Hexane Extract of *Thymus serpyllum* L.: GC-MS Profile, Antioxidant Potential and Anticancer Impact on HepG2 (Liver Carcinoma) Cell Line

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**Abstract**—The present study was designed to scrutinize anticancer activity of 5 different extracts of *Thymus serpyllum* in MCF-7, MDA-MB-231, HepG2, HCT-116, PC3 and A549 and to assess the antioxidant activity. Identification of the phytochemicals present in the most active extract of *Thymus serpyllum* was conducted using gas chromatography coupled with mass spectrophotometry and antioxidant activity was measured by using DPPH radical scavenging assay. Anticancer impact of the extract in terms of IC50 was evaluated using MTT cell viability assay. Results revealed that the hexane extract showed the best anticancer activity in HepG2 (Liver Carcinoma Cell Line) with an IC50 value of 23 ± 0.14 µg/ml followed by 25 µg/ml in HCT-116 (Colon Cancer Cell Line), 30 µg/ml in MCF-7 (Breast Cancer Cell Line), 35 µg/ml in MDA-MB-231 (Breast Cancer Cell Line), 57 µg/ml in PC3 (Prostate Cancer Cell Line) and 60 µg/ml in A549 (Lung Carcinoma Cell Line). GC-MS profile of the hexane extract showed the presence of 21 compounds with carvacrol, thymol and thymoquinone being the major compounds. Phenolics such as Vitamin E, terpinen-4-ol, borneol and phytol were also identified. Hence, here we present the first report on cytotoxicity and acute toxicity of hexane extract of *Thymus serpyllum* extract in HepG2 cell line and rats, respectively, with a robust anticancer activity with an IC50 23 ± 0.14 µg/ml.

**Keywords**—Anticancer activity, Antioxidant activity, GC-MS profile, HepG2 cancer cell line, Hexane extract.

I. INTRODUCTION

Cancer is the leading cause of death that claims 3,500 million lives worldwide each year [1] with mortality rate estimated at 2-3% [2], [3]. Statistics have estimated that cancer will cause 83.2 million deaths from 2005 to 2015 worldwide. Breast cancer is the most common of all other cancer-related deaths in women that claims 480,000 deaths with lung cancer being responsible for 1.3 million deaths annually [4]. Hepatocellular carcinoma is the most public cancer and third largest cause of cancer related deaths worldwide with about 500,000 new cases annually.

Free radicals play a critical role in the development of tissue damage during pathological events [5]. Reactive oxygen species (ROS) are the products of naturally occurring metabolic processes in our body. Healthy cells are capable of maintaining a balance between production of these ROS and distinct defense systems. Disruption of this balance ensues oxidative damage due to accretion of free radicals, known as oxidative stress, that leads to diseases like cancer, arteriosclerosis, cardiovascular damage and even aging [6]–[8]. They are closely involved in the pathology of various diseases, including cancer. Antioxidants are the chemical compounds which are capable of scavenging these free radicals and thereby are the key players in protecting the human body against such diseases. Recently, increased interest has been seen in naturally-occurring antioxidants that can prevent the human body from oxidative damage. Strong antioxidants and phenols have been reported in the extracts of *Thymus serpyllum* [7], [9].

Chemotherapy and radiation therapy are on top of the list of strategies that have been adopted to combat this deadly disease, however, chemotherapy is the most popular one specifically known for its effectiveness against most of the cancer types but resistance of stubborn cancer cells limits successful aftermaths in most of the cases. This is mainly because of the incapability of certain drugs to discriminate between normal and cancerous cells, and some serious side effects as deadly as congestive heart failure [4].

Medicinal plants used in folk medicine and their biologically active compounds used in conventional medicine constitute the basis for the treatment of a variety of diseases, cancer being one of them. Clinical applications have demonstrated their therapeutic potential for any decades. About 40% of all the available anticancer drugs are plant-derived or their mimics, such as, vincblastine (from *Catharanthus roseus*), paclitaxel (from *Taxus brevifolia*) and...
podophyllotoxin (from *Podophyllum emody*) have been used as anticancer agents [10].

*Thymus serpyllum* L. (wild thyme) of family Lamiaceae (Labiateae) is a famous thyme with perennial, herbaceous shrub that is native to southern and southern-eastern Europe. It grows wild or is cultivated across Spain, France, Italy, Yugoslavia, Greece, Central European countries, Turkey, Israel, Iran, Morocco and North America [11]. It is a highly aromatic herb used extensively in folk medicine [12] Thymus serpyllum possesses strong antioxidant properties, and this is correlated to the presence of essential oils [7]. The most abundant constituent of essential oil is thymol (30-75%) followed by carvacrol, p-cymene, 5-terpinene and 1,8 – cineole (Thyme). Thyme and thyme oil have strong antioxidant properties [13]-[17]. Of various medicinal plant volatiles tested, thyme oil was found to be the most effective antioxidant [18]. Recent studies show that the aqueous extract of *Thymus serpyllum* has strong growth inhibitory property in Colon (HCT-15) [19] and Breast cancer (MCF-7) cell lines [9].

This study was conducted to assess cytotoxic impact of 5 *Thymus serpyllum* extracts in 6 cancer cell lines (MCF-7, MDA-MB-231, HEPG2, HCT-116, PC3 and A549), to determine the antioxidant potential of *Thymus serpyllum* extracts and to analyze GC-MS profile of the most cytotoxic extract of *Thymus serpyllum*.

II. RESULTS AND DISCUSSION

A. Cytotoxic Activity of *Thymus serpyllum* against Human Cancer Cell Lines

To investigate the potential of hexane extract against cancer cell lines, 6 human cancer cell lines were screened to assess the cytotoxic impact, that is, MCF-7, MDA-MB-231, HEPG2, HCT-116, PC3 and A549 cancer cell lines. The cytotoxicity was calculated in terms of the IC50 value which defines the amount of the drug capable of imposing inhibitory effect on 50% of the cells. The IC50 recorded for each cell line varied from 22 ± 0.57 µg/ml to 200 µg/ml, depending upon the cell line and the extract (Table I). Among all the five cancer cell lines (MCF-7, MDA-MB-231, HCT-116, PC3, HepG2 and A549), the hexane crude extract showed the best activity against HepG2 (Liver Carcinoma Cell Line) with an IC50 value of 22 ± 0.57 µg/ml (Table I), while the aqueous extract exhibited poor cytotoxicity with an IC50 values > 200 µg/ml in all the screened cell lines as compared to the other extracts of *Thymus serpyllum*.

In the light of the previous studies, essential oil extracted from *Thymus serpyllum* showed significant antiproliferative activity with an IC50 of 7.02 ± 0.07 µg/ml in HCT-15 (Colon Cancer) cell line [19], however, the same extract gave an IC50 of 52.69 ± 3.28 µg/ml in MCFl7 (Human Breast Cancer) cell line [19]. Similarly, another study suggested that the essential oil content of *Thymus serpyllum* inhibited proliferation of the MCF-7 (Human Breast Cancer) cell line with an IC50 of 95.8 ± 2.8 µg/ml. The present study showed a good anticancer impact with an IC50 of 23 ± 0.14 by hexane extract.

B. Chemical Constituents in Hexane Extract of *Thymus Serpyllum* 

The results obtained by GC-MS chemical analysis of hexane extract of *Thymus serpyllum* are presented in Fig 1. And Table II.

In total, 21 compounds were identified. The GC-MS analysis showed major portion of the chemical profile accounting for essential oils. The GC-MS analysis showed the presence of 3 major compounds, carvacrol, thymol and thymoquinone (TQ) in hexane extract of *Thymus serpyllum*. Thymol and carvacrol were the main oxygenated monoterpene identified in the hexane extract of *Thymus serpyllum*.
Quite a number of studies have been reported on the chemical composition of the oils from the plants belonging to the genus *Thymus* [20], [21]. *Thymus serpyllum* being one of them. However, few studies have investigated the chemical profile of *Thymus serpyllum* [22]. This, perhaps, is the first study to report the anticancer potential and chemical profile of the hexane extract of *Thymus serpyllum*.

1. Thymol, Carvacrol and Thymoquinone: Major Compounds of the Hexane Extract

Among all the compounds identified by the GC-MS analysis, thymol, carvacrol and thymoquinone were the major compounds in the hexane extract of *Thymus serpyllum* (Fig. 1). Carvacrol (2-methyl-5-(1-methylthyl)-phenol) is major monoterpenene phenol that is present as an active constituent of the essential oil of thyme group [23]. Carvacrol is a chief monoterpenoic phenol that is believed to hamper cancer advancement and development [24]. Studies have suggested robust role of carvacrol as an anticancer agent [23], [25]-[27]. These major monoterpenes have also been reported to have potential antioxidant capacities. Carvacrol (Fig. 1) has also been reported to exhibit potential anticancer role by inhibiting cell proliferation and preventing metastasis in DEN-induced hepatocellular carcinogenesis [24]. Recently, carvacrol has been reported to have anti-inflammatory activities on lipopolysaccharide-induced endotoxemia and acute lung injury via inhibition of inflammatory cytokines TNF-α IL-6 and IL-1β production in mice.

Thymol (Fig. 1) is a naturally occurring phenolic compounds that is known for its antioxidant, antimicrobial, anti-inflammatory activities [20]-[22], [28]-[30]. Thymol has also been reported as an anti-cancer agent [22], [31]. Previously, anti-cancer activity of thymol and carvacrol has been reported in HepG2 cell line [22]. The study suggested moderate anti-cancer impact on HepG2 cell lines with an IC50 of 53.09 µg/ml and 60.01 µg/ml by thymol and carvacrol, respectively. However, when they compared the cytotoxicity imposed by the crude extract it revealed that thymol and carvacrol alone were more capable of exhibiting cytotoxic impact as compared to crude extract. Interestingly, our study showed potential anticancer activity with an IC50 23 µg/ml. Thymol has been studied thoroughly for its anticancer effects by inducing apoptosis in cancer cells [32]-[36]. The best anti-cancer activity reported is 22 µg/ml [35].

Among the essential oils with promising health benefits, thymoquinone (Fig. 1) is no exception. Accumulating studies regarding its biological activity signifies its potential as an antioxidant, antimicrobial and anticancer agent [37]-[46]. It has also been shown to have anti-inflammatory activities [47]. Moreover, anti-toxic effects [48] and *in vivo* diabetic neuropathy [49] have also been studied. It’s also an important constituent of a *Nigella Sativa* [50], [51]. Recently, antioxidant effects of TQ have also been studied in brain tissue *in vivo* [37]. TQ has been extensively investigated in tumor xenograft mice models for colon, prostate, pancreatic and lung cancers [52] [53], [54]. TQ has also been shown with

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**TABLE III**

**REPRESENTATION OF CHEMICAL COMPOUNDS FOUND IN THE HEXANE EXTRACT OF *THYMUS SERPYLLUM* BY GC-MS**

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>Formula</th>
<th>Retention Time (s)</th>
<th>Similarity</th>
<th>Weight</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzene, 1-methyl-3-(1-methylethyl)-</td>
<td>C20H14</td>
<td>926.85</td>
<td>971</td>
<td>134</td>
<td>1.11650</td>
</tr>
<tr>
<td>2</td>
<td>Borneol</td>
<td>C10H16O</td>
<td>1261.05</td>
<td>926</td>
<td>154</td>
<td>0.15435</td>
</tr>
<tr>
<td>3</td>
<td>Terpinen-4-ol</td>
<td>C10H14O</td>
<td>1285.35</td>
<td>886</td>
<td>154</td>
<td>0.38518</td>
</tr>
<tr>
<td>4</td>
<td>a-Terpineol</td>
<td>C10H14O</td>
<td>1315.75</td>
<td>906</td>
<td>154</td>
<td>0.33226</td>
</tr>
<tr>
<td>5</td>
<td>Benzene 2-methoxy-4-methyl-1-(1-methylethyl)-</td>
<td>C11H14O2</td>
<td>1411.25</td>
<td>955</td>
<td>164</td>
<td>0.42899</td>
</tr>
<tr>
<td>6</td>
<td>Thymoquinone</td>
<td>C10H14O2</td>
<td>1444.55</td>
<td>950</td>
<td>164</td>
<td>7.61290</td>
</tr>
<tr>
<td>7</td>
<td>Thymol</td>
<td>C10H14O</td>
<td>1540.20</td>
<td>956</td>
<td>150</td>
<td>34.0650</td>
</tr>
<tr>
<td>8</td>
<td>Carvacrol</td>
<td>C10H18O</td>
<td>1559.65</td>
<td>954</td>
<td>150</td>
<td>29.7630</td>
</tr>
<tr>
<td>9</td>
<td>Thymol Acetate</td>
<td>C11H16O2</td>
<td>1655.40</td>
<td>950</td>
<td>192</td>
<td>0.28583</td>
</tr>
<tr>
<td>10</td>
<td>Carvacrol Acetate</td>
<td>C11H16O2</td>
<td>1691.60</td>
<td>944</td>
<td>192</td>
<td>0.06958</td>
</tr>
<tr>
<td>11</td>
<td>N,N-Dimethyl-4-nitroso-3-(trimethylsilyl)aniline</td>
<td>C21H27NOSi</td>
<td>1719.75</td>
<td>747</td>
<td>222</td>
<td>0.17758</td>
</tr>
<tr>
<td>12</td>
<td>p-tert-Butyl catechol</td>
<td>C10H14O2</td>
<td>1830.75</td>
<td>788</td>
<td>166</td>
<td>8.6299</td>
</tr>
<tr>
<td>13</td>
<td>Bisabolene-(1\beta)&gt;</td>
<td>C20H24</td>
<td>1949.45</td>
<td>916</td>
<td>204</td>
<td>0.76580</td>
</tr>
<tr>
<td>14</td>
<td>2(4H)-Benzo[diso]annulene,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-</td>
<td>C20H18O2</td>
<td>1984.85</td>
<td>899</td>
<td>180</td>
<td>0.20541</td>
</tr>
<tr>
<td>15</td>
<td>Thymohydroquinone</td>
<td>C10H14O</td>
<td>2030.25</td>
<td>840</td>
<td>166</td>
<td>0.19202</td>
</tr>
<tr>
<td>16</td>
<td>Phytol</td>
<td>C20H40</td>
<td>3323.30</td>
<td>926</td>
<td>296</td>
<td>0.15488</td>
</tr>
<tr>
<td>17</td>
<td>Octacosane</td>
<td>C32H64</td>
<td>3770.25</td>
<td>958</td>
<td>394</td>
<td>0.18559</td>
</tr>
<tr>
<td>18</td>
<td>2-methyloctacosane</td>
<td>C32H66</td>
<td>3840.35</td>
<td>931</td>
<td>408</td>
<td>0.31617</td>
</tr>
<tr>
<td>19</td>
<td>Hexacosane</td>
<td>C32H64</td>
<td>3956.30</td>
<td>959</td>
<td>366</td>
<td>1.16390</td>
</tr>
<tr>
<td>20</td>
<td>Heptacosane</td>
<td>C32H66</td>
<td>4127.25</td>
<td>961</td>
<td>380</td>
<td>6.2985</td>
</tr>
<tr>
<td>21</td>
<td>Vitamin E</td>
<td>C20H30O</td>
<td>4355.50</td>
<td>838</td>
<td>430</td>
<td>0.22860</td>
</tr>
</tbody>
</table>
enhanced therapeutic effects in synergy with other conventional drugs [47]. It synergizes with DNA-damaging agent “cisplatin” to inhibit cellular viability [55].

Thus, it could evidently be established that the cytotoxicity imposed on cell viability by hexane extract of *Thymus serpyllum* is relatable to the presence of thymol, carvacrol and thymoquinone. Most of the literature suggests strong anticancer activity of these compounds both *in vitro* and *in vivo*.

![Structural formula of compounds](image)

**Fig. 1** Structural description of the major compounds found in the hexane extract of *Thymus serpyllum*. A. Thymol b. Thymoquinone and c. Carvacrol

**C. Antioxidant Activity**

1. Free Radical Scavenging Potential
2. The hydrogen atoms or the electron donation abilities of the samples were measured via bleaching of a purple-colored methanol solution of DPPH. The percent scavenging ability of each extract [hexane, dichloromethane, ethyl acetate, methanol and ascorbic acid (control)] are represented in Table II. Ascorbic acid was used as control. The results demonstrated the antioxidant potential in a decreasing fashion as Ethyl Acetate > Hexane Extract > Methanol Extract > Dichloromethane. However, all the extracts showed an increase in the antioxidant potential with increasing concentration, particularly, ethyl acetate extract exhibited a strident increase Fig 3. Whereas, hexane extract manifested a gentle increase in the antioxidant potential with increasing concentration followed by methanol and dichloromethane extracts Fig 3. Hence, it could be established from the obtained results that the ethyl acetate extract of *Thymus serpyllum* possessed the highest antioxidant capacity (IC_{50}: 1739.06 ± 1.00 µg/mL) of all the extracts analyzed (hexane, methanol and dichloromethane).

Heretofore, there are a number of studies that reported antioxidant potential of essential oils of *Thymus serpyllum* [56]-[60]. The antioxidant potential has been attributed to the presence of mono-terpenes, such as carvacrol and thymol. Similar to the antioxidant effect of these compounds, our results suggest that the anticancer potential of hexane extract more or less rests on the antioxidant activity. Interestingly, ethyl acetate fraction of *Thymus serpyllum* extract exhibited the best antioxidant capacity but poor cytotoxic impact in the screened cell lines. Similarly, dichloromethane extract demonstrated poor antioxidant capability compared to other extracts but manifested better cytotoxic results, in fact, closer to the best effects showed by hexane extract Table 2 and Fig. 2. Thus the results suggest that the cytotoxicity imposed by the extracts of *Thymus serpyllum* are independent of the antioxidant capacities of these extracts.
Fig. 2 Free Radical Scavenging Potential of Hexane, Dichloromethane, Ethyl Acetate and Methanol Extracts (Ascorbic Acid-Control)

TABLE IV
ANTIOXIDANT ACTIVITIES OF HEXANE, DICHLOROMETHANE, ETHYL ACETATE AND METHANOL EXPRESSED AS IC_{50} (µG/ml)

<table>
<thead>
<tr>
<th>Samples</th>
<th>R² Values</th>
<th>IC_{50} (µG/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>0.8687</td>
<td>3887.6 ± 0.02</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>0.8563</td>
<td>3887.6 ± 0.02</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>0.5643</td>
<td>3887.6 ± 0.02</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.8628</td>
<td>3887.6 ± 0.02</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>0.4225</td>
<td>3887.6 ± 0.02</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of three independent experiments.

II. EXPERIMENTAL SECTION

A. Chemicals and Instrumentation

Analytical grade n-hexane, Dichloromethane (D.C.M), Ethyl Acetate, Methanol DPPH (2,2-diphenyl-1-pierylhydrazyl), Ascorbic Acid were all obtained from Sigma-Aldrich (Karlsruhe, Germany). For the anticancer potential assaying of the plant extract, all cell lines were purchased from ATCC. All spectrophotometric data were acquired using a Jasco V-530 UV-vis spectrophotometer (Jasco International Co., Ltd., Tokyo, Japan).

B. Cell Lines Culture and Maintenance

Invasive and metastatic human breast cancer cell line MDA-MB-231 which is known to cause tumors in athymic mice and the ER positive noninvasive breast cancer cell line MCF-7 along with HepG2 (Liver Carcinoma), HCT-1116 (Colon Cancer), PC3 (Prostate Cancer) and A549 (Lung Carcinoma) cell lines were obtained from ATCC. These cell lines were maintained in RPMI 1640 media containing either 10% fetal calf serum (FCS) or serum free medium (0.1% BSA supplemented with L-glutamine and antibiotics). Cell cultures were maintained in plastic flasks and incubated at 37°C in a humidified chamber containing 5% CO2. Cells were washed with chilled phosphate-buffered saline (PBS), harvested by scraping with a rubber policeman, and homogenized in ice cold PBS [61]-[63].

C. Preparation of Thymus Serpyllum Extracts with 5 different Solvents (Hexane, Dichloromethane, Ethyl Acetate, Methanol and Water)

In lieu with the fact that the chemical composition mainly depends on geographical and climatic conditions of growth [64], plant material (Thymus serpyllum L.) was collected from an altitude 16000-18000 ft. above the sea level during the flowering period (June-August). The plant material was dried under shadow conditions. For the purpose of extract preparation, 60 g of plant material (flowers and leaves of Thymus serpyllum L.) were grounded and weighed. This plant material was then soaked in 800ml of n-hexane for 72 hr at room temperature with occasional shaking. The solution was then filtered using a filter paper (Whatman No. 1, UK). This was repeated until a colorless solution was obtained. The residue (plant material) was subsequently soaked in dichloromethane, ethyl acetate, ethanol and methanol using the same methodology used for n-hexane. All 4 obtained extracts were concentrated to dryness using a rotary evaporator. Whereas, extraction with water was achieved by boiling the plant material for 30 min in water which was then filtered and the extract was dried in a freeze dryer. All 5 crude extracts were stored at 4°C till further use.

D. Gas Chromatography and Mass Spectroscopic Analysis (GC-MS)

The GC–MS analysis of the hexane extract was performed on an Agilent-Technologies 7890A Network gas chromatographic (GC) system, equipped with an Agilent-Technologies LECO PENGASUS HT High Throughput TOF-MS Mass selective detector and Agilent-Technologies 7693 series auto-injector. Compounds were separated on Rxi-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm; USA). A sample of 1.0 µl was injected in the split mode with split ratio 1:10. For GC/MS detection, an electron ionization system, with ionization energy of 70 eV, was used. The column oven temperature programme, initial 40°C hold 5 minutes, 4°C/min 160°C hold 10 minutes and 5°C/min 280°C hold 10minutes. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Mass scanning range was 50–1000 m/z while the injector and MS transfer line temperatures were set at 250 and 300°C, respectively.
E. Determination of Antioxidant Activity

1. DPPH Radical Scavenging Assay

The free radical scavenging potential of the hexane extract was assessed by measuring its ability to scavenge 2,2'-diphenyl-1-picrylhydrazyl stable radicals (DPPH). The DPPH assay was performed as described by [65]. The samples (from 4 to 20 µg/ml) were mixed with 1 ml of 90 µmol/L DPPH solution and made up with 95% methanol to a final volume of 4 ml. Synthetic antioxidant (Ascorbic acid) was used as control. After 30 min incubation period at room temperature, the absorbance was recorded at 515 nm. Percent radical scavenging concentration was calculated using the following formula:

\[ \text{Radical Scavenging} \% = 1 - \left( \frac{A_{\text{blank}} - A_{\text{samp}}}{A_{\text{blank}}} \right) \]

F. MTT Assay for Cytotoxicity

The cytotoxic effect of all 5 extracts (hexane, dichloromethane, ethyl acetate, methanol and water) was determined by MTT assay. The mechanism underlying this colorimetric method is the conversion of the yellow tetrazolium bromide (MTT) to the purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells. Six cell lines (MCF-7, MDA-MB-231, HCT-116, PC3, HepG2 and A549) were seeded at the density of 1 × 10⁴ cells/ml in 96-well plate and incubated for 24 h at 37 °C with 5% CO₂ saturation. Following incubation, each cell line was treated with hexane, dichloromethane, ethyl acetate, methanol and water (dissolved in 1% DMSO) at different concentration and incubated for another 24 and 48 h. Following the treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl was added into each well to dissolve the purple formazan formed. The absorbance was recorded at 570 nm. Results were expressed as percentage of control giving percentage cell viability after exposure to test agent. The strength of cell growth inhibition for test agent was expressed as IC₅₀ value, which is defined as the concentration that causes a 50% loss of cell growth. Viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells.

G. Statistical Analysis

All the experiments were performed in three replicate and the data is presented as mean values ± standard deviation of triplicate findings. Statistical analysis of the data was performed by Analysis of Variance (ANOVA) using SPSS software and a probability value of \( p \leq 0.05 \) was considered to show a statistical significant difference among mean values.

H. Conclusion

Here, we present the first study regarding the cytotoxic impact of hexane extract of Thymus serpyllum on 6 cancer cell lines (MDA-MB-231, MCF-7, HepG2, HCT-116, PC3 and A549). The hexane extract manifested strong anticancer potential in HepG2 cell line with an IC₅₀ of 22 ± 0.02 µg/ml. The chemical composition of the hexane extract highlighted the presence of many essential oils with 3 major compounds carvacrol, thymol and thymoquinone. Hence, the study suggests that essential oils of Thymus serpyllum inflicts robust cytotoxicity in HepG2 cell line. Based on our study, the hexane extract of Thymus serpyllum should be further investigated for any improved cytotoxic effects on Liver Carcinoma cell line that shall be demonstrated by drug formulation.

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