ANTIPLASMODIAL ACTIVITY OF POPULUS TREMULA GROWING IN GEORGIA

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Georgia is very rich botanically, with about 4400 species of vascular plants occurring in its 76,400 square kilometers. Of these, about 380 species, or 9%, are endemic to Georgia. Topographical and climatic conditions produce a mosaic of habitat types in Georgia. Genus Populus in the world is presented by 35 species [1]. Species of Populus studied till present, are distinguished with containment of polyphenol: phenolic acids, phenylpropanoids, flavonoids, flavons, chalcons, leikoantocyanidines, tannins [2].

On the basis of modern pharmacological investigations were attested the antioxidant, antiviral, fungicidal and anti-inflammatory activities of phenolic compounds contained in Populus species [3].

In spite of the global world program for the eradication of malaria, the countries of Asia, Africa and South America still are the active hotbeds of malaria, which cause the spreading of the disease all over the world. The international relations and process of migration help the disease to spread. Nowadays the imported malaria is the problem of many countries and the problem of Georgia too. During the last 5 years when hundreds of cases of malaria, caused by Plasmodium falciparum were fixed in Georgia, in the region near the Azerbaijan boundary, they paid much attention to the prophylactic and treatment of malaria. In many countries the treatment of malaria is complicated because in last years the existence of resistant Chloroquine and Mefloquine Plasmodiums was proved. A lot of substances were proposed for treatment of malaria, but very little of them had the action against the disease [4,5].

The object of the study of the present project is the research of antimalarial activity from Georgian flora.

MATERIAL AND METHODS

Plants materials. Buds of Populus tremula were collected in Georgia (Kojori) in April 2011 and identified by Dr. Tsiala Gviniaashvili, a botanist from the Institute of Botany. Voucher specimens N 9594 were deposited in the Herbarium at the Department of Pharmacognosy and Botany, Faculty of Pharmacy, Tbilisi State Medical University.

Preparation of extracts. Crude plant extracts were prepared by submitting 15-50g of air-dried powdered plant material to a sequential extraction procedure with 150-500 mL of n-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) for 48 h, at
room temperature. After filtration, the extracts were fully dried, under reduced pressure at 40-45 °C, by using a rotatory evaporator, and then stored at low temperature (4°C) until their use in antimalarial assays.

**High-performance Liquid Chromatography (HPLC).** Samples of the extracts (1 mg) were percolated through modified silica gel cartridges (Separsol SI C18) and the solvent was evaporated. The dry residues were dissolved in 1 ml of mobile phase and filtered through a 0.45 μm filter before injection. An Agilent 1290 HPLC system (Agilent Technologies) operated by Windows NT based ChemStation software was used. The HPLC equipment was used with a diode array detector (DAD). System consisted of a binary pump, degasser and auto sampler. The column used was a ZORBAX Eclipse C18 : 4.6 mm×250 mm, 5 μm equipped with a ZORBAX Guard Cartridge. The mobile phase consisted of two solvents: Solvent A, water/formic acid (95:5; v/v) and Solvent B, acetonitrile. Phenolic compounds were eluted under the following conditions: 0.5 mL/min flow rate and the temperature was set at 25 °C, isocratic conditions from 0 to 3 minute with 0% B, gradient conditions from 0% to 5% B in 12 min, from 5% to 15% B in 18 min, from 15% to 25% B in 24 min, from 25% to 50% B in 30 min, followed by washing and reconditioning the column. The ultra-violet-visible spectra (scanning from 200 nm to 900nm) were recorded for all peaks (Fig.1). The identification of phenolic compounds were obtained out by using authentic standards and by comparing the retention times and ultra-violet-visible spectra while quantification was performed by external calibration with standards.

**Figure 1**

Typical HPLC-RP Chromatogram of polyphenols in ethyl acetate extract from P. tremula

Antiplasmodial activity. Cultivation of parasites. Continuous *in vitro* culture of asexual erythrocytic stages of moderately chloroquine-resistant *Plasmodium falciparum* line from Colombia (FCB 1) was maintained at 37 °C and under an atmosphere of 5% CO₂, 5%O₂ and 90% N₂. The host cells were human red blood cells (A or O Rh+). The culture medium was constituted by RPMI supplemented by 32 mM NaHCO₃, 25mM HEPES, 1.67 g/l glucose, 30
mg/l antibiotics (streptomycin-penicillin) and 10% human pooled serum. Drug-sensitivity assays were carried out on 96-well micro-plates. The micro-plates were titrated with two-fold serial dilutions of each extract as well as the control. The strain was plated (in duplicate) using a synchronized culture at ring-stage parasites, with a parasitaemia between 0.6–0.8%. Each well received 10 µL of parasite-loaded erythrocytes, 5% haematocrit, and 90 µL of the different drug dilutions.

**Drug solution.** The compounds and extract (1 mg) were dissolved in 50 µl of dimethylsulfoxide (DMSO). 10 µl of this solution was diluted with 1ml medium to give a stock solution.

**RESULTS.**

By optimizing extraction, separation and measurement condition, we have achieved a reliable, reproducible and accurate methodology for quantitative determination of polyphenols from Populus buds. This methodology should meet the need for an implementation of quality control in Populus products.

Eight extracts from P.tremula were screened for their potential antimalarial properties against chloroquine-sensitive *P. falciparum* strain. The extracts were prepared by sequentially extracting the plant material with *n*-hexane, dichloromethane, ethyl acetate and methanol (see experimental section). The antimalarial activity of extracts was defined according to the IC50 values obtained. An extract showing an IC50 value ≤ 5 µg/ml was classified as highly active. Extracts with IC50 values ≥ 10 µg/ml and ≤ 50 µg/ml were considered moderately active and those with IC50 values > 50 µg/ml inactive.

The IC50 values of plants extracts shown in table 1 demonstrated that they possess an antimalarial activity.

Total phenolic content of different *P. tremula* fractions were solvent dependent. Aqueous fractions of *P. tremula* showed higher amounts of phenolics while their counterparts showed lower phenolic content. The content of total phenolics in aqueous fractions decreased in the order of ethyl acetate (418±5.4 mg/g) > methanol (303±5.4 mg/g) > DMC (46.4±3.1 mg/g) > *n*-hexane (22.0±1.5 mg/g) fraction.

**Table 1.**

*In vitro* antimalarial activity (IC50 values) of the plant extracts against *Plasmodium falciparum*

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Extract IC50 (µg/ ml)</th>
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Plant extracts showed moderate to good antiparasitic effects. Promising antiplasmodial activity was found in the extracts from flower buds, 50% inhibitory concentration (IC50) \(3.4\ \pm\ 0.9\ \mu g/ml\) (ethyl acetate extract), \(5.2\ \pm\ 1.2\ \mu g/ml\) (methanol extract). Moderate activity (20–35 \(\mu g/ml\)) was found in DMC and \(n\)-hexane extract. Cytotoxicity study with P. tremula flower and leaf bud extracts showed good therapeutic indices. These results demonstrate that flower and leaf ethyl acetate and methanol extracts of P. tremula may serve as antimalarial agents even in their crude form. The isolation of compounds from P. tremula to be of special interest for further antimalarial studies.

<table>
<thead>
<tr>
<th></th>
<th>(n)-hexane</th>
<th>DMC</th>
<th>EtOAc</th>
<th>MeOH</th>
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<tr>
<td>Populus tremula</td>
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<tr>
<td>(flower buds)</td>
<td>23.6 ± 3.6</td>
<td>22.1 ± 3.4</td>
<td>3.4 ± 0.9</td>
<td>5.2 ± 1.2</td>
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<tr>
<td>Populus tremula</td>
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<tr>
<td>(leaf buds)</td>
<td>28.7 ± 3.9</td>
<td>27.1 ± 3.8</td>
<td>5.7 ± 1.4</td>
<td>6.8 ± 1.5</td>
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</table>

REFERENCES
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SUMMARY

According to the WHO data up to 2 million people are at risk of malaria infection. Up to 3 million die by malaria every year. At the moment local case of malaria in Georgia are recorded in Kakheti and Lower Kartli.

An in vitro study of the polyphenols of a poplar (Popoulus tremula) showed high activity and a perspective for more chemical and pharmacological study. The antimalarial effect of different fractions of a poplar species (Popoulus tremula) widespread in Georgia, was in vitro studied on a chloroquine resistant strain of Plasmodium falciparum. The fractions were gathered by liquid-liquid extraction with solvents of different polarity. The polyphenol composition of these fractions was studied with HPLC. It was found that the object of the research has a strong antimalarial effect. The ethyl acetate and methanol extracts presented the highest effect, with there IC$_{50}$(µg/ml) being 3.4 ± 1.1 and 5.2 ± 1.3 respectively.

KEY WORDS: Populus tremula, antiplasmodial assay, polyphenols

ИЗУЧЕНИЕ ПРОТИВОПЛАЗМОДЕИВОГО ЭФФЕКТА РАСПРОСТРАНЕННОГО НА ТЕРРИТОРИИ ГРУЗИИ ТОПОЛЯ (POPULUS TREMULA)

Дж. Кучухидзе, М. Джохадзе, Т. Муртазашвили, Н. Кипиани, В. Кипиани

РЕЗЮМЕ

По данным ВОЗ до 2-x миллиардов жителей Земли находятся под риском заражения малярией. Около 3-x миллионов людей ежегодно погибают от малярии. На данный момент, местные случаи инфицирования малярией зарегистрированы в Грузии в основном в Кахетии и Нижней Картли.

Предварительное фармакологическое изучение in vitro полиfenолов распространенного в Грузии тополя (Popoulus tremula), показало высокую активность и перспективу для химического и фармакологического изучения.
Изучено in vitro противомалярийное действие различных фракций представителя грузинской флоры – тополя (Popouls tremula) на хлорохин резистентный штамм Plasmodium falciparum. Фракции получены жидкость-жидкостной экстракцией растворителями различной полярности. Методом ВЭЖХ изучен состав полифенольных фракций. Установлено, что объект изучения обладает высоким противомалярийным действием. Выраженным действием обладают этилацетатные и метанольные экстракты, IC\textsubscript{50} (µg/ml) которых составляет 3.4 ± 1.1 и 5.2 ± 1.3 соответственно.

**КЛЮЧЕВЫЕ СЛОВА:** популус тремулла, антиплазматический анализ, полифенолы

saqarTveloSi gavrcelebuli verxvis (popouls tremula) antiplazmodiuri moqmedebis Seswavla

\[ j. kuWuxiZe, m. joxaZe, T. murTazaSvili, n. yifiani, v. yifiani \]

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**sakvanZo sityvebi:** populus tremula, antiplazmodiuri analizi, polifenolebi