Investigation of Cytotoxic Effects of *Inula Viscosa* Extract

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**Abstract.** *Inula viscosa* is a type of Asteraceae family, which widely used for medicinal plant to treatment of different diseases. *Inula viscosa* contains many biological compounds, such as monoterpenes, sesquiterpenes, diterpenes, flavonoids. Our study aimed to investigate the cytotoxic effects of *Inula viscosa extract* on MCF-7 (breast carcinoma), C6 (glioblastoma cancer), MG63 (bone osteosarcoma), cancer cells and L929 (mouse fibroblastoma) cell lines. Cytotoxic effects of the *Inula viscosa* extracts were performed by XTT assay. In this study, *Inula viscosa* extracts showed selective cytotoxic effect on MCF-7 compared than L929 cells. The *Inula viscosa* extracts appear to be a promising source of new anticancer agent. Further studies are needed to identify the cytotoxic activity mechanisms on these cancer cell lines.

**Keywords:** Cancer, Cytotoxic Effect, *Inula Viscosa*.


**Anahtar Kelimeler:** Kanser, Sitotoksik Etki, *Inula Viscosa*.

1. **INTRODUCTION**

Cancer is a pathological condition that occurs in a genetic and developmental process, resulting in loss of cells’ excessive proliferation and apoptosis functions. Some cancer types cause death and are one of the most searched health problems for treatment. Lung, stomach, colon, liver and breast cancers are reported to be the most common causes of death [1-4]. Despite the fact that billions of dollars are spent for cancer research every year, the question of how exactly the cancer has developed remains unanswered. Despite advances in methods such as surgery, chemotherapy,
radiation radiotherapy, hormone replacement for cancer treatment, the expected improvement in advanced disease is not at the desired level [5]. Chemotherapy is a frequently used method for cancer. It has disadvantages such as ineffectiveness; serious toxicity and multiple drug resistance reduce the percentage of success [4]. Therefore, new strategies are needed to defeat resistance against anticancer drugs [5].

*Inula viscosa* is type of Asteraceae family. *Inula helenium L.*, *Inula racemosa* Hook.f and *Inula britannica L.* species are used in medicine. *Inula viscosa* is a perennial herbaceous plant that profusely colonizes sub-nitrophile and sub-saline soils in abandoned and plowed fields in the Mediterranean region [6-7]. Inula species include many bioactive compounds, such as monoterpenoids, sesquiterpenoids, flavonoids, and glycosides [8]. It has many biological activities, such as antiinflammatory, anthelmintic, antipyretic, antiseptic, antiphlogistic, and antitumor [9-11]. It is widely used in traditional medicine for treatment of different diseases especially cancer treatment [12]. In this study, we aim that cytotoxic effects of *Inula viscosa* was determined on bone osteosarcoma (MG63), glioblastoma (C6), breast (MCF-7) cancer cells.

### 2. MATERIALS AND METHOD

#### 2.1. Materials

Dimethylsulfoxide (DMSO), ethanol purchased from Sigma (St. Louis, USA). Cell Proliferation Kit II (XTT), Dulbecco’s Modified Eagle’s Medium (high glucose) from Sigma (St. Louis, MO, USA). Penicillin, streptomycin and trypsin from Gibco (Paisley, England), Eagle's Minimum, fetal bovine serum (FBS) from Biochrom (Berlin, Germany), phosphate buffer saline (PBS) tablet from Medicago (Uppsala, Sweden). *Inula Visiosa* was collected from Akhisar district of Manisa province, Turkey (Table 1).

#### 2.2. Plant Extraction

##### 2.2.1. Infusion extract (Yield: 3.08 %)

*Inula viscosa* powder (50 g) was added to boiled water (1L) and stirred for 1h. The mixture was filtered through Whatman No.1 filter to remove insoluble particles (should have same text type and size of other parts of manuscript). The water extract was lyophilized (Cryodos 80, 75°C, 5 m³/h). Infusion extract is yield 3.08 % [13].

##### 2.2.2. Boiling extract (Yield: 9.1%)

*Inula viscosa* powder (50 g) and 1 liter of water boiled for 1h. The mixture was filtered through Whatman No.1 paper. The water extract was lyophilized (Cryodos 80, 75°C, 5 m³/h). Boiling extract is yield 9.1% [14].

#### 2.2.3. Cell Culture

All cell lines were purchased from (ATCC). The medium environment that we used in our study was DMEM, containing 10% fetal bovine serum (FBS), 1% L-glutamine, 100 IU/mL penicillin and 10 mg/mL streptomycin. Cell lines produced using DMEM would be reproduced at 37°C, in an oven with 95% humidity and 5% CO₂.

#### 2.2.4. Cytotoxic Effect

Cytotoxic effects of *Inula Viscosa* extract on MCF-7, C6, MG63, and L929 cells were determined by XTT (2, 3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenyl amino) carbonyl]-2H-tetrazolium hydroxide) assay for 24 h after treatment. XTT solution was prepared by mixing XTT agent (Labelling reagent)/ activation agent (electron coupling reagent) at 50/1 ratio. The intensity of the orange color resulting from formazan is proportional to the number of live cells. The cell viability was determined according to the intensity of the orange color observed, which was measured 475 nm [15]. All absorbance was compared to control samples (without any compound) which represented 100% viability. Cell viability was determined as in Eq1.

\[
\text{Cell viability} = \frac{(A_s-Ab)}{(Ac-Ab)} \times 100
\]

\[A_s: \text{Absorbans Sample}\]
\[A_b: \text{Absorbans Blank}\]
\[A_c: \text{Absorbans Viable cell (control)}\]

#### 2.2.5. Statistical Analysis

Data were expressed in the form of arithmetic mean ± standard deviation (x ± SD).

| Table 1. Collection information of the *Inula Visiosa* |
|---------------------------------|-------------|-------------|-----------------|----------------|
| **Plant** | **Collection site** | **Altitude** | **Collection period** | **Voucher number** |
| *Inula* | Turkey: Manisa-Akhisar district | 1100 m | 15.08.16 | H.B.Karayel (GOPU 7690) |
| *Viscosa* | | | | |
3. RESULTS AND DISCUSSION

*Inula viscosa* is widely used for treatment different diseases. When the literature is examined, *Inula viscosa* extracts showed potent antifungal, anti-inflammatory, and antioxidant activities (16). Haoui demonstrated that; 23 compounds of *Inula viscosa* essential oil were identified. GC-Ms analysis of several studies showed that *Inula viscosa* contains biological many compounds, including flavonoid, terpenoids, isocostic acid and tomentosin agents [17]. Many studies have highlighted the biological activity of tomentosin [6].

Recently, determine the cytotoxic and antiproliferative effects of plants have become the main strategy for new anticancer agent. Our study, we investigated different concentrations (100-1.56 μg mL⁻¹) of *Inula vacosa* extracts on MCF-7, C6, MG63, and L929 for 24 h.

IC₅₀ values are shown in Table 2. Cytotoxic effect of infusion extract is better than boiling extract. We have found a significant cytotoxic effect of *Inula viscosa* L. extracts on against MCF-7 cells. This activity was mainly attributed to the presence of tomentosin, a sesquiterpene lactone [18-19]. As a control group, L929 cells were used.

Table 2. IC₅₀ values of *Inula Viscosa*

<table>
<thead>
<tr>
<th></th>
<th>L929</th>
<th>MCF-7</th>
<th>MG63</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion</td>
<td>38.33±1.05</td>
<td>18.76±1.64</td>
<td>20.67±1.11</td>
<td>25.47±0.69</td>
</tr>
<tr>
<td>Boiling</td>
<td>57.33±0.95</td>
<td>25.76±1.83</td>
<td>28.98±0.62</td>
<td>35.76±1.31</td>
</tr>
<tr>
<td>Cis-platin</td>
<td>20.04±1.78</td>
<td>8.34±0.54</td>
<td>10.54±1.62</td>
<td>12.11±1.42</td>
</tr>
</tbody>
</table>

Figure 1. The cytotoxic effect after the treatment with the extract (50 μg mL⁻¹) for 24 h. Bars indicate mean ± standard deviation. All comparisons were made relative to untreated control cells.

Microscopic images of the MCF-7 cells are shown below with boiling and infusion extracts in Figure 2.

Figure 2. Cytotoxic effects a. without extract, b. infusion extract treatment, c. boiling extract treatment on MCF-7 cells.

Obtained results showed that boiling and infusion extracts are exhibited cytotoxic activities on MCF-7 for 24 h. Other studies have reported that plants of *Inula* exhibit in vitro cytotoxic effects on various cancer cells [20-21]. The *Inula helenium* extract showed a highly selective cytotoxicity effects different cancer cell lines (HT-29, MCF-7, Capan-2 and G1) [22]. Another study, Rozenblat et al. showed that tomentosin and inuviscolide inhibited the growth of three human melanoma cell lines [18]. Our cytotoxic effects of results were different from the previous reports. This situation may arise from harvested region, chemical composition of the plants, climate [6].

4. CONCLUSION

It was observed that *Inula viscosa* extract has high cytotoxic effects on MCF-7 cells. Thus, *Inula viscosa* might be evaluated candidates to develop natural derived therapeutics. Moreover, it may be also an alternative additive for foods. There is a need for further studies in this area.

REFERENCES


