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# The potential of herbal medicine for prostate cancer therapy as an alternative of the common cancer treatments

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## Abstract

Prostate cancer is the second most common cause of cancer death worldwide in men. Common cancer therapies such as chemotherapy, hormonotherapy, surgery and immunotherapy have annoying side effects on patients. Moreover, the medicines using for the common cancer therapies reported as a case of environment polluters. Recently, herbal medicine received great attention for cancer therapy owing to their minimal side effects and eco-friendly properties. In this research, *Teucrium hyrcanicum* was investigated as a novel herbal medicine for prostate cancer cells (PC-3) treatment. First the flavonoid content and other components of *T. hyrcanicum* were measured by GC-MS analysis. Then, to evaluate the anticancer potential of *T. hyrcanicum* for prostate cancer removing, MTT assay, Woundhealing Assay, Clonogenic Assay were analyzed before and after treatment with different concentrations of *T. hyrcanicum*. The results revealed that *T. hyrcanicum* has components such as Skatole, Indole and Tetrodecanoic acid that indicated its high potential of anticancer property. The results of MTT, Woundhealing and colonogenic assay proved that methanolic extraction of *T. hyrcanicum* effectively removed prostate cancer cells (PC-3). This work suggests *T. hyrcanicum* as a promising herbal medicine for prostate cancer treatment.

**Key words:** Herbal medicines, *Teucrium hyrcanicum*, Prostate cancer therapy, Anticancer drugs



## 1. Introduction

Cancer is the second cause of death in the world which still people are not able to overcome it entirely [1]. Cancer can be categorized as the complicated diseases related to abnormal growth of cells in the form of tumors which can remain into the original place of formation at the beginning. However, they can move to another parts of body to attack other organs which calls metastasis [2]. It has been reported that the number of cancer cases increases up to 50.5 million within the next 5 years. While, 20.6 million out of this number (40 %) includes breast, prostate, colorectal and lung cancer [3]. Chemotherapy, hormone therapy, surgery and radiation are the common treatments for various cancers [4]. Antineoplastic drugs (ADs) such as cyclophosphamide (cyc), 5-fluorouracil (5-Fu), cisplatin and doxorubicin have been widely used for cancer treatments [5]. Despite their importance for treatment, the presence of anticancer drugs in the aquatic environment raises significant concerns because of the probability of the adverse ecological effects and their potential as a source of pollution [6]. For example, in France 13 tons of anticancer drugs were produced in 2005 and it had increased to 17.5 tons by 2008 [7]. This can negatively effective on polluting different organisms, animals and environment especially aquatic environment [5, 6]. On the other hand, these drugs have annoying side effects on the cancer cases including nausea, vomiting, loss of appetite, general malaise, hair loss, rash, hot flashes, and anemia [8].

Meanwhile, herbal medicines or phytomedicines which are extracted from plants have received great attention owing to their natural base, low cost and secondary metabolisms [9]. Considering the above mentioned unfavorable side effects of ADs, approximately 75% – 80% of people prefer to use the herbal medicines to treat their diseases such as cancers [10]. Up to now, *Teucrium* species (*Lamiaceae* family) [9], *Wedelia chinensis* (*Asteraceae* family) [11], *Plumbago zeylanica* (*Plumbaginaceae* family) [12] have been widely investigated as herbal medicines for different cancer treatment. Among them, *Teucrium* species showed a higher anti-cancer potential due to having higher level of flavonoid content [13,14]. However, to the best of our knowledge no study has yet been reported about the usage of *teucrium hyrcanicum* for cancer treatment.

Therefore, this study investigates the potential of *teucrium hyrcanicum* as a novel herbal medicine for prostate cancer treatment instead of common cancer treatments. To do this, the effect of different dosage of *T. hyrcanicum* on prostate cancer cells (PC-3) was investigated using flavonoid content, GS-MS, MTT, wound healing and clonogenic analyses.



## 2. Materials and methods

### 2.1. Cell line and cell culture

Human prostate cancer cell line was obtained from the national cell bank of Iran (NCBI), Pastor, Tehran, Iran. PC-3 cells were cultured in the RPMI-140 medium (sigma, Germany), which was supplemented with 10 % (v/v) fetal bovine serum (FBS, Invitrogen) and 1 % × penicillin streptomycin solution. The cells were kept in a humidified environmental cell incubator with 5 % CO<sub>2</sub> and 95 % air at 37 °C.

### 2.2. Extraction and stock

Aerial parts of *Teucrium hyrcanicum* were collected from Mazandaran province at the north part of Iran, in June 2020. To extract the stock solution, *T. hyrcanicum* mixed with methanol (Merck) solvent using Soxhlet extractor and shaker with were used. Then, 400gr of plant dried by freeze-drier. Finally, 20mgr of dried plant solved into 20ml methanol and stored in -200C refrigerator until using for follow experiments.

### 2.3. Flavonoid content

Flavonoid content was measured with Aluminium Chloride (AlCl<sub>3</sub>) according to colorimetric method. In detail, 150 ml of AlCl<sub>3</sub> (10 %) and 75µl NaNO<sub>2</sub> (5%) was added to 25, 50, 100 µg/ml methanolic plant extraction. After 6min, 500µl NaOH was added to all tubes. After 20 min, the absorbance was measured at 510 nm wavelength. The blank sample consisted of the extraction and methanol mixture without AlCl<sub>3</sub>. Standard curve was plotted with Quercetin, then the total flavonoid content determined as mg of quercetin as equivalents (QE0/g of extract).

### 2.4. GC-MS

To analysis the compounds of *Teucrium hyrcanicum* by GC-MS, 20 µg of dried extract was solved into 200µL methanol. Helium was used as carrier gas at a rate of 1 ml/min. The temperature program was as follows: initial temperature was 50 °C for 4 min, increased to 260 °C at a rate of 5 °C/min and held for 13min. The identification of each metabolite was confirmed by the comparing mass fragments with the mass spectra data with those of an NIST, Wiley mass spectral library.

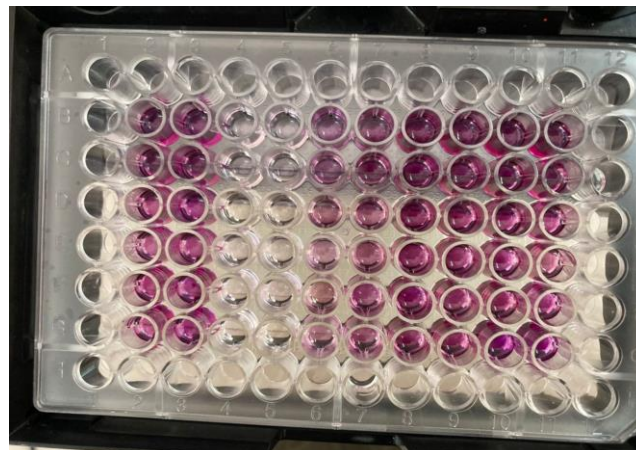


## 2.5. MTT Assay

MTT Assay was used to measure cancer cells survival after treating by different concentrations of *T. hyrcanicum*. The PC-3 cells (100 $\mu$ l growth medium) were cultured in 96-well plate (5000 cells each well). After 2 days, the cells treated by 2, 5, 10, 25, 50, 75 and 100  $\mu$ g/ml (both 24 and 48h). Then, the cells medium was removed and 5  $\mu$ l MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) was added to each well. After 2-3 hours, the MTT solution was replaced by 100  $\mu$ l DMSO. Absorbances were measured by Eliza-reader in 590 nm wavelength. Eq. (1) was used to determine the percentages of cell viability:

$$\text{Cell viability (\%)} = (\text{treated samples absorbance/control absorbance}) \times 100 \quad (1)$$

IC50 amount was measured by GraphPadPrism V2.0.8 software.



**Figure. 1.** MTT assay samples

## 2.6. Wound healing Assay

For cell motility determination, when they reached 90%-100% confluency in 6-well culture plate, they scratched across the center of the well. Then cells treated with 10,20,30,45,60,70  $\mu$ g/ml concentrations for 48 hours. The migration area was measured with ImageJ software.

## 2.7. Clonogenic Assay

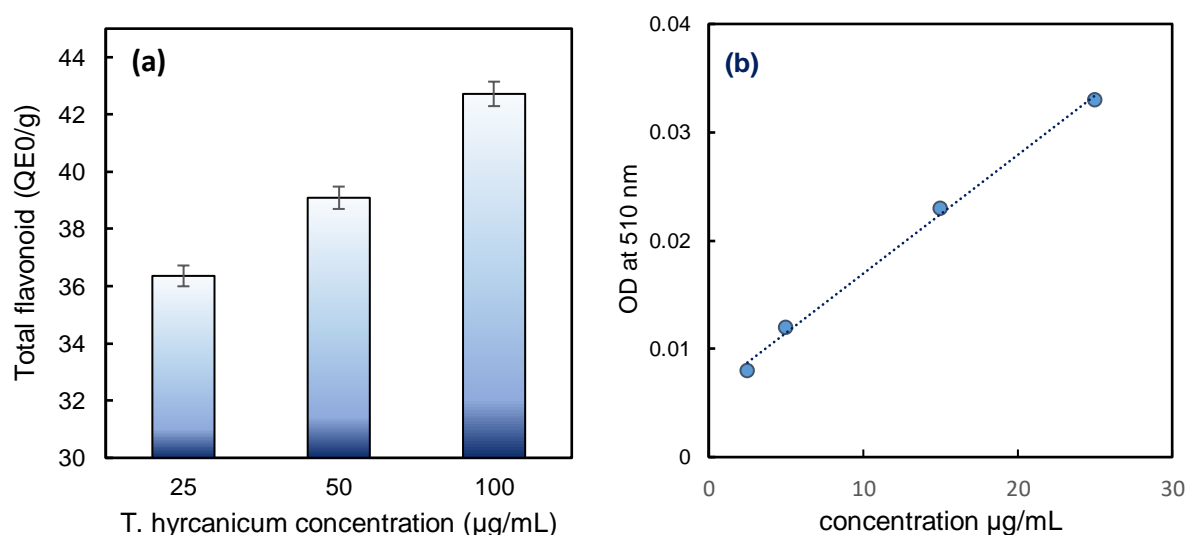
Cells were seeded in 24-well plates .Then, they treated by 2, 5, 10, 15, 25  $\mu$ g/ml, the treatment repeated every 48h, after 7days cells were stained. Fixator (methanol: acetic acid) was used for fixing the colonies. Then, fixator was removed and 200 $\mu$ l crystalviolet added to wells for 2h. The number of colonies were counted for each concentration.



### 3. Results and discussion

#### 3.1. Flavonoid content test results

Figure 2 demonstrates the amount of flavonoid for various concentrations of *Teucrium hyrcanicum* which was plotted based on Quercetine standard curve (Figure 2b). It was observed that the total flavonoid concentration increased from 30 to 43 QE0/g (up to 17%) when the *Teucrium hyrcanicum* increased from 25 to 100  $\mu\text{g}/\text{L}$  which indicated that *Teucrium hyrcanicum* has a great potential as herbal medicine.



**Figure 2:** Flavonoid content of the *Teucrium hyrcanicum* extraction with various concentration

#### 3.2. GS-MS analysis results

Table 1 illustrates the result of GC-MS analysis which was ordered based on the compounds amount. More than 40 compounds were recognized for the *Teucrium hyrcanicum*. The first 6 compounds include more than 70% of the plant extraction. According to the GC-MS results, *Teucrium hyrcanicum* contains high amount of sesquiterpens and mono or diterpens which proves the anti-cancer ability of the *Teucrium hyrcanicum*.



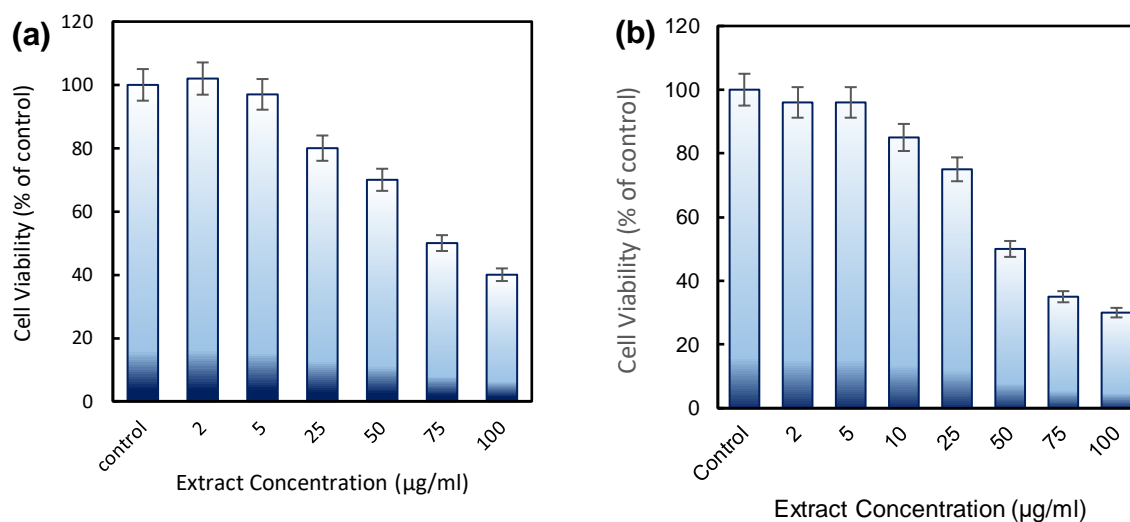
**Table1:** GC-MS analysis of *Teucrium hyrcanicum* extraction

| N. | Formulation                                                     | percentage | R.T*   | Compound                             |
|----|-----------------------------------------------------------------|------------|--------|--------------------------------------|
| 1  | C <sub>11</sub> H <sub>21</sub> ClO                             | 48.21      | 45.049 | Undecanoyl chloride                  |
| 2  | C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>                  | 20.36      | 45.801 | Di-n-octylphthalate                  |
| 3  | C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O                | 4.15       | 44.391 | L-Tryptophanol                       |
| 4  | C <sub>8</sub> H <sub>7</sub> N                                 | 3.39       | 44.8   | Indole                               |
| 5  | C <sub>9</sub> H <sub>9</sub> N                                 | 3.21       | 49.861 | Skatole                              |
| 6  | C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>                  | 3.06       | 33.551 | Tetradecanoic acid                   |
| 7  | C <sub>5</sub> H <sub>12</sub> O                                | 1.9        | 8.208  | Isoamylol                            |
| 8  | C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O                  | 1.6        | 13.307 | Picolinamide                         |
| 9  | C <sub>6</sub> H <sub>5</sub> NO                                | 1.3        | 13.04  | 2-Pyridinecarbox aldehyde            |
| 10 | C <sub>14</sub> H <sub>22</sub> O                               | 1.23       | 29.052 | 2,6-di-tert-butylphenol              |
| 11 | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>                  | 1.2        | 36.903 | Palmitic acid, Ethyl ester           |
| 12 | C <sub>5</sub> H <sub>11</sub> N                                | 0.9        | 38.684 | Azacyclohexane                       |
| 13 | C <sub>20</sub> H <sub>40</sub>                                 | 0.82       | 38.574 | Phytol                               |
| 14 | C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>                  | 0.57       | 39.182 | Octadecanoic acid                    |
| 15 | C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>                  | 0.5        | 39.562 | Ethyl octadecanoat                   |
| 16 | C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>                  | 0.5        | 36.253 | Dibutyl phthalate                    |
| 17 | C <sub>2</sub> H <sub>6</sub> O <sub>3</sub> S                  | 0.46       | 6.613  | Dimethyl sulfite                     |
| 18 | C <sub>16</sub> H <sub>26</sub> N <sub>4</sub> Si <sub>2</sub>  | 0.74       | 47.852 | Phenyl-2, 4-Pyrimidinediamineditrens |
| 19 | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>                  | 0.35       | 38.887 | Methyl 9,12-heptadecadienoate        |
| 20 | C <sub>15</sub> H <sub>22</sub> O <sub>2</sub>                  | 0.3        | 35.814 | Arteannuic acid                      |
| 21 | C <sub>3</sub> H <sub>7</sub> ClO <sub>2</sub>                  | 0.21       | 6.838  | Methoxyacetyl chloride               |
| 22 | C <sub>7</sub> H <sub>14</sub> O <sub>4</sub>                   | 0.2        | 9.281  | Diethoxymethyl acetate               |
| 23 | C <sub>5</sub> H <sub>13</sub> NO                               | 0.18       | 6.769  | Aminoethyl isopropyl                 |
| 24 | C <sub>32</sub> H <sub>67</sub> O <sub>4</sub> P                | 0.17       | 41.993 | Dihexadecyl hydrogen phosphate       |
| 25 | C <sub>14</sub> H <sub>28</sub> O                               | 0.15       | 35.501 | Tetradecanal                         |
| 26 | C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>                   | 0.15       | 37.789 | Tetrt-pentanoic acid                 |
| 27 | C <sub>37</sub> H <sub>76</sub> O                               | 0.15       | 37.989 | 1-Heptatriacotanol                   |
| 28 | C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>                   | 0.13       | 28.707 | Caproic acid                         |
| 29 | C <sub>12</sub> H <sub>25</sub> Cl                              | 0.12       | 28.858 | N-Dodecylchloride                    |
| 30 | C <sub>3</sub> H <sub>7</sub> BrO                               | 0.12       | 6.714  | 1-romo-2-propanol                    |
| 31 | C <sub>5</sub> H <sub>10</sub> O                                | 1          | 28.756 | Butane                               |
| 32 | C <sub>3</sub> H <sub>7</sub> ClO                               | 0.09       | 6.976  | Propene chlorohydrin                 |
| 33 | C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>                  | 0.09       | 35.942 | Palmitic acid, methyl ester          |
| 34 | C <sub>9</sub> H <sub>7</sub> NO <sub>2</sub>                   | 0.07       | 36.118 | 2-arboxyindole                       |
| 35 | C <sub>3</sub> H <sub>6</sub> O <sub>3</sub>                    | 0.06       | 8.352  | Methoxyacetic acid                   |
| 36 | C <sub>7</sub> H <sub>4</sub> FNS                               | 0.05       | 8.564  | Isothiocyanic acid                   |
| 37 | C <sub>12</sub> H <sub>22</sub> O                               | 0.05       | 34.809 | Cyclododecanone                      |
| 38 | C <sub>28</sub> H <sub>20</sub> S                               | 0.05       | 39.402 | Thiophene, tetraphenyl               |
| 39 | C <sub>5</sub> H <sub>5</sub> N                                 | 0.04       | 8.69   | Pyridine                             |
| 40 | C <sub>15</sub> H <sub>17</sub> N                               | 0.04       | 22.551 | Benzeneethanamine                    |
| 41 | C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>                  | 0.04       | 35.147 | 7-Methanoa zulene-8-methanol         |
| 42 | C <sub>27</sub> H <sub>48</sub> O                               | 0.04       | 39.098 | Cholestan-3-ol                       |
| 43 | C <sub>13</sub> H <sub>16</sub> Cl <sub>2</sub>                 | 0.04       | 39.452 | 1-3,3- Dichloro-1-propynyladamentane |
| 44 | C <sub>9</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> Si | 0.03       | 38.988 | 1-minopropylsilatrah                 |
| 45 | C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>                   | 0.03       | 40.111 | 4-Ethoxy-3-hydroxy benzaldehyde      |



### 3.3. MTT assay

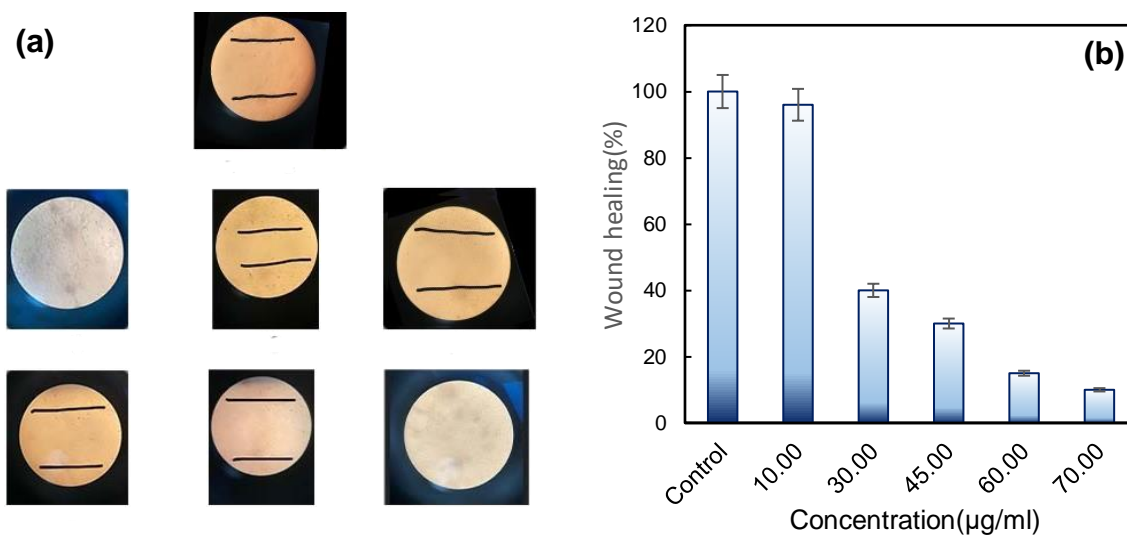
Figure 3 shows the results of MTT assay for 24 and 48 h. According to the results, the IC<sub>50</sub>s for 24h and 48h were measured as 65.48 and 43.87  $\mu\text{g/ml}$ , respectively. As can be seen from figure 3, in the concentrations of 75 and 100 $\mu\text{g/ml}$ , the cell viability were below to 40%. The results revealed that *Teucrium hyrcanicum* extraction could successfully remove prostate cancer cells at higher concentrations. Since, more effectiveness was observed during 48h, this IC<sub>50</sub> was used for the other tests.



**Figure 3:** MTT results of the *T. hyrcanicum* extraction with various concentrations at (a) 24 h and (b) 48 h.

### 3.4. Wound healing

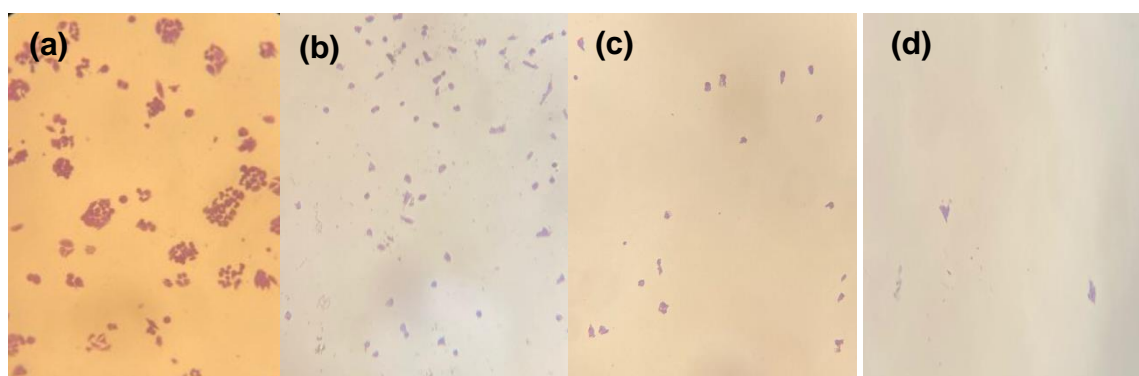
The pictures and the results of wound healing analyses are presented as Figure 4. Based on the pictures, for control (no treatment) and 10  $\mu\text{g/ml}$  concentration of *Teucrium hyrcanicum* no wounds were observed because the cancer cells were still have their migration ability. In the case of 30  $\mu\text{g/ml}$  concentration of *Teucrium hyrcanicum*, the surface of the wound decreased below 50 %. While, this number decreased to below 20 % when the concentration of *Teucrium hyrcanicum* was higher than 60  $\mu\text{g/L}$ . The wound healing results revealed that the *Teucrium hyrcanicum* plant extraction could efficiently affect the prostate cancer cells migration disability.



**Figure 3:** Wound healing (a) picture and (b) percentages of the *T. hyrcanicum* extraction with various concentrations.

### 3.5. Clonogenic assay

The ability of creating colonies by cancer cells was measured after 10, 25 and 45 (IC<sub>50</sub>) µg/ml. According to the clonogenic assay results, in the control sample there was too many colonies. However, as the concentration of *Teucrium hyrcanicum* increased, the number of colonies decreased too. For the concentration close to IC<sub>50</sub> and higher, no colonies were observed by the light microscopy which indicated the *Teucrium hyrcanicum* extraction had notable effect on the prostate cancer cells colonies.



**Figure 4.** Microscopic images of the colonies in (a) the control sample (without treatment) that include too many and different concentration of (b) 10, (c) 25 and (d) 45 µg/mL



## 4. Conclusion

Due to the negative side effects and environmental pollutions of the common anticancer drugs, focusing on herbal medicine proved to be better for both patient and environment. This study investigated *Teucrium hyrcanicum* as a novel herbal medicine for prostate cancer treatment which is an environmental friendly approach and more useful for people. The results of this work proved that *Teucrium hyrcanicum* extraction contains high amount of flavonoids (sub agent of phenol). The GC-MS results also shows high amount of sesquiterpenes and mono or di-terpenes that are anti-cancer property of *Teucrium hyrcanicum*. This novel species of teucrium like similar species including *teucrium polium*, *teucrium parviflorum*, *teucrium arduini* and *teucrium flavum* which indicates high anticancer potential. In addition, the results of colonogenic assay and wound healing assay confirmed the result of MTT assay.

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