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Antimicrobial activity against periodontopathogenic bacteria, antioxidant and cytotoxic effects of various extracts from endemic *Thermopsis turcica*

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PEER REVIEW

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Comments

This is a well-organized study in which the authors investigated the antimicrobial activity of water, ethanol, methanol, *n*-hexan and EtAc extracts of *T. turcica* against *A. actinomycetemcomitans* and *P. gingivalis* as oral microaerophilic and anaerobic bacterial strains and evaluated the antioxidant potential and cytotoxic effects against human cancer (DU145, PC-3, K-562, HL60) and HGF cells. It has very good scope to further continue this work with more advanced tools to prove such plant potentials.

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ABSTRACT

Objective: To investigate the *in vitro* antimicrobial potential of *Thermopsis turcica* Kit Tan, Vural & Küçüködük against periodontopathogenic bacteria, its antioxidant activity and cytotoxic effect on various cancer cell lines.

Methods: *In vitro* antimicrobial activities of ethanol, methanol, ethyl acetate (EtAc), *n*-hexane and water extracts of *Thermopsis turcica* herb against periodontopathogenic bacteria, *Aggregatibacter actinomycetemcomitans* ATCC 29523 and *Porphyromonas gingivalis* ATCC 33277 were tested by agar well diffusion, minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Antioxidant properties of the extracts were evaluated by 1,1-diphenyl-2-picryl-hydrazyl radical scavenging activity and β-carotene bleaching methods. Amounts of phenolic contents of the extracts were also analysed by using the Folin-Ciocalteu reagent. Additionally, cytotoxic activity of the extracts on androgen-insensitive prostate cancer, androgen-sensitive prostate cancer, chronic myelogenous leukemia and acute promyelocytic leukemia human cancer cell lines were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Human gingival fibroblast cells were used as a control.

Results: Our data showed that EtAc extract had the highest antimicrobial effect on *Aggregatibacter actinomycetemcomitans* (MIC: 1.562 mg/mL, MBC: 3.124 mg/mL) and *Porphyromonas gingivalis* (MIC: 0.781 mg/mL, MBC: 1.562 mg/mL). In antioxidant assays, EtAc extract exhibited also the highest radical scavenging activity [IC_{50}=(30.0±0.3) μg/mL] and the highest inhibition [(74.35±0.30)%] against lineloic acide oxidation. The amount of phenolic content of it was also the highest [(162.5±1.2) μg/mg gallic acid]. In cytotoxic assay, only ethanol [IC_{50}=(80.00±1.21) μg/mL] and EtAc extract [IC_{50}=(70.0±0.9) μg/mL] were toxic on acute promyelocytic leukemia cells at 20-100 μg/mL (P<0.05). However, no toxic effect was observed on human gingival fibroblast cells.

Conclusions: According to our findings, owing to its antioxidant and cytotoxic potential, EtAc extract might include anticancer agents for acute promyelocytic leukemia.

KEYWORDS

Thermopsis turcica, Antimicrobial activity, Periodontopathogenic bacteria, Antioxidant effect, Phenolic content, Cytotoxic effect, Human gingival fibroblast, Acute promyelocytic leukemia

1. Introduction

Turkey has significant diversity of plant and rich flora. Its floral diversity is resulted from locating in intersection of three phytogeographic region (Mediterranean, Irano-Turanian and Euro-Siberian), being a bridge between

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flora of Southern Europe and Southwest Asia, being an origin and diversification center of numerous plant genus and possessing high endemism rate of plant species, probably concerning about ecological and phytogeographic diversity[1]. Medicinal plants play an important role in amelioration of some diseseaes such as infectious disease in Turkey[2,3]. Their parts used as a drog (flower, leave, seed, root, bark, *ect.*) ameliorate disease due to their effective compounds[4,5]. Their usage has increased on account of their low adverse effects and healthful features all over the world[6].

Plants of the genus *Thermopsis* (Fabaceae) includes poisonous and harmful species with low feeding value. However, this genus has been used as a source of traditional oriental medicines and considered to be medicinal plants all over the world[7-9]. It belongs to Fabaceae family, including 25 species. *Thermopsis turcica* (*T. turcica*) (Kit Tan, Vural & Küçüködük) is an unique endemic species of this genus in Turkey. It spreads narrowly between Eber Lake and Akşehir Lake in inner part of West Anatolia of Turkey[10,11]. It is known as Eber Sarısı or Piyan[9]. Although it is categorized as critically endangered by the Red Data Book[12], the investigation of its biological activities might increase importance of *T. turcica* conservation in nature.

Thermopsis species have been constantly investigated in several areas. Total flavonoid contents of some *Thermopsis* species contain six flavonoid components: formononetin, chrysoeriol, apigenin, luteolin, thermopsoside and cynaroside. *Thermopsis* extracts could be considered potential hypolipidemic and antisclerotic agent[13-16]. *Thermopsis alterniflora* contains alkaloids, flavanoids, vitamin C, macro and microelements and its air-dried aerial part is used as a medicinal raw for a cytisine preparation[17].

There are limited investigations about *T. turcica* in literature. Studies of *T. turcica* concern with its antimicrobial activity, morphology and anatomy, mutagenic potential, determination of its propagation by using conventional and *in vitro* techniques and also investigation of its free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status[8,9,18-20].

The aim of this study was to investigate the antimicrobial activity of water, ethanol, methanol, *n*-hexane and ethyl acetate (EtAc) extracts of *T. turcica* against *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) and *Porphyromonas gingivalis* (*P. gingivalis*) as oral microaerophilic and anaerobic bacterial strains and evaluate the antioxidant potential and cytotoxic effects against human cancer [androgen-insensitive prostate cancer cells (DU145), androgen-sensitive prostate cancer cells (PC-3), promyelocytic leukemia (K-562), acute leukemia (HL60)] and human gingival fibroblast (HGF) cells.

2. Materials and methods

2.1. Chemicals

Chloroform, Folin-Ciocalteu's phenol reagent, ethanol, methanol and *n*-hexane were purchased from Merck (Darmstadt, Germany). 3-[4,5-Dimethylthiazole-2-yl]-2,5-diphenyltetrazoliumbromide (MTT), Tween 40, dimethylsulphoxide (DMSO), ethyl acetate (EtAc), ethylenediaminetetraacetic acid, β-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), gallic acid, 2,6-di-tert-butyl-4-methylphenol (BHT) and linoleic acid were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum were purchased from Gibco BRL (Gaithersburg, MD, USA). All other chemicals were analytical grade and obtained from either Sigma or Merck.

2.2. Plant material

T. turcica was collected from Afyon: Sultandağı, Dereçine, Kavaklı köyü, Köprülüöz, 965 m, near Akşehir Lake in Turkey during the flowering period in May 2011, 38°29′56″ N, 31°18′49″ E, Mecit Vural 10392 & Osman Tugay. Plant material was deposited at the herbarium of Gazi.

2.3. Preparation of Thermopsis extracts

The collected plants were dried at room temperature for 4-7 d. The dried plants (30 g) were ground into powder and extracted three times with 450 mL of ethanol, methanol, EtAc or water at ambient temperature for 24 h. The extracts were filtrated and concentrated to dryness with rotary evaporator at 40 °C. The dried extracts were stored in the dark at 4 °C until used.

2.4. Antimicrobial activity

2.4.1. Bacterial strains

A total of two microbial strains were used in the study. *A. actinomycetemcomitans* (ATCC 29523) and *P. gingivalis* (ATCC 33277) which were taken from microbiology laboratory of Gazi University, Faculty of Dentistry were used in the study. The strains were kept in Gazi University, Faculty of Dentistry, Medical Microbiology laboratory and cryopreserved at −86 °C. For experiments bacteria were inoculated onto Colombia agar (Merck, Germany) plates supplemented with hemine (5