Antioxidant, antimicrobial and anticarcinogenic activities of *Sambucus ebulus* L. flowers, fruits and leaves

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**ABSTRACT:** The aim of this study was to evaluate the antioxidant, antimicrobial and anticarcinogenic activities of methanol extract obtained from flowers, fruits and leaves of *Sambucus ebulus* L. Free radical scavenging activities and total phenolic contents of the species were assayed by DPPH method and Folin–Ciocalteu method, respectively. The antimicrobial activity of the extracts were tested by the micro-broth dilution method against seven microbial species. L929 (ATCC, CCL-1) mouse fibroblast cell line was used for the determination of in vitro citotoxicity activity and HeLa (ATCC, CCI-2) human cervix adenocarcinoma cell line was used for the determination of anticarcinogenic and growth inhibition activity. Leaves of *Sambucus ebulus* L. showed the strongest antioxidant activity and also leaves of plant has highest phenolic content, *Sambucus ebulus* L. extracts were found to have no significant activity against any strains of bacteria. Only *Sambucus ebulus* fruit extract has shown moderate effect against *Candida albicans*. In 10μg/mL concentration the highest anticarcinogenic activity were found in leaves extract, in addition that none of the extracts were not found cytotoxic.

**KEY WORDS:** *Sambucus ebulus*, antioxidant, antimicrobial, anticarcinogenic, cytotoxic

**INTRODUCTION**

Oxidation is a life-sustaining process for living organisms. It provides energy for the biological processes. In addition that oxygen-centered free radicals can lead to cell death and tissue damage. Free radicals are responsible for many diseases, such as cancer, diabetes, aging etc. Therefore, there is an increasing interest in natural antioxidants such as polyphenols which are found in wide range of plants.

Microbial drug resistance is a worldwide problem in medicine. Natural products provide unlimited opportunities for emerging and efficient additives and drug treatments because of their unmatched range of chemical diversity. For this reason there is a necessity to discover new compounds from plants which can be used in the treatment of resistant microbial strains(1,2).

Cancer is a deadly disease and it strikes more people each passing day. Therefore most researchers study on cancer treatments and recent studies concentrate on correlation of herbal medicine and cancer therapy. Plants are naturally rich in bioactive compounds and they can be a good source for anticancer drugs. MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay is a quantitative procedure measure cell proliferation, cell viability and cytotoxicity. This procedure is based on reducting of tetrazolium salt which catalysis by mitochondrial activity(3,4,5).

The genus *Sambucus* L. (Caprifoliaceae) is represented by 2 species *Sambucus nigra* L. and *Sambucus ebulus* L. in Turkey (6). Hippocrates, Theophrastus, Discorides and Galen regarded *Sambucus nigra* as one of nature’s greatest remedial plant
(7). Both Commission E and WHO monographs include Sambucus flos as diaphoretic and expectorant for treatment of common cold(8,9). S.nigra and S. ebulus are used for common cold, inflammation, rheumatism, burns and infectious wounds, in addition that both of them are used as diuretic, expectorant, diaphoretic and also in cancer treatment as traditionally in Turkey(10,11,12,13,14).

**MATERIALS AND METHODS**

**Plant material**

S. ebulus L was collected from Çatalca, İstanbul-Turkey in Autumn 2009, and identified by Dr. Gizem Bulut. A voucher specimen was deposited in the Herbarium of Faculty of Pharmacy, Marmara University (MARE: 11718).

**Plant extraction**

10 g of air dried leaves, fruits and flowers have been extracted by maceration with methanol until solvent found to be colorless at room temperature. All extracts were filtered, dried under vacuum and stored in refrigerator for further analysis.

**Determination of radical scavenging activity by DPPH method**

Antioxidant activity of Sambucus ebulus leaves, fruits and flowers were determined with DPPH method using BHT and Ascorbic acid as standards. Free radical scavenging capacity of extracts and standards were evaluated according to Ozsoy et al. (15).

The percent scavenging activity of extracts and standards against DPPH were calculated according to the following formula:

$$\text{Percent scavenging activity} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

where A0 was the absorbance of the control (containing all reagents except the test compounds), and A1 is the absorbance of the extract / standard.

**RESULTS AND DISCUSSION**

**Antimicrobial Activity**

The antimicrobial activity of the methanolic extracts were tested against six bacteria (Staphylococcus aureus ATCC 6538, Staphylococcus epidermidis ATCC12228, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 4352 Pseudomonas aeruginosa ATCC 27853, Proteus mirabilis ATCC 14153) and one yeast (Candida albicans ATCC 1023) by the microbroth dilutions technique strictly following the recommendation of National Committee for Clinical Laboratory Standards (NCCLS). Ciprofloxacin and Fluconazole were used as the reference compounds for bacteria and fungi (17,18).

**Anticarcinogenic and growth inhibitor activities**

were done by ISO-10993-5 protocol) and HeLa (ATCC, CCI-2) human cervix adenocarcinoma cell line were used for the determination of anticarcinogenic and growth inhibition activities. Anticarcinogenic and growth inhibitor activities were done by the modified method of Beekman et al. (5).

The MTT metabolic assay was carried out in 96-well tissue culture plate seeded 5x10^3 cells/well. 100mM containing L-Glutamine without antibiotics Eagle’s MEM (Minimum Essential Medium) and RPMI 1640 medium with 10% FBS (Fetal Bovine Serum) was used for producing cells and during the procedure of the assay(3,4,5).

**Statistical analysis**

The data were reported as means±standard deviations and analysed by one-way analysis of variance (ANOVA) followed by the Tukey’s multiple comparison tests using GraphPad Prism 5. Differences between means at p<0.05 level were considered significant.

**Total Phenolic Contents (TPC)**

The total phenolic contents of extracts were calculated using the equation obtained from the standard curve of gallic acid graph (y= 2.8541x +0.04147 ve R^2=0.9874). Leaves of plant have the highest total phenolic contents. There is a correlation between antioxidant activity and total phenolic content of plant showed very strong antioxidant activity, also leaves showed stronger antioxidant activity than BHT.

**TABLE 2. Total Phenolic Contents (mg/mL)**

<table>
<thead>
<tr>
<th></th>
<th>Flowers</th>
<th>Fruits</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Phenolic Contents (mg/mL)</td>
<td>49.72±4.28</td>
<td>24.28±3.18</td>
<td>57.66±4.96</td>
</tr>
</tbody>
</table>

- Each value in the table is represented as mean ± SD (n = 3).
- Different letter superscripts in the same row or column indicate significant differences (P <0.05).
**TABLE 3. Antimicrobial activity results of extracts**

<table>
<thead>
<tr>
<th>Extracts and standards</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sa</td>
</tr>
<tr>
<td>S. ebulus flowers</td>
<td>-</td>
</tr>
<tr>
<td>S. ebulus fruits</td>
<td>-</td>
</tr>
<tr>
<td>S. ebulus leaves</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25µg/ml</td>
</tr>
<tr>
<td>Fluconazol</td>
<td>-</td>
</tr>
</tbody>
</table>

*S.a: S. aureus; S.e: S. epidermidis; E.c: E. coli; K.p: K. pneumoniae; P.a: P. aeruginosa; P.m: P. mirabilis; C.a: C. albicans*

**FIGURE 1.** Cell viability and growth inhibition

**MTT Assay**

**Evaluation of Cytotoxicity:** All extracts were determined in 10µg/mL concentration. In those concentration, maximum growth inhibition (29.19%) was found in flower extract. Although flower extract has shown the highest cytotoxic activity, all results were under the value of 50%, so none of the extracts in 10µg/mL concentration were found cytotoxic.

**Evaluation of Anticarcinogenic Activity:** All extracts were determined in 10µg/mL concentration. In those concentration minimum cell viability (66.19%) and maximum growth inhibition 33.81% were found in leaves extract and this value of 33.81% is a significant value for anticarcinogenic assays. The statistical evaluation of the obtained data is significant (p<0.05).

**CONCLUSION**

This study clearly indicate that *S. ebulus* fruits, flowers and leaves extracts can be used as a source of antioxidant. Especially leaf extract has very strong antioxidant activity, stronger than BHT which has used as a standart. In addition that none of the tested ex-tracts were found cytotoxic in 10 µg/ml concentration. The strongest anticarcinogenic activity was found in leaf extract. The value of 33.81% is a significant value for anticarcinogenic assays. In addition, assays can be tested in increasing concentration. There was not any antimicrobial activity of *Sambucus ebulus* extracts. Only *Sambucus ebulus* fruit extract has shown moderate effect against *Candida albicans*.

**ACKNOWLEDGEMENTS**

The authors are grateful to Dr. Gizem Bulut from the Faculty of Pharmacy of Marmara University for the identification of the plant. This research was financially supported by the Marmara University Scientific Research Committee (Project No: SAG-C-YLP-0160510-0120)

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**Sambucus ebulus L. bitkinisinin çiçek, meyve ve yapraklarının antioksidan, antimikrobiyal ve antikarsinojenik aktiviteleri**

ÖZET: Bu çalışmanın amacı Türkiye’de yetiştirilen *Sambucus ebulus L.* bitkinisinin çiçek, meyve ve yapraklarından hazırlanan metanol ekstrerlerinin antioksidan, antimikrobiyal ve antikarsinojenik aktivitelerini değerlendirilmektir. Ekstrelerin serbest radikal süpürücü aktivite tayinleri DPPH metodu ile, toplam fenolik madde miktar tayini ise Folin–Ciocalteu metoduyla, antimikrobiyal aktivitesi 7 mikroorganizma türune karşı mikro dilüsyon yöntemyle, antikarsinojenik aktivite tayini ise L929 (ATCC, CCL-1) fare fibroblast hücre hattı ve HeLa (ATCC, CCLI-2) kanser hücre hattı kullanılarak MTT yöntemiyle sitotoksik etkinlikleri araştırılmış üzere test edildi. DPPH radikal süpürücü aktivite tayininde en yüksek aktiviteyi yaprak ekstresi gösterdi ayrıca toplam fenolik madde miktarı da en yüksek oranda bu ekstrede bulundu. Antimikrobiyal aktivite tayininde sadece meyve ekstresi *Candida albicans’a* karşı orta derecede etki göstermiş. Antikarsinojenik aktivite test sonuçlarına göre ise yaprak ekstresi en yüksek değeri göstermiş, hiçbir ekstre sitotoksik bulunmamıştır.

ANAHTAR KELİMELER: *Sambucus ebulus*, antioksidan, antimikrobiyal, antikarsinojenik, sitotoksik
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