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Antioxidants And Anticancer Activity of Two Extracts of Thyme Leaves Based On Partition Coefficient

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Abstract

Thymus vulgaris Linn. contains phytochemicals that may exhibit cytotoxic and cytogenic activities. Different solvents have different abilities in dissolving plant bioactive compounds. The current study aimed to identify the profile of bioactive compounds in thyme leaves and their anticancer properties. The extraction was done using two solvents on the same amount of thyme, depending on the partition coefficient (log p). These solvents were acetone (**ATE**) and ethanol 80% (**ETE**), respectively. The approximate chemical composition of thyme (*Thymus vulgaris* L.) leave extract (**TLE**) was studied. Phytochemical screening of studied extracts revealed some differences in their constituents. The extracts were investigated for total polyphenols and total flavonoid, and the scavenging activity of thyme (*Thymus vulgaris* L.) leaf extracts against 1,1-diphenyl-2 picrylhydrazyl radical (DPPH) and **β-Carotene** free radicals was determined. LC/MS-MS polyphenolic fractions in studied extracts were carried out, and the cytotoxic effect of studied extracts against human cell lines HCT 116 and PC3 occurred. The results showed that the major components in ATE were (naringenin 8019.63 mg/ellagic acid 9.97 mg/ chlorogenic acid 3.6 mg/ quercetin 821.95 mg/ rutin 271.06 mg/ and apigenin 29.96 mg); in ETE, they were (naringenin 2027.04mg/ gallic acid 41.96mg/ apigenin 14.53mg/ chlorogenic acid 82.87mg/ ellagic acid 3.74 mg/ rutin 613.63mg/ and quercetin 220.58mg). Data showed that ACETON extract of thyme (ATE) possessed good potent inhibitory activities against HCT 116 and PC3 cell lines. The anticancer effect of acetone extract of thyme (ATE) encourages the use of it as protective agents for normal cell lines, while the other samples showed the lowest effect on HCT 116 and PC3 cell lines. We conclude that TLE offers great promise in the creation of phytomedicine with anticancer qualities, and drugs developed from TLE may serve as an alternative medicinal source due to their anticancer activities.

Keywords, thyme, Antioxidant, Antitumor, LC/MS-MS

1. Introduction

The interest in natural substitutes for synthetic chemicals is consistently growing. [1]. the vast biodiversity of herbal plants around the globe showcases their ability to adapt to diverse environments and accounts for their extensive phytochemical variety. [2, 3]. The components obtained from medicinal plants' roots and aerial parts include primary and secondary metabolites. Secondary metabolites, often known as phytochemicals, encompass a wide range of natural compounds. These compounds are integral to plant adaptation, playing crucial roles in response to both abiotic and biotic stress factors. [1, 2, 4]. Secondary metabolites include phenolic acids, flavonoids, alkaloids, steroids, saponins, tannins, glycosides, lignins, phenylpropanoidglycerols, isoprene-derived terpenoids like monoterpenes, diterpenes, sesquiterpenes, and triterpenes. These phenolic compounds, including flavonoids, perform a wide range of functions in plants, from free radical scavenging and signaling to modulating auxin transport and contributing to plant defense systems. [4]. Flavonoids and phenolic acids, which are the most abundant and extensively studied plant-produced polyphenols, boast a variety of industrial uses in cosmetics,

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therapeutics, and food sectors. [5-9]. Thyme boasts a rich history in traditional medicine, where it is utilized to address various ailments, including respiratory conditions such as whooping cough, bronchitis, and asthma, through various forms like tea, ointments, tinctures, syrups, or steam inhalation. [10]. Thyme, with thymol as its main component, is known to combat stomach and intestinal fungi and enhance appetite due to its antibacterial and antiparasitic qualities. Recent research on Thymus vulgaris has uncovered its analgesic and antipyretic properties, demonstrated through thyme extract effects in mice. Transitioning from a conventional herb to an important medicinal plant in rational phytotherapy, thyme provides an abundance of iron, calcium, manganese, vitamin K, and aids in improving blood circulation and vitality. [10].

A variety of extraction methods are available for isolating phenolic compounds from plants, each offering different efficiencies and resulting in distinct compounds. These methods encompass Subcritical water, Ultrasonic-assisted extraction, Supercritical extraction, and Microwave-assisted extraction, among others. Conventional extraction of thyme yielded twenty-two polyphenols, while ultrasonic-assisted extraction revealed twenty-five. Regardless of the method, the predominant phenolic compounds in thyme were kaempferol-3-O-rutinoside, p-coumaric acid-4-O-glucoside, and feruloyl glucose. The choice of extraction technique markedly influenced the composition of the thyme extract. Compounds like apigenin-7-O-glucoside, kaempferol, eugenol, and homovanillyl alcohol were unique to conventional extraction, while oleuropein, quercetin-3-O-rutinoside, 4-vinylguaiacol, and chrysoeriol-7-O-(6-malonyl-apiosyl-glucoside) were specific to ultrasonic-assisted extracts.[11].

The active biochemical compounds found in Thyme species comprise flavonoids like thymonin, cirsilineol, and 8-methoxycirsilineol, along with caffeic acid, triterpenoids, aliphatic aldehydes, long-chain saturated hydrocarbons, and Labiatae tannin, known as rosmarinic acid. [12, 13].

Thymus Vulgaris L. also contains phenolic acid, terpenoids, and flavonoid glycosides [14]. The primary phenolic compounds in Thymus vulgaris, carvacrol and thymol, make up approximately 20–55% of the oil extracted from thyme. Many studies have demonstrated that thyme's volatile oil, a key essential oil, is utilized in cosmetics for its antioxidant and preservative properties, and also in the food industry. [15]. Carvacrol and thymol are recognized for their antimicrobial, antioxidative, antibacterial, antitussive, antispasmodic, and expectorant properties. [16, 17]

In this research, we used the solvent extraction method in sequence, where we discovered phenolic chemical compounds that worked effectively as antioxidants and anticancer.

2. Experimental

2.1. Materials and Chemicals

Thyme leave has been obtained from a local market (Zagazig, Egypt). All solvents used throughout the present work were obtained from different companies.1, 1-Diphenyl-2picrylhydrazyl (DPPH), β-carotene, quercetin, gallic acid and Tert-butyl hydroquinone (TBHQ), were purchased from Sigma (St. Louis, MO, USA). HCT116 cell (human colon cancer cell line), and PC3 cells (prostate carcinoma cell) were obtained from VACSERA Tissue Culture Unit (Giza, Egypt).

2.2. Preparation of Thyme extracts (TLE).

Thyme leaves were dried in a vacuum oven (Thermo Fisher Scientific Inc., Japan) at 45°C for 72 hours and milled into a fine powder in a mill (Retsch, Model ZM 1000, Haan, Germany).
Then after, sequential extraction was preformed according to [18, 19] and with some modification. dried thyme (100 g) was extracted using extraction ratio (1:10) (plant: solvent) by acetone and ethanol 80%, respectively. The extraction was done by using magnetic stirrer at room temperature followed by filtration through Whatman No.1 filter paper. The extraction period was 1 h for every solvent. The residues were re-extracted under the same conditions, then acetone (ATE) and ethanol (ETE) combined filtrate was evaporated in a rotary evaporator (BÜCHI- Rotavapor R-124 & water bath-B-480) then the residue was freeze-dried. The dried extracts after evaporation of solvents were weighed to determine the extraction yield and stored at -20◦C until further use.

2.3. Phytochemical analysis of (TLE)

2.3.1. Determination of total phenolic compounds

The concentrations of TPCs in thyme leaves extracts (TLE) were measured by a UV spectrophotometer(Jenway-UV-VIS Spectrophotometer) as described by **[20]** The used reagent was Folin-Ciocalteu reagent. To 0.5 mL of diluted extract (1 mg in 1 mL solvent), 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water), and 2 mL of Na_2CO_3 (7.5 %) were added.

For a control sample, 0.5 mL of distilled water was used. The samples were incubated at 50˚C for 5 min then cooled. The absorbance was measured at 760 nm. TPC expressed asgallic acid equivalent (GAE) was calculated using the following linear equation based on the calibration curve:

$$y = 0.024x + 0.095$$
$$R^2 = 0.9937$$

y = absorbance, x = concentration (mg GAE/g extract), and R^2 = correlation coefficient make up the equation.

2.3.2. Determination of total flavonoids.

Quantifcation of the yellow color produced by the interaction of total flavonoid (TF) contents with aluminum chloride (AlCl3) was measured as described by [**21**] with some modifcation. A 0.5 mL of TLE solution (10 mg in 10 mL solvent) was mixed with a 3 mL aliquot of 10 g/L AlCl3 ethanolic solution and after one hour, the absorbance was measured at 420 nm. TF contents expressed as quercetin equivalent (QE) was measured depending on the calibration curve using the following equation:

$$y = 0.0148x - 0.0135$$
$$R^2 = 0.9996$$

where R2 is the correlation coefficient, y is the concentration (µg QE), and x is the absorbance.

2.3.3. LC/MS-MS for thyme leaves extract (TLE).

The analysis of ATE and ETE was conducted using liquid LC/MS-MS with an ExionLC AC system for separation and a SCIEX Triple Quad 5500+ MS/MS system equipped with electrospray ionization (ESI) for detection. ZORBAX SB-C18 Column (4.6×100 mm, 1.8 µm) was used to separate the samples. The mobile phases consisted of two eluents: acetonitrile (LC grade) and 0.1% formic acid in water. The mobile phase program was established as follows: 2% B from 0 to 1 minute, increasing to 60% B from 1 to 21 minutes, maintaining 60% B from 21 to 25 minutes, then returning to 2% B from 25.01 to 28 minutes. The flow rate was set to 0.8 ml/min, with a 3 µl injection volume. For the MRM study of chosen polyphenols, both positive and negative ionization modes were used in the same run, using the following parameters: Curtain gas at 25 psi; ion spray voltage at 4500 for positive and -4500 for negative modes; source temperature at 400°C; ion source gases 1 and 2 at 55 psi, delustering potential at 50; collision energy at 25; and collision energy spread at 10.

2.4. Antioxidant activity Determination of TLE.

2.4.1. DPPH radical-scavenging activity

The electron donation ability of the obtained extracts was measured by bleaching of the purple-coloured solution of DPPH. One hundred microliter of each extract (10 mg extract / 10 ml solvent) was added to 3 ml of 0.1 mm DPPH [**22**] dissolved in acetone and ethanol according to the solvent used for extraction. After incubation period of 0, 30, 60 and 120 min at room temperature, the absorbance was determined against a control at 517 nm **[23]**. The Percentage of antioxidant activity of DPPHfree radical was calculated as follows:

Antioxidant activity (Inhibition) % = [(A control – A $_{TLE}$)/A control] × 100

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

2.4.2. β-Carotene/linoleic acid bleaching

The ability of extracts and synthetic antioxidants to prevent the bleaching of β-carotene was assessed **[24]**. In brief, 4 mg of β-carotene in 20 mL of chloroform, 400 mg of linoleic acid and 4 g of tween 20 were placed in a round-bottom flask. After removal of the chloroform, 1 L of distilled water was added, and the resulting mixture was stirred vigorously. Aliquots (3.5 mL) of the emulsion were transferred to tubes containing extracts. Immediately after mixing 0.5 mL of TLE (10 mg extract /10 mL solvent), an aliquot from each tube was transferred to a cuvette, the absorbance at 470 nm was recorded (Abs^0). The remaining samples were placed in a water bath at 50°C for 2 h, then the absorbance at 470 nm was recorded (Abs^{120}). A control with no extract was also analyzed. Antioxidant activity was calculated as follows

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

Where $\mathbf{Abs^0}_{TLE}$ is the absorbance of TLE at 0 time, $\mathbf{Abs^{120}}_{TLE}$ is the absorbance of TLE after 120 minutes, $\mathbf{Abs^0}_{control}$ is the absorbance of control at 0 time, and $\mathbf{Abs^{120}}_{control}$ is the absorbance of control after 120 minutes

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Antitumor Activity determination of TLE

2.5. Determination of sample cytotoxicity on cells (MTT protocol)

The effect of TLE concentrations ranging from 31.25-1000 µg/mL on human cell line viability was tested in vitro using the MTT-assay. Normal cells (Vero cells) and cancer cells (HCT 116 and PC3) were obtained from the VACSERA Tissue

Culture Unit in Giza, Egypt. A 96-well tissue culture plate was filled with 1 X 105 cells/ml (100 µl/well) and cultured at 37°C for 24 hours to generate a full monolayer. The growing medium was then extracted from the microtiter plates. The confluent cell monolayer was washed twice with washing medium. Two-fold dilutions of the test sample were produced in RPMI medium with 2% serum. 0.1 ml of each dilution was applied to separate wells, with three wells serving as controls, containing only maintenance media. The plate was incubated at 37°C for evaluation. Cells were examined for toxicity markers such as partial or total monolayer loss, rounding, shrinkage, or granulation. An MTT solution (5mg/ml in PBS) supplied by BIO BASIC CANADA INC was prepared. Each well received 20 µl of this solution. The plate was shaken at 150 rpm for 5 minutes to completely mix the MTT and medium. It was then incubated at 37°C with 5% CO_2 for 1-5 h to metabolize the MTT. The media was discarded, and the plate was dried with paper towels as needed. Formazan, a metabolic product of MTT, was resuspended in 200 µl DMSO. The plate was shaken at 150 rpm for 5 minutes to properly mix the formazan and solvent. The optical density was measured at 560nm and subtracted from the background at 620nm, which should immediately correlate with cell quantity. The percentages of cell viability and cytotoxicity were computed using the following formulas:

Cell viability (%) = (Abs $_{TLE}$ /Abs control) x 100
The following formula was used to compute the cytotoxic activity (%) of the tested substance:

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

The TLE concentration that inhibits growth by 50% is known as the IC50 value.

2.6. Statistical analysis

Experiments were done in triplicate, and findings were expressed as mean ± standard error. ANOVA variance analysis was performed using the general linear models (GLM) method of statistical analysis system software (SAS version 9.1, SAS Institute, 2003). Significant differences were defined at a p-value of less than 0.05.

3. Results and discussion

3.1. Yield, TPCs and TFs for TLE

The yield of TLE recovered from different solvents under study ranged from 5 to 18.36 %. ETE the highest amount (18.36 g ETE/100 g) followed by ATE (5.92 g ATE/100 g). Variation in the extraction yields from different solvents is attributed to the differences in the polarity of constituents found in plant materials**[25].**

The results showed that TPCs from ATE (ca. 143.67 mg GAE g^{-1} ATE) was high than ETE (108.42 mg GAE g^{-1} ETE). Flavonoids have a broad spectrum of biochemical and biological activities. Hence, Thyme extracts were assayed for total flavonoid levels. Ethanol was the best solvent for extracting flavonoids from Thyme (29.93 mg QE g^{-1}ETE), followed by acetone (28.58 mg QE g^{-1} ATE).

Table 1 yield (%), TPCs (mg GAE/g TE) and TFs (mg QE/g TE) of TLH

TLE	Solvent log *p*	Extract yield %	TPCs mg GAE/g TE	TFCs mg QE/g TE
ATE	0.20	5.92 **b**	143.67 **a**	28.58 **c**
ETE	0.07	18.36 **a**	108.42 **b**	29.93 **b**
Total		24.28	252.09	58.51
***P*-value**		≤ 0.001	≤ 0.001	≤ 0.001

Means denoted by different letters indicate significant differences using Tukey's HSD at a significance level of >0.001.

3.2. LC/MS-MS for TLE

Analysis of ATE and ETE of Thyme revealed the presence of 18 compounds. As depicted in Table 2, the major components in ATE were (naringenin 8019.63 mg, ellagic acid 9.97 mg/chlorogenic acid 3.6 mg/quercetin 821.95 mg/rutin

271.06 mg and apigenin 29.96 mg), in ETE were (naringenin2027.04mg/gallic acid 41.96mg/apigenin 14.53mg/chlorogenic acid 82.87mg/ellagic acid 3.74 mg/rutin 613.63mg and quercetin 220.58mg).

Table 2 phenolic and flavonoid compounds in ATE and ETE by using (LC//MS-MSms-----).

Compounds	log p	Retention Time (RT)	TLE	
			ATEµg/g	ETEµg/g
Gallic acid	0.42	3.93	9.97	41.96
3.4-Dihydroxybenzoic acid	0.81	5.76	9.40	14.76
Chlorogenic acid	-0.75	7.36	3.6	82.87
Methyl gallate	0.69	7.48	0.25	0.48
Caffeic acid	1.42	8.08	21.85	36.44
Syringic acid	0.95	8.42	4.27	14.86
Coumaric acid	1.54	9.57	24.31	19.98
Rutin	-1.97	9.73	271.06	613.63
Ellagic acid	1.05	9.95	3.74	17.02
Ferulic acid	1.42	10.27	9.85	16.06
Kaempferol	0.75	15.36	97.53	18.61
Quercetin	0.35	13.59	821.95	220.58
Apigenin	1.9	15.05	4.27	14.53
Naringenin	1.63	14.99	8019.63	2027.04
Luteolin	1.51	13.53	127.62	74.98
Hesperetin	-0.69	15.62	2.25	2.86
Vanillin	1.6	9.58	5.21	7.17
Apigenin	2.84	15.05	4.27	14.53
Catechin	0.4	7.38	1.27	0.47

3.3 The antioxidant activity of TLE

The oxidation process in cells is influenced by three factors: free radicals, active oxygen, and metals [**26**]. Therefore, a single assay is insufficient to test the antioxidant activity of plant extracts, as various tests may yield inconsistent results. Consequently, multiple tests utilizing different mechanisms are recommended. To evaluate the antioxidant capacity of thyme extracts, we employed several methods, including DPPH•, and the β-carotene/linoleic acid bleaching test.
Figure 1 depicts the antiradical activity of TLE as measured by DPPH. 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 (Figure 1). It has been shown that the antioxidant capacity of plant extracts is attributable to the concentration of phenols in the extract[**27**].

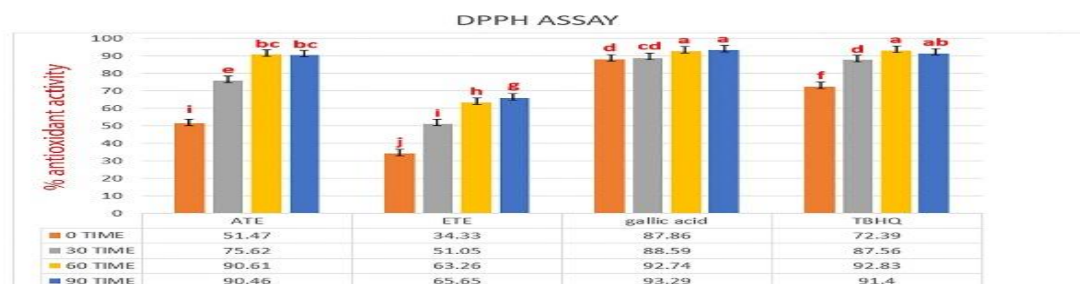

Fig 1: Antioxidant activity of TLE against DPPH• as compared with TBHQ and gallic acid
Means ± SD followed by different letters differ significantly according to Tukey's HSD test (P < 0.01). Uppercase letters indicate significant differences in the main effect, while lowercase letters indicate significant differences in the interaction effect.

Figure 2 shows that TLE scavenged linoleate-derived radicals, inhibiting β-carotene bleaching. At 200 mg/mL, ATE had the lowest activity (49.04), followed by ETE (44.83), whereas TBHQ (32.05) and gallic acid (18.91) had higher activity. According to the bleaching test results, TLE can quench free radicals in a complicated heterogeneous medium. This shows that the extracts might be useful as natural antioxidant preservatives in emulsions.

Phenolic compounds and flavonoids have antioxidative properties in biological systems, functioning as scavengers of singlet oxygen and free radicals **[28].** The content of phenolic chemicals is strongly related to antioxidant activity [**29]** In biological systems, free radicals are commonly referred to as reactive oxygen species (ROS), which are the most physiologically important free radicals. Cells produce ROS such as the hydroxyl radical, hydrogen peroxide, and superoxide anion**[30].**

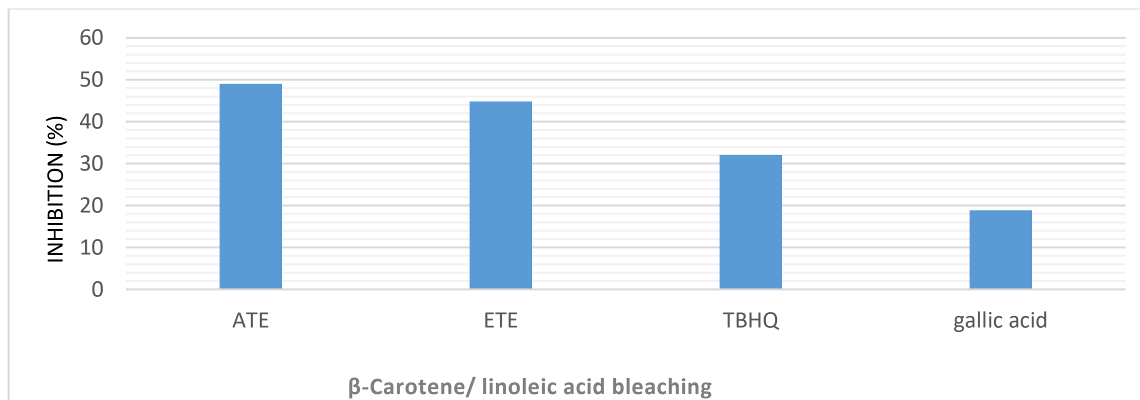

Fig 2: - Inhibition of TLE in β-carotene-linoleic acid emulsion as compared with TBHQ and gallic acid

3.4. Cytotoxicity effect of Thyme 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 indicated in tables (3,4,5 and 6), figures (3,4,5,6,7,8,9 and 10). The plant extract's antiproliferative efficacy on cancer cell lines was assessed in terms of IC50 values. IC50 is the inhibitory concentration that inhibits 50% of the cancer cell population.

The extracts resulted in varying degrees of inhibition in cell growth, depending on the type of extract and cell line. Phenolic compounds derived from medicinal plants are bioactive and significantly contribute to cancer prevention. Their mode of action is both complementary and overlapping, encompassing antioxidant activity, free radical scavenging, and modulation of carcinogen metabolism. These actions modify crucial cellular and molecular pathways associated with carcinogenesis—a complex process that includes the transformation, survival, proliferation, angiogenesis, and metastasis of tumor cells[**31**].

The anticancer properties of thyme and its primary derivatives are associated with various mechanisms, such as antioxidant effects, antiangiogenic properties, epigenetic actions, immune response regulation, and anti-inflammatory responses. These also include altering hormone signaling, modifying specific metabolic pathways, and boosting the expression of tumor-suppressor genes. Thyme is mainly acknowledged for its potential as an anticancer agent, predominantly due to its antioxidant capabilities. It can target free radicals and defend against the oxidative damage they cause to DNA, proteins, and lipids**[32]**, while, as later discovered, thyme compounds are capable of exerting a cytotoxic impact precisely through the generation of reactive oxygen species (ROS).

Thyme extracts have anti-tumor effects through a variety of mechanisms, including cell growth inhibition (antiproliferative activity), apoptosis induction, generation of intracellular reactive oxygen species (ROS), mitochondrial membrane depolarization, activation of Baxproapoptotic mitochondrial proteins, and interactions with caspase or poly-ADP ribose polymerase[**33**].

Elbe and her coworkers **[34]** Thymol'santiproliferative and proapoptotic abilities have been investigated using non-small-cell lung carcinoma, highly metastatic breast adenocarcinoma, and prostate cancer cell lines. Thymol has cytotoxic effects on several tumor types in a dose-dependent and time-dependent manner.

It selectively inhibited the proliferation of cancer cells and induced apoptosis, although the exact mechanism remains unclear. It is likely related to the hydrophobic nature of the thymol molecule and its ability to increase the permeability of the cancer cell plasma membrane.

Several preclinical investigations have emphasized T. vulgaris' anticancer potential, with the plant demonstrating significant free radical scavenging action and proapoptotic effects.**[35]** in the human breast cancer T47D cell line. In a colorectal HCT116 cancer cell culture, *T. vulgaris* extract was found to suppress proliferation in a concentration- and time-dependent manner [**36**]. A decrease in proliferative rate is associated with increased apoptosis, as seen by increased caspase 3/7 activation. Furthermore, *T. vulgaris* inhibits the migratory and invasive capacities of HCT116 cells. *T. vulgaris* extract has also been demonstrated to reduce tumors in human leukemia THP-1 cells **[37]**

Table 3 Percent cell viability of ATE extract of HCT 116 cell line.

ID	Conc. µg/mL	Viability %	Toxicity %	IC50µg/mL
HCT 116	--------	100	0	
ATE	1000	5.058717254	94.94128275	158.61
	500	5.329719964	94.67028004	
	250	25.56458898	74.43541102	
	125	50.04516712	49.95483288	
	62.5	79.94579946	20.05420054	
	31.25	97.2899729	2.7100271	

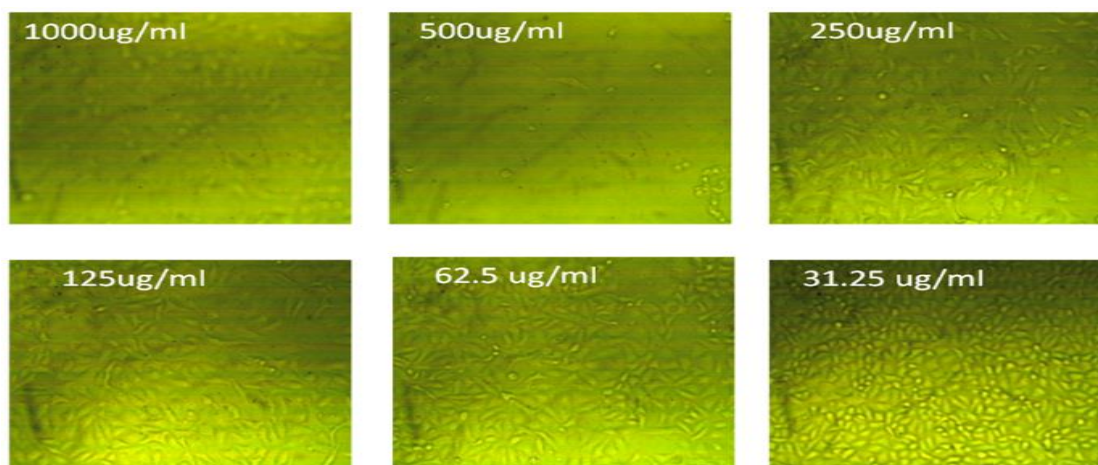

Fig 3: - Effect of ATE extract on human colon cancer cell line (HCT 116).

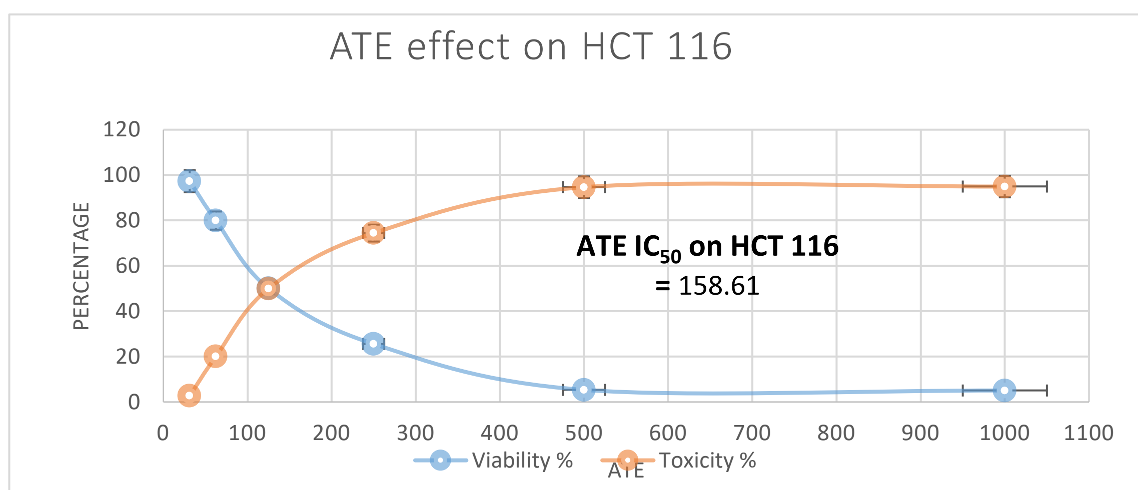

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Fig 4: - Percent cell viability and toxicity of ATE extract of HCT 116 cell line

Table 4 Percent cell viability of ETE extract of HCT 116 cell line.

ID	Conc. μg/mL	Viability %	Toxicity %	IC50 μg/mL
HCT 116	--------	100	0	
	1000	5.149051491	94.85094851	
	500	23.66757001	76.33242999	
ETE	250	52.30352304	47.69647696	330.72
	125	95.84462511	4.155374887	
	62.5	99.36766034	0.632339657	
	31.25	99.63866305	0.361336947	

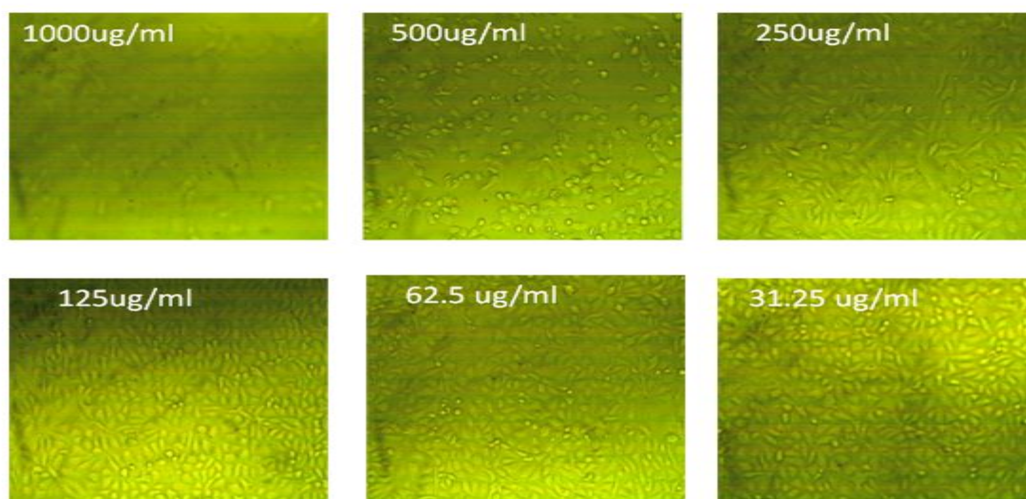

Fig 5: - Effect of ETE extract on human colon cancer cell line (HCT 116).

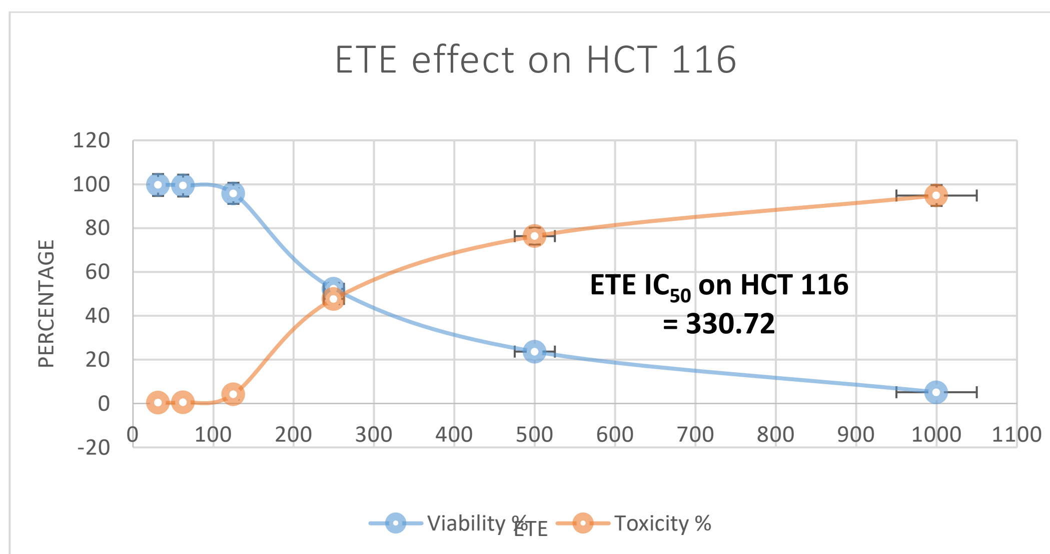

Fig 6: - Percent cell viability and toxicity of ETE extract of HCT 116 cell line

Table 5 Percent cell viability of ATE extract of PC 3 cell line.

ID	Conc. µg/mL	Viability %	Toxicity %	IC50 µg/mL
PC3	--------	100	0	
ATE	1000	4.675324675	95.32467532	97.33
	500	4.848484848	95.15151515	
	250	13.67965368	86.32034632	
	125	29.43722944	70.56277056	
	62.5	76.27705628	23.72294372	
	31.25	98.0952381	1.904761905	

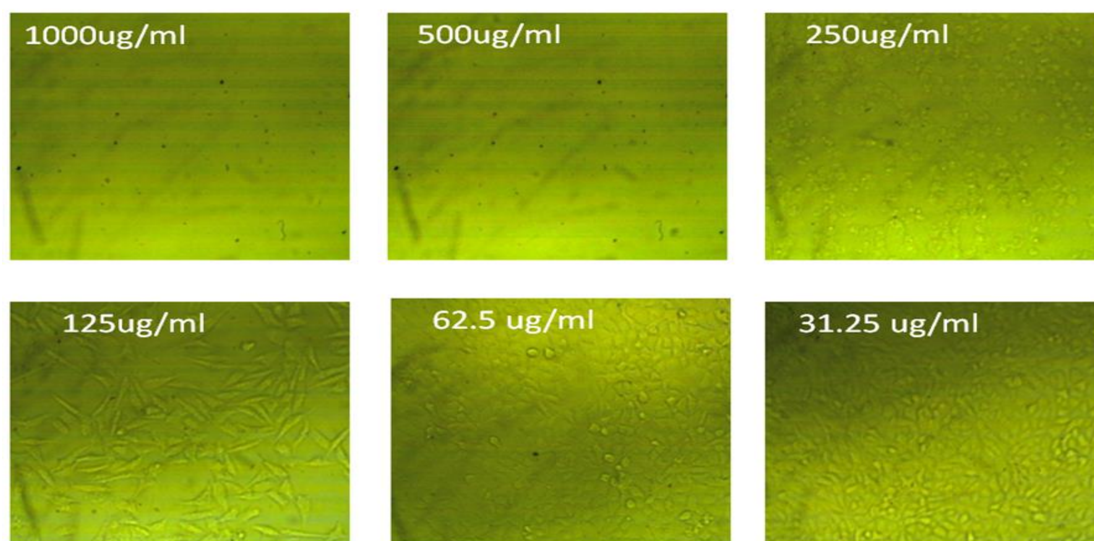

Fig 7: - Effect of ATE extract on human prostate cancer cell line (PC3).

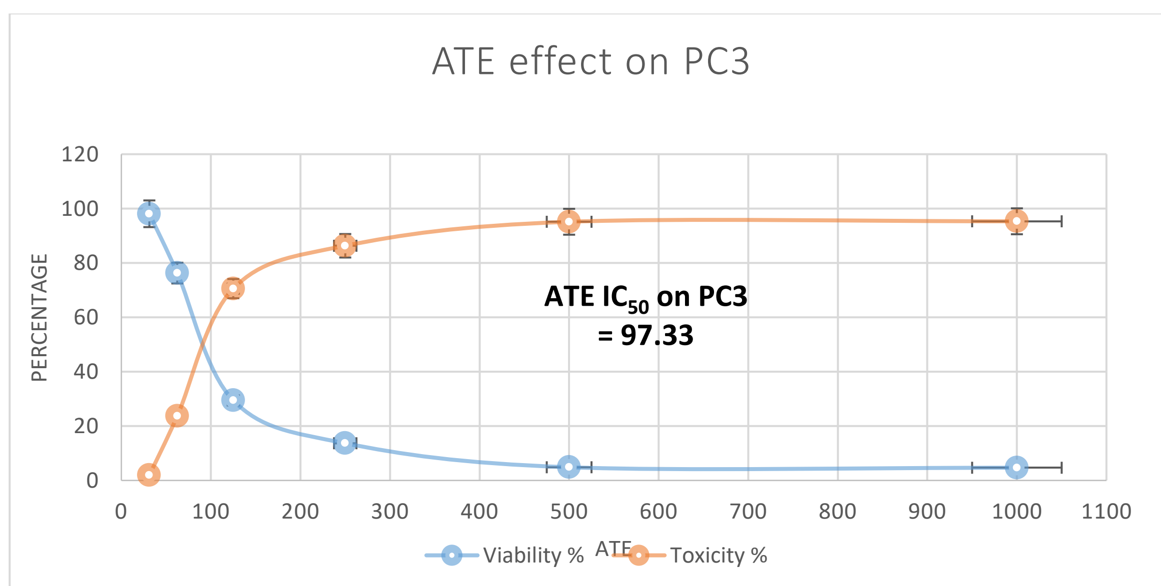

Fig 8: - Percent cell viability and toxicity of ATE extract of PC3 cell line.

Table 6 Percent cell viability of ETE extract of PC3 cell line.

ID	Conc. µg/mL	Viability %	Toxicity %	IC50µg/mL
PC3	--------	100	0	
ETE	1000	5.108225108	94.89177489	115.53
	500	5.021645022	94.97835498	
	250	25.19480519	74.80519481	
	125	44.25108225	55.74891775	
	62.5	81.9047619	18.0952381	
	31.25	99.82683983	0.173160173	

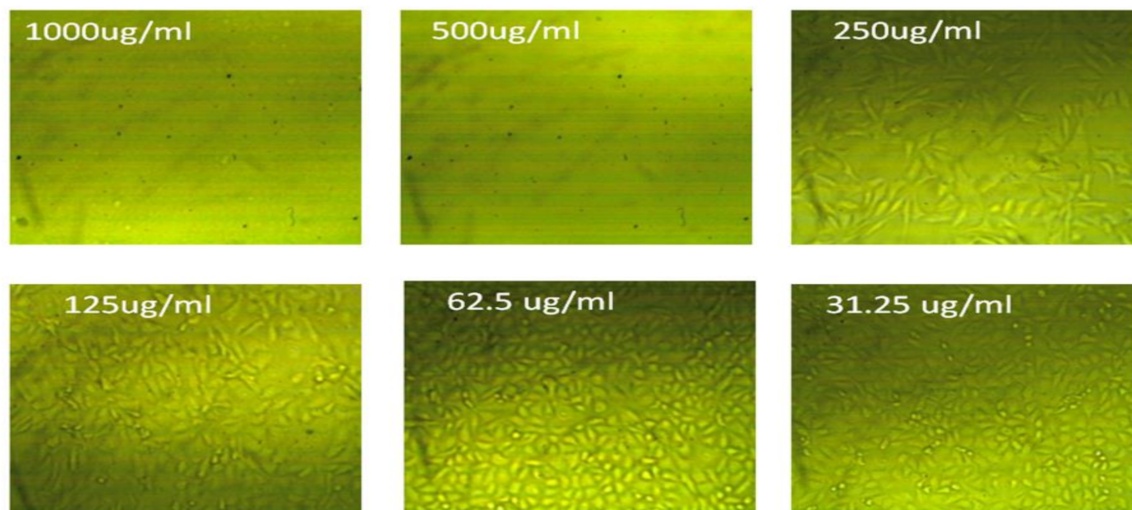

Fig 9: - Effect of ETE extract on human prostate cancer cell line (PC 3).

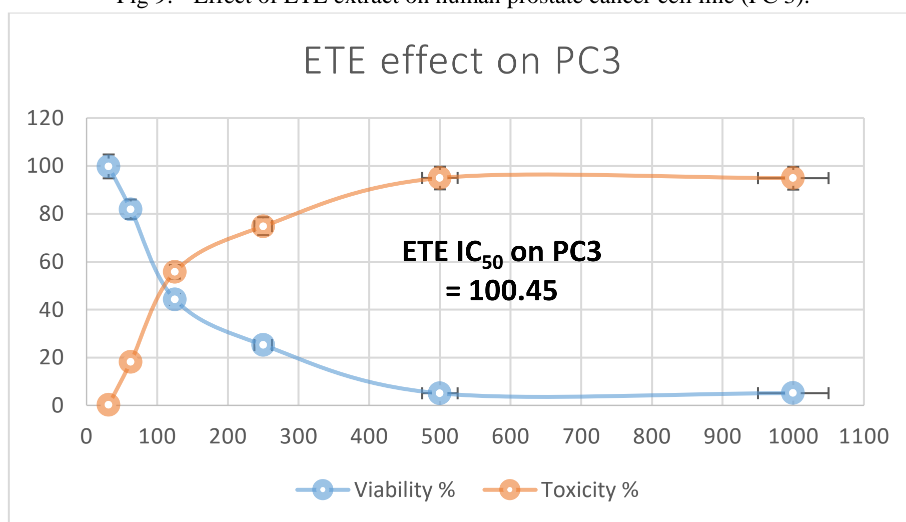

Fig 10: - Percent cell viability and toxicity of ETE extract of PC3 cell line.

4. Conclusion

Chemical composition constituents of THYME (TLE) indicated the presence of phenolic components such as naringenin, ellagic acid, quercetin, rutin and apigenin. (TLE) has antioxidants, and anticancer activity. TLE offers great promise in the creation of phytomedicine with anticancer qualities, and drugs developed from TLE may serve as an alternative medicinal

source due to their anticancer activities. This study also found that the acetone and ethanol extracts of TLE had the highest anticancer activity against (HCT 116) human colon and human prostate (PC3) cancer cell lines.

5. References

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