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ANTIOXIDANT AND ANTITUMOR ACTIVITY OF THYME LEAVES WATER EXTRACT

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ABSTRACT: Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases. In this work, thyme (*Thymus vulgaris* L.) leave water extract (TLE) was investigated for total phenolic compound total flavonoid, and the cytotoxic effect of studied extracts against human cell lines HCT 116 and PC3 occurred. The results showed that the extract had high content of these parameters. Scavenging activity of thyme (*Thymus vulgaris* L.) leaves water extract against DPPH and β-Carotene free radicals were determined and the results showed that TLE were characterized by a high content of antioxidants compound. Data showed that water extract of thyme (TLE) possessed good potent inhibitory activities against HCT 116, and PC3 cell lines. The anticancer effect of water extract of thyme (TLE) encourages the use of it as protective agents for normal cell line.

Key words: Thyme, antioxidant, antitumor.

INTRODUCTION

The human body constantly creates free radicals culminating in an "oxidative stress" when their elimination by antioxidant defense mechanisms is not sufficient **(Badarinath *et al.*, 2010)**. Oxidative stress contributes to the pathogenesis of many human diseases; therefore, the intake of antioxidative agents is important for the prevention of chronic diseases **(Armstrong, 2010)**. Antioxidants play an important role in preserving food too. In food industry widely used synthetic antioxidants as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are very effective because of their low cost, high thermal stability and efficiency but they are instable and they can play role as promoters of carcinogenesis **(Al-Menhali *et al.*, 2015; Brewer and Safety, 2011; Miladi *et al.*, 2013; Yasin and Abou-Taleb, 2007)**.

Due to these reasons, there is a growing interest in the study of natural additives as potential antioxidants **(Jorge *et al.*, 2015)**. The presence of antioxidants in many spices gives them food-preserving properties too, especially in preventing oxidation of lipids **(Yasin and Abou-Taleb, 2007**). Nevertheless, the use of synthetic antioxidants in the food industry has been questioned regarding its innocuousness. Studies about spices and aromatic herbs have been widely emphasized, can act as an alternative to prevent the oxidative deterioration of food and reduce the use of synthetic antioxidants **(Gallego *et al.*, 2013)**.

The use of natural antioxidants from food plants has the following advantages: They are accepted by the consumers; they are considered safe; they do not need safety tests; they have functional and acceptable sensory properties **(Vábková and Neugebauerova, 2012)**. Studies found in literature have demonstrated that the spices belonging to the Lamiaceae family, as well as their extracts and essential oils, are efficient antioxidants **(Gallego *et al.*, 2013; Grigore *et al.*, 2010; El-Guiche *et al.*, 2015; Pecarski *et al.*, 2014; Sofiane *et al.*, 2015; Villanueva Bermejo *et al.*, 2015; Ghandchi**

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and Jamzad, 2015). Herbs are usually considered as plants with aromatic properties and are mainly used in spicy foods and for preparation of herbal teas in folk medicine **(Sofiane *et al.*, 2015)**. Medicinal plants have always been considered as a source of health **(Pogacnik and Ulrih, 2011)**.

Thymus vulgaris L. (thyme), locally known "zaatar" or "zaitra', a member of the Lamiaceae family, is widely used in medicine for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative and diuretic properties. The aromatic and medicinal properties of the genus Thymus have made it one of the most popular plants worldwide. Thymus species are commonly used as herbal tea, flavoring agents (condiment and spice) and medicinal plants6 and have high levels of antioxidant activity and phenolic substance contents **(Yen *et al.*, 1997; Zheng *et al.*, 2001)**. Thyme contains phenolic and flavonoids **(Haraguchi *et al.*, 1996; Miura *et al.*, 2002)**. The flavonoids have anti-inflammatory effects, they reduce the peroxidation of lipids **(González-Segovia *et al.*, 2008)** and they have anticarcinogenic effects.

The aim of this study was to determine the antioxidant, total flavonoid, total phenolic, total alkaloid and antitumor activity of thyme leaves extract.

MATERIALS AND METHODS

Plant Materials

Thyme leaves (*Thymus vulgaris*) have been gained from a local market (Zagazig, Egypt).

Chemicals

β-carotene, 1, 1-Diphenyl-2, picrylhydrazyl (DPPH), tert-butyl hydroquinone (TBHQ), gallic acid and quercetin, all phenolic compounds were purchased from Sigma (St. Louis, MO, USA). Other chemicals such as solvents were analytical grade. HCT 116 cell (human colon cancer cell line), and PC3 cells (prostate carcinoma cell) were obtained from VACSERA Tissue Culture Unit (Giza, Egypt).

Preparation of Thyme leave water extract (TLE)

Thyme was dried in a vacuum oven (Thermo Fisher Scientific Inc., Japan) at 45◦C for 3 days and grounded to a fine powder in a miller at 4000 rpm (IKA Werke, Germany). Thereafter, sequential extraction of plants was performed. Milled plants (100 g) were extracted using extraction ratio (1:10) (plant: solvent) by distilled water, Water extract was freeze-dried by using Heto PowerDry LL3000 Freeze Dryer (Thermofisher Scientific, Waltham, Massachusetts, USA). The dried extract after freeze-driedof solvent were weighed to determine the extraction yield and stored at -20◦C until further use.

Phytochemical analysis of Thyme leave water extract (TLE)

Determination of total phenolic compounds

Total phenolic compounds of TLE were determined according to the method described by **Škerget *et al.* (2005)**.

Determination of total flavonoids

Total flavonoid contents of (TLE) were determined according to the method described by **Ordonez *et al.* (2006)**.

Antioxidant activity eetermination of Thyme leave water extract

DPPH radical-scavenging activity

The DPPH radical-scavenging activity of the extracts (TLE) was assessed following the method described in reference **(Gülcin, 2012)** The percentage of antioxidant activity against the DPPH free radical was calculated as follows:

$$\text{Antioxidant activity (Inhibition) \%} = [(A_{control} - A_{TLE})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction, and A_{TLE} is the absorbance in the presence of TLE. TBHQ and gallic acid were used as a positive control. TLE were analyzed in triplicate.

β-Carotene/linoleic acid bleaching

The ability of extracts and synthetic antioxidants to prevent the bleaching of β-carotene was assessed (**Dastmalchi *et al.*, 2007**).

$A_{control}$ with no extract was also analyzed. Antioxidant activity was calculated as follows:

Antioxidant activity (%) = [1−(Abs^{0} $_{TLE}$ − Abs^{120} $_{TLE}$)/ (Abs^{0} $_{control}$ − Abs^{120} $_{control}$)] × 100

Where Abs^{0}_{TLE} is the absorbance of (TLE) at 0-time, Abs^{120}_{TLE} is the absorbance of (TLE) after 120 min, $Abs^{0}_{control}$ is the absorbance of control at 0-time and $Abs^{120}_{control}$ is the absorbance of control after 120 min.

Antitumor Activity Determination of Thyme Leave Water Extract

Determination of sample cytotoxicity on cells (MTT protocol)

The impact of TLE from different, at a 31.25-1000 µg/mL concentration range, on human cell line viability was assessed in vitro using MTT-assay. Normal cells (Vero cells) and cancer cells (HCT 116, and PC3) were obtained from VACSERA Tissue Culture Unit (Giza, Egypt). A 96-well tissue culture plate was inoculated with 1 X 10^5 cells/ml (100 µl/well) and incubated at 37°C for 24 hours to form a complete monolayer. The growth medium was then removed from the microtiter plates. The confluent cell monolayer was washed twice with washing media. Two-fold dilutions of the test sample were prepared in RPMI medium containing 2% serum (maintenance medium). 0.1 ml of each dilution was added to different wells, with three wells serving as controls containing only maintenance medium. The plate was incubated at 37°C for examination. Cells were inspected for signs of toxicity, such as partial or complete monolayer loss, rounding, shrinkage, or granulation. An MTT solution (5 mg/ml in PBS) provided by BIO BASIC CANADA INC was prepared. 20 µl of this solution was added to each well. The plate was placed on a shaker at 150 rpm for 5 minutes to mix the MTT with the media thoroughly. It was then incubated (37°C, 5% CO_2) for 1-5 h to metabolize the MTT. The media was discarded, and the plate was dried on paper towels if needed. Formazan (the metabolic product of MTT) was resuspended in 200 µl DMSO. The plate was again placed on a shaker at 150 rpm for 5 minutes to mix the formazan with the solvent thoroughly. The optical density was read at 560nm, subtracting the background at 620 nm, which should correlate directly with cell quantity **(**Van de Loosdrecht, **Beelen *et al.*, 1994)**. The percentage of cell viability and cytotoxicity was calculated using the following formulas:

Cell viability (%) = (Abs $_{TLE}$ /Abs $_{control}$) x 100

Cytotoxic activity (%) of the tested substance was calculated following the formula:

Cytotoxic activity (%) =100 %- cell viability (%)

The TLE concentration producing 50% growth inhibition is termed IC_{50}.

Statistical Analysis

Experiments were tested in triplicate and the results were expressed as mean ± standard error and statistically analyzed with ANOVA variance analysis through general linear models (GLM) method of statistical analysis system software (SAS version 9.1, **SAS Institute, 2003**). Significant differences were used to set at p value < 0.05.

RESULTS AND DISCUSSION

Yield, TPCs and TF for Thyme Leave Water Extract

Phenolic compounds, extensively researched and reported by **(Rice-Evans *et al.*, 1996; Mattei *et al.*, 1998)** possess at least one aromatic ring with hydroxyl groups known as reducing agents. These natural antioxidants, including phenolics and flavonoids, exhibit a broad range of pharmacological effects such as anti-allergic, antibacterial, anti-inflammatory, neuroprotective, and anticancer properties, and also shield plants from pathogenic microbial attacks. The medicinal properties of Plants exist because of phytochemicals. These phytochemicals are secondary metabolites that are produced in sufficient amount under stressed conditions, allowing the plant to protect itself from detrimental environmental effects. Consuming phytochemicals through diet may offer health advantages, such as protection against chronic degenerative conditions including cardiovascular, neurodegenerative diseases, and cancer.

The study indicated that the phenolic content was 55.08 mg GAE/g and the flavonoid content

was 20.61 mg QE/g. Phenolic compounds have been demonstrated to inhibit the cyclooxygenase and lipoxygenase pathways **(Ferrandiz *et al.*, 1991; Ferrandiz *et al.*, 1990)**. Flavonoids have been shown to block the Ornithine decarboxylase enzyme, a rate-limiting enzyme in polyamine biosynthesis that is related with DNA synthesis and cell proliferation in numerous tissues, thereby impeding cell proliferation **(Tanaka *et al.*, 1997; Makita *et al.*, 1996)**. Additionally, flavonoids can inhibit the growth of microorganisms by depolarizing their membranes and inhibiting DNA, RNA, and protein synthesis (**Dzoyem *et al.*, 2013**). Investigating the flavonoids and phenols in this plant could further reveal the medicinal properties of thyme.

Antioxidant Activity of TLE

Plant materials include many phenolic compounds that contain hydroxyl groups (-OH) conjugated to aromatic rings **(Gülçin *et al.*, 2006a)**. These phenolic compounds block chain oxidation reactions by chelating metals or donating hydrogen atoms. Therefore, these plant metabolites act as reducing agents, metal chelators, singlet oxygen quenchers, and antioxidants. Many studies have shown that the phenolic contents of plants display some antioxidant properties **(GÜLÇİN *et al.*, 2010; Gülçin *et al.*, 2005)** Free radicals or ionic radicals are highly reactive species that are responsible for many cell disorders due to their effects on proteins, lipids, and DNA, **(Köksal *et al.*, 2016)** The radical scavenging activity of a compound indicates its antioxidant activity and ability to inhibit the initiation of an oxidation chain. DPPH and the β-carotene/linoleic acid bleaching test have been widely used to determine the radical scavenging activity of a compound. **(Bursal and Gülçin, 2011; Gülçin, *et al.*, 2006b**).

Fig. 1 displays the results of TLE DPPH• antiradical actions. The results showed that all TLE have antiradical action. When compared to TBHQ and gallic acid, extracts with a high concentration of TPCs demonstrated strong antiradical activity (Fig. 1). It has been observed that the antioxidant potential of plant extracts is attributable to the concentration of phenols in the extract **(Heim *et al.*, 2002)**.

Also as shown in Fig. 2, TLE prevented bleaching of β-carotene by scavenging linoleate-derived radicals. Scavenging linoleate-derived radicals resulted in a higher concentration of β-carotene (85.13a) than TBHQ (31.3) or gallic acid (18.1). Phenolic chemicals and flavonoids have been linked to antioxidative activity in biological systems, serving as scavengers of singlet oxygen and free radicals **(Sharma *et al.*, 2015)**.

The presence of phenolic compounds is greatly linked to antioxidant activities **(Shahwar *et al.*, 2010)** In biological systems, free radicals are often referred to as reactive oxygen species (ROS), which are the most biologically significant free radicals. ROS produced in cells include the hydroxyl radical, hydrogen peroxide, and superoxide anion **(Pryor *et al.*, 2006)**.

Cytotoxicity Effect of Thyme Leaves Extract (TLE) on (HCT 116) Human Colon and Human Prostate (PC3) Cancer

The MTT assay was used to assess the cytotoxic effect of thyme extracts against (HCT 116) Human colon and human Prostate (PC3) cancer cell lines with different concentrations (31.25-1000 µg/mL) as reported in Tables 1 and 2 and Figs. 3, 4, 5 and 6. The antiproliferative activity of the plant extract on cancer cell lines were expressed in IC_{50} value. IC_{50} is the inhibitory concentration that causes 50% inhibition of the cancer cell population.

Thyme has been suggested as an anti-cancer agent. Thymol can inhibit CRC cell proliferation and induce apoptosis **(Zeng *et al.*, 2020**). Thyme extracts exhibited significant cytotoxicity and cytogenic effect, as well as inducing cell cycle arrest on a number of cancers **(Adham *et al.*, 2020; Fathima *et al.*, 2017)**. Furthermore, thyme methanol extract has been able to prevent DNA damage due to chemotherapy agents **(Salmani *et al.*, 2015)**

Thymus vulgaris L. is a herb rich in essential oil and contains oxygenated monoterpenes and monoterpene hydrocarbons as its major chemical components. Specifically, thymol, carvacrol, *p*-cymene, borneol, *trans*-caryophyllene, and *cis*-sabinene hydrate are present at the highest concentrations **(Noroozisharaf *et al.*, 2018; Pérez López *et al.*, 2015)**. Furthermore,

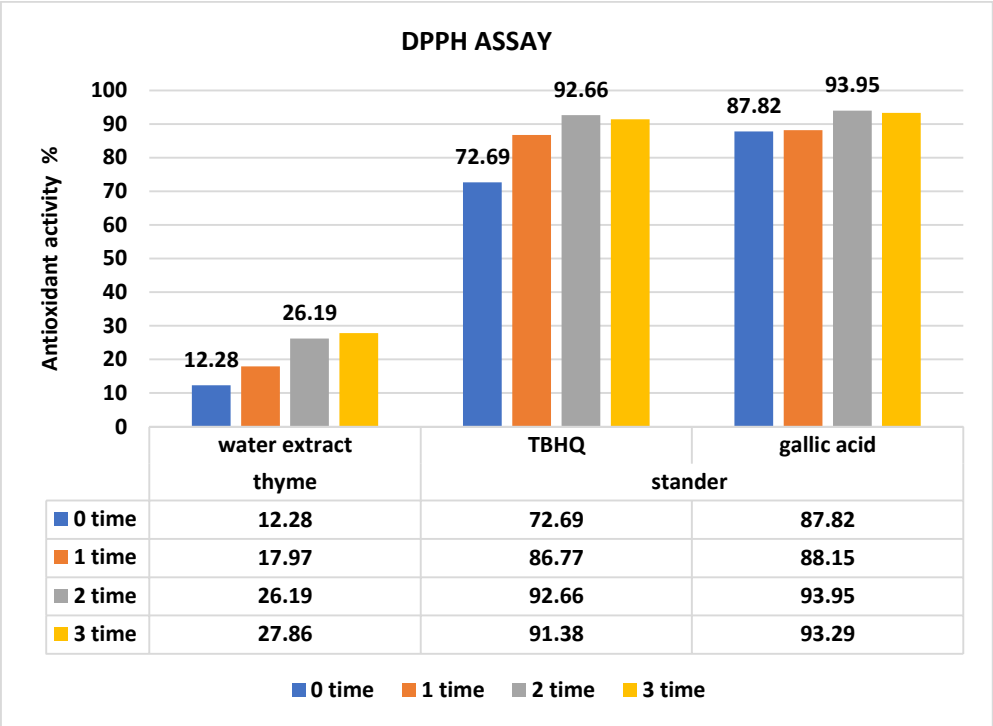

Fig. 1. Antioxidant activity of TLE against DPPH$^{\bullet}$ as compared with TBHQ and gallic acid

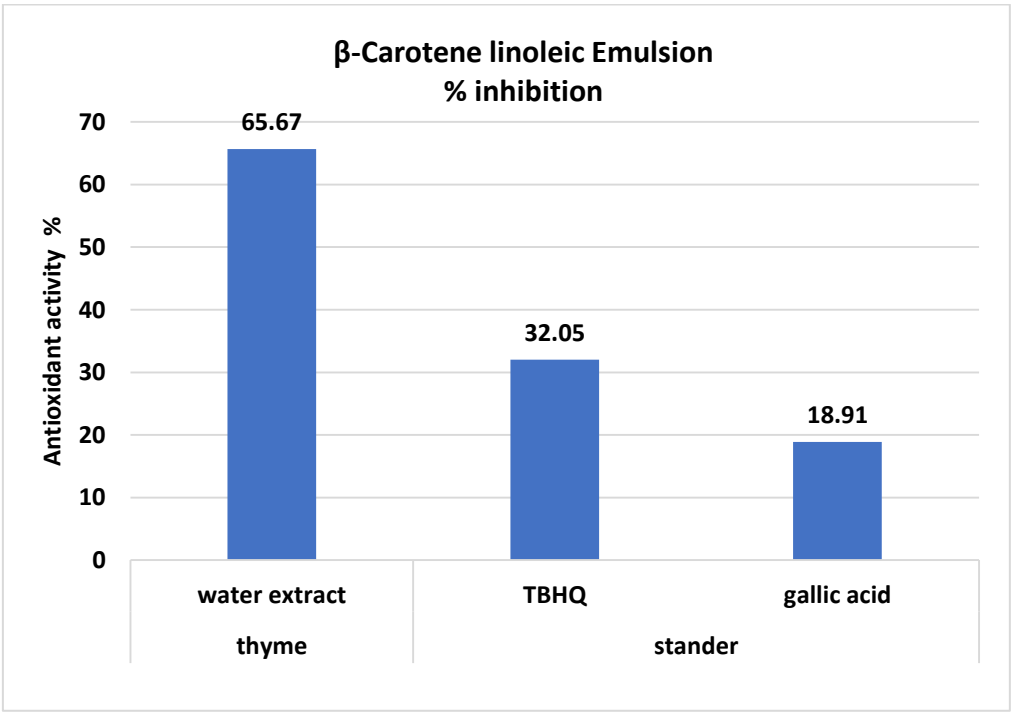

Fig. 2. Inhibition of TLE in *β*-carotene-linoleic acid emulsion as compared with TBHQ and gallic acid

Table 1. Percent cell viability of TLE extract of HCT 116 cell line

ID	Conc. mg/mL	Viability %	Toxicity %	IC_{50} mg/Ml
HCT 116	--------	100	0	
	1000	7.678410117	92.32158988	
	500	21.58988257	78.41011743	
TLE	250	79.76513098	20.23486902	363.29
	125	92.95392954	7.046070461	
	62.5	98.2836495	1.716350497	
	31.25	99.90966576	0.090334237	

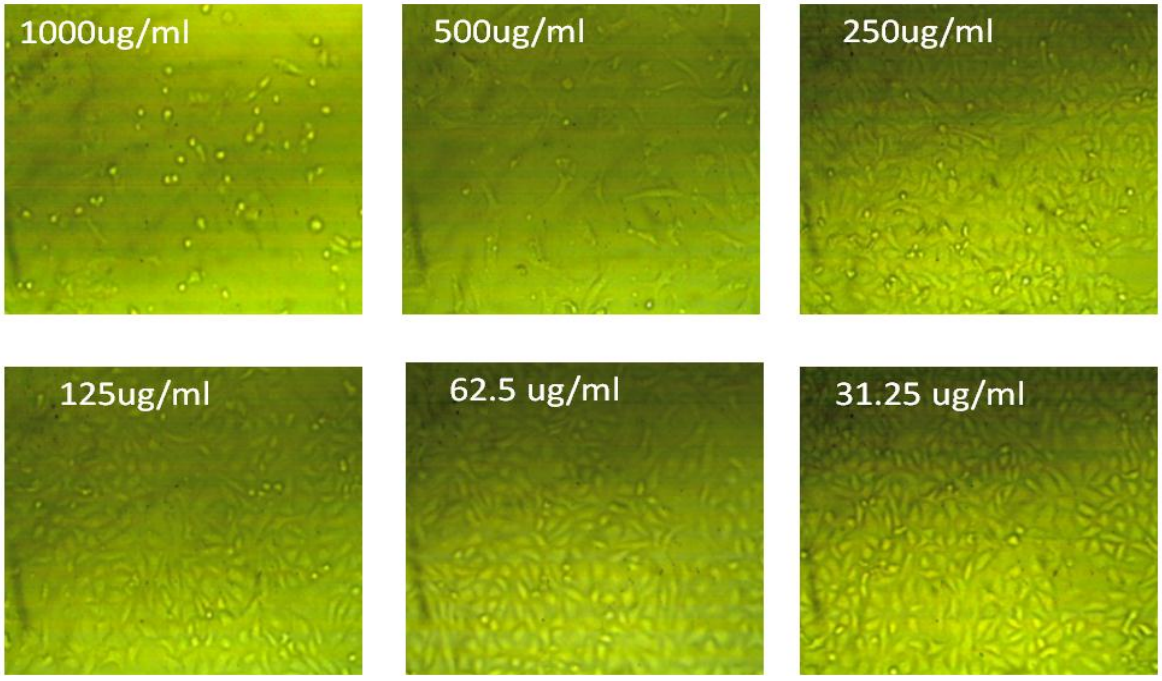

Fig. 3. Effect of TLE extract on human colon cancer cell line (HCT 116)

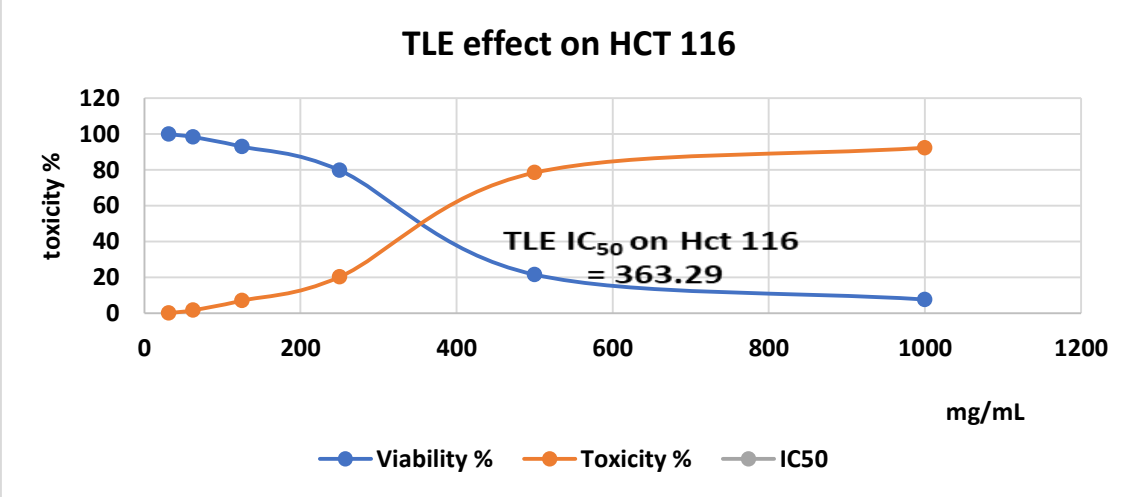

Fig. 4. Percent cell viability and toxicity of TLE extract of HCT 116 cell line

Table 2. Percent cell viability of TLE extract of PC 3 **cell line**

ID	Conc. mg/mL	Viability %	Toxicity %	IC_{50} mg/Ml
PC3	--------	100	0	
	1000	4.761904762	95.23809524	
	500	4.848484848	95.15151515	
TLE	250	26.23376623	73.76623377	166.39
	125	52.81385281	47.18614719	
	62.5	87.61904762	12.38095238	
	31.25	99.56709957	0.432900433	

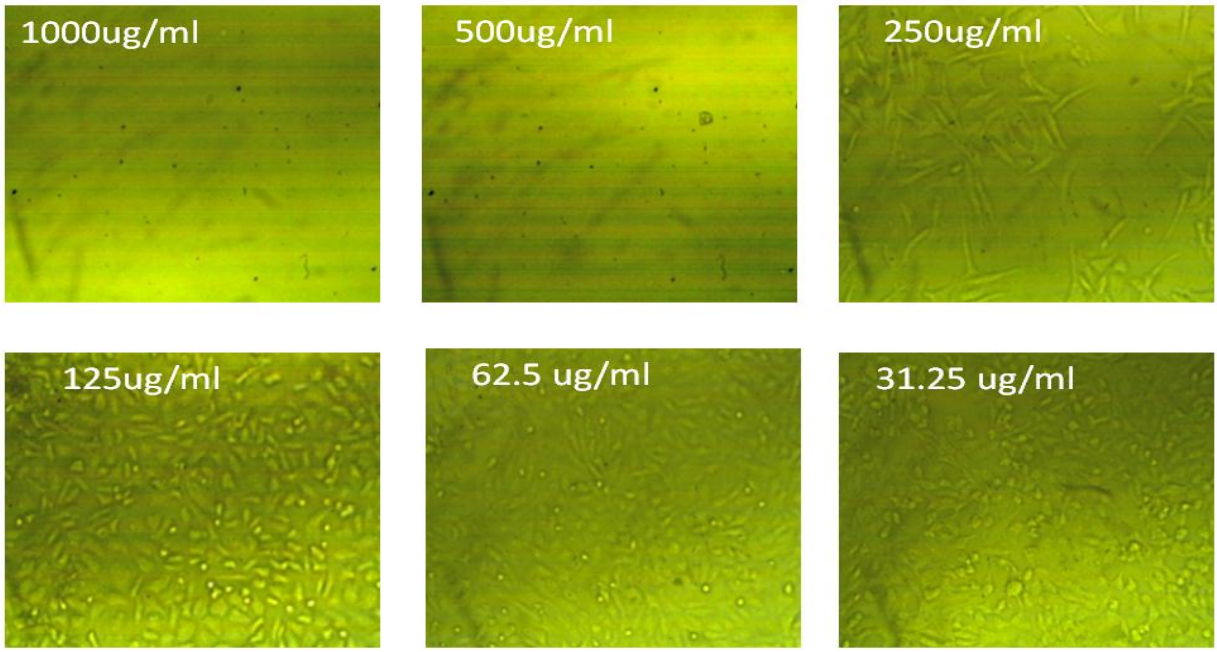

Fig. 5. Effect of TLE extract on human prostate cancer cell line (PC 3)

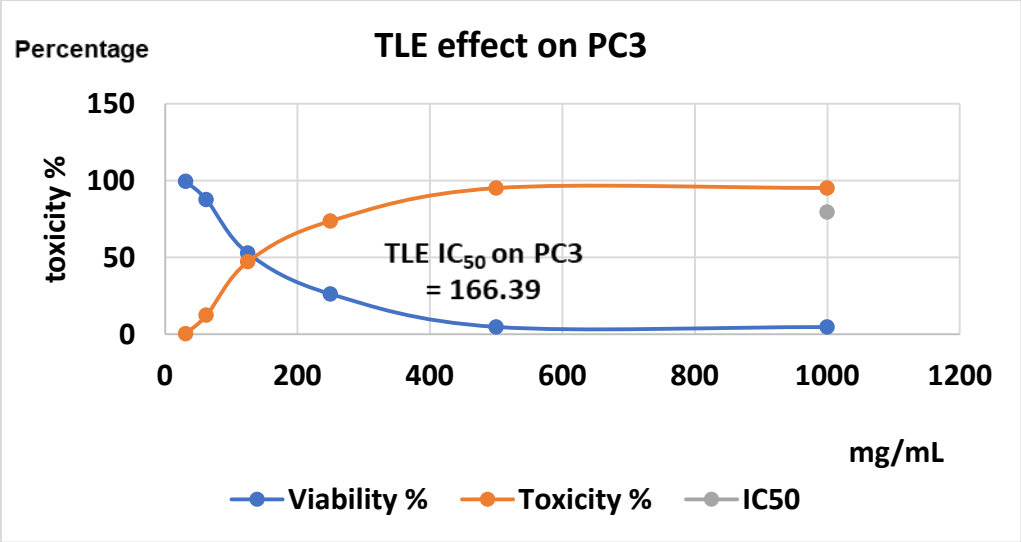

Fig. 6. Percent cell viability and toxicity of TLE extract of PC3 **cell line**

Thymus spp. contain phenolics represented by rosmarinic acid and flavonoid derivatives **(Vila, 2002)**. These phytochemicals categorize *T. vulgaris* amongst plant foods with the highest antioxidant activity (**Bentayeb *et al.*, 2014**). There are several preclinical studies pointing to the anticancer potential of *T. vulgaris*. For example, the aforementioned herb has demonstrated significant free radical scavenging activity and proapototic effects **(Heidari *et al.*, 2018)** in the human BC T47D cell line. In a colorectal HCT116 cancer cell model, *T. vulgaris* extract was shown to inhibit proliferation in a concentration- and time-dependent manner **(Al-Menhali *et al.*, 2015)**. A decrease in proliferation rate has been associated with elevated apoptosis as evidenced by increased caspase-3/7 activity. In addition, *T. vulgaris* decreases the migratory and invasive capacities of HCT116 cells. Tumor inhibitory effects of *T. vulgaris* extract have also been observed against human leukemia THP-1 cells (**Ayesh *et al.*, 2014**). Finally, *T. vulgaris* essential oil has been observed to significantly inhibit growth of human oral cavity squamous cell carcinoma. This effect is accompanied by the regulation of N-glycan biosynthesis and extracellular signal-regulated kinase 5 (ERK5) and interferon signaling **(Sertel *et al.*, 2011)**.

Conclusion

Chemical composition constituents of THYME (TLE) indicated the presence of phenolic components. (TLE) has antioxidants, and anticancer activity.

Developing phytomedicine with anticancer properties, TLE-derived drug may have potential for an alternative medicinal source due to its anticancer activity. This study also indicated that TLE water extract has potential anticancer activity against (HCT 116) human colon and human prostate (PC3) cancer cell lines.

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النشـــاط المضـــاد للأكســـدة والمضـــــاد للأورام فـي مسـتخلص أوراق الـزعتـر المائيـــة

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لقد جذبت البوليفينولات النباتية اهتمامًا متزايدًا نظرًا لخصائصها المضادة للأكسدة القوية وتأثيراتها الملحوظة في الوقاية من مختلف الأمراض المرتبطة بالإجهاد التأكسدي . في هذا العمل، تم دراسة المستخلص المائي لزعتر من حيث إجمالي المركبات الفينولية وإجمالي الفلافونيدات، وكذلك التأثير السمي للمستخلصات المدروسة ضد خطوط الخلايا البشرية أظهرت النتائج أن المستخلص كان يحتوي علي نسبه عاليه من هذه المعاييرو تم تحديد نشاط استخراج اوراق الزعتر ضد الجذور الحرة وأظهرت النتائج أن المستخلص المائي لاوراق الزعتر تتميز بمحتوى عالٍ من مركبات مضادات الاكسدة.

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