CHAPTER 2

LITERATURE REVIEW

2.1 *Salmonella* background.

Salmonella is a gram-negative anaerobic bacterium in the form of a facultative that is generally 2-5 micron in length and 0.5-1.5 micron in width and is driven by peritrichous flagella. Salmonella genome size varies between serovars with a range of 4460 to 4857 kb. Salmonellae belongs to the family Enterobacteriaceae and pathogens are medically important for both human and italic animals (Grimont *et al*., 2007).

Typhoid fever is commonly knows as a worldwide infection caused by *Salmonella typhimurium* bacteria. The disease is transferred from water, food, milk, fruits and vegetables which are contaminated by *Salmonella Typhimurium*. It is also transferred by operators of health and food handlers which are contaminated by *Salmonella Typhimurium* bacteria. *Salmonella Typhimurium* can be mechanically transported from dirt to food by insects like flies, reptiles such as turtles, lizards, and snakes and common pets *(Birgitta et al., 2005*). The World Health Organization predicted the 12-week infection rate of about 12.6 million typhoid fever causing nearly 600,000 people died every year (WHO, 2003).

Typhoid common therapies are antimicrobialagens such as ampicillin, chloramphenicol, and cotrimoxazole or trimethoprim-sulfamethoxazole which is generally used to treat typhoid fever suffered by adults and children who also include carriers, functioned as an alternative medicine to quinolones and ampicillin. Cotrimoxazole restricts the folic acid synthesis which is required by the bacteria to extract nucleic acid. Typhoid fever which is known as enteric fever is one life-threatening chronic illness which is triggered by bacteria of Salmonella Typhimurium (Kotton et al., 2007). This sickness is the most often found fever after malaria, specifically in the tropical regions *(Wilcocks and Manson-Bahr, 1972).* This is possible because *S. Typhimurium* escapes from the cells of macrophage to enter the spleen, liver and other organs in which the bacteria can survive before going back to enter blood *(Jones and Falkow, 1996).*

The rat thypoid model is an experimental model widely used for human typhoid fever. In the previous study experiment, when mice were administered with *Salmonella Typhimurium* at a subletal dose, the infection developed for several weeks

in several clearly identifiable stages. During a natural infection, the early phases of the invasion of bacteria to the intestinal wall were followed by their localization in the cell, perhaps macrophages, first in the local lymph nodes and immediately dominated in the liver and spleen. *(Benjami.et al.1990 and Hohmann et al., 1978).*

2.2 Pathogenesis Of *S.Typhimurium* In mice

Salmonella typhimurium can pass the barrier of epithelial by passively transporting it with the help of dendritic cells extending the pseudopods among the local epithelial cells, or by invading it actively. After the bacteria reach the lower intestine, they stick to the membrane of mucous to attack the cells of epithelials. A site in which this occurs is the Peyer microfold (M) patch cell situated in the small intestine where the bacteria are able to pass through the barrier in the epithelial continuing to the follicle as well as mesenteric lymph nodes that underlie in the lymphoid tissue. During the ongoing bacteremia, secondary infection can happen because of the bacteria spreading to other organs like spleen, gallbladder, and liver. In acute *S. Typhimurium* infection cases, the gallbladder serves as a reservoir *(Hurley et al., 2014).*

After a mouth infection case*, Salmonella Typhimurium* invades epithelial and M cells before going through Peyer's patch, nodes of mesenteric lymph, lymph vessels which then enter the bloodstream. Alternative invasion mechanisms have been described in which Salmonella is covered by mucosal dendritic (DC) cells and after that travelled to the bloodstream from the gastrointestinal tracts by phagocytes of CD18 *(Mastroeni and Me'nager 2003).* The last infection phase is identified by generating immune responses that are able to remove *S. Typhimurium* and then is resistant to reinfection *(Mittru¨cker et al., 2000).*

The clear mechanisms to improve susceptibility of neonatal and dangerous life-threatening illness development are basically still covered. It was hypothesize dthat major protective mediators existing in adults might not be available in newborns because of the their immune system immaturity. On the other hand, other may be the causes like gastrointestinal differences of the population *(Cebra et al., 1999).* Followed by a few-day phase, bacterial intracellular multiplication appears and the bacteria titer in the spleen and liver enhancement. In this case, it is worth noted that *S. Typhimurium* is able to insert and survive in both phagocytic and nonphagocytic cells. Furthermore, the growth intracellularly becomes particularly essential in the time of infection as mutants failing the survival in cells of the host are highly

attenuated in vivo. In the mice, about 108 bacteria appear as a critical burden to survive. If the bacterial titer reaches the threshold, the host organism can no longer resist the infection. As a result, endotoxin shock, secondary bacteremia, and quick death occur. Conversely, during nonfatal infections, rats limit the bacterial titer to some degree. The next infection phase is characterized by splenomegaly, generalized macrophages mediated by immune suppression, and high number of bacteria, relying on the used mice and *S. Typhimurium* strains, which may last one or some weeks. The last infection phase can be identified by the adaptive immune response which is able to remove *S. Typhimurium* and provide long-term immunity to defend from reinfection *(Willi et al., 2000).*

The existence of pathogenic microorganisms is commonly shaped largely by their interactions with allied host species. The initial step of any infection is colonization. As for bacteria which are enteropathogenic, it is a daunting work because the targetted organ hosts have already been colonized by solid microbial communities, microflora, or 'microbiota'. The intestinal colonization of microbiota starts directly just after birth and ends a lifetime. In healthy intestines, microbiota remains fairly stable, and the gross composition of it is the same as other individuals at higher taxonomic levels, even in humans and mice *(ley et al.2006).*

The ecosystem of intestine exists due to the symbiotic that involves interaction between the microbiota and the host organisms. Yet, the composition of microbiota is influenced by the available nutrients, local pH, and it may also be affected by the host immune system *(Suzuki K et al.2004).* In a study, mice were treated with three different antibiotic regimens which had been commonly applied to inhibit microbiota in mice. There were enteric and inflammatory infection models. The effect on colonization of intestinal microbes by some dominant bacterial swamps was evaluated *(Salzman et al., 2001).*

2.3 The Immune Response Of *S. Typhimurium*

Salmonella is the most successful enteric pathogen as it has established a strategy to overcome the imajority of mmune defenses used by the host during different disease phases *(Broz et al., 2012).* Systemic *S. Typhimurium* infection starts on the seventh day after the beginning infection *(Ulhaq et al., 2009).* In addition, the various phases of the infection of *S. Typhimurium* are represented in the various mechanisms of innate and obtanined immunity which cause this response. Bacteria. The innate immune mechanism possesses the foundamental function to identify and combate microbial invaders and notify the system of adaptive immune to their

existence. The innate immune process is carried out by cells that are relatively not limited to the specificity of pathogens, including NK cells, macrophages, and neutrophils in the initial response to *S. typhimurium* as shown in Figure 2

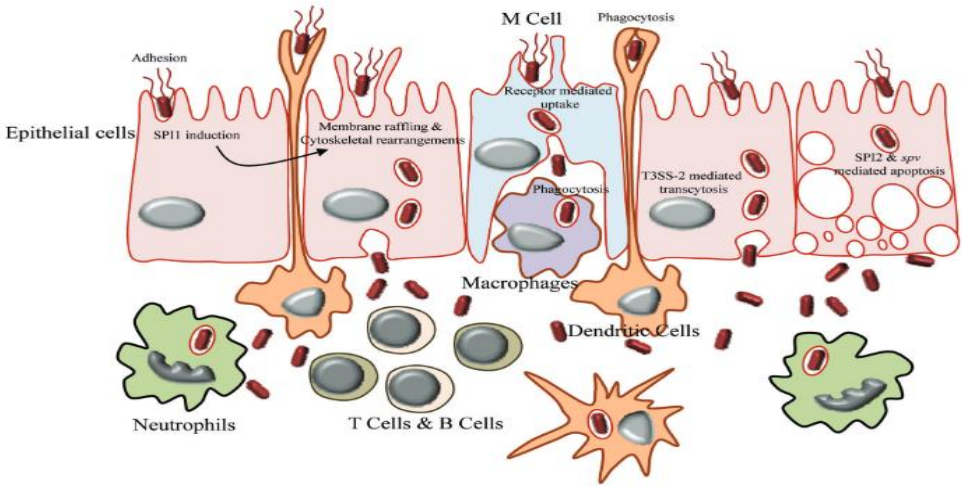

Figure 2.1: The Immune Response of *S. Typhimurium (Garai, et al, 2012).* The immune response of *S.* Typhimurium is noticed by the host's immune system by using TLRs which initiate the innate immune response. The TLRs respond the PAMPS situated on the pathogen surface. This recognition lets the innate immune system to start its response, which cause the macrophage and cytokine activation and recruitment such as IFN-γ as an important cytokine to activate macrophage and to make early resistance of host to *S. Typhimurium* .

The various *S. Typhimurium* infection phases are represented in various innate and immune mechanisms that contribute to the response to these bacteria. Then it differs in its interests during different stages of infection. During the early stages, phagocytes are essential for controlling Salmonella infections, and neutrophil granulocytes and macrophages are essential for the infected mice to survive. Macrophage fagocytize S. typhimurium. Additionally, this process is strengthened by receptor-mediated uptake after opsonizing Salmonella with antibodies or complemnt *(Mittru¨cker et al., 2000),*

Existing data indicate that antibodies or T cells themselves are only able to provide a mediocre protection level from salmonellosis. transfer of immune passive serum or B cells themselves may cause the mice resistant to salmonella or malignant bacteria susceptible to malignant organisms. In addition, immune cell iand mmune transfers are able to protect mice from the infection of low-dose virulent Salmonella

or to infections sufficiently of virulent organisms In contrast, immune serum transfers and T cells can naturally protect susceptible mice from highly malignant *salmonella*, suggesting that humoral and cell-mediated immunities are needed for the animals to be resistant to malignant salmonella. Additionally, besides the production of antibodies, B cells show other various functions in the system of immune, which include presentation of antigen and production of cytokines. The usage of an attenuated bacterium makes it possible for the analysis of immune responses to *S. typhimurium* in susceptible mice *(Mittru¨cker et al., 2000).*

2.4 Pathomechanism of *S.Typhimurium* infection

After absorption and colonization of the small intestine as shown in Figure 2.1, bacteria pass through the intestinal mucosa. *Salmonella* enters macrophages and dissiminates through blood to the liver and spleen where it grows intracellularly. The *Salmonella* intracellular niche is an improvised phagolysosome called Salmonago containing vacuoles (SCV). The entry and survival strategies within the target cell correspond to the cell type and depend on the temporal expression of certain genes by Salmonella. Targeted cells include M. cells Epithelial cells, macrophages, neutrophils, monocytes, dendritic cells, granulocytes, B cells, and T cells *(Garai et al., 2012).*

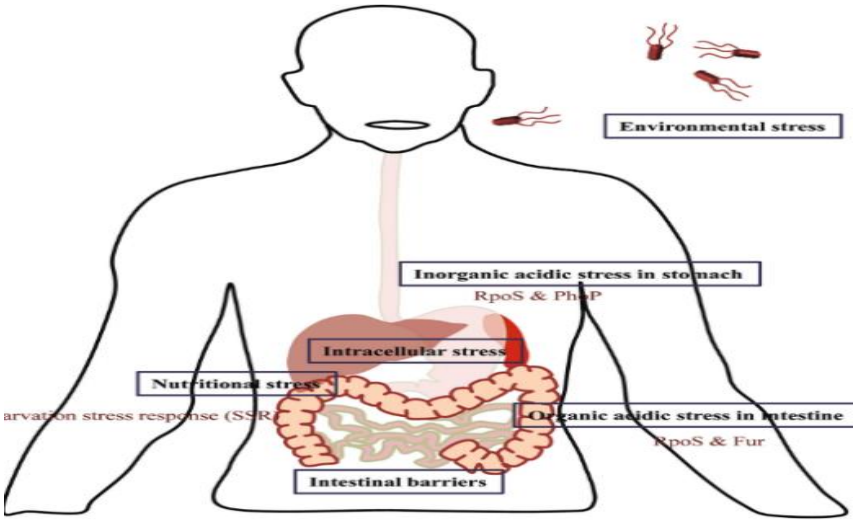

Figure 2.2 Pathogenesis of S.*Typhimurium* (Garai, et al., 2012). After ingestion orally and colonization of the small intestine, *S. Typhimurium* inserts the intestinal epithelium and goes into the pore of Peyer, the lymphoid structure lining the intestine. From the Peyer patch, S. *Typhimurium* enters the nodes of mesenteric lymph, and from that area the bacteria dissiminate through the efferent lymphocytes to the circulatory mechanism, which causes transient bacteremia. Bacteria are quickly excreted from the blood by phagocytes in the spleen and liver, and most bacteria are killed by these cells while bacterial intracellular multiplication happens and bacterial colonies in the spleen and liver enhance.

Salmonella enters M cells and epithelial cells and goes through Peyer patches, mesenteric lymph nodes, lymph vessels into the bloodstream. Alternative invasion mechanisms have been presented in which Salmonella is covered by mucosal dendritic (DC) cells and then moved from the gastrointestinal tract to the blood stream by phagocytes of CD18 (*Mastroeni and Me'nager 2003).* The final stage of infection is characterized by a generation of immune responses that are capable of removing S. *typhimurium*, and is resistant to reinfection *(Mittru¨cker et al., 2000).*

During a natural infection with *S. Typhimurium*, only a small percentage of bacteria pass across the intestinal epithelium, reaching the bloodstream through the gastrointestinal tract. The initial stage of Salmonella infection is usually completed in some hours when the natural response to bacterial challenge involves the introduction of bacterial components, such as LPS and DNA, and cell activation as a result of such encounters. The release of inflammatory mediators results in infiltration of various cell types to the site of infection and strengthening of the response. Uptake and bacterial damage by phagocytic cells also facilitate host protection. The innate immune process is performed by cells that are relatively not limited to the specificity of pathogens, including neutrophils, macrophages, and NK cells *(Alun et al., 2002).*

S. Typhimurium has come as a pathogenic bacterial model o. Using bioluminescent imaging, researchers can estimate that local tissue is influenced by Salmonella concentrations *(Guydish et al., 2005).*

Salmonella enterica serotype *Thyphimurium* (*Salmonella Typhimurium*) is the agent of the cause of typhoid fever murine. After oral retrieval, S. *typhimurium* passes through the intestinal epithelium through the M cell and enters the Peyer's pores. From there, the bacteria spread through the mesenteric lymph nodes to the spleen and liver, where they replicate. The first stage of infection is performed by the manufacturing of inflammatory cytokines and phagocyte *activation (Makela¨et al.,. 2007).*

2.5 Role IL12 Cytokines on Salmonella infection

Salmonellas enter dendritic cells and macrophages, in which the bacteria can reproduce and increase their number. Simultanously, they trigger an immune response that happens in some phases and ultimately control the infection with the assistance of CD4 T cells. Initially, lipoproteins and peptidoglycan on bacterial surfaces ligate receptor in macrophages and dendritic cells. Upon entering the cell, this returns TLRS *(Murphy et al., 2012).*

TLR is the first critical line of defense to combat bacteria attackers and plays an important role in microbial sensing *(Lizard et el.,, 2013).* Especially TLR and mannose receptors, and their ligation help to stimulate the production of nitric oxide with cells, which are hazardous to bacteria. Signals by TLR stimulate the production of IL12, which then encourages NK cells to generate IFN-γ in the early immune response stage. IL12 also stimulates the antigen-specific CD4 cells to produce IFN-γ *(Murphy et al., 2012).* IFN-γ is important during the early bacterial growth phase by limiting the bacterial multiplication rate *(Muotiala et al., 1990).*

In contrast, dendritic cells, macrophages and other cells secrete cytokines that mediate many cellular reactions of innate immunity. In congenital immunity, the main source of cytokines is dendritic cells and macrophages that are activated by the introduction of microbes, the binding of bacterial components such as LPS or viral molecules such as double-stranded RNA to TLR dendritic cells and macrophages that are powerful cytokim stimulative secretions. By cell *(Abbas and Lichtman, 2004).*

At the same time, cytokines are also released from immune-mediated cells. In this adaptive immunity type, the main source of cytokines is helper. T Lymphocytes Cytokines are taken in small amounts in response to external stimuli and bind to high affinity receptors in target cells. IFN γ macrophage activity becomes more effective in killing microbial phagocytes. Thus, NK cells and macrophages function cooperatively to remove the intercellular microbes. Macrophages ingest microbes and produce IL12, IL12 activates NK cells to secrete IFN γ, and IFN γ in turn activates macrophages to kill swallowed microbes *(Abbas and Lichtman, 2004).*

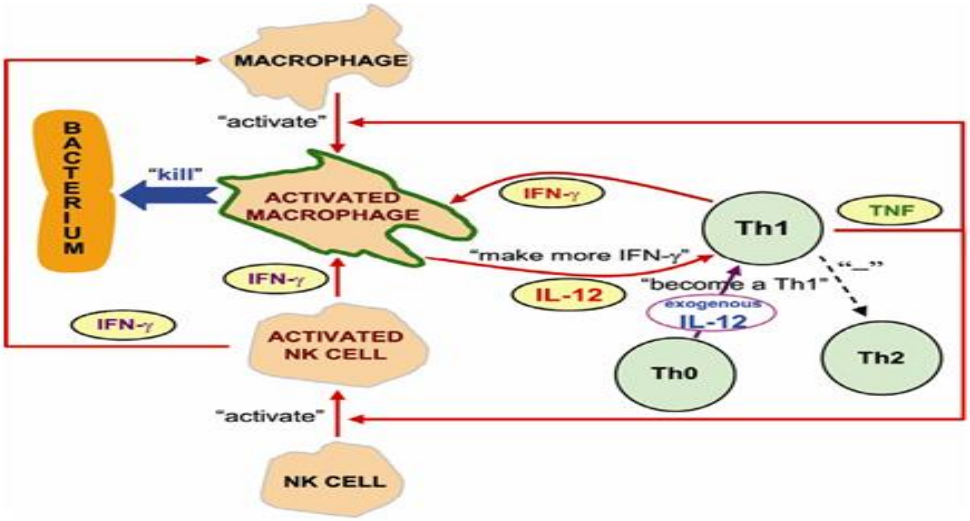

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Figure 2.3 The function of IFN-γ and IL12 to overcome intracellular bacteria *(Hamza et al.,2010)*.

TH1 cells generate IFN-γ cytokine which activates phagocytes to kill ingested microbes and stimulate the production of antibodies that promote the consumption of microbes by phagocytes. ABCs antigen that presents cells finding microbes secretes the IL12 cytokine stimulating naïve CD4 T cells to distintc into IFN -γ secreting Th1 cells and increasing IFN-produksi production. IFN-γ activates macrophages to kill the swallowed ABC, IFN-y, and IL 12 microbes.

IL-12 has many biological activities, and this is a key factor that drives Th1 responses and IFN production. Early application or production of IL-12 during infection can activate macrophages and enhance cell-mediated immunity while forming the main antigen - from specific immune responses. As a result, IL-12 may play a key role in the protection of bacterial and viral infections, and IL-12 immunotherapy may be important in the treatment of diseases where the Th1

response is desirable. While cytokines including IL-12 have short half-lives in vivo and the development of advanced drug delivery systems *(Haynie et al., 2005; Jiang et al., 2009).*

2.6 *Thymus vulgaris*

Thymus vulgaris is an aromatic plant that belongs to the family Lamiaceae, which is used for medicinal purposes and spices almost everywhere in the world *(R. MORALES et al., 2002).* In Romania, the genus of Thymus consists of one species cultivated as an aromatic plant (*Thymus vulgaris* L.) and 18 other wild species *(R. MORALES et al., 2007).*

2.6.1 Botanic description

Thymus vulgaris is known locally as "zaatar" or "zaitra ', a member of the Lamiaceae family, widely used in folk medicine of Morocco for its properties such as expectorants, antispasmodic, antibronkolitik, carminative, anthelmintic, antitussives and diuretic. The nature of aromatic and medicinal of the genus Thymus has made it one of one plant of the most popular around the world. Species thymus is usually used as a herbal tea, flavoring agent (condiments & spices) and medicinal plants *(Stahl-Biskup and Saez, 2002).* The essential oils and plant extracts have been used for thousands of years, especially in the preservation food, medicine, alternative medicine and natural therapy *(Lis-Balchin & Deans, 1997).* it has long been known that some essential oils of plants showed the efficacy of antimicrobial *(Finnemore, 1926).* and it needs to investigate these plants scientifically, which has been used In traditional medicine to improve the quality of health services Essential oil is a potential source of antim compounds New ikroba especially against pathogenic bacteria *(Prabuseenivasan et al., 2006).*

2.6.2 Scientific classification

Kingdom:	Plantae
(unranked):	Angiosperms, Eudicots, Asterids
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Thymus*
Species:	*T. vulgaris*

Bionomial name: *Thymus vulgaris*

Figure 2.4: *Thymus vulgaris.(http://sophy.u-3mrs.fr/)*.

2.6.3 Major Chemical Constituents

Thymus vulgaris possesses about 2.5% but not less than 1.0% essential oil. The essential oil composition fluctuates depending on the chemotype under consideration. The main components of Thyme are thymol (European pharmacopoeia, 2nd ed. Strasbourg, Council of Europe, 1995). And carvacrol (Materia medika Indonesia, Volume Jakarta, IV Department of Health, Republic of Indonesia., 1980). Up to 64% of oil, along with linalool, p-cymol, cymene, thymene, α-pinene, apigenin, luteolin and 6-hydroxyluteolin glycosides and flavonoids di-, tri and tetrametoksilat, everything was substituted in the 6 - position (for example 5, 4'-dihydroxy-6,7-dimetoksiflavon, 5,4'-dihidroksi- 6,7,3'-trimetoksiflavon and derivatives 8-metoksilasi 5,6,4'-trihydroxy-7, 8,3'-trimethoxyflavone) (European pharmacopoeia, 2nd ed Strasbourg, Council of Europe, 1995). *Thymus vulgaris* essential oil (TEO) is a monoterpene mixture. The main compound of this oil is a natural terpenoids and phenol thymine isomer carvacrol (CVL) (Amiri, 2012; B. Nickavar *et al*., 2005), Terpenoids, aglycone flavonoid, flavonoid glycosides and phenolic acids are also found in the Thymus species.

Thyme (Thymus vulgaris L., Lamiaceae), a small subshrub originating in the western Mediterranean region of Europe, has a long history of use and is a species that varies chemically (A. ZarzueloandE.Crespo *et al.,* 2002).

Thyme leaves (*Thymus vulgaris*) can be used fresh or dried as a spice. Thyme also has various beneficial effects, including antiseptic, carminative, antimicrobial, and antioxidant effects *(R. Baranauskiene, et al., 2003).* Recently, in our laboratory showed that the constituent elements, thymol and carvacrol*, Thymus vulgaris* L. essential oils present on the immune response *(F. C. Fachini-Queiroz, et al ., 2012).*

2.7 Essential oil molecules

1) -**Terpenes**: Terpenes are hydrocarbons formed through a combination of several isoprene units (C5H8). The most common dipes are monoterpenes (C10H16) and sesquiterpenes (C15H24), but longer chains, such as diterpenes (C20H32), triterpenes (C30H40) and so on. Among terpenes, p-Cymene, limonene, terpinene, sabinene and pinene are the most famous. This in vitro test indicates that terpenes exhibit ineffective antimicrobial activity when used as a single compound. *(Filomena et al., 2013).*

2) -**Terpenoid**: Terpenoid terpene with an extra oxygen molecule that has a methyl group or they are transferred or issued by a specific enzyme *(Caballero et al., 2003),* thymol, carvacrol, linalool, menthol, geraniol, linalyl acetate, citronellal and piperitone is terpenoids The most common and famous. The antimicrobial activity of most terpenoids is related to their functional groups, and the phenolic terpenoid hydroxyl groups and the presence of delocalized electrons are important elements for their antimicrobial action. For example, carvacrol is more effective than other EOs, such as p-cymene. (*Dorman et al., 2000; Ultee et al., 2002; Ben Arfa et al., 2006).*

3) -**Phenylpropenes**: Fenilpropen so named because it contains six carbon aromatic phenol group and propene propene three carbon from cinnamic acid, which is produced in the first stage phenylpropanoid biosynthesis. These compounds represent a fraction of EOs. Eugenol, isoeugenol, vanillin, safrole and cinnamaldehyde are the most widely studied phenylpropene. Most of the antimicrobial activity of these molecules is given by the free hydroxyl groups *(Filomena et al., 2013).*

Thyme contains many active ingradients including thymol, carvacol and flavonoids. The main chemicals of thyme are essential oils (borneol, carvacol, linalool

and thymol), tannin principle, saponin and triterpen acid (shabnum et al., 2011). Thymol is part of a class of natural compounds known as biocides, with strong antimicrobial properties when used alone or with other biocides such as carvacrol. In addition, natural biocidal agents such as thymol can reduce bacterial resistance to common drugs such as penicillin *(Palaniappan et al., 2010).*

Numerous studies have demonstrated antimicrobial effects of thymine, ranging from inducing antibiotic susceptibility on drug-resistant pathogens to the potent antioxidant properties (Zarrini G *et al.,* 2010; Ündeğer *et al.,* 2009). Some researchers suggest that natural biocides such as thymol and carvacrol reduce bacterial resistance to antibiotics through synergistic effects *(Palaniappan et al., 2010).*

Thymol (2-isopropyl-5-methylphenol) is the main monoterpene phenol, isomeric with carvacrol, which is found in thyme extracts. These compounds have shown anti-inflammatory, immunomodulating, antioxidant, antibacterial and antifungal properties *(P. C. Bragae et al., 2006; H. Tian et al., 2006).* Clinical trials were conducted on 12 healthy volunteers. Each subject received a dose of TP Bronchipret tablet, which is equivalent to 1.08 mg of thymol. No thymol is detected in plasma or urine. However, the metabolites of thymol sulfate and timol glucuronide are found in the urine. Thymol sulfate, but not thymol glucuronide, is detectable in plasma. Peak plasma concentration is achieved after 2.0 hours. The average terminal elimination half-life is 10 hours. Thymol sulfate can be detected up to 41 hours after administration. Urinary excretion can be followed for 24 hours. The second amount of thymid sulfate and glucuronide excreted in 24 hour urine was 16% of the dose *(Sahelian et al., 2016).*

Carvacrol and carvacrol bearing essential oils, havCarvacrol occurs in aromatic plants and in many essential oils of the Labiatae family, includine emerged for their wide spectrum antimicrobial activity and have been investigated by a large number of researchers worldwide. However, the susceptible microorganisms are far too many to be dealt with, as they include microorganisms that belong to the Gram-positive bacteria, Gram-negative bacteria, molds and yeasts. The activity of carvacrol is extended to drug-resistant microorganisms, strains with a particular significance for pathogenesis as currently are difficult to treat *(Papalia et al., 2012).* it also have anti biofilm action as mentioned by previous research *(Dalleau et al., 2008).*

Essential oils and plant extracts have been used for thousands of years, especially in the preservation of foods, medicines, alternative medicine and natural

therapies *(Lis-Balchin & Deans, 2011).* It has long been known that some essential oils of plants exhibit antimicrobial properties *(Finnemore et al., 2008).* And it is necessary to investigate these plants scientifically, which have been used in traditional medicine to improve the quality of health care. Essential oils are potential sources of new antimicrobial compounds especially against pathogenic bacteria *(Prabuseenivasan et al., 2006).*

2.8 Mechanism of Action and The target sites of *Thymus vulgaris* against the bacterial cell

Alcohol extracts are more efficient at various pathogenic bacteria and mixed extracts have very high antibacterial activity *(Al-Saimary et al., 2006).* The T. vulgaris extract component has activity against various targets, especially the membranes and cytoplasm, and in some cases, they completely alter cell morphology, some thyme components, such as carvon, thymol and carvacrol, lead to increased intracellular ATP concentrations, an associated event With microbial membrane destruction *(Helander et al., 2014).*

2.9 Immunomodulator

The term " immunomddoulation " means immune change. Responsiveness Increased immune responsiveness is called immunostimulation and decreased immune responsiveness called i2mmunosuppressin *(Mukherjee et al., 2014).* The immune system is part of the body to detect pathogens by using specific receptors to generate an immediate response with activation. From immune component cells. They modulate and define the system *(Kumar et al, 2011).* Immunomodulatory effects can be predicted to there which is stimulation. Emphasis and narrowing of the immune system *(Yeap et al., 2011).* Immunomodulators are used in practice to stimulate and normalize the activity of the immune system. By increasing T cell immunity. Reduce or inhibit suppressant activity. Stimulaling natural killer cells (NKcells) and interferon production as well as inducing the production of specific cytokines by activated target cells *(Gabius et al., 2003; Stanlove et al., 2005; aLam et al., 2010)*