Nocardia asteroids
Proteus sp.
Pseudomonas aeruginosa
Salmonella sp.
Salmonella cholerae-suis
Salmonella typhi
Salmonella typhimurium
Serrata marcescens
Shigella sp.
Staphylococcus aureus
Streptococcus agalactiae, Str. dysgalactiae, Str. Uberis
Streptococcus faecalis
Streptococcus mutans
Streptococcus pneumonia
Streptococcus pyogenes
Trichophyton rubrum, T. tonsurans, T. mentagrophytes var.
Vibrio choleriae

Honey was reported to inhibit the growth of *Rubella* virus *in vitro* (Zeina *et al.*, 1996), three species of the *Leishmania* parasite (Zeina *et al.*, 1997) and *Echinococcus* (Kilicoglu *et al.*, 2006). Mundo *et al.*, (2004) collected honey samples from different sources and evaluated against food spoilage organisms and pathogens namely, *Alcaligenes faecalis, Aspergillus niger, Geotrichum candidum, Penicillium expansum, Lactobacillus acidophilus, P. fluorescens, B. cereus, E. coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* Ser. Typhimurium, *S. aureus, S. aureus* 9144 and *B. stearothermophilus*. The results showed that *B. stearothermophilus* was highly sensitive to honey, *A. faecalis* and *L. acidophilus* were less sensitive, and the fungi *A. niger, P. expansum, G. candidum* and *S. aureus* were unaffected to honey. Taormina *et al.*, (2001) observed that honey from six different sources in USA showed inhibitory activity against *E. coli* O157:H7, *Shigella sonnei*, *S. typhimurium*, *L. monocytogenes*, *B. cereus* and *S. aureus;* the activity was related to hydrogen peroxide and non-peroxide components. The antibacterial agent hydrogen peroxide is produced by honey glucose oxidase (White *et al*., 1963), and the production capacity depends on catalase activity of honey (Dustmann, 1971).

Malaysian honey has been reported to have antibacterial activity (Aljadi and Yusoff, 2003; Tan *et al*., 2009). Tan *et al*., (2009) evaluated the antimicrobial activity of Malaysian tualang honey from different aromatic and medicinal plants against multidrug resistant bacteria isolated from human; all honey samples showed strong activity against *E. coli, S. aureus, B. subtilis* and *Pseudomonas aeruginosa.* Recently, Abd-Rahman, (2011) observed that tualang honey obtained from seven different sources in Malaysia showed good inhibitory activity against *E. coil, S. typhimurium, Klebsiella pneumonia, B. cereus,* slight inhibitory activity towards *S. aureus*, and no activity against *S. epidermidis.* Voidarou *et al*., (2011) observed that almost all of the honey samples coniferous, thyme, citrus, and polyfloral honey showed antibacterial activity on the clinical bacteria such as *S. aureus, S. typhimurium, Streptococcus pyogenes, B. subtilis* and *B. cereus*. Similarly, Manuka honey from New Zealand showed similar antibacterial effects against those bacteria (Mandal & Mandal, 2011).

Substances of botanical origin of honey could contribute to the antimicrobial effects of honey (Molan, 1997; Bogdanov, 1997). Different chemical substances such as aromatic acids (Russell *et al*., 1988), unknown compounds with different chemical properties (Bogdanov, 1997) and phenolics and flavonoids (Cushnie & Lamb, 2005; Weston *et al*., 1999) are detected present in honey. It was reported that gelam honey and coconut honey produced in Malaysia contained non-peroxide factors, such as phenolic acids and these honey were shown to inhibit the growth of *E. coli* and *S. aureus* (Aljadi & Yusoff, 2003). Low pH of honey can also contribute to the antibacterial activity as observed by Yatsunami & Echigo, (1984).

2.3.8.1 Oral health

Caries are inhibited by honey because of its antimicrobial activity (Steinberg *et al*., 1996; Molan, 2001b) and could induce a carioprotective effect (Sela *et al*., 1998;

Edgar & Jenkins, 1974). The potential of honey as function of food for oral health was studied by University of Illinois at Chicago that focus on four different areas that include the honey effects on the growth and cariogenic properties of plaque bacteria, the formation and acidity of human dental plaque, the use of honey treatment on caries formation and aphthous ulcers (Bogdanov *et al.*, 2008). Inhibition of the growth and acid production of *Streptococcus mitis*, *Streptococcus sobrinus* and *Lactobacillus caseii* was reported from oral intake of honey (Bogdanov *et al.*, 2008). Similarly, manuka honey was reported to have antibacterial activity and positive effects against dental plaque development and gingivitis (English *et al.*, 2004). It can be concluded that honey is not as caiogenic as other sugars.

2.3.8.2 Gastroenterology

According to the Muslim the Holy Hadith, dating back to the 8th century A.D. Prophet Mohamed s.a.w. recommended honey against diarrhea (Al-Bukhaari, 1994). Honey was used as cure for diarrhea in the (ca. 25 A.D.) by the Roman physician Celsus in 1935 (cited by Bogdanov *et al.*, 2012). Gastro-intestinal disorders such as peptic ulcers, gastritis and gastroenteritis can be prevented by applying honey and this has been reported in various books and publications from Eastern Europe (Potschinkova, 1992; Cherbuliez & Domerego, 2003) and from Arabic countries (Salem, 1981).

Honey shows good inhibitory activity against peptic ulcers and gastritis caused by *Helicobacter pylori* (Al Somal *et al.*, 1994). Honey acted against gastric ulcers experimentally induced by indomethacin alcohol in rats (Ali, 1997; Gharzouli *et al.*, 2002). Honey has a stimulatory effect on the sensory nerves in the stomach that respond to capsaicin (Ali, 1997; Al Swayeh & Ali, 1998). Another mechanism of action has been suggested, postulating that this effect is due to the antioxidant activity of honey. Honey intake in rats prevented indomethacin-induced gastric lesions in rats by decreasing the ulcer index, microvascular permeability, and myeloperoxidase activity of the stomach (Nasuti *et al.*, 2006). Gastric juice acidity was reduced by 56% using dandelion honey (Baltuskevicius *et al.*, 2001). The ingestion of glucose and fructose mixture was faster than the ingestion of honey (Pokorn & Vukmirovic, 1978).

Human digestion is also significantly affected by honey and that was link to oligosaccharides. These honey constitutions have prebiotic effects, similar to that of fructo-oligosaccharides (Sanz *et al.*, 2005; Yun, 1996). The oligosaccharide panose was the most active oligosaccharide. Bifidobacteria and lactobacilli increased the probiotic activity by oligosaccharides in a synergistic mode of action (Ustunol, 2000). Five human bifidobacteria were stimulated in growth by sour-wood, alfalfa and sage origin honey (Shin & Ustunol, 2005). *Lactobacillus acidophilus* and *L. plantarum* were increased *in vivo* and *in vitro* by honey and that was due to the sucrose (Shamala, 2000). The duration of bacterial diarrhea was shortened clinically in infant and children by honey (Haffejee & Moosa, 1985).

2.3.8.3 Wound and burn healing

Honey was used as treatment for wounds and sores in ancient times. Nowadays the extensive effect of honey in wound healing confirms the antimicrobial agent and promoter of healing (Molan, 2002). Wound multitude types were successfully treated with honey dressings (Molan, 2001a). Therapeutic effects observed attributed to using honey as a wound dressing include rapid healing, clearance of infection, reduction of inflammation, stimulation of the healing process, cleansing action on wounds, stimulation of tissue regeneration, and the comfort of the dressings due to lack of adhesion to the tissues (Molan, 2001a). In recent years, honey has been rediscovered as a treatment for wound (Molan, 2001b). Laboratory research has verified its effectiveness against many pathogens that cause wounds infection, including some antibiotic resistant bacteria such as MRSA (Methicillan resistant *Staphylococcus aureus*) and VRE (Vancomycin resistant enterococci) (Allen *et al.*, 2000; Cooper *et al.*, 2000a & b).

Burns are possible to be cured by honey better than traditional dressings (Postmes, *et al.*, 1997). Several studies from India has reported that dressing with pure unprocessed, undiluted honey obtained from hives showed positive impact over conventional medical treatments such as OpSite® (Subrahmanyam, 1993a), Silver sulfadiazine (Subrahmanyam, 1997) and traditional, low-cost treatments such as boiled potato peels (Subrahmanyam, 1996). Comparing different dressings elaborated that honey is an effective dressing which makes healing faster, enhance formation of tissue, reducing inflammation and scaring (Cowley, 1990). Other advantages are the low coast of honey and easy dressing. On top of that, skin grafts were significantly cured at room temperature in honey (Subrahmanyam, 1993b).

Honey can be effective in curing infecting non-healing skin wounds (Mclnerney, 1990; Somerfield, 1991). Honey was evaluated on Fournier's gangrene with topical unprocessed honey and showed fast healing (Efem, 1991; Hajase *et al.*, 1996). Ampicillin and nitrofuazone were compared with honey in animal study using buffalo and, honey was more effective than tested antibiotics (Gupta, *et al.*, 1992; Kumar *et al.*, 1992).

Rozaini *et al.*, (2004) reported that topical application of honey on burn wounds can improved healing with regards to the tensile strength property. Nasir *et al.*, (2010) observe that Malaysian tualang honey has a bactericidal and bacteriostatic effect against wound and burn organisms, it is useful as a dressing as it is easy to apply and less sticky compared to manuka honey. However, for Gram positive bacteria, tualang honey is not as effective as silver-based dressing or medical grade honey dressing.

Honey was evaluated in surgical dressing for vulvectomies because of its bactericidal properties and there was significant success in treating ulcerations following radical surgery for carcinoma of the breast and varicose veins (Bulman, 1955). Other researchers reported using undiluted honey following radical operations for carcinoma of the vulva and the result showed that there were no infections, minimal debridement and less staying in hospital (Cavanagh *et al.*, 1970). Honey is recommended to be applied on wounds especially surgical wounds because of the low cost of honey (Ustunol, 2000).

2.4 Lactic acid bacteria (LAB)

LAB is Gram-positive microorganisms, prefer anaerobic conditions but are aerotolerant, acid-tolerant, and strictly fermentative. This groups of bacteria is nonpathogenic and save to use with the status of General Recognize as Safe (GRAS), acid resistant, bile tolerant and produce antimicrobial substances, including organic acids and hydrogen peroxide and bacteriocins (biologically active protein) (Dunne *et al*., 1999). These organisms produce lactic acid as the major end product during the fermentation of carbohydrates (Axelsson, 1998). LAB are used in the production of foods prepared by lactic fermentation such as dairy products, fermented vegetables, fermented meats, and sourdough bread (Hammes & Hertel, 2003). The most important genera are *Lactobacillus*, *Lactococcus*, *Enterocococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, and *Bifidobacterium*. *Bifidobacterium* shares certain physiological and biochemical properties with LAB and some common ecological niches such as the gastrointestinal tract. Therefore, for practical and traditional reasons, *Bifidobacterium* is considered a part of the LAB group.

Members of the LAB are usually divided into two groups based on their carbohydrate metabolism. The homofermentative group consisting of *Lactococcus*, *Pediococcus*, *Enterococcus*, *Streptococcus* and some lactobacilli utilize the glycolytic pathway to transform a carbon source mainly glucose into lactic acid (Axelsson, 1998). And heterofermentative bacteria produce equimolar amounts of lactate, CO_2, ethanol or acetate from glucose using phosphoketolase pathway. Members of this group include *Leuconostoc*, *Weissella* and some lactobacilli. The species belonging to *Enterococcus* genus are frequently found in traditional fermentations and may be included as a component of some mixed starters. However, their deliberate utilization in dairy fermentations still remains controversial, especially since some of the species have now been recognized as opportunistic human pathogens associated with hospital-acquired- and urinary tract infections (Franz *et al*., 1999).

2.4.1 Antibacterial activity of lactic acid bacteria

Several strains of bacteria were isolated from honey and demonstrated antimicrobial activity against both gram negative and positive pathogenic and spoilage bacteria (Aween *et al*., 2010; Ibarguren *et al*., 2010; Lee *et al*., 2008). Olofsson & Vasquez, (2008) isolated novel LAB in the genera *Lactobacillus* and *Bifidobacterium* from honeybee stomach, and the same isolates were also detected in honey. Lactic acid bacteria (LAB) are known for their antimicrobial activity specially lactobacilli (Klaenhammer, 1993). Coconnier *et al*., (1997) isolated *L. acidophilus* strain LB from human that showed antimicrobial activity against *K. pneumoniae*, *Enterobacter* spp., *S.* Typhimurium, *E.coli*, *L. monocytogenes*, *S. flexneri* and *P. aeruginosa*. Recently, Fathabad & Eslamifar *et al.*, (2011) reported that strain of *Lactobacillus paraplantarum* isolated from tea leaves has antimicrobial activity against *E. coli* and *S.* Typhimurium.

It is believed that naturally occurring lactic acid bacteria from different sources are known to produce different compounds that have the ability to inhibit the growth of both bacteria and fungi (Muhialdin *et al.*, 2011a & b). There are many reports on the production antimicrobial compounds by lactic acid bacteria (LAB) isolated from fruits and fermented food (Lindgren & Dobrogosz, 1990; Stiles, 1996; Muhialdin *et al.*, 2011a & b). LAB also produce various antimicrobial compounds, which can be classified as low-molecular-mass (LMM) compounds such as hydrogen peroxide (H2O2), carbon dioxide (CO2), diacetyl (2,3- biutanedione), and high-molecular-mass (HMM) compounds like bacteriocins (Jay,1982; Klaenhammer, 1988; Piard & Desmazeaud , 1991), those compounds can be used in many fields such as health care, pharmaceutical and preservation.

LABs have a wide range of antimicrobial activities, among these activities, the production of lactic acid and acetic acid is obviously the most important. On the other hand, certain strains of LAB are known to produce bioactive molecules like ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, reuterin, and reutericyclin. Many strains also produce bacteriocins and bacteriocin-like molecules that display antibacterial activity (De Vuyst & Vandamme, 1994).

2.4.2 Antibacterial compounds produced by LAB

The productions of lactic acid by lactic acid bacteria cause the reduction of the pH and in the end inhibition of spoilage microorganisms. On top of the pH effect, the undissociated acid collapses the electrochemical proton gradient, causing bacteriostasis and death of bacteria (Eklund, 1989).The production of lactic acid and acetic acid of lactic acid bacteria is very important. For example, strains of *Lactobacillus plantarum*, isolated from sourdough and grass silage, display antifungal activity, due to the production of organic acids, other low-molecular-mass metabolites, and/or cyclic dipeptides (Lavermicocca *et al*., 2000; Schnürer & Magnusson, 2005). The effect of acetic an probionic acid are also related to the inter action of the cell membrane and it depends on the reduction of the pH caused by lactic acid (Eklund, 1989).

Bacteriocins could be produced by many Gram positive and negative bacteria (Riley & Wertz, 2002). Bacteriocins which are produced by LAB have received particular attention because of their potential application in the food industry as natural preservatives (Ennahar *et al*., 1999). Bacteriocins produced by lactic acid bacteria are small, ribosomally synthesized, antimicrobial peptides or proteins that possess activity towards very much related to Gram-positive bacteria, whereas producer cells are immune to their own bacteriocin(s) (De Vuyst & Vandamme, 1994; Cotter, *et al*., 2005; Klaenhammer, 1988). The antibacterial spectrum frequently includes spoilage organisms and pathogens such as *Listeria monocytogenes* and *S. aureus*. Bacteriocins which produced by lactic acid bacteria have the potential to cover a very broad field of application including medical sector and food industry observe by. The mechanism of action of bacteriocin are causing pore in the cell membrane of the bacteria which cause the leakage of cytoplasm and finally death (De Vuyst & Leroy, 2007).

Lactic acid bacteria are well known producer of antimicrobial compounds especially bacteriocins which have high antimicrobial activity (Jay, 1982; Klaenhammer, 1993; Piard & Desmazeaud ,1991). Bacteriocins produced by *L. acidophilus* U1 isolated from pygmy goat meat showed antimicrobial activity against *E. coli* but did not inhibit the growth of *S.* Typhimurium (Ogunbanwo & Okanlawon, 2008). Oh *et al*., (2000) observed that bacteriocin from *L. acidophilus* 30SC obtain from dairy microbiology laboratory did not inhibit the growth of rang of Gram negative bacteria including *K. pneumoiae*, *E. coli* and *S. typhimurium*.

2.4.2 Identification of lactic acid bacteria

The identification of lactic acid bacteria was established many years ago because of the need to determine the strains that can be used in the industry to characterize the properties and the marketing value of the strain, and above of that is to confirm the safety of the strain to be used in the food application or even in pharmaceutical application. Historically, the identification of LAB was by using phenotypic and chemicals methods. These methods are based on the activity of the LAB and the different carbohydrate fermentation, hetero or homo fermentation, gas production, motility and spore producing.

In the last twenty years researches have to develop fast and reliable methods to identify LAB and these methods are used to reduce the time of the identification process. Some of mentioned methods have been developed by Biotech (Gen2 and Gen3) and API center (BioMérieux, France) and the principle of the test is based on the carbohydrate fermentation which is provided in each well and this takes 24 to 48 h, conceders faster than other chemicals methods. Phenotypic characterization based on sugar fermentation may not always offer sufficient basis for the reliable identification of LAB as observed by several researchers (Nigatu, 2000; De Angelis *et al.*, 2001; Muyana *et al.,* 2003; Ashmaig *et al.*, 2009).

Recently, the molecular biology has developed very fast and that had high impact on the microbiology world. Using the gene sequencing is the most reliable method of identifying the bacteria. 16S rDNA is one of these methods and it has been used for many cases for the identification of the bacteria especially LAB. Six isolates of LAB were isolated from several samples of honey and these isolates; H006-A, H006-C, H008-D, H008-E, H009-F and H010-G were identified as (*Lactobacillus acidophilus*) by using API 50 CHL. The primers 16S.S: (5-AGAGTTTGATCCTGGCTC-3) and 16S.R. (5-CGGGAACGTATTCACGG-3) were used to identify the isolates and the results were uncultured bacteria. LAB isolated from ripe mulberries collected in Taiwan were identified by using 16S rDNA, and then sequenced with the following primer: (5′-CTGCTGCCTCCCGTAG-3′) and the isolates were *Lactobacillus plantarum*, *W. cibaria* and *Lactococcus lactis* subsp. *Lactis*. That shows the use of the 16S rDNA is good for almost lactic acid bacteria species. The molecular identification is based on the similarity with other sequences within the data base. Although it is very useful and simple method for the identification of genus and species of bacteria, it does not allow differentiation of subspecies (Du Plessis *et al.*, 2004; Moreno-Arribas & Polo, 2008).

The 16S rRNA gene sequence has been widely used as a molecular method to identify the bacteria especially LAB bacteria. Lim *et al*., (2009) reported that *Lactobacillus brevis*, *Enterococcus faecium* and *Pediococcus acidilactici* were isolated from children faces and identified using primers: 27f (5 '-AGAGTTT-GATCMTGGCTCAAG-3') and1525r (5'-AAGGAGGTGWTCCARCC-3'). The two universal primers 27F (5_-GCCTTGCCAGCCCGCTCAGTCAGAGTTTGATCCTG-GCTCAG-3_) and 338R (5_- GCCTCCCTCGCGCCATCAGNNNNNNNNCATGC-TGCCTCCC-GTAGGAGT-3_) were used for PCR amplification to identify LAB isolates from vagina and the isolates identified as; *L. crispatus*, *L. iners*, *L. gasseri*, *L. jensenii* and *Streptococcus* reported by Forney *et al*., (2010).

The RAPD-PCR technique has been described as a useful technique for both identification and finger typing for bacteria (Du Plessis & Dicks, 1995; Rodas *et al*., 2005). It is very useful tool to distinguish between the strains that they are much related. Ashmaig *et al*., (2009) isolated LAB from fermented camel's milk and identified the isolates using API 50 CHL and RAPD-PCR analysis using primers: 5′-GTT GCG ATC C-3′, 5′-CAA ACG TCG G-3′ and 5′-AGG GGT CTT G-3′.