CHAPTER II

LITERATURE REVIEWS

2.1 Beehive

Bees are social insect. In the wild, they create elaborate nests called hives containing up to 20,000 individuals during the summer months. (Domestic hives may have over 80,000 bees.) The central feature of the bee hive is the honeycomb. This marvel of insect engineering consists of flat vertical panels of six-sided cells made of beeswax. House bees take the beeswax and form it with their mouths into the honeycomb. The cells within the comb are used to raise young and to store honey and pollen. The comb is two-sided, with cells on both sides as shown in figure 2.1. The cells are perfectly uniform in shape. Not only that, but the combs are built a precise distance apart depending on whether they are meant to contain food or young bees. The nursery area of the hive is called the brood comb, and that is where the queen lays her eggs [4].

The beehive products are divided into two groups: the products that bees imported raw materials from outside the hives such as honey, bee pollen, propolis and the products that bees produced themselves such as royal jelly, beeswax and bee venom.

Sumonthip preliminary study and a search for antibacterial activity in beehives and propolis (samples from different geographic origins of Thailand) against *S. aureus* and *E. coli* by agar diffusion method and accompanied by TLC. The results showed that both beehive and propolis extracts inhibited *S. aureus* and *E. coli*. The results of TLC experiments indicates that extract from beehive give positive reactions to the testing reagents which as flavonoids, lipids, sugars and amino acids the extract from propolis indicates positive reaction to the testing reagents in flavonoids, lipid, sugars and anthraquinones [5].

Figure 2.1 Characterization of beehive
(www.gotoknow.org/blog/orchidman1/50048)

2.1.1 Honey [6]

Honey is a sweet, thick syrup made by honeybees from nectar. Nectar contains approximately 70% water and 17% honey. After the bee has collected the nectar, she will store it in a special organ called the honey sac. Enzymes in the honey sac change large complex sugars in nectar, called polysaccharides, into simple sugars called monosaccharides. Upon return to the hive, the foraging bee will transfer the partially converted nectar to house bees, which will then remove most of the water through evaporation.

When the water content of the honey has been reduced to less than 20%, the bees will store the honey in the cells of the wax comb. The excess moisture is removed by evaporation as a result of the rapid fanning of the bees wings over the nectar cells in the hive. Since honey contains so little water, microbes such as bacteria and molds cannot grow in it. For this reason, honey can be stored for a long time. Honey contains simple sugars that are readily absorbed by the body. Pollen grains and a wide variety of other substances such as minerals and vitamins are also presented in small quantities.

Differences in honey colour, aroma and taste are determined by the flower source, not by the honeybees. When honey has been left standing in a cool place, sugar crystals form. This is a natural process and can be "undone" by

warming the honey. The bees use the honey as a carbohydrate source to feed themselves.

Figure 2.2 Characterization of honey

(www.burnoutcig.com/products_new....p/page/6)

2.1.1.1 Composition of honey. [7]

The precise composition of honey varies according to the plant species on which the bee forages, but the main constituents are the same in all honeys. The average composition of honey is given in Table 2.1.

Table 2.1 Average composition of honey

Component	Average (%)
Moisture	17.20
Fructose	38.19
Glucose	31.28
Sucrose	1.31
Disaccharides, calculated as maltose	7.31
Higher sugars	1.50
Free acid as gluconic	0.43
Lactone as Gluconolactone	0.14
Total acid as gluconic	0.57
Ash	0.169
Nitrogen	0.041

2.1.1.2 The benefits of honey

Antibacterial activity of honey

Hydrogen peroxidase which is produced by the glucose oxidase in honey could be the inhibitory substance against bacteria. However, it is known that honey itself, as well as bacteria produce a catalase that eliminates hydrogen peroxide. But although activity of the catalase with high concentrations of hydrogen peroxide, it is of low activity with physiological levels [8].

Lavie P. Les. found an additional group of lightsensitive, heat-stable antibacterial factors in honey which inhibited the growth of *Bacillus subtilis, B. alvei, Escherichia coli, Pseudomonas pyocyanes, Salmonella* and *Staphylococcus aureus* [9].

Gastroenteritis

Infections of the intestinal tract are common throughout the world, affecting people of all ages. The infectious diarrhoea exacerbates nutritional deficiencies in various ways, but as in any infection, the calorific demand is increased. Pure honey has bactericidal activity against many enteropathogenic organisms, including those of the *Salmonella* and *Shigella* species, and enteropathogenic *E. coli* [10].

In vitro studies of *Helicobacter pylori* isolates which cause gastritis have been shown to be inhibited by a 20% solution of honey. Even isolates that exhibited a resistance to other antimicrobial agents were susceptible [11].

Gastric ulcers

Clinical and animal studies have shown that honey reduces the secretion of gastric acid. Additionally, gastric ulcers have been successfully treated by the use of honey as a dietary supplement. An 80% recovery rate of 600 gastric ulcer patients treated with oral adminstration of honey has been reported. Radiological examination showed that ulcers disappeared in 59% of patients receiving honey [12].

A controlled clinical trial demonstrated the use of fructose in the treatment of acute alcoholic intoxication. A small but significant increase ocurred

in the rate of fall of blood-ethanol levels and it was concluded that fructose may be beneficial in shortening the duration of alcoholic intoxication [13].

Wound healing properties

Patients suffering from wound breakdown after operation for carcinoma of the vulva were treated by pouring honey into the wounds twice daily. The wounds became bacteriologically sterile within 3-6 days and *in vitro* studies of bacteria cultured from the wounds showed that undiluted honey not only failed to sustain growth of the bacteria (*Proteus mirabilis, P. aeruginosa, E.coli, Streptococcus faecalis, Clostridium perfingens* and *S. aureus*), but actually killed them. The in vitro anti-fungal activity of honey has been also tested on *Candida albicans, C. pseudotropicalis, C. stellatoidea* and *C. tropicalis*. They were all found to be susceptible [14].

In a clinical study involving 59 patients with wounds and ulcers, most of which had failed to respond to conventional treatments, 15-30 ml fresh honey was applied daliy. The bacteria isolated from 58 of these wounds (*E. coli, S. aureus, P. mirabilis*, mixed coliforms, *Klebsiella* species, and *S. faecalis*) were all susceptible to honey *in vitro*. One other bacteria, *P. pyocyanea* did not undergo complete lysis *in vitro* tests but it was completely sterilised *in vivo*. In one case in which the patient had a Buruli ulcer infected with *Mycobacterium ulcerans*, honey treatment was ineffective an *in vitro* tests showed the mycobacteria to be resistant to honey [15].

Diabetes

Bornet *et al.*(1085) demonstrated the sucrose or honey at breakfast have no additional acute hyperglycaemic effect over and isoglucocidic amount of bread in type II diabetic patients [16]. In addition, a clinical study by Katsilambros NL *et al.* (1988) indicated that honey could be a suitable sweetner for the type II diabetic diet since fat-rich foods added to honey result in higher triglyceride and insulin serum concentration [17].

Honey also can be use in beauty industry. Because honey is a strong anti-inflammatory agent that prevents infections and reduces the existing ones

when applied on problem, blemish or sensitive skin. It also has antibacterial properties used for preparation of body scrub recipes that cleanse the body and leave it bacteria free. In general, honey is effective in beautifies and soothes the skin, clears many skin disorders. Besides this, honey also can be replace with table sugar for who was in diet. Because honey is fat free and natural sweetener. Fructose and glucose play an important role in processing fatigue during exercise and are quickly absorbed by the body. The developer of the diet, who discovered that the athletes eating foods rich in fructose (such as honey) burnt more fat and had increase stamina. Honey has also been found to keep levels of blood sugar fairly constant compared to Table sugar [18].

2.1.2 Bee pollen

Bee pollen is the male seeds of a flower blossom which has been gathered by the worker bees and to which special elements from the bees has been added. The bees collected and stored the pollen in their hives. It is collected on the legs of bees and formed into granules as they move from the flower to flower looking for nectar. The bees secrete nectar and special enzymes into the flower pollen to create what we know as "bee pollen". The pollen is usually collected by placing a special device at the entrance of the beehives that brushes it from the hind legs of the bees into a collection vessel [19].

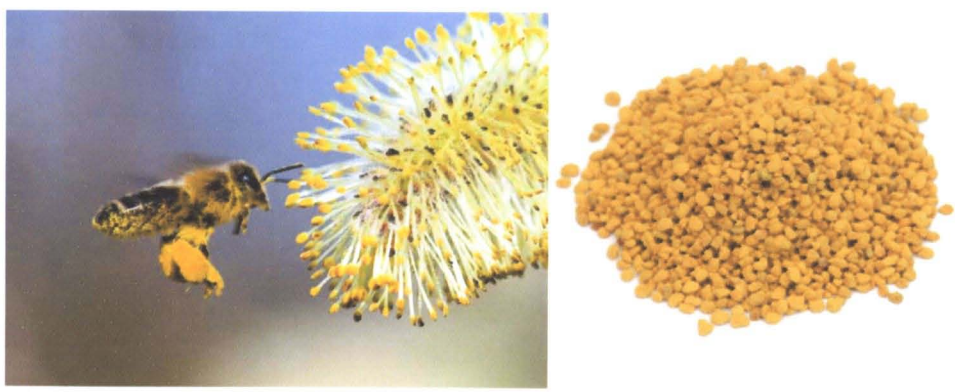

Figure 2.3 Characterization of bee pollen
(www.natures-desktop-hd.com/bee-d...ying.php)

2.1.2.1 The compositions of bee pollen [20]

Table 2.2 Percentage composition of bee pollen

Composition	Unit
Amino acid	**(%)**
- Arginine	4.4-5.7
- Histidine	2.0-3.5
- Isoleucine	4.5-5.8
- Leucine	6.7-7.5
- Lysine	5.9-7.0
- Methionine	1.7-2.4
- Phenylalanin	3.7-4.4
- Treonine	2.3-4.0
- Tryptophan	1.2-1.6
- Valine	5.5-6.0
Minerals	**(%)**
- Potassium	20-45
- Magnesium	1-12
- Calcium	1-15
- Copper	0.05-0.08
- Iron	0.01-0.3
- Silicon	2-10
- Phosphorus	1-20
- Sulfur	1
- Manganese	1.4

Table 2.2 (Continued.) Percentage composition of bee pollen

Composition	Unit
Vitamins and Hormones	**(µg/g)**
- Thiamine	5.75 – 10.8
- Riboflavin	16.3 – 19.2
- Nicotinic acid	98 – 210
- Pyridoxine	0 – 9
- Pantothenic acid	3.51
- Biotin	0.1 – 0.25
- Folic acid	3.4 – 6.8
- Lactoflavine	0.2 – 1.7
- α / β Carotene (A)	avg. 1.53
- B_2	16.3 – 19.2
- C	152 – 640
- D	0.2 – 0.6
- E	0.1 – 0.32
- Inositol	30 – 40
- B_{12}	avg. 0.0002
Pigments	
- Flavoxanthin	
- Xanthophyll eposides	
- Carotene	
- Epiphisic Cardenoids	

Table 2.2 (Cont.) Percentage composition of bee pollen

Composition	Unit
Pigments	
- Flavonols	
- Ethylic Ether	
- Quercetin	
- Zeaxanthine	
- Lycopene	
- Crocetin	
Other Components	**(%)**
- Water	3 – 4
- Reducing sugars	7.5 – 40
- Non-reducing sugars	0.1 – 19
- Starches and other carbohydrates	0 – 22
- Etheric extract	0.9 – 14
- Proteins	7 – 35
- Free amino acids	10
- Human Growth Hormone Factor	Not measured
- Hormones (gonadatropic estrogenic)	Not measured
- Rutin	Not measured
- Ash	1 - 7

2.1.2.2 Benefits of bee pollen

Antioxidant/anti-aging

The oxidative damage caused by free radicals have been implicated in quite a number of disease processes, and is the primary factor in aging. Antioxidants are capable of providing protection, sometimes significant protection, against this oxidative damage. Interestingly, bee pollen appears to provide significant antioxidant activity, which may explain its traditional use as an anti-aging food [21]. One animal study demonstrated that bee pollen (as well as beta-carotene oil), was able to abolish the effects of harmful ionizing radiation on the brain. This was a function of bee pollen's antioxidant properties [22]. Xrays can activate lipid peroxidation (i.e., free radical activity) in the liver, and adversely affect liver glutathione (i.e., antioxidant) systems. Animal research has shown that bee pollen is able to normalize the activity of important glutathione system enzymes in the liver [23]. Another study demonstrated that bee pollen was able to markedly decrease lipoperoxide levels in animals fed a limited diet, compared to animals not receiving bee pollen [24].

Free radicals can also contribute towards lipofuscin, also known as age pigments and liver spots. These are commonly seen as small brownish spots on the back of hands on elderly people. Liver spots are actually an outward sign of internal toxic accumulation of lipofuscin; including, but not limited to vital nerve centers such as the brain. Such toxic accumulation of lipofuscin can block nutrient absorption in the cells. Animal research has shown that bee pollen markedly reduces lipofuscin in the cardiac muscle (heart), significantly inhibits the increase of lipofuscin in cardiac muscle, liver, brain and adrenal gland cells [25].

Increase red blood cells & hemoglobin

Traditionally, bee pollen has been used as a food to help increase energy levels. One possible explanation for this use is that bee pollen helps to increase red blood cells, and the hemoglobin component of red blood cells. Since it is the hemoglobin in red blood cells that carry oxygen for energy metabolism, this may explain the relationship between bee pollen and energy. For example, in one animal study, bee pollen resulted in increases in hemoglobin and serum iron [26]. In a study

on humans, bee pollen and several other Chinese herbs were found to increase the number of red blood cells [27].

2.1.3 Propolis [28]

Propolis is a natural resin from buds of conifer and poplar trees that create by bees to build their hives. It is also sticky glue that bees use to seal hive hole or cracks. This natural glue is cleverly utilized by the bees to provide exterior protection to the hive against the invasion of any outside contaminants. Normally propolis is dark brown color, but it can be found in green, red, black and white hues, depending on the sources of resin found in the particular hive area.

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Figure 2.4 Characterization of propolis
(www.addwellsuccess.com/more-abou...ok-like)

2.1.3.1 The composition of propolis [28-29]

Propolis consists of resins, waxes, volatile oils, pollen, vitamin A, B complex, E, minerals, waxes, amino acid, ethanol and highly active ingredients known as flavonoids or bioflavonoids. Other components include cinnamic acids, vanillin, caffeic acids, pinocembrin, tetochrysin, chrysin, isalpinin, galangin and ferulic acids.

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2.1.3.2 Benefits of propolis

Antibacterial/antifungal activity

The antimicrobial activity of EEP (ethanol extract of propolis) showed activity against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis, Bacillus subtilis, Bacillus cereus* and *Bacillus liqueiniformis*) and fungi (*Candida albicans*), whereas, no activity against Gram-negative bacteria (*Salmonella enteritidis*, *Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris*) [30].

In-vitro research has demonstrated that propolis has significant antibacterial activity, and also helps to reduce oxidation potential [31]. Other research has also verified that the growth of bacteria (particularly Gram-positive bacteria) is inhibited by propolis [32-34].

In addition to its antibacterial properties, propolis has also been found to have antifungal effects against *C. albicans* [35].

Dental research

Dental studies have also been conducted on the value of propolis, including its antibacterial properties. In one study, propolis was found to inhibit certain enzymes and bacteria that are chief culprits in the formation of dental caries (cavities) [36]. Other research on propolis has also demonstrated a similar antibacterial effect, including a reduction of bacteria in saliva [37].

Another dental-related value of propolis is its desensitizing properties for teeth. In one study, propolis was administered to 26 women over a period of four weeks. The women were tested for teeth sensitivity by two methods: 1) cold air stimuli, and 2) subjective reporting of pain. Eighty five percent of the subjects in this study reported that they were highly satisfied; the propolis had significant desensitizing effects on their sensitive teeth [29].

Anti-viral activity

Besides its antibacterial properties, propolis has also demonstrated significant antiviral properties particularly where cold viruses are

concerned. For example, in one study, preschool and school children were treated with propolis during the cold season. Favorable effects of propolis treatment were observed, including a lowering of the number of cases common cold with acute or chronic symptoms, and decrease and suppression of the viruses and other microbes in the upper airways [38].

In-vitro research has also shown that the flavonoids found in propolis caused a reduction of intracellular replication of herpes-virus strains [39].

2.1.4 Royal jelly

Royal jelly is a substance produced by worker honey bees. If fed to an ordinary female bee in the larval stage, royal jelly will transform her into the queen bee. As a queen, she will grow 1½ times normal size, become extremely fertile and lay over a thousand eggs each day. Incredibly, she may live over five years while all the other bees live only a few weeks. The only difference is that she receives royal jelly while the others don't [21].

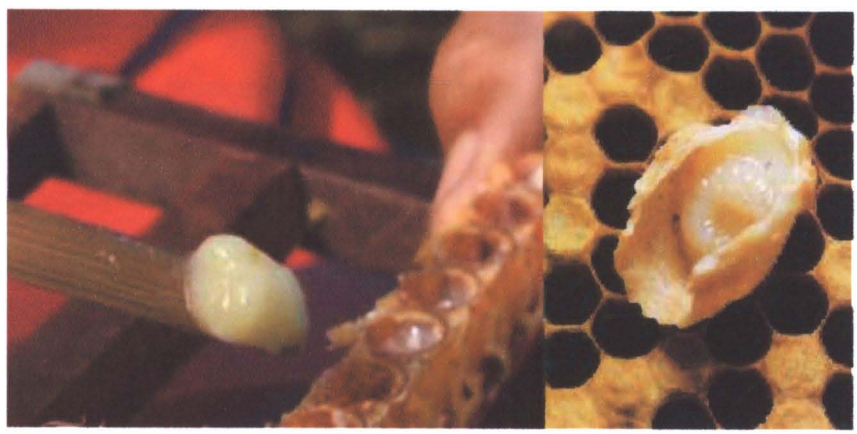

Figure 2.5 Characterization of royal jelly
(www.thailanna.co.th/index.php%3F...3D197829)

2.1.4.1 The composition of royal jelly [21, 40]

Table 2.3 Percentage composition of royal jelly

Composition	Percentage
1. Proteins	12
2. Sugar	12
3. Fat and variable amounts of minerals (calcium, iron, potassium, copper, silicon and sulfur), vitamins (B-1, B-2, B-6, C, E, niacin, biotin, inositol, folic acid and pantothenic acid) and pheromones	6
4. 10-hydroxy-trans-(2)-decanoic acid (HAD)	15
5. B vitamins and pantothenic acid	
6. Amino acids	
7. Lipids	
8. Sterols	
9. Phosphorous (acetylcholine)	

2.4.1.2 Benefits of royal jelly

Scientific research on royal jelly has revealed that it possesses antitumor activity in experimental mouse leukemias [41]. Additional antibacterial activity against Gram-positive bacteria, but not against Gram-negative bacteria [42]. Furthermore, research with chronically diabetic rats demonstrated that anti-inflammatory action and is able to augment wound healing [43]. Royal jelly has also been shown to prevent the cholesterol-elevating effect of nicotine [44] and has lowered serum cholesterol in animal studies [45]. Some research has also demonstrated that can lower cholesterol levels in humans [46-47]. Cholesterol – lowering research has shown that the typical dose used for this purpose is 50-100 mg daily [48].

Some of the many reported benefits of royal jelly [40] :

- Helps promote mental alertness
- Helps combat dizziness, buzzing in the ears

- Found to be helpful to people who suffer from nervousness, anxiety and memory loss
- Found to greatly improve energy levels thereby helping eliminate fatigue
- Fortifies your natural immune system to help your body fight infections and diseases.
- Treating bronchial asthma and liver disease.
- Helps enhance sexual desire and performance.
- Treating stomach ulcer and bone fractures
- Have the ability to restore the skin 's youthfulness and improve the skin beauty. It is because royal jelly is rich in nucleic acids and gelatin, is the one of the precursors of collagen. Collagen is famous as anti-aging element

2.1.5 Beeswax [49]

Beeswax is the material used by honeybees in the construction on their combs. Normally beeswax is produced by young worker bees between 12-17 days old in the form of thin scales secreted by glands on the ventral surface of the abdomen. However some older bees can produce wax, dependent upon the state of their wax gland development.

Pure beeswax, normally is in pale yellow color, is sometimes nearly white and the different in color is depend to pollen consumed by the bees. The beeswax have very stable property. Its chemical characteristic keeps unchanged even long time replacement, it is resistant to hydrolysis and nature oxidization and is completely insoluble in water, less digestive acids or juices can dissolve of it except larvae of wax moth. Being a nature lipid that contains in various of hydrocarbons, acids or hydroxyl-acids, alcohols and pigment which comes from pollen and propolis, its stable property is very welcome.

Figure 2.6 Characterization of beeswax
(www.learners.in.th/blog/episky-beebee/277165)

2.1.5.1 The composition of beeswax

Beeswax consists mainly of ester of higher fatty acids and alcohols. Apart of ester, beeswax contains small quantities of hydrocarbons, acids and other substances [50].

Table 2.4 Percentage composition of beeswax [51]

Components	Percentage
Ester	67
Hydrocarbons	14
Free acids	12
Alcohols	1
Others	6

2.1.5.2 Benefits of beeswax [49]

The uses of beeswax are many. Some major uses of beeswax are cosmetics and candle making. Some minor uses are lotions, cold creams, ointments, salves, lipsticks, rouges, coatings for electrical apparatus, floor and furniture polishes, leather polishes, art and craft items, crayons, inks. Besides this external use, beeswax also offers many medical benefit as below:

- Helps in swelling and infected wounds speedy
- Helps to reduce infections and diseases

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- Helps in treatment of cancer
- Helps to increase sexual desire and performance

2.1.6 **Bee venom** [52]

Bee venom, also called "Apitoxin" which is made up of enzymes, proteins and amino acids. Bee venom is produced in the venom gland of female bees (workers and queens) from the time they are 14 days old and stored in their venom sac (0.3 mg per bee). It is a bitter colorless clear liquid, with a sweet taste and little bitter. Bee venom is soluble in water, insoluble in alcohol and ammonium sulphate. Apitoxin is acidic. It is similar to snake venom and nettle toxin. Apitoxin can be deactivated with ethanol.

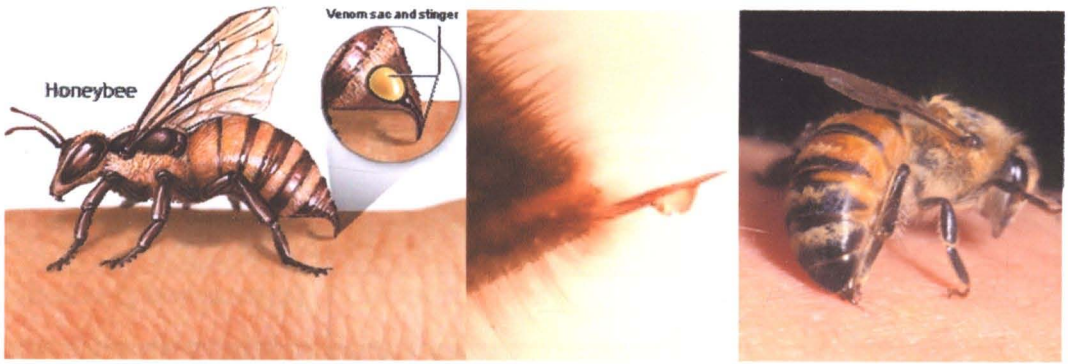

Figure 2.7 Characterization of bee venom
(www.honeyhivetaupo.com/category....yid%3D52)

2.1.6.1 **The compositions of bee venom** [53]

Table 2.5 The compositions of bee venom dry matter

Substance group	Component	% dry of weight
Proteins (Enzymes)	Phospholipase A2	10-12
	Phospholipase B	1
	Hyaluronidase	1-2
	Phosphatase	1
	α - Glucosidase	0.6

Table 2.5 (Continued.) The compositions of bee venom dry matter

Substance group	Component	% dry of weight
Peptides	Pamine	1-3
	Minimine	2
	Adolapine	0.5-1
	Procamine A, B	1-2
	Protease inhibitor	0.1-0.8
Phospholipids		1-3
Biogenic amines	Histamine	0.5-2
	Dopamine	0.2-1
	Noradrenalin	0.1-0.5
Amino acids	Aminobutyric acid, α-amino acids	1
Sugars	Glucose, fructose	2-4
Volatiles (pheromones)	Complex ethers	4-8
Minerals	P, Ca, Mg	3-4

2.1.6.2 Benefits of bee venom [52]

Bee venom has multiple effects on the body:

- Improves blood circulation
- Support physical strength and well being
- Support to join mobility
- Has anti-oxidant effect
- Fortifies the natural immune system to increase antibody against infections and diseases
- Promotes mental alertness
- Helps in maintain lower cholesterol
- Combats dizziness, buzzing in the ears

- Helpful to people who suffer from nervousness, anxiety and memory loss
- Improves energy levels thereby helping eliminatefatigue
- Helps in enhance sexual desire and performance
- Helps in grow of smooth and healthy skin

2.2 Bacteria causing skin diseases

Skin is a barrier between the internal organs and the environment. Bacteria are always around us. Including, our skin is host of bacteria most of which are beneficial. The friendly flora in our gut, more than 200 species of bacteria reside within the tissues exposed to the external environment. Skin infections result from these bacteria when the integrity of the skin breaks down or when the immune defense system is weak. Skin infections can occur on the skin surface or deeper within the skin tissue. Bacteria that cause infections of the skin are as follows.

2.2.1 *Staphylococcus aureus* [54-55]

Scientific classification

Domain	:	Bacteria
Kingdom	:	Eubacteria
Phylum	:	Firmicutes
Class	:	Bacilli
Order	:	Bacillales
Family	:	Staphylococcaceae
Genus	:	*Staphylococcus*
Species	:	*aureus*
Binomial name	:	*Staphylococcus aureus*

2.2.1.1 Morphology [56]

S. aureus is Gram-positive, spherical cells about 1 μm in diameter arranged in irregular clusters and found free-living in the environment. Grow most rapidly at 37°C but form pigment best at room temperature (20-25 °C). Colonies on solid media is gray to deep golden yellow.

2.2.1.2 Infections [57]

In humans, staphylococci produce a variety of infections that involve any and all tissues of the body. The disease states are characterized by pus formation. They include carbuncles, deep tissue abscesses, empyema, endocarditis, furuncles, boils, impetigo contaginosa, meningitis, osteomyelitis, pneumonia and wound infections. In recent years, staphylococcal infections have gained additional significance because of the appearance of drug-resistant mutants of *S. aureus*.

2.2.1.3 Drug susceptibility [58]

When penicillin was first introduced, the vast majority of strain of *S. aureus* were susceptible. Depending upon the geographic area, as many as 50 percent of strains occurring in the population-at-large are now penicillin-resistant, and 80 percent of strains responsible for hospital-acquired disease may be resistant. Resistance is due to the plasmid mediated enzyme penicillinase, which is a β-lactamase that splits that β-lactam ring of the penicillin nucleus [59]. In severe staphylococcal disease, unless the strain is sensitive to penicillin,penicillinase-resistant penicillins such as oxacillin and methicillin are the drugs of choice. In patients allergic to penicillin, cephalosporin derivatives may be used. Combined therapy with an aminoglycoside such as gentamicin is often employed, but there is no clear evidence that such combinations are more effective than the use of the bactericidal penicillins or cephalosporins alone. Vancomycin is used in patients who are allergic to the penicillins and cephalosporins.

2.2.2 Methicillin-resistant *Staphylococcus aureus* (MRSA)

2.2.2.1 Morphology

S. aureus is a Gram positive, coagulase positive coccus in the family *Staphylococcaceae.* MRSA is a particular strain of it [60].

Methicillin is an antibiotic that was commonly used to treat *S. aureus* infections. Although methicillin was very effective in treating most *S. aureus* infections some *S. aureus* bacteria developed resistance to methicillin and can no

longer be killed by this or similar antibiotics. These resistant bacteria are called methicillin-resistant *Staphylococcus aureus* or MRSA. [61].

2.2.2.2 Infections

In people, *S. aureus* is an opportunist. MRSA can cause the same types of infections as other isolates of *S. aureus*. This organism can be involved in a wide variety of skin and soft tissue infections including impetigo, folliculitis, furunculosis, cellulitis, abscesses and wound infections. MRSA can also cause invasive infections such as pneumonia, endocarditis, septic arthritis, osteomyelitis, meningitis and septicemia [62-69].

MRSA has also been detected in cases of staphylococcal scalded skin syndrome in infants and adults [70-73]. This disease, which is caused by strains that carry exfoliative toxins is characterized by widespread blistering and loss of the outer layers of the epidermis [62]. Staphylococcal scalded skin syndrome usually occurs in children. In adults, this disease is generally associated with immunosuppression [71].

2.2.2.3 Drug resistance [58]

Penicillinase plasmids in staphylococci have created a less serious clinical problem than the R factors in gram-negative bacteria, because multiple resistance is not usually transmitted by penicillinase plasmids. Antibiotics that are resistant to penicillinase can therefore successfully treat infections caused by penicillinase-producing staphylococci. An exception to this is the resistance to methicillin in 10 to 17 percent of staphylococcal infections in England, France and Switzerland occurring in hospitalized patients with chronic debilitating diseases [74]. This resistance may cross over to other penicillinase-resistant penicillins and is not caused by inactivation of the drug. Because methicillin-resistant staphylococci are confined to extremely debilitated patients, they have probably lost virulence, even though they cause serious and even fatal infections. They are becoming a problem in the Unitated States, where such strains have been isolated recently in growing numbers.

2.2.3 Streptococcus pyogenes (Beta-Hemolytic Streptococcus group A) [55,75]

Scientific classification

Domain	:	Bacteria
Kingdom	:	Eubacteria
Phylum	:	Firmicutes
Class	:	Cocci
Order	:	Lactobacillales
Family	:	Streptococcaceae
Genus	:	*Streptococcus*
Species	:	*pyogenes*
Binomial name	:	*Streptococcus pyogenes*

2.2.3.1 Morphology [76]

The group A streptococcus bacterium (*Streptococcus pyogenes*, or GAS) is a Gram-positive, nonmotile, nonsporeforming coccus that occurs in chains or in pairs of cells. Individual cells are round-to-ovoid cocci, 0.6-1.0 μm in diameter. Streptococci divide in one plane and thus occur in pairs or (especially in liquid media or clinical material) in chains of varying lengths. The metabolism of *S. pyogenes* is fermentative; the organism is a catalase-negative aerotolerant anaerobe (facultative anaerobe), and requires enriched medium containing blood in order to grow. Group A streptococci typically have a capsule composed of hyaluronic acid.

The type of hemolytic reaction displayed on blood agar has long been used to classify the streptococci. β-hemolysis is associated with complete lysis of red cells surrounding the colony, whereas α-hemolysis is a partial or "green" hemolysis associated with reduction of red cell hemoglobin. Nonhemolytic colonies have been termed γ -hemolytic.

2.2.3.2 Infections [57]

Erysipelas

This disease occurs world-wide, but with greater frequency in the temperate zone. Erysipelas is an acute infection arising as a complication of

surgery or of accidental wounds. Usually victims of the disease show a history of minor injuries. Group A streptococci are the ones most often found causing this disease.

Scarlet fever

Various strains of GAS are known to cause scarlet fever. Many of these organisms also produce other infections, including septic sore throat, erysipelas, tonsillitis, puerperal fever, and wound abscesses. Typically, scarlet fever is an acute inflammation of the upper respiratory tract that may be accompanied by a generalized rash. In recent years this infection appears to be milder in its effects. The term scarlatina is often used to designate the milder forms of scarlet fever. The various effects of the infection are directly related to a toxin produced by streptococci infected by a temperate bacterial, virus. The toxin usually spreads from the infected site to other parts of the body.

Transmission : Scarlet fever infection is spread by means of droplet nuclei, aerosols, contaminated food and water and direct contact with carriers, individuals in the acute stage of the disease. Fomites have been implicated, but it is believed such contaminated objects are not commonly involved.

2.2.3.3 Drug susceptibility [58]

Unlike staphylococci, no group A streptococcus strain has been resistant to penicillin. This makes penicillin the drug of choice in the treatment of streptococcal infections and in prevention of rheumatic fever. The MIC for penicillin G is usually 0.001 μg/ml. About 40 percent of strains are resistant to tetracycline and sulfadiazine. Resistance to erythromycin is uncommon and is mediated by a plasmid. Streptococci are resistant to polymyxins, kanamycin and streptomycin.

2.2.4 *Escherichia coli* [55,77]

Scientific classification

Domain	:	Bacteria
Kingdom	:	Eubacteria
Phylum	:	Proteobacteria
Class	:	Gamma proteobacteria
Order	:	Enterobacteriales
Family	:	Enterobacteriaceae
Genus	:	Escherichia
Species	:	coli
Binomial name	:	*Escherichia coli*

2.2.4.1 Morphology

E. coli is a Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped and are about 2.0 μm long and 0.5 μm in diameter with a cell volume of 0.6 – 0.7 μm. Optimal growth of *E. coli* occurs at 37°C but some laboratory strains can multiply at temperatures of up to 49°C.

Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine.

2.2.4.2 Infections [78]

E. coli infections include septic arthritis, endophthalmitis, suppurative thyroiditis, sinusitis, osteomyelitis, endocarditis, skin and soft-tissue infections (especially in patients with diabetes), acute bacterial meningitis, pneumonia, intra-abdominal infections, enteric infections and urinary tract infections.

2.2.4.3 Drug sensitivity [58]

Except for benzyl penicillin, Enterobacteriaceae are intrinsically sensitive to achievable serum and tissue concentrations of most antimicrobials when they

are first introduced. Resistant strains are selected quickly, however, because the Enterobacteriaceae in the normal intestinal tract are frequently exposed to antibiotics directed another, more invasive bacteria. Furthermore, some genera of Enterobacteriaceae are intrinsically resistant to certain antimicrobials. Rapid selection of resistant mutants may occur when Enterobacteriaceae are exposed to nalidixic acid, rifampin or the aminoglycosides as single antimicrobial therapy. All Enterobacteriaceae are intrinsically resistant to achievable levels of erythromycin and the other macrolides.

Most of the genetic determinants of resistance, however are carried on transferable plasmids that have been responsible for the appearance of an increasing number of multiresistant Enterobacteriaceae. The use of antibiotics for treatment of disease and as food additives in animal breeding has exerted a powerful selection pressure for antibiotic-resistant strains. When the use of antibiotics is restricted, the number of resistant strains in that area decreases [79].

Thus, the antimicrobial sensitivities of individual species of this important family of bacteria are unpredictable. They vary from broad sensitivity to complete resistance to the tetracyclines, aminoglycosides, the sulfonamides, cephalosporins, polymyxins, chloramphenicol, ampicillin and carbenicillin. For this reason, antimicrobial sensitivity tests must be conducted quickly and accurately to ensure effective therapy against enterobacterial infections.

2.2.5 *Psuedomonas aeruginosa* [55, 80]

Scientific classification

Domain	:	Bacteria
Kingdom	:	Eubacteria
Phylum	:	Proteobacteria
Class	:	Gamma proteobacteria
Order	:	Pseudomanadales
Family	:	Pseudomanadaceae
Genus	:	Pseudomonas
Species	:	aeruginosa
Binomial name	:	*Pseudomonas aeruginosa*

2.2.5.1 Morphology [81]

P. aeruginosa is a gram-negative, rod-shaped, asporogenous and monoflagellated bacterium that has an incredible nutritional versatility. It is about 1-5 μm in length and about 0.5-1.0 μm in breadth and is an obligate aerobe, which means it requires oxygen and uses aerobic respiration as its choice of metabolism. Due to its capability to synthesize arginine, *P. aeruginosa* can also proliferate in anaerobic conditions. This, then, makes *P. aeruginosa* a very ubiquitous microorganism, for it has been found in environments such as soil, water, humans, animals, plants, sewage, and hospitals. In all oligotropic aquatic ecosystems, which contain high-dissolved oxygen content but low plant nutrients throughout, *P.aeruginosa* is the predominant inhabitant and this clearly makes it the most abundant organism on earth.

2.2.5.2 Infections

P. aeruginosa has been largely ignored as a causative agent of several specific skin diseases. This has been reported to produce a variety of skin infections associated with epidermal destruction, such as with burns. *P. aeruginosa* septicemia may develop from these cases or from changes in the normal human flora or immune mechanisms of the individual, brought on by the use of antibiotics or drugs capable of suppressing antibody production. In healthy persons the pathogenic effects of this organism are limited. Examples of these dermatologic infections include "green nail" syndrome and toe web infections [57].

Further *P. aeruginosa* produces infection of wounds and burns, giving rise to blue- green pus; meningitis, when introduced by lumbar puncture; and urinary tract infection, when introduced by catheters and instruments or in irrigating solutions. Involvement of the respiratory tract, especially from contaminated respirators, results in necrotizing pneumonia. The bacterium is often found in mild otitis externa in swimmers. It may cause invasive (malignant) otitis externa in diabetic patients. Infection of the eye, occurs most commonly after injury or surgical procedures [56].

2.2.1.3 Drug susceptibility [58]

Most strains of *P. aeruginosa* are susceptible to one or more of the aminoglycosides, amikacin, gentamicin, kanamycin, netilmicin, sisomycin, and tobramycin [82]. Resistance to one aminoglycoside is not necessarily accompanied by resistance to others. Resistance, when present, may be attributed either to failure of the agent to enter the bacterium or to enzymatic inactivation. Both chromosomal and plasmid DNA are involved in the mediation of resistance.

The uniform resistance to penicillin appears referable to both exclusion by the cell membrane and chromosomally mediated β-lactamase. Penicillin derivatives such as carbenicillin and ticarcillin presently are effective therapeutic agents since they do cross the membrane and are relative resistant to the β-lactamase. Mucoid strains are exceptional in that they frequently are resistant to both carbenicillin and tobramycin but are susceptible to tetracycline [83]. Most strains of *P. aeruginosa* are susceptible to polymyxin B and E (colistemethate) but are resistant to other antimicrobials.

Table 2.6 Features of selected bacterial diseases associated with the skin [57]

Disease	Causative Agent	General Features of the Disease	Possible Treatment
Boils, carbuncles	*S. aureus*	Localized swollen areas of tissue destruction in deeper skin layer ; may lead to bloodstream invasion ; fever and general malaise	Appropriate antibiotics ; for penicillin-resistant organisms, semi-synthetic penicillins not affected by penicillinase
Erysipelas	GAS	Fever, headache, stinging or itching at site of infection developing into widespread thickened, reddened areas	Penicillin, or erythromycin, symptomatic
Furuncles	*S. aureus*	Localized swollen areas which develop soft centers and eventually discharge pus	Same as listed earlier for this organism
Green-nail syndrome and toe web infection	*P. aeruginosa*	Greenish discoloration of nail plate. Formation of thick, white scaling areas between toes	Sulfonamides, carbenicillin, gentamicin
Impetigo contagiosa	*S. aureus,*	Crust, scabs; localized pain and fever accompany the disease	Same as listed earlier for this organism
	GAS	Less severe from than for *S. aureus*	Same as listed earlier for this organism

Table 2.6 (Continued.) Features of selected bacterial diseases associated with the skin

Disease	Causative Agent	General Features of the Disease	Possible Treatment
***Pseudomonas* pyoderma**	*P. aeruginosa*	Eroded and macerated skin surface, producing a bluish green pus and grape odor.	Same as listed earlier for this organism
Scarlet fever	GAS	Fever, headache, sore throat, vomiting, raised reddened rash, "strawberry tongue"; peeling of body surface and tongue may occur	Same as listed earlier for this organism