Chapter 1

Research Background

The interest in natural antioxidants for the prevention and treatment of many health conditions and diseases have increased in recent years. It is mainly due to their ability to protect organisms and cells from oxidative stress damage responsible for aging, degenerative diseases, and cancer (Yanishlieva et al., 1999; Li et al., 2014). The main role of antioxidants to combat such conditions is by scavenging free radicals and maintaining body redox balance. Natural antioxidants have the advantage over synthetic antioxidants due to their potential health benefits, in addition to the increased public awareness and health-related issues consideration regarding the latter alternative. Natural antioxidants are believed to be free from side effects, while synthetic antioxidants are often linked to adverse effects, such as enhancing tumor growth and increasing body weight (Pokorný & Parkányiová, 2004). Thus, the determination of novel natural antioxidants becomes the main focus of natural product development in this present time (Bhuyan & Basu, 2017).

Natural antioxidants can be found abundantly in different plants and seeds phenolic extracts. Specifically, aromatic plants have been regarded as one of the best sources of antioxidants from their phenolic extracts. Thyme is an aromatic plant member of the vast Lamiaceae family, utilized for its medical and spice purposes worldwide. Other than its culinary applications, it has been historically used for its antimicrobial, antitussive, spasmolytic, and antioxidant activity (Begrow et al., 2010; Alu'datt et al., 2015). Its antioxidant content is especially high due to the presence of its essential oils, characterized by a large number of monoterpenes, such thymol and carvacrol (Kosińska & Andlauer, 2012). As the interest of thyme increases, production also shows promising economic advantages of thyme in this present time. For example, the world thyme production was 14000 ton in 2008, most of which is produced by Mediterranean countries. Furthermore, Turkey has been reported to have the highest share of export, reaching 50% of world trade, equaling to 9.7 thousand tons and $43 million revenue in 2008 (Aslan & Mevlut, 2017). Another Mediterranean country which utilizes thyme plant

to a great extent is Tunisia. In Tunisia, the genus *Thymus* (Lamiaceae) is mainly represented by *Thymus capitatus*, a perennial, herbaceous shrub commonly used as a spicy herb and also locally known as "zaaˆtar" (Boutanirou et al., 2007). Tunisian thyme essential oils and their components have been gaining interest due to their relatively safe status, wide acceptance by consumers, and their potential as a multi-purpose functional plant (Ormancey, Sisalli, & Coutiere, 2001).

Various studies have investigated the antioxidant activity potential of different thyme plants and compared them with other *Thymus* species or other herbaceous plants. In all cases, different thyme plant species, were found to express different antioxidant activities depending on its total phenolic content and main phenolic constituents (Hazzit et al., 2006; Durgadevi & Kalava, 2013; Prasanth et al., 2014). A study by Gedikoğlu et al. (2019) reported that their *T. vulgaris* essential oil expressed higher DPPH radical scavenging activity compared to different thyme plants such as the *T. mastichina, T. zygis, T. capitatus,* and *T. spathulifolius.* However, another study by Ballester-Costa et al. (2017) found that *T. capitatus* had the highest FRAP values in comparison with other *Thymus* species, followed by *T. zygis, T. mastichina,* and *T. vulgaris*, respectively. This suggests that *T. capitatus* extracts may be useful for its ferric reducing capacity in therapeutical application. In addition, the most recent review of *T. capitatus* by Bouyahya et al. (2020) revealed differences not only in antioxidant activity, but also in antibacterial, antifungal, antiviral, and antiparasitic activity, all of which are suggested to be determined by the phenolic compound and its composition of the *T. capitatus* extract. Therefore, it is important to investigate the antioxidant activity and the chemical composition specific to *T. capitatus* phenolic compounds in regard to these findings.

While many researches have shown the potential of antioxidants coming from different sources such as plants and seeds, there are still little-known effects of the compounds to the real human in *in vivo* conditions. Many compounds cannot develop their biological activities and/or become increasingly toxic in a specific amount or dose. In order to test the toxicity to human consumption, Caco-2 and RAW 264.7 cell models have been used extensively for their ability to mimic human cells (Bailey et al., 2004; Lea et al., 2015). Caco-2 is the most specialized cell type currently

used in the studies of *in vitro* modelling of the human small intestines. It has the unique ability to reach differentiation into monolayer representing the human intestinal epithelial cells after reaching confluence (Lea et al., 2015; Verhoeckx et al., 2015). On the other hand, RAW 264.7 cells are macrophage-like, leukemia virus transformed cell line derived from BALB/c mice, which has the ability to produce inflammatory-related cytokines when they are stimulated (Bailey et al., 2004). As both cell lines have similarities to the human cells, toxicity tests applied to these cells may become a high-throughput *in vitro* method of testing a substance's toxicity, including antioxidant compounds. Toxicological effect can be determined via cell viability assessment through such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Riss et al., 2016).

In this study, the antioxidant activity and toxicological effect of two *T. capitatus* extracts from Tunisia was evaluated based on *in vitro* system. The total phenolic content of the thyme extracts was quantified using the Folin-Ciocalteau (FC) method determination. Meanwhile, their antioxidant activities were determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and 2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays. Additionally, to further evaluate toxicological effects with regard to human consumption, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was also performed using Caco-2 and RAW 264.7 cells. The objective of this study was to assess the antioxidant activities and toxicological effects of *T. capitatus* from Tunisia with antioxidant assays and MTT assay, respectively. We hypothesized that the ethanolic extract will show antioxidant activities and toxicological effects in concentration-dependent manner. Finally, the results of the study are aimed to improve knowledge regarding *T. capitatus* for better nutraceutical research in the future.

Research questions:

- How are the antioxidant activities of both Tunisian thyme extracts?
- How are the toxicological effects of both Tunisian thyme extracts towards Caco-2 and RAW 264.7 cells?