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RESEARCH PAPER

Biological activities of aerial parts extracts of endemic *Tanacetum argenteum* subsp. *argenteum*

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ABSTRACT

The objective of the present study was to investigate the antimicrobial and anti-proliferative activities of *n*-hexane, chloroform, methanol and aqueous methanol extracts obtained from *Tanacetum argenteum* subsp. *argenteum* aerial parts. Anti-proliferative activity was tested *in vitro* against four human cancer cell lines (A549: lung adenocarcinoma, Hela; cervix adenocarcinoma, HT-29: colon adenocarcinoma, MCF-7; breast adenocarcinoma) using MTT assay. Antimicrobial activity was assessed by micro-broth dilution technique against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, and *Candida albicans* ATCC 10231. Hexane extract inhibited proliferation of HT-29 and MCF-7 by 75 and 74% while chloroform extract against the same cancer cell lines displayed inhibition of 89 and 73% at the concentration of 30 µg/mL, respectively. Also, chloroform extract at the same concentration showed significant anti-proliferative activity against A-549 and HeLa with inhibition values of 75% and 62%. Chloroform extract exhibited moderate antibacterial activity against *Staphylococcus aureus* and *S. epidermidis* with the MIC values of 625 µg/mL. Methanol and aqueous methanol extracts showed weak antimicrobial activity against *Staphylococcus epidermidis* and *Candida albicans* with MIC values of 1250 µg/mL. The results showed that *n*-hexane and chloroform extracts have significant anticancer activity against cancer cell lines used in this study.

Key Words: Asteraceae, antimicrobial activity, anti-proliferative activity, *n*-hexane extract, chloroform extract

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Introduction

Tanacetum L. is an important genus of Asteraceae family which is represented by about 160 species worldwide. The genus is also represented with forty six species in Turkey (1). *Tanacetum* species are used in the treatment of arthritis, fever, migraine, menstrual disorders, stomach-ache, toothache, and insect bites in the folk medicine (2). Also, it is reported that different *Tanacetum* species have anticancer, antimicrobial, anti-inflammatory and antioxidant activity (3, 4). This species contain essential oils, flavonoids and sesquiterpene lactones as secondary metabolites (2, 5, 6).

A literature survey revealed several reports on chemical composition, antimicrobial, and anticancer activities of essential oil and various extracts of *Tanacetum argenteum*. One of those studies reports anticancer activity of methanol extract from *Tanacetum argenteum* subsp. *argenteum* leaves

against MCF-7 (7), whereas the other explains antibacterial, anticandidal and antioxidant effects of *Tanacetum argenteum* subsp. *flabellifolium* aqueous methanol extract (1). Also, terpenic compounds were isolated from *Tanacetum argenteum* subsp. *flabellifolium* in a previous study (8). Furthermore, Tabanca *et al*. (2007) and Polatoğlu *et al.* (2010) reported chemical composition and antimicrobial activities of essential oils from *Tanacetum argenteum* subsp. *argenteum* and *Tanacetum argenteum* subsp. *flabellifolium* (2, 6).

However, based on our knowledge, the current study is the first to investigate the antimicrobial and anti-proliferative activities of various extracts from *Tanacetum argenteum* subsp. *argenteum* aerial parts against different microbial strains and cancer cell lines.

Material and methods

Plant material

Plant samples were collected in the flowering periods from the Doğanşehir district of Malatya province of Turkey in 2012 and were identified by Dr.Şükran Kültür, a botanist of the Faculty of Pharmacy, University of Istanbul. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE No: 98969).

Extraction

Dried and powdered aerial parts of *T.argenteum* subsp. *argenteum* (10 g) were extracted with 3×100 mL MeOH, using an ultrasonic bath. After filtration and evaporation, the residue (TAM) was dissolved in 50 mL 50 % aqueous methanol, and subjected to solvent-solvent partition between *n*-hexane (3×50 mL) and chloroform (3×50 mL). The n-hexane, chloroform and aqueous methanol extracts of *T.argenteum* subsp. *argenteum* obtained by this method were coded as TAH, TAC and TAAM, respectively. All extracts were stored under refrigeration for further analysis.

Antimicrobial activity

Antimicrobial activity against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153 and *Candida albicans* ATCC 10231 were determined by the microbroth dilutions technique using the Clinical Laboratory Standards Institute (CLSI) recommendations (9, 10). Mueller-Hinton broth for bacteria, RPMI-1640 medium buffered to pH 7.0 with MOPS for yeast strain were used as the test medium. Serial two-fold dilutions ranging from 2500 µg/mL to 2.4 µg/mL were prepared in medium. The inoculum were prepared using a 16-18 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent to a 0.5 McFarland standard, diluted in broth media to give a final concentration of $5x10^5$ cfu/mL for bacteria and $0.5x10^3$ to $2.5x10^3$ cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporations. The trays containing Mueller-Hinton broth were incubated at 35°C for 18-20 h and the trays containing RPMI-1640 medium were incubated 35°C for 46-50 h. The minimum inhibitory concentrations (MIC) were defined as the lowest concentration of compound giving complete inhibition of visible growth. Levofloxacin and fluconazol were used as reference antibimicrobials for bacteria and yeast, respectively. Also as control, antimicrobial effects of the dimetil sulfoxid was investigated against test microorganisms. According to values of the controls, the results were evaluated.

Cytotoxic activity

The extracts were tested for anti-cancerogenic activities. Cell viability and anti-cancerogenic activity, and growth inhibition profile of the extracts were analyzed using the ISO-10993-5 *in vitro* cytotoxicity test-MTT assay (11).

Results

The percentage yields of TAM, TAH, TAC, and TAAM extracts obtained from *T.argenteum* subsp. *argenteum* aerial parts were found to be 23.1, 1.74, 2.59, and 14.74%.

All extracts, except for TAAM, at the concentration of 100 µg/mL inhibited growth of four cancer cell lines in range of 63 to 93% in compared to a control. TAH (MCF-7: 74%; HT-29: 75%) and TAC (A-549: 75%; HeLa: 62%; HT-29: 89%; MCF-7: 73%) extracts at the concentration of 30 µg/mL showed a strong anti-proliferative activity against tested cancer cell lines (Figure 1).

TAC presented moderate activity against *Staphylococcus aureus* (MIC: 625 µg/mL) and *Staphylococcus epidermidis* (MIC: 625 µg/mL) while TAM and TAAM showed weak antimicrobial activity against *Staphylococcus epidermidis* and *Candida albicans* with MIC values of 1250 µg/mL (Table 1).

Table 1. The MIC values (**µg**/mL) of different ectracts of *T.argenteum* subsp. *argenteum* against various microorganisms.

Extracts / Standards	MIC values (µg/mL)							
	S.a**	S.e	E.c	K.p	P.a	P.m	E.f	C.a
TAH***	-*	-	-	-	-	-	-	-
TAM	-	1250	-	-	-	-	-	1250
TAC	625	625	-	-	-	-	-	-
TAAM	-	1250	-	-	-	-	-	1250
Levofloxacin	0.25							
Fluconazole								1

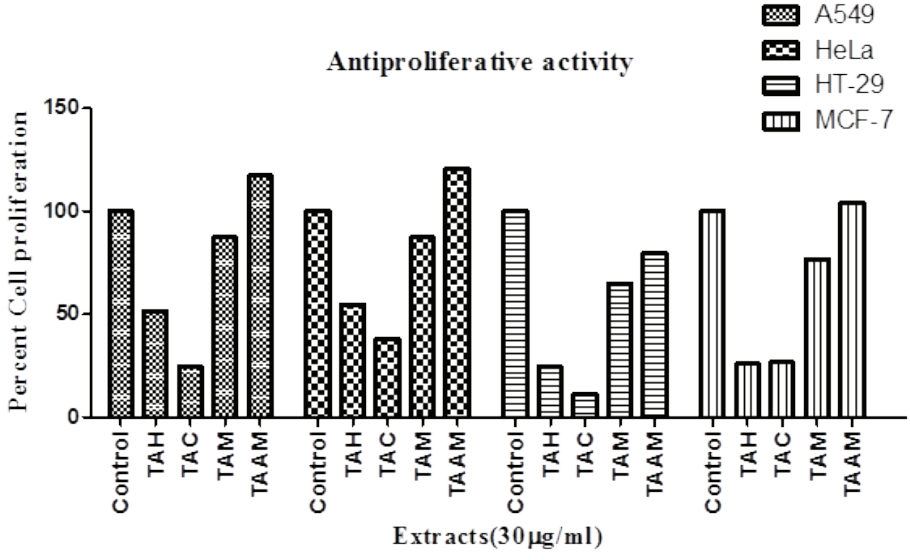
* not active (>1250 **µg**/mL)
** S.a: *Staphylococcus aureus* ATCC 29213, S.e: *Staphylococcus epidermidis* ATCC 12228, E.c: *Escherichia coli* ATCC 25922, E.f: *Enterococcus faecalis* ATCC 29212, K.p: *Klebsiella pneumoniae* ATCC 4352, P.a: *Pseudomonas aeruginosa* ATCC 27853, P.m: *Proteus mirabilis* ATCC 14153, C.a: *Candida albicans* ATCC 10231.
*** Hexane, chloroform, methanol and aqueous methanol extracts of the aerial parts of *T.argenteum* subsp. *argenteum* was coded as TAH, TAC, TAM and TAAM, respectively.

Discussion

The anti-proliferative and antimicrobial activity of different extracts of *T. argenteum* subsp. *argenteum* were evaluated on four cancer cell lines and eight microorganisms, respectively.

Both extracts, TAH and TAC extracts, inhibited growth of HT-29 and MCF-7 cancer cell lines by more than 50% at the concentration of 30 µg/mL. In addition, TAC at this concentration inhibited more than 50% proliferation of A-549 and HeLa cancer cell lines. The criteria of cytotoxicity activity for the crude extracts, as established by the American National Cancer Institute (NCI) is an IC_{50}<30 µg/mL in the preliminary assay (12). Based on this information, it could be asserted that TAH and TAC extracts have strong activity against cancer cell lines in current study. Yumrutas *et al.* (2015) reported that the methanol extract from *T. argenteum subsp. argenteum* leaves inhibited growth of MCF-7 cancer cell line by 47.42% at the concentration of 100 µg/mL. This value was lower than the value in our present study (64.76%) (7). This difference could be explained based on the fact that different extraction method was used. Several studies were recorded in the literature on anticancer activity of different *Tanacetum* species. In one of those studies, it was shown that *Tanacetum parthenium* ethanol extract has mild cytotoxic activity against HeLa cells with IC_{50} value of 153.71 µg/mL (13). In an another study, Karimian *et al.*

Figure 1: Anti-proliferative activity of *T.argenteum* subsp. *argenteum* extracts at the concentration of 30 µg/mL



* Hexane, chloroform, methanol and aqueous methanol extracts of the aerial parts of *T.argenteum* subsp. *argenteum* was coded as TAH, TAC, TAM and TAAM, respectively.

(2015) reported that *T. polycephalum* leaves hexane extract had selective cytotoxic effects against different cancer cell lines and 8β-hydroxyl-4β,15- dihydrozaluzanin C, a tricyclic sesquiterpene lactone with cytotoxic effect, had been isolated from *T. polycephalum* leaves hexane extract using a bioassay-guided approach (14). In particular, sesquiterpene lactones and flavonoids are known to be potential anticancer agents (15-17). Therefore, they could be responsible for cytotoxic effects of hexane and chloroform extracts from *T. argenteum* subsp. *argenteum*.

The antimicrobial activity of plant extracts is considered to be significant (MIC< 100 µg/mL), moderate (100<MIC≤ 625 µg/mL) or weak (MIC> 625 µg/mL) as described by Kuete (2010) (18). The current study showed that TAC had moderate activity against *Staphylococcus* strains. This finding is similar to study conducted by Unal *et al.* (2008) who showed that *T. aucheranum* aerial parts chloroform extract have antibacterial activity against *S. aureus*. However, it has been found that *T. aucheranum* aerial parts chloroform extract (125 µg/mL) had higher MIC value than T. *argenteum* subsp. *argenteum* aerial parts chloroform extract, reported in the present study, against *S. aureus* (19). Also, it is known that some flavonoids and sesquiterpene lactones have antimicrobial activity (20, 21). Thus, the antimicrobial activity of chloroform extract of the *T. argenteum* subsp. *argenteum* could be due to the presence of various secondary metabolites as flavonoids and sesquiterpene lactones in chloroform extract.

The results showed that TAH and TAC were good candidates for further activity-guided fractionation in the search for new active antitumor compounds.

Endemik *Tanacetum argenteum* subsp. *argenteum* bitkisinin toprak üstü kısımlarından elde edilen ekstrelerin biyolojik aktiviteleri

ÖZ

Bu çalışmanın amacı, *Tanacetum argenteum* subsp. *argenteum* bitkisinin toprak üstü kısımlarından elde edilen *n*-hekzan, kloroform, metanol ve sulu metanol ekstrelerinin antimikrobiyal ve antiproliferatif aktivitelerini araştırmaktır. Ekstrelerin antiproliferatif aktivitesi dört farklı insan kanser hücre hatlarına (A549: Akciğer adenokarsinoma, Hela; Serviks adenokarsinoma, HT-29: Kolon adenokarsinoma, MCF-7; Meme adenokarsinoma) karşı MTT yöntemiyle test edilmiştir. Ekstrelerin antimikrobiyal aktivitesi ise *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, ve *Candida albicans* ATCC 10231 suşlarına karşı mikro-broth dilüsyon yöntemiyle araştırılmıştır. Kloroform ekstresi 30 µg/mL konsantrasyonda HT-29 and MCF-7 hücre hatlarının gelişimini sırasıyla % 89 ve % 73 oranında durdururken, *n*-hekzan ekstresi ise aynı konsantrasyonda bu hücre hatlarının gelişimini sırasıyla % 75 ve % 74 oranında inhibe etmiştir. Aynı zamanda kloroform ekstresi A-549 and HeLa hücre hatlarının gelişimi sırasıyla % 75 ve % 62 oranında durdurarak önemli bir antiproliferatif aktivite göstermiştir. Kloroform ekstresi, *Staphylococcus aureus* and *S. epidermidis*'e karşı 625 µg/mL'lik *MİK* değerleriyle orta derecede antibakteriyel aktivite göstermiştir. Metanol ve sulu methanol ekstreleri ise 1250 µg/mL'lik *MİK* değerleriyle *Staphylococcus epidermidis* ve *Candida albicans* suşlarına karşı zayıf antimikrobiyal aktivite göstermiştir. Bu sonuçlar, *n*-hekzan ve kloroform ekstresinin bu çalışmada kullanılan insan kanser hücre dizilerine karşı önemli bir antikanser aktiviteye sahip olduklarını gösterdi.

Anahtar Kelimeler: Asteraceae, antimikrobiyal aktivite, antiproliferatif aktivite, *n*-hekzan ekstresi, kloroform ekstresi

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