SHORT SCIENTIFIC REPORT

Kvaratshkelia T., Jokhadze M., Mshvildadze V.,

Legault J., Kuchukhidze J.

Cytotoxic activity of the biological active compounds from the buds of *Populus tremula* L. growing in Georgia

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Populus tremula L. (*Salicaceae*) is tree growing in deciduous forests of Georgia. The genus *Populus* L. contains more than 40 species widespread in Europe and Asia. The species of *Populus* have a long history in traditional medicine, with uses in many areas. In traditional medicine is also used the bark of the members of Gen. *Populus*, which was assigned with astringent, anti-inflammatory, anti-rheumatic and antiseptic properties [1-7]. The medical applications of *Populus* buds' products are very extensive, because of a wide range of pharmacological and physiological actions [8]. Phytochemical investigations of *Populus* species have shown that they contain mainly polyphenolic compounds and terpenoids [9-11].

In the present paper we report the results of simultaneous determination of compounds extracted by solvents of different polarities from buds of *Populus tremula* and their *in vitro* cytotoxic activity.

Buds of *poplar* species were collected in April 2019 from trees growing in the village Kojori (Georgia). The buds (10 g) were extracted with 50 ml of n-hexane at the room temperature along 4 h under the periodic stirring. After this the solvent was evaporated, the raw materials were washed with 15 ml of n-hexane, and the solvent was concentrated from the combined extracts to a volume of 0.5 ml under vacuum on a rotor evaporator.

After extraction with hexane, the dry buds were extracted with 25 ml of Ethyl acetate and mixture was stored for 4 h. Obtained extract was filtered; the residue was washed with 10 ml of ether and evaporated to dryness under vacuum. To the dry residue 100 ìl of BSTFA with 1% TMS (Sigma-Aldrich) were added. The resulting solution was heated for 1 h at 60 ^{0}C.

Hexane extracts and solutions of TMS derivatives were analyzed by GC-MS (Agilent technologies 7890B, MS 5977A) instrument supplied with a HP-5ms Ultra Inert 30m x 0.25mm x 0.25μm fused silica capillary column.

Helium flow rate through the column was 1 ml min^{-1} with a 1:50 split. The injector temperature was 280 ^{0}C. Hexane and Ethyl acetate solutions were separated in the temperature programming regime from 60 to 280 ^{0}C at a rate 4^{0}C min^{-1}; 280 to 320 ^{0}C at a rate 5^{0}C min^{-1}.

Under the above conditions a mixture of C_8-C_{18} n-alkanes was separated, and their retention times were determined. Linear retention indices were calculated from the results of the chromatography of these mixtures and extracts, and after integration the fraction of each component in the total ion current (TIC) was calculated.

Identification of components

To identify the mixture components, mass spectral data and calculated retention indices (RI) were used. Mass spectrometric identification was carried out with the aid of an automated system, which formed a part of the instrumentation used. Identification consists in the comparison of mass spectra recorded during the analysis and those contained in the instrument library.

Retention indices of components of Hexane extract was compared with those reported by Adams (1995). The RI values for TMS were determined from the analysis of derivatives of authentic commercial preparations or taken from different papers (Tanaka and Hine, 1982; Tuchman et al., 1984; Lefevere et al., 1989; Greenaway and Whatley, 1991; Greenaway et al., 1992a, 1992b; English et al., 1992). When not less than three RI values are given for the same compound in different sources they were randomized.

Cell culture

The human lung carcinoma A549, colorectal adenocarcinoma DLD-1 and skin fibroblast WS1 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, USA). Cells lines were grown in minimum essential medium containing Earle's salts (Mediatech Cellgro Herndon, USA), supplemented with 10% fetal calf serum (Hyclone, Logan, USA), solution of vitamins, sodium pyruvate, nonessential amino acids, 100 IU of penicillin and 100 lg mL of streptomycin (Mediatech Cellgro). Cells were cultured at 37^0C in a humidified atmosphere containing 5% CO_2.

Cytotoxicity assay

Exponentially growing cells were plated at a density of 5 103 cells per well in 96-well microplates (BD Falcon) in culture medium (100 lL) and were allowed to adhere for 16 h before treatment. Then, cells were incubated for 48 h in the presence or absence of 100 lL of increasing concentrations of extract, fraction or pure compounds dissolved in culture medium and DMSO. The final concentration of DMSO in the culture medium was maintained at 0.25% (v/v) to avoid toxicity. Cytotoxicity was assessed using the resazurin reduction test [12]. Fluorescence was measured on an automated 96-well Fluoroskan Ascent Fl™ plate reader (Labsystems) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxicity was expressed as the concentration of drug inhibiting cell growth by 50% (IC50).

About 50 compounds present in hexane extract from buds of *P. tremula* in amounts of not less than 0.1% of TIC. The compounds belong to several groups.

The first group contains the aromatic compounds - benzyl alcohol, 2- phenylethanol, eugenol, 2-hydroxybenzaldehyde, methyl acetophenone, and ethyl benzoate. The second group is formed by sesquiterpene hydrocarbons and sesquiterpenoids. Among sesquiterpenoids in buds'

tertiary bicyclic alcohols with a structure of azulene type prevail. The third group is formed by esters of cinnamic acid.

The components detected in Ethyl acetate extract can also be divided into several groups. One of them consists of polyol. The main fraction of the Ethyl acetate extract consists of acidic compounds of aliphatic and aromatic series. The former is represented by saturated and unsaturated mono-, dicarboxylic and hydroxycarboxylic acids. Aromatic acids are represented by two groups of compounds. One of them includes benzoic, 4-hydroxybenzoic, and 4-hydroxyphenyl acetic acids. The other one is formed by cinnamic acid and its derivatives.

The cytotoxicity of the Hexane and Ethyl acetate extracts of the buds of *P. tremula* was evaluated against A549, DLD-1 and WS1 cell lines and the results are displayed in Table 1. Cytotoxic studies show, that the Hexane and Ethyl acetate

extracts of the buds of *P. tremula* grown in Georgia specific cytotoxic activity in cancer cells: A-549 (lung carcinoma), DLD-1 (intestinal adenocarcinoma), WS-1 (human fibroblasts).

Table. Cytotoxic activity of the Hexane and Ethyl acetate extracts of the buds of *P. tremula*

	Resazurine	Resazurine	Resazurine	Hoechst	Hoechst	Hoechst
Samples	**A-549**	**DLD-1**	**WS-1**	**A-549**	**DLD-1**	**WS-1**
Hexane extract	43±1 µg/ml	30±3 µg/ml	57±7 µg/ml	35±3 µg/ml	28±4 µg/ml	71±8 µg/ml
Ethyl acetate extract	103±10 µg/ml	81±2 µg/ml	138±11 µg/ml	67±9 µg/ml	50 ± 2 µg/ml	118±30 µg/ml
Etoposide	>50 µM	12±2 µM	>50 µM	18±µM	4,1±0,8 µM	>50 µM

The analysis of these data shows the phytochemical characteristics of the buds *P. tremula.* The extraction by a non-polar solvent made it possible to broaden greatly the list of components. Moreover, sample preparation for extraction with Hexane is much less laborious and reagents used for derivatization are not needed. The components can be divided into several groups. The first group consists of aromatic compounds. The second group is formed by sesquiterpene hydrocarbons and sesquiterpenoids.

The components detected in Ethyl acetate extracts can also be divided into several groups. One of them consists of polyols. The main fraction of the Ethyl acetate extract consists of acidic compounds of aliphatic and aromatic series. The former is represented by saturated and unsaturated mono-, dicarboxylic and hydroxycarboxylic acids.

Cytotoxic studies show, that the Hexane and Ethyl acetate extracts of the buds of *P. tremula* grown in Georgia have specific cytotoxic activity in cancer cells: A-549 (lung carcinoma), DLD-1 (intestinal adenocarcinoma), WS-1 (human fibroblasts).

References:

1. Stanescu, U.; Hancianu, M.; Cioanca, O.; Aprotosoaie, A.C.; Miron, A. Medicinal Plants From A to Z, revised and added, 3rd ed.; Polirom: Ia¸si, Romania, 2018; ISBN 978-973-46-7240-0.

2. Victor Kuete, Ean-Jeong Seo, Benjamin Krusche et al. Cytotoxicity and Pharmacogenomics of Medicinal Plants from Traditional Korean Medicine. Evid Based Complement Alternat Med. 2013; 2013: 341724

3. Scaysbrook T., Greenaway W., and Whatley F. R. (1992), Relation of "antimicrobial" compounds present in poplar bud exudates to disease resistance by poplars. Z. Naturforsch. 47c, 197-200.

4. Hancianu, M.; Stanescu, U.; Aprotosoaie, C. Pharmaceutical, Pharmacological and Clinical Bases of Phytotherapy; "Gr. T. Popa": Ia¸si, Romania, 2008; Volume 3, ISBN 978-973-7682-68-0.

5. Royer, M.; Houde, R.; Viano, Y.; Stevanovic, T. Non-wood Forest Products Based on Extractives-A New Opportunity for the Canadian Forest Industry Part 1: Hardwood Forest Species. J. Food Res. 2012, 1, 8.

6. Bouchard, M. Identification D'utilisations pour le Bouleau à Papier et Le peuplier Faux-Tremble; Enseignement Supérieur, Recherche, Science et Technologie: Quebec, QC, Canada, 2013; pp. 145.

7. Stanescu, U.; Hancianu, M.; Cioanca, O.; Aprotosoaie, A.C.; Miron, A. Medicinal Plants From A to Z, revised and added, 3rd ed.; Polirom: Ia¸si, Romania, 2018; ISBN 978-973-46-7240-0.

8. Chi, Y.S.; Jong, H.G.; Son, K.H.; Chang, H.W.; Kang, S.S.; Kim, H.P. Effects of naturally occurring prenylated flavonoids on enzymes metabolizing arachidonic acid: Cyclooxygenases and lipoxygenases. Biochem. Pharmacol. 2001, 62, 11851191.

9. Greenaway,W.; May, J.; Scaysbrook, T.; Whatley, F.R. Identification by gas chromatography-mass spectrometry of 150 compounds in propolis. Z. Nat. Sect. C J. Biosci. 1991, 46, 111121.

10. Greenaway, W. Compositions of Bud and Leaf Exudates of Some Populus Species Compared. Z. Nat. C 1992, 47, 329334.

11. Jerkovic, I.; Mastelic, J. Volatile compounds from leaf-buds of Populus nigra L. (Salicaceae). Phytochemistry 2003, 63, 109113

12. O'Brien, J., Wilson, I., Orton, T., Pognan, F., 2000. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur. J. Biochem. 267, 54215426

SUMMARY

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Populus tremula L. (*Salicaceae*) is a tree growing in the deciduous forests of Georgia. The aim of the study was the simultaneous determination of compounds extracted by solvents of different polarities from buds of *Populus tremula* and their *in vitro* cytotoxic activity. The components containing in Hexane extract consists of aromatic compounds, sesquiterpene hydrocarbons and sesquiterpenoids. The components containing ethyl acetate extract consist of polyols. The main fraction of the ethylacetate extract consists of acidic compounds of aliphatic and aromatic series. The former is represented by saturated and unsaturated mono-, dicarboxylic and hydroxycarboxylic acids. Cytotoxic studies show, that the Hexane and Ethyl acetate extracts of the buds of P. tremula grown in Georgia have specific cytotoxic activity in cancer cells: A-549 (lung carcinoma), DLD-1 (intestinal adenocarcinoma), WS-1 (human fibroblasts).