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ORIGINAL RESEARCH

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 anticarsinogenic 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) mouce fibroblast cell line was used for the determiation of in vitro citotoxicity activity and HeLa (ATCC, CCl-2) human cervics 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.* exracts 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, anticarsinogenic, cytotoxic

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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 Sambuci 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 standarts were evaluated according to Ozsoy et al. (15).

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

DPPH radical-scavenging activity (%) = [(A0–A1)/A0]×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.

Determination of Total Phenolic Contents (TPC)

Total phenolic contents of the MeOH extracts were measured using Folin–Ciocalteau reagent according to Gao et al.(16). Gallic acid was used as a standard and the total phenolics were expressed as mg GAE / g plant extract.

Determination of 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 Comittee for Clinical Laboratory Standarts (NCCLS). Ciprofloxacin and Fluconazole were used as the reference compounds for bacteria and fungi (17,18).

Determination of MTT assay

S. ebulus extracts were tested for their cytotoxic, anticarcinogenic and growth inhibition activities. L929 (ATCC, CCL-1) mouce fibroblast cell line was used for the determination of *in vitro* cytotoxicity activity (*In vitro* cytotoxic activity test was done by ISO-10993-5 protocol) and HeLa (ATCC, CCl-2) human cervics 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.

RESULTS AND DISCUSSION

Radical scavenging activity by DPPH method

IC_{50} value represents concentration of extract which is required to scavenge 50% of DPPH free radical, therefore low IC_{50} value indicate strong antioxidant activity. Flowers and fruits of plant showed weak antioxidant activity, but leaves of plant showed very strong antioxidant activity, also leaves showed stronger antioxidant activity than BHT.

TABLE 1. IC_{50} values (mg/mL) of extracts

	Flowers	Fruits	Leaves	BHT	AA
IC_{50} mg/ml	$6,614^{a}\pm0,674$	$8,895^{b}\pm1,391$	$1,655^{c,d}\pm0,437$	$2,263^{c}\pm0,123$	$0,024^{d}\pm0,003$

BHT: Butil Hidroxy Toluen, **AA:** Ascorbic Acid
- 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)

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 R2=0,9874). Leaves of plant have the highest total phenolic contents. There is a correlation between antioxidant activity and total phenolic content of leaves extract.

TABLE 2. Total Phenolic Contents (mg/mL)

	Flowers	Fruits	Leaves
Total Phenolic Contents (mg/g)	$49,72^{a}$ + 5,281	$24,28^{b}$ ± 1385	$57,66^{c}$± 4,966

- 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).

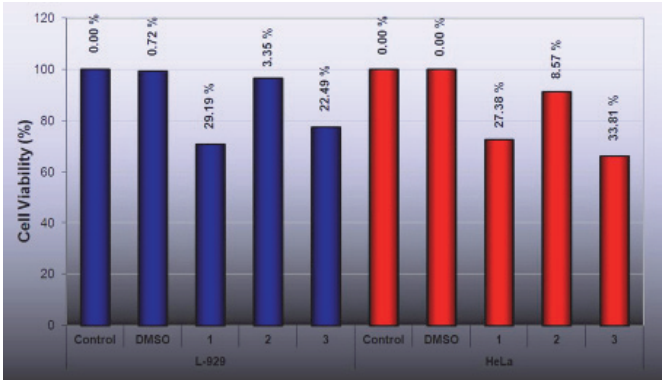
Antimicrobial Activity

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*.

TABLE 3. Antimicrobial activity results of extracts

Extracts and standards	Microorganisms						
	Sa	Se	Ec	Kp	Pa	Pm	Ca
S.ebulus flowers	-	1250 µg/ml	-	-	-	-	-
S.ebulus fruits	-	-	-	-	-	-	312 µg/ml
S.ebulus leaves	-	1250 µg/ml	-	-	-	-	-
Ciprofloksasin	0.25 µg/ml	-	-	0.625 µg/ml	1 µg/ml	-	-
Flukonazol	-	-	-	-	-	-	1 µg/ml

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



1: Flowers, 2: Fruits, 3: Leaves

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 not 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*.

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

ÖZET: Bu çalışmanın amacı Türkiye'de yetişen *Sambucus ebulus L.* bitkisinin çiçek, meyve ve yapraklarından hazırlanan metanol ekstrelerinin antioksidan, antimikrobiyal ve antikarsinojenik aktivitelerini değerlendirmektir. Ekstrelerin serbest radikal süpürücü aktivite tayinleri DPPH metodu ile, toplam fenolik madde miktar tayini ise Folin–Ciocalteu metoduyla, antimikrobiyal aktivitesi 7 mikroorganizma türüne karşı mikro dilüsyon yöntemiyle, antikarsinojenik aktivite tayini ise L929 (ATCC, CCL-1) fare fibroblast hücre hattı ve HeLa (ATCC, CCl-2) kanser hücre hattı kullanılarak MTT yöntemiyle sitotoksik etkinlikleri araştırmak 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 göstermiştir. 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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