Anticancer Activity of *Micromeria fruticosa* and *Teucrium polium* Growing in Lebanon

Mohammad Al-Hamwi¹, Maha Aboul-Ela¹, Abdalla El-Lakany¹, Salam Nasreddine²

**ABSTRACT**

The anticancer activities of two Lebanese plant extract *Micromeria fruticosa* (A) and *Teucrium polium* (B) and their fractions were tested against MCF7 and A549 cancer cell lines using MTT assay. Separation was carried out through column chromatography and TLC analysis. Results showed that both plants possess a promising and dose dependent inhibitory activity with IC50 of extract A 28.52 and 26.47 μg/ml on MCF7 and A549 cells respectively, and that of extract B was 41 and 279 μg/ml on MCF7 and A549 cell lines, respectively. More interestingly, combination treatment with either extracts A or B and cisplatin, significantly boosted the cytotoxic effect of cisplatin against the two cancer cell lines. Further studies are recommended to determine the active components in both plants responsible for these activities and explore their interactions at molecular level.

**INTRODUCTION**

Medicinal plants have been widely utilized for tumor and cancer prevention. Over 60% of today’s anticancer chemotherapeutic drugs originate from natural sources as plants. Family Lamiaceae (or Labiatae), is a flowering plant that has roughly 87 MG) and Teucrium species were reported to grow widely in the mountain region. However, none of these plants were evaluated for its anticancer or other biological effect. In our previous work, the volatile oil of *Micromeria fruticosa* was analyzed and evaluated for its antimicrobial activity. Accordingly, this study aimed to evaluate the in vitro anticancer activities of *M. fruticosa* and *T. polium*, grown in Lebanon, against human breast cancer cell lines (MCF7) and human lung carcinoma cell lines (A549). In addition, we also aimed to determine their beneficial use as adjuvant to anticancer drugs.

**MATERIALS AND METHODS**

**Plant materials**

The aerial parts of *Micromeria fruticosa* and *Teucrium polium* were collected from a rocky mountain in the Lebanese Bekaa valley (1300m above sea level) during their full flowering period on July 2017. The plants were authenticated by Dr. Ali Chakas (Botanist from the Lebanese University, Faculty of Science). A voucher specimen of each plant was deposited in the herbarium of the Faculty of Pharmacy at the Beirut Arab University.

**Preparation of plant extract**

The air-dried and ground aerial parts of *M. fruticosa* and *T. polium* were subjected to excessive alcoholic extraction at room temperature until exhaustion.
The residues were removed by filtration. *M. fruticosa* extract (A) and that of *T. polium* (B) were concentrated using a rotary evaporator under reduced pressure at 35−40°C and then lyophilized into powders.

**Chemicals**

All organic solvents for column chromatography (CC) and thin-layer chromatography (TLC) (petroleum ether, ethyl acetate, and methanol) were analytical grade and purchased from Sigma-Alrich™ (Germany). The alumina used was Neutral alumina 507 (for CC) and Silica-gel GF254 (for preparative TLC) that were purchased from Fluka® (Switzerland). TLC was performed on pre-coated silica gel 60 F254 purchased from ALUGRAM® SIL G (Germany). Methanol was used for recrystallization and LC-MS, while UV analysis was HPLC-grade purchased from ALUGRAM® SIL G (Germany).

**Separation of crude extract**

Crude extracts of A and B were separated using column chromatography (silica gel, 350 g, column diameter 3.5 cm). Elution was carried out with gradient mixtures of petroleum ether (PE) and ethyl acetate (eluents from 20% to 100% EtOAc), and then continued with EtOAc and methanol (from 5% to 60% methanol) to give 22 fractions (A1 - A22) for *Micromeria fruticosa* (extract A) and 43 fractions (B1 - B43) for *Teucrium polium* (extract B). Each fraction was analyzed using TLC analysis, detected by UV lamp, and sprayed with different spray reagents to be visualized and to suggest the chemical class. Based on TLC analysis (RF, shape, and spot color), similar fractions were combined together for further separation through CC to yield the sub fractions. The physical and chemical class of each fraction are shown in Table 1.

**Cell culture**

Human breast cancer cell lines (MCF7) and human lung carcinoma cell lines (A549) were purchased from the American Type Culture Collection (ATCC; Manassas, VA) and maintained in DMEM high glucose media (Sigma Chemical Company) supplemented with 0.1mg/ml streptomycin, 100 U/mL penicillin, and 10% fetal bovine serum (FBS). Cell lines were maintained at 37°C under an atmosphere containing 5% CO2.

**Table 1**: Physical and chemical properties of the separated fractions.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Elution solvent</th>
<th>TLC screening</th>
<th>Number of compounds</th>
<th>Expected chemical class</th>
</tr>
</thead>
<tbody>
<tr>
<td>A7</td>
<td>(PE/ EtOAc80:20)</td>
<td>Yellow with conc NH3</td>
<td>1 major 2 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>A9</td>
<td>(PE/ EtOAc70:30)</td>
<td>Yellow with conc NH3</td>
<td>1 major 1 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>A10</td>
<td>(PE/ EtOAc65:35)</td>
<td>Yellow with conc NH3</td>
<td>1 major 1 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>A11</td>
<td>(PE/ EtOAc70:30)</td>
<td>Yellow with conc NH3</td>
<td>1 major 1 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>A15</td>
<td>(PE/ EtOAc50:50)</td>
<td>Yellow with conc NH3</td>
<td>2 major 1 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>B2</td>
<td>PE/ EtAc70:30</td>
<td>Yellow with conc NH3</td>
<td>1 major 2 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>B5</td>
<td>PE/ EtAc60:40</td>
<td>Yellow with conc NH3</td>
<td>1 major 2 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>B10</td>
<td>PE/ EtAc30:70</td>
<td>Orange with Dragendorff’s</td>
<td>1 major</td>
<td>Alkaloid</td>
</tr>
<tr>
<td>B12</td>
<td>PE/ EtAc25:75</td>
<td>Yellow with conc NH3</td>
<td>2 major 3 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>B13</td>
<td>PE/ EtAc20:80</td>
<td>Yellow with conc NH3</td>
<td>1 major 2 minor</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>B14</td>
<td>(PE/ EtAc15:85)</td>
<td>Yellow with conc NH3</td>
<td>1 major 1 minor</td>
<td>Flavonoid</td>
</tr>
</tbody>
</table>

**Treatment of cells**

A 1mg/mL stock solution of lyophilized plant extracts in DMSO was prepared, while the stock solution of the chemotherapeutic drug Cisplatin (CDDP) was diluted in NaCl. Solutions were immediately sterilized by filtration through a sterile membrane filter with a porosity of 0.2 micron. Different concentrations were prepared by diluting the stock solution with DMSO. Cells were plated in 96-well micro titer plates, at a concentration of 105 cells/well, and incubated in a humidified environment of 37°C with a 5% CO2 incubator for cell adhesion. The anti proliferative activity was carried out by measuring the cell viability of the MCF7 and A549 cell lines 72 hours after the treatment with increasing concentrations of total extracts A and B (10, 20, 30, 40, 50, and 100 μg/ml), their separated fractions (100, 150, 200, and 250 μg/ml), and cisplatin (2, 4, 8, 10, 20, and 40 μg/ml). Similarly, MCF-7 and A549 cells were treated with combinations of total extracts A and B (20 μg/ml) and cisplatin (2 μg/ml). Untreated cells (the control) for plant extracts received DMSO, while cisplatin received NaCl.

**Cell viability assay**

Cells were plated in 96-well plates at a density of 10,000 cells/well and treated with tested compounds and total extracts at different concentrations for 72 hours. A 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) was added to evaluate mitochondrial viability 27. After 4hrs of incubation at 37°C, the supernatants were removed and 100 μl of isopropanol−HCl was added to each well to solubilize the formazan crystals. The optical density, determined at 595 nm, measured the signal and the background. The experiment was repeated three times independently, each time in triplicates. The percentage of viability for each sample was calculated using the obtained OD values within the following formula: Percentage of viability (%) = OD sample/OD control × 100. The cytotoxicity of all organic solvents for column chromatography (CC) and thin-layer chromatography (TLC) (petroleum ether, ethyl acetate, and methanol) were analytical grade and purchased from Sigma-Aldrich® (Switzerland). TLC was performed on pre-coated silica gel 60 F254 that were purchased from Fluka® (Germany). The alumina used was Neutral alumina 507 (for CC) and Silica-gel GF254 (for preparativeTLC) that were purchased from Fluka® (Germany). Methanol was used for recrystallization and LC-MS, while UV analysis was HPLC-grade purchased from ALUGRAM® SIL G (Germany).

**Statistical analysis**

All results were presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPadPrism 8 (GraphPad Software Inc., CA, USA). Two-way ANOVA was used to calculate sample probability values (p); p ≤ 0.05 was considered statistically significant. Groups that are significantly different from the control are indicated in the Figures as * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, and p ≤ 0.0001.

**RESULTS**

Both the total extracts of *Micromeria fruticosa* (A) and *Teucrium polium* (B) and their separated fractions were found to reduce cell survival of MCF7 and A549 cancer cells.

The MTT assay was used to determine the proliferation rate of cancerous cell lines (MCF7 and A549) treated with different concentrations of total plant extracts A and B and their fractions. Results revealed that the cytotoxic activity of the total extract of the two plants were higher than their separated fractions. IC50 of extract A was 28.52 ± 1.455 and 26.47 ± 1.423 μg/ml on MCF7 and A549 cell lines, respectively. While IC50 of extract B was 41.07 ± 1.614 and 27.97 ± 1.447 μg/ml on MCF7 and A549 cell lines, respectively (Table 2 and Figure 1). Most of the separated fractions from A and B showed dose-dependent inhibitory activity on the cancerous cell lines. The most effective fraction isolated from extract A was ‘A15’ (IC50=186.67 ± 2.271 on MCF7), and the most effective fraction isolated from extract B was ‘B’ (IC50=170.8 ± 2.232 and 141.9 ± 2.152 on MCF7 and A549 cells, respectively) (Table 2).
Al-Hamwi, et al.: Anticancer Activity of **Micromeria fruticosa** and **Teucrium polium** Growing in Lebanon

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MCF7 $IC_{50}$ ± SEM (µg/ml)</th>
<th>A549 $IC_{50}$ ± SEM (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total extract A</td>
<td>28.52 ± 1.455</td>
<td>26.47 ± 1.423</td>
</tr>
<tr>
<td>A7</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>A9</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>A10</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>A11</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>A15</td>
<td>186.7 ± 2.271</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Total extract B</td>
<td>41.07 ± 1.614</td>
<td>27.97 ± 1.447</td>
</tr>
<tr>
<td>B2</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>B5</td>
<td>170.8 ± 2.323</td>
<td>141.9 ± 2.152</td>
</tr>
<tr>
<td>B10</td>
<td>223.7 ± 2.350</td>
<td>208.7 ± 2.319</td>
</tr>
<tr>
<td>B12</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>B13</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>B14</td>
<td>&gt;250</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>14.74 ± 1.168</td>
<td>5.299 ± 0.7242</td>
</tr>
</tbody>
</table>

Table 2: IC50 values of total extracts A and B and their fractions (µg/ml).

**Figure 1:** Total extract A and B and their separated fractions enhance the inhibition of proliferation in MCF7 and A549 cancer cell lines. A, F. MCF7 and A549 cells were incubated for 72 hrs with different concentrations (0-250 µg/ml) of total extract A and B and fractions. Cell viability was estimated by MTT test. Percent of the viability is calculated through the formula: % viability = OD (optical density) of treated cells / OD of non-treated cells × 100. Each column represents different concentrations of extracts. (A) Represents the total extract A and B in MCF7; (B) represents the total extract A and B in A549; (C) represents the separated fractions of extract A in MCF7; (D) represents the separated fractions of extract B in MCF7; (E) represents the separated fractions of extract A in A549; (F) represents the separated fractions of extract B in A549. Experiments were conducted in triplicates and results represent the mean ± SEM (standard error of the mean) of n=3 independent experiments. The resultant P-value was expressed as * P <0.05; ** P < 0.01; P*** <0.001 was considered to be statistically highly significant and **** P < 0.0001 extremely significant (Two-way ANOVA). Ctr, control; Ext A, **Micromeria fruticosa**; Ext B, **Teucrium polium**.
low concentrations (20 μg/ml) of total plant extracts. The doses for treatment, cells were treated with a low dose of cisplatin 2 μg/ml and greater anticancer effect on the cell lines (A549 and MCF7) than single treatment. The results demonstrated that the combination treatment significantly enhanced the inhibitory effects on cell viability compared to total extract or cisplatin treatments alone in the MCF7 and A549 cell lines (Figure 3A and 3B). Taken together, these results showed that total extracts A and B boosted the cytotoxic effect of cisplatin against the two cancer cell lines.

**DISCUSSION**

Due to the damaging side effects of chemotherapy, alternative modalities to prevent and treat malignancies are highly demanded and desired. Screening of medicinal plants have been a promising approach to complement or possibly reduce the adverse side effects of chemotherapy. This study showed that both *Micromeria Fruticosa* and *Ticorium Polium* extract have dose-dependent inhibitory effect on the cancerous cell lines MCF7 and A549, with IC50 of 28.52 ± 1.455 and 26.47 ± 1.423μg/ml for extract A, 41.07 ± 1.614 and 27.97 ± 1.447μg/ml for extract B on MCF7 and A549 cell lines, respectively. In addition, both plant extracts A and B significantly potentiated the inhibitory effect of cisplatin on cell viability compared with total extract or cisplatin alone on MCF7 and A549 cell lines. According to American National Cancer Institute, an IC50 of 30 μg/ml or less has been identified as the criteria to determine significant cytotoxic activity of a plant extract. Interestingly, our results identified lower values with IC50 of 28.52 ± 1.455 and 26.47 ± 1.423μg/ml for extract A, 41.07 ± 1.614 and 27.97 ± 1.447μg/ml for extract B on MCF7 and A549 cell lines respectively. The cytotoxic activity of *T. polium* were reported against melanoma (IC50 value 91.2 μg/ml) and human breast adenocarcinoma MDA-MB-361 cells (IC50 value of 200 μg/ml) but no data were found against MCF7 or A549 cell lines. Other studies reported that extract of *M. fruticosa* exerted ant proliferative activity with IC50 of 175 and 200 μg/ml against U-87 MG (glioblastoma multiform) cell lines.

Accordingly, the inhibition effect of Lebanese plant species (*Micormeria* & *Ticorium*) were much potent than other plants like *Salvia* (IC50=57-76 μg/ml) against human melanoma cell lines (A375) and human foreskin fibroblast (HFF) cell lines. The differences in IC50 between current results and previously reported studies may be attributed to the methods of extraction, different ecological factors, and cell type specificity. The augmentation of anti-proliferative activity of Cisplatin when co-administered with Micromeria or Teurcium extract suggested the plant as a potential adjuvant remedy in cancer therapy. Further studies are needed to explore the bioactive components in both plants responsible for this cytotoxic activity and possible mechanism of interactions.

**CONCLUSION**

This is the first study to report the anticancer activity of *Micromeria fruticosa* and *Ticorium polium* plants in Lebanon. Both plants showed high inhibitory activity against MCF7 and A549 cancer cell lines with IC50 below 30μg/ml. More interestingly, both plants significantly boosted the activity of cisplatin at low doses, suggesting the plants as new potential remedies in the cancer therapy.

**REFERENCES**


GRAPHICAL ABSTRACT

ABOUT AUTHORS

Mohamad Al-Hamwi, is a graduate of the Faculty of Pharmacy, Beirut Arab University, Master degree in Pharmacognosy and Medicinal plants, pharmaceutical sciences department, faculty of Pharmacy, BAU. Ph.D. candidate in pharmaceutical sciences department faculty of Pharmacy, BAU.

Prof Maha Aboul Ela. is a graduate of the Faculty of Pharmacy, University of Alexandria, Egypt. She has done her Ph.D. at the Department of Pharmacognosy, Faculty of Pharmacy, the University of Alexandria in collaboration with the Institute for Organic Chemistry, Technical University of Berlin, Germany. She has more than fifty publications in national and international journals and is the head of the pharmaceutical sciences department faculty of Pharmacy, Beirut Arab University.

Prof Abdalla El-Lakany, is a graduate of the Faculty of Pharmacy, Alexandria University. Based on academic excellence, he was granted a Ph.D. scholarship from the Faculty of Pharmacy, Alexandria University. He has published several research articles in the medicinal plant domain. Currently, he is the dean of the Faculty of Pharmacy at Beirut Arab University.